EXHIBIT 31

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NOVA SCOTIA AQUACULTURE REVIEW BOARD

Applications by KELLY COVE SALMON LTD. for a BOUNDARY AMENDMENT and TWO NEW MARINE FINFISH AQUACULTURE LICENSES and LEASES for the cultivation of ATLANTIC SALMON (Salmo salar) - AQ#1205x, AQ#1432, AQ#1433 in LIVERPOOL BAY, QUEENS COUNTY.

Affidavit of Jonathan W. Carr

Affirmed January 19, 2024.

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NSARB 2023-001

NOVA SCOTIA AQUACULTURE REVIEW BOARD

Applications by KELLY COVE SALMON LTD. for a BOUNDARY AMENDMENT and TWO NEW MARINE FINFISH AQUACULTURE LICENSES and LEASES for the cultivation of ATLANTIC SALMON (Salmo salar) - AQ#1205x, AQ#1432, AQ#1433 in LIVERPOOL BAY, QUEENS COUNTY.

Affidavit of Jonathan W. Carr

I, Jonathan Weldon Carr, of the Town of St. Andrews, in the Province of New Brunswick, AFFIRM AS FOLLOWS:

1. I have been asked to review and provide an expert opinion regarding impacts on wild Atlantic salmon that are likely to result from the approval of the application by Kelly Cove Salmon Ltd. ("**KCS**") for a boundary amendment to marine finfish licence and lease AQ#1205, and for new marine finfish licences and leases AQ#1432 and AQ#1433 (the "**Applications**") on behalf of the intervenor, Protect Liverpool Bay Association.

2. Together with Dr. Stephen Sutton and Heather Perry, I have co-authored a report detailing our analysis and conclusions regarding impacts of the Applications on wild Atlantic Salmon (the "**Report**"), which is attached to my affidavit as **Exhibit "A".**

3. I am the Vice President of Research and Environment at the Atlantic Salmon Federation ("**ASF**"), where I have been employed for almost 30 years. My qualifications as a subject matter expert on the protection, conservation and recovery of wild Atlantic salmon are set out in my Curriculum Vitae, attached as **Exhibit "B".** As outlined in my CV, I have specific expertise with respect to the impacts of open net pen salmon farming on wild Atlantic salmon populations.

4. My co-author Dr. Stephen Sutton's qualifications as a subject matter expert on wild Atlantic salmon are set out in his CV attached as **Exhibit "C"**, which he provided to me and which I believe is true and accurate. Dr. Sutton is the Director of Public Policy at ASF and has held that position since 2015.

5. My co-author Heather Perry's qualifications are set out in her CV attached as **Exhibit "D",** which she provided to me and which I believe is true and accurate. Ms. Perry is a biologist with ASF and has held that position since 2021.

6. The Report represents my objective opinion with respect to the likely impacts on wild Atlantic salmon resulting from KCS's Applications. I have exercised my professional judgement to the best of my training, knowledge and ability regarding the data, analysis and conclusions set out in the attached Report. The Report includes all data that is relevant to my expert opinion and highlights any information that could reasonably lead to a different conclusion. I am prepared to testify before the Aquaculture Review Board, comply with the Board's directions, and apply independent judgement when assisting the Board.

7. I affirm this affidavit in support of the Report and in support of Protect Liverpool Bay Association's intervention before the Aquaculture Review Board and for no other or improper purpose.

Affirmed before me on this)
19 day of January, 2024)
at St. Andrews, New Brunswick)
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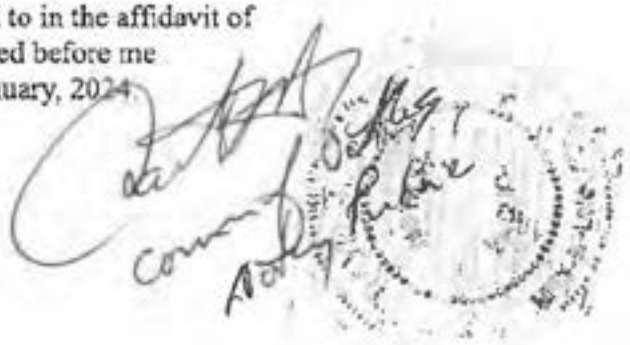
A Commissioner of Oaths in and for the) Jonathan W. Carr
Province of New Brunswick)

David R. Ames K.C.
Solicitor & Commissioner of Oaths
Notary Public

This is **Exhibit "A"** referred to in the affidavit of
Jon W. Carr, affirmed before me
this 19th day of January, 2024



Report for the Aquaculture Review Board

Respecting an application by Kelly Cove Salmon Ltd. for an amendment to finfish licence and lease AQ #1205 and for new licences and leases #AQ 1432 and #AQ 1433 in Liverpool Bay, Nova Scotia

Jonathan Carr, M. Sc.

Stephen Sutton, Ph. D.

Heather Perry, B. Sc.

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Scope of the Report

We have been asked by the intervenor Protect Liverpool Bay Association to review and provide an expert opinion regarding impacts on wild Atlantic salmon resulting from the application by Kelly Cove Salmon Ltd. for two new marine finfish licences and leases at Brooklyn (#1433) and Mersey Point (#1432) as well as an expansion of the lease and licence at Coffin Island (#1205) in Liverpool Bay. We have reviewed the Application Package, Report on Outcomes of Consultation, and Report on Performance Review for the application, as well as the draft chapter on the Medway River watershed produced by the Nova Scotia Salmon Association and exhibited to the affidavit of Dr. Edmund Halfyard.

In this document, we limit our opinion to the following questions:

1. What impacts, if any, will the proposed sites have on the survival of wild Atlantic salmon?;
2. Will the proposed expansion impede wild Atlantic salmon recovery efforts?; and
3. Are there steps the applicant could take to avoid or mitigate impacts on wild salmon in the event the application is approved?

As such, we focus primarily on the information provided in Section 7 of the Application (The Sustainability of Wild Salmon), the section of the Report on Consultation containing the DFO Letter of Advice and CSAS Science Report 2021/nnn,the Report on Performance Review and the affidavit of Dr. Edmund Halfyard.

Summary of Findings

Based on our review of these materials we offer the following opinions:

1. In the absence of effective protective measures, the proposed expansion will likely impede the recovery of wild Atlantic salmon. No evidence or rationale is provided to establish that proposed mitigation measures will effectively protect wild salmon from the negative impacts acknowledged by the applicant, the DFO and the scientific literature.

2. Based on available information, our expert opinion is that the existing farm #1205 has likely had a negative impact on wild Atlantic salmon, and the expansion of #1205 and new sites #1432 and #1433 will greatly increase the likelihood and magnitude of those impacts. In combination with existing documented threats, this significant expansion of open net pen salmon farms in Liverpool Bay will likely lead to the extirpation critically endangered salmon in the rivers in closest proximity to Liverpool Bay, including the Medway, Petite, and LaHave Rivers.

3. If the application is approved, there are several actions the applicant should take to avoid, mitigate, and monitor the impacts on wild salmon. The use of sterile fish, monitoring of local rivers for escapes and genetic introgression, monitoring of wild salmon for increased sea lice and disease loads, and triggers for responses to sea lice and disease outbreaks that are specifically designed to protect wild salmon. Even if these mitigation measures are required and implemented, the proposed expansion would still pose a serious threat to wild salmon.

We arrived at these conclusions based upon the following:

1. **A substantial body of peer-reviewed scientific literature demonstrates the impacts of open net pen salmon aquaculture on wild salmon.**

The applicant, the federal Department of Fisheries and Oceans (DFO), and the Nova Scotia Department of Fisheries and Aquaculture (NSDFA) have all recognized the threats to wild Atlantic salmon posed by salmon aquaculture. In Appendix 1 we provide a brief review of the relevant literature which demonstrates at least five pathways through which aquaculture impacts wild salmon: 1) Farmed salmon escape and interbreed with wild salmon; 2) Sea lice proliferate in salmon farms and are transmitted to wild fish; 3) Salmon farms and escaped fish have negative ecological interactions with wild salmon; 4) Diseases and pathogens proliferate in salmon farms and are transmitted to wild fish; and 5) Salmon farms alter the local environment thereby changing the selective pressures to which locally-adapted wild populations are subject. As noted in Appendix 1, numerous studies have directly linked these impacts to declines in the abundance of wild salmon. The literature also indicates that the presence and magnitude of these impacts can vary from location to location depending on a range of environmental variables, farm characteristics, and farming practices. The magnitude of impacts on wild populations is related to the biomass of farmed salmon in net-pens, the distance from net-pens to rivers and the size of wild populations (Keyser et al. 2018, Tab 20; DFO 2021; Diserud et al. 2022).

While the literature cannot be used to draw definite conclusions about the impacts of specific sites such as AQ #1205x and those proposed at Mersey Point (AQ#1433) and Brooklyn (AQ# 1432), it does strongly suggest that impacts on wild salmon are typical when domesticated salmon are farmed in open net pens in proximity to wild populations.

The scale of this expansion is expected to considerably increase the likelihood and magnitude of negative impacts to wild salmon, given the critically low abundance of the Southern Uplands populations, which include all salmon in mainland Nova Scotia, and are in closest proximity to Liverpool Bay (DFO 2021, Diserud et al. 2022). None of the materials we reviewed from the applicant or DFA contained any empirical evidence speaking to the impact of aquaculture operations in Liverpool Bay on wild salmon. In the absence of such evidence, the precautionary principle dictates that we must follow the large and increasing volume of science that demonstrates with certainty the negative impacts of salmon aquaculture on wild salmon.

2. **Aquaculture has been implicated in the decline of salmon in the Bay of Fundy and Southern Uplands of Nova Scotia.**

As noted by the applicant, wild salmon in the Bay of Fundy and Southern Uplands region of Nova Scotia have declined significantly and have been assessed as Endangered by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC), meaning they face imminent extirpation or extinction. The Inner Bay of Fundy populations have been listed as such under the federal Species at Risk Act (SARA) while listing decisions are pending for the Outer Bay of Fundy and Southern Uplands populations. The primary underlying cause of these declines is a decrease in marine survival due to changed ocean conditions which has affected wild Atlantic salmon across their range (Thorstad et al. 2021, Tab 30). On Canada's east coast, observed declines in areas where salmon aquaculture is present are an order of magnitude greater than observed declines in areas where aquaculture is absent, suggesting that wild

salmon populations already made vulnerable by low marine survival are unable to cope with the additional stressors imposed by the impacts of aquaculture (Ford and Myers 2008). In all areas of eastern Canada where aquaculture and wild salmon co-occur, wild salmon populations have been assessed by COSEWIC as Endangered or Threatened (including South Newfoundland and Eastern Cape Breton). Sufficient research has not been conducted to estimate the magnitude of the impact of aquaculture on wild salmon throughout eastern Canada. However, Fisheries and Oceans Canada recognizes open net pen salmon aquaculture as a threat to wild salmon in all areas where it occurs, including the Inner and Outer Bay of Fundy and Southern Uplands of Nova Scotia (DFO 2008, p.34; DFO 2013a, p.40 (Tab 10); DFO 2013b, p.20; DFO 2014a, p.17; DFO 2014b, p.25).

3. In the absence of information to demonstrate otherwise, the applicant's proposed projects in Liverpool Bay will most likely have significant adverse impacts on the survival and recovery of Atlantic salmon.

Based on the information reviewed in 1 and 2 above and in Appendix 1, it is clear that the proposed expansion of salmon aquaculture in Liverpool Bay poses a significant risk to wild salmon, particularly on those from the Medway River that are likely to have the greatest exposure to the open net-pens. No information has been provided to indicate whether impacts of the existing AQ #1205 site on wild salmon have been considered to assess threats posed by the proposed expansion. Information required to make such an assessment would include: numbers of escapes annually, sea lice counts in the farm and on wild fish, records of disease outbreaks, surveys of local rivers for escapes, and testing of wild populations for genetic introgression. It is our opinion that if the project proceeds as described there will be significant adverse impacts on surrounding wild salmon populations and ongoing recovery efforts leading to further declines and increasing the risk that those populations will become extirpated.

We note that the applicant has described minimal risk to wild salmon in relation to the proposed expansion. However, important information is omitted from their discussion. We outline our concerns about the information provided by the proponent in the paragraphs below.

a. Identification of vulnerable salmon populations is incomplete.

The applicant provides a general overview of the status of wild salmon in the Southern Uplands populations but does not identify all populations of salmon that inhabit waters in proximity to the proposed new and expanded sites at some point of their life cycle. Region-wide electrofishing surveys captured juvenile salmon the Medway, Petite and LaHave Rivers, all within 40km or less of Liverpool Bay. Near-shore habitat along the East coast of Nova Scotia supports the growth and maturation of wild salmon and effectively serves as a critical migration corridor to migrant salmon from the Bay of Fundy, Gulf of Maine and Southern Uplands salmon populations (Hubley et al 2008, Tab 17; Lacroix and Bradford 2013, Tab 22; DFO 2021). Lacroix and Bradford (2013, Tab 22) emphasize the need to protect that habitat from aquaculture developments as part of the recovery strategy for the endangered populations.

b. The proponent has not sufficiently assessed the impacts of the project on ongoing recovery efforts.

The discussion of restoration efforts in Section 7.2.2 is centered around a stocking program that KCS has supported in the Bay of Fundy, and that has the potential to be expanded to a similar effort on the

Medway River. The program grows salmon smolt in open net-pens to the adult stage before releasing them back into the wild, to bolster wild populations. There is no evidence from their existing program that wild salmon production has increased due to their efforts (Roth 2023). A substantial body of scientific evidence shows that stocking programs are more likely to harm wild salmon recovery than help (DFO 2018, McMillan et al 2023, Tab 23). In cases where hatchery supplementation or support for wild salmon stock recovery is considered, it should only be used as a tool in combination with other restorative efforts, and only as a temporary measure until stock recovery is achieved (Carr et al. 2015, Tab 8). It is unclear whether this program has been initiated on the Medway, however it would not address impacts to wild salmon from the proposed new and expanded aquaculture sites in Liverpool Bay.

The applicant references critically low abundances of salmon in many Southern Upland populations and briefly acknowledges active restoration projects on several rivers in the Southern Upland region, though no information is provided about the nature or outcomes of those efforts and how they will be affected by the projects. Their discussion of major threats is limited to river acidification, which is one of several threats to these populations including habitat fragmentation and degradation, pollution, aquaculture interactions and poor marine survival, none of which preclude the recovery of those populations (COSEWIC 2011).

The mean annual pH of 5.0 on the Mersey River does not indicate its potential for recovery. The applicant uses no scientific literature to support the claim that pH 5.0 is the lethal to Atlantic salmon. Gjedrem & Rosseland (2011, Tab 14) identified a critical pH of 4.7 for North American salmon, and juveniles show behavioural avoidance of river stretches with low pH during periods of high acidification (Kroglund et al 2008). Laudon et al. (2002), who reported the mean annual pH of 5.0 on the Mersey River, also acknowledged a rapid improvement of anthropogenically driven acidification following the curtailment of SO2 emissions. Several studies have demonstrated the benefits of limestone applications in restoring river pH to conserve local populations and their distinct genetics which, in Nova Scotian populations, have adapted a tolerance to low pH (Watt 1986; Gjedrem & Rosseland 2011, Tab 14).

Intensified aquaculture in Liverpool Bay would very likely impede the success of any future restoration of wild salmon to the Mersey River, where salmon would be required to travel within meters of the open net-pens at Mersey Point and Brooklyn to migrate in and out of the river (DFO 2021). Despite acidification, many Southern Upland rivers continue to produce and support wild salmon. For instance, in the Medway River, referenced in Section 7.2.2 of the application, the average watershed pH is 4.81, with 79/105 spatial habitat units ranked as ‘Marginal habitat’ for Atlantic salmon with a pH between 4.8-5.29, and a wild population still exists in that river (Affidavit of Dr. Edmund Halfyard). The applicant provided a smolt wheel that collected wild salmon smolt from the Medway River between 2021 and 2023.

The Doelle-Lahey panel stated that the need for better management of other threats such as river acidification and marine mortality does not justify the escalation of threats posed by salmon aquaculture (Doelle & Lahey 2014). Rather, stress induced by acidified river water act cumulatively with stress experienced in the marine environment, resulting in reduced tolerance to sea lice and pathogens for smolt passing aquaculture farms; therefore, intensifying aquaculture operations is likely to further reduce marine survival (Finstad et al. 2007; Krkosek et al. 2007; Krkosek et al. 2013).

c. *The applicant has used very limited information in discussing the potential impacts to wild salmon populations.*

As discussed in Appendix 1, there is a substantial body of peer-reviewed scientific evidence demonstrating

at least five pathways of impact that need to be considered. The discussion in Section 7.2.1 of the Application references none of that literature and is therefore inadequate to inform the reader about potential impacts or to demonstrate that the applicant adequately understands the potential for the farm to impact wild salmon. The discussion about potential impacts does not provide an adequate basis for developing avoidance, mitigation, or monitoring strategies.

KCS and DFO identify priority objectives to reduce the risk of impacts of salmon aquaculture on wild salmon populations which included improved containment and fish health management, and the use of local stocks. These mitigation measures are developed based on advice provided by DFO in 1999 and updated in 2008, which predates most of the science that has been conducted on wild salmon-aquaculture interactions (as outlined in Appendix 1) and therefore does not incorporate up-to-date information and best practices.

d. The applicant's proposed mitigation efforts are not based on current "best practices."

The mitigation efforts proposed by the applicant and any new or revised Farm Management are not based on more recent best-practice guidelines (e.g., the "Guidance on Best Management Practices to address impacts of sea lice and escaped farmed salmon on wild salmon stocks" developed by NASCO and the International Salmon Farmers Association in 2010 (NASCO 2010, Tab 26) or the standards developed by the Aquaculture Stewardship Council (ASC 2019, Tab 1) (see point 5 below). In any event, best practices would not eliminate the risks of open net pens to wild salmon survival and recovery.

Regardless, the mitigation measures that are actually proposed for the new sites as referenced in Section 7.2.3.1 to Section 7.2.3.4 , fail to demonstrate the implementation of any of the priority mitigation measures they identified and are thus insufficient to demonstrate the reduction of risk to wild salmon populations in the area.

e. Density-dependent proliferation of sea lice and pathogens in open net-pens pose significant threats to the survival and recovery of wild Atlantic salmon.

Concerns for increased exposure of wild salmon to sea lice from open net-pen farms are dismissed on the premise that current sea lice levels are below treatment thresholds. However, a review of self-reported sea lice counts in Canada found significant increases during months audited by the Department of Fisheries and Oceans, which suggests that sea lice levels are frequently underreported (Godwin et al 2021).

Current low levels of sea lice in Nova Scotia aquaculture farms do not predict future levels as farmed salmon production increases and other influential factors change (Doelle and Lahey 2014; DFO 2021). It was noted by both the DFO and the Doelle-Lahey Panel that current low prevalence of sea lice is at least in part due to the relatively limited scale and wider distribution of the aquaculture industry in Nova Scotia and the risk of sea lice may increase and become difficult to control if salmon farming intensifies (Doelle & Lahey 2014; DFO 2021). The unnatural concentrations of salmon in net-pens creates a reservoir for parasites and pathogens to proliferate, and elevated levels of sea lice can be detected on wild salmon up to 30km from an open net pen (Thorstad et al. 2015, Tab 29). Increased exposure to sea lice in areas where salmon aquaculture occurs has been demonstrated to significantly reduce marine survival of wild salmon by as much as 39% (Krkosek et al. 2007; Thorstad et al. 2015, Tab 29; ICES 2016, Tab 18; Bohn et al. 2020; Dempster et al. 2021, Tab 9; Johnsen et al. 2021).

The statement that farmed salmon are more vulnerable to Infectious Salmon Anemia (ISA) than wild

salmon neglects valid concerns about the threat of pathogen transfer between wild and farmed salmon. The likelihood of transmission between wild and farmed salmon depends on a number of factors such as hydrographic regimes, migration routes of wild salmon, and shedding rates (Bakke and Harris 1998, Tab 3; Krkosek et al. 2017). Farmed salmon may be more susceptible to infection given the densities at which they are kept, and sublethal effects on wild salmon may be more detrimental to wild salmon given the impacts to fitness and their ability to navigate environmental pressures in the wild (Ibieta et al. 2011; Miller et al. 2014). Quantification of pathogen levels in wild salmon is complicated by premature mortality before sampling (Bakke and Harris 1998, Tab 3). Many pathogens persist at low levels in the wild, however their potential to become significant threats is magnified when they proliferate in densely stocked salmon cages and disseminate to wild salmon along their marine migrations (Krkosek 2006; Miller et al. 2014, Tab 24; Bouwmeester et al. 2021, Tab 5). The rate of infectious disease spread is one to two orders of magnitude faster in aquatic environments compared to on land, particularly when multiple reservoirs of hosts exist within an area (McCallum et al. 2003; Miller et al. 2014, Tab 24). The intensification and expansion of open net-pen aquaculture coupled with changing sea conditions elevate risks of pathogen emergence and transfer between farmed and wild Atlantic salmon (Krkosek et al. 2006; Cohen 2012; Miler et al. 2014; Bouwmeester et al. 2021, Tab 5).

f. The potential for escapes and harmful genetic introgression is significant and has not been adequately assessed.

Research has shown that when salmon are reared in open nets, escapes will occur. Even with the strongest containment and management plans it is inevitable that fish will escape (i.e. because of containment failure or human error). DFO acknowledges that escapes of Atlantic salmon from finfish aquaculture occur regularly in Atlantic Canada and the true number of escapees are estimated to significantly exceed the number reported (DFO 2021).

The report on performance review for site AQ #1205 notes a single suspected escape event, but provides no information about the suspected number of escapees or their fate. Response and contingency plans are limited to efforts to repair the source of the breach, and no attention is given to mitigating post-escape impacts to endangered wild salmon populations such as using sterile fish and monitoring local rivers for escapes and genetic introgression.

The state of Maine has one of the most stringent containment management plans and auditing systems in place, yet salmon still escape from the Cooke-owned open net pen farms in that region. The proposed Liverpool Bay expansion would be larger than Cooke's salmon production in Maine. In Maine, some escapes have not been reported (escaped unnoticed), but have been traced back to the site of origin due to the legislated reporting and tracking mechanisms in place. This is the only genetic marking program that exists where regulations stipulate that salmon must be traced to the 'cage site' or origin (not just to the company). The infrastructure and containment measures outlined in Section 7.2.3.1 and 7.2.3.2 of the Application materials do not demonstrate any improvements that have been made to infrastructure that would prevent the escapes that continue to occur from existing containment infrastructure for net pens in Maine. Therefore, it is highly likely that escapes will occur from the Liverpool Bay net pen operations with the proposed mitigative measures. Based on research elsewhere (including the example from Maine), it is highly likely that salmon escapes have gone unnoticed at the AQ#1205 site in the past.

The situation on the Magaguadavic River provides another relevant example. There has been a monitoring program in place at the fish ladder on the St. George hydropower dam near the mouth of this river since

1992. Varying levels of escapes have been reported at this facility every year since 1992, with numbers ranging from 3 to 1200 per annum (Jon Carr email Sept 11 2023, Tab 1). Most of the escapees documented in that river were from unreported escape events from aquaculture sites. Research on the Magaguadavic points to salmon aquaculture as being a primary source for the extirpation of wild salmon in that river due to interbreeding (Bourrett et al. 2011, Tab 4).

DFO has acknowledged that escaped salmon can enter rivers 200-300km from their source and that escapees from farms in Liverpool Bay may interbreed with salmon from any of the Southern Upland rivers (DFO 2021. As such, salmon that escape from farms in Liverpool Bay pose a considerable threat to the locally adapted genetics in all critically endangered Southern Upland populations (Gibson et al. 2011,Tab 15; Bourrett et al. 2011,Tab 4; DFO 2021).

4. The existing regulatory framework is insufficient to protect wild Atlantic salmon from the impacts of aquaculture.

As demonstrated by our review of the relevant literature (Appendix 1), significant impacts of salmon aquaculture on wild salmon have been documented throughout the North Atlantic, including eastern Canada. In all jurisdictions where impacts have been demonstrated, the aquaculture industry is heavily regulated. In some jurisdictions, regulations provide equal or better protection to wild salmon than those in place in Nova Scotia (Anon. 2016). For example, Norway boasts one of the most stringent sea lice management programs which sets aquaculture production limits based on monitored levels of sea lice from salmon farms on wild salmon. Yet, the impacts of sea lice on wild salmon remain a major concern in Norway (Einum et al. 2023, Tab 12). In the Bay of Fundy, despite the existence of farm management plans, codes of containment, and escape reporting requirements, farm escapees continue to be detected annually at the monitoring facility on the Magaguadavic River. Likewise, despite strict federal and provincial regulation on the importation of foreign genetic strains of salmon, genes from European salmon have recently been detected in the Inner Bay of Fundy live gene bank program. This genetic material is believed to have come from illegal importation of European salmon by the aquaculture industry (DFO 2018; O'Reilly et al. 2018). Significantly increased sea lice counts during months audited by the DFO highlight weaknesses in environmental policies and enforcement that incentivize inaccurate reporting (Godwin et al 2021). While the applicant will be required to comply with all applicable provincial and federal regulations, this does not prove that the farm will have no impact on wild salmon or salmon restoration efforts, or that any impacts will be limited to acceptable levels.

5. In the event the applications are approved, there are several actions the applicant should take to monitor and reduce impacts of the farm on wild Atlantic salmon, though no suite of mitigation measures can eliminate those risks aside from restricting open net-pen development.

The North Atlantic Salmon Conservation Organization (NASCO) is an intergovernmental organization established by international convention in 1984 with Canada as a founding member. NASCO's objective is to conserve, restore, enhance and rationally manage Atlantic salmon though international co-operation, taking account of best available scientific information. NASCO and its Parties (including Canada) recognize the impacts of aquaculture on wild salmon and the need to take effective action to avoid and mitigate these impacts (NASCO 2020b). In 2010 NASCO, in collaboration with the International Salmon Farmers Association (ISFA), agreed to goals for protecting wild salmon from aquaculture and developed a series of best management practices to guide government and industry efforts to address

impacts of aquaculture on wild stocks. The agreed goals are:

a. 100% of farms to have effective sea lice management such that there is no increase in sea lice loads or lice-induced mortality of wild salmonids attributable to the farms; and
b. 100% farmed fish to be retained in all production facilities.

NASCO's Williamsburg Resolution (NASCO 2006, Tab 25) and Guidance on Best Management Practices (NASCO 2010, Tab 26) documents outline a range of actions that should be taken to protect wild salmon e.g., the use of sterile fish, mandatory reporting of all escapes, monitoring of local rivers for escapes and genetic introgression, monitoring of wild salmon for increased sea lice loads, and triggers for responses to sea lice and disease outbreaks that are specifically designed to protect wild salmon. NASCO recently reviewed Canada's efforts to implement the Best Management Practices and meet the agreed goals. Their review concluded that Canada has made no progress towards meeting these goals and that Canada has proposed no acceptable management actions to address the issues of escapes and sea lice for the 2020-2024 period (NASCO 2020a).

Likewise, the Aquaculture Stewardship Council's Salmon Standard outlines a management framework to address the key negative environmental and social impacts associated with the salmon aquaculture industry, including the health and genetic integrity of wild salmon populations e.g., maximum sea lice loads within farms during sensitive periods for wild fish, monitoring of sea lice levels on wild salmon, and capping the number of escapes permitted. The Standard also requires that farm operators have an evidence-based understanding of salmonid migration routes, migration timing and stock productivity in major waterways within 50 kilometres of the farm (ASC 2019, p.24, Tab 2).

In order to protect wild salmon from the impacts of expanded aquaculture operations in Liverpool Bay, if the proposed expansion at AQ#1205, and new farms at Mersey Point and Brooklyn are approved, the applicant should be required to implement the NASCO/ISFA Best Management Practices and ASC standards necessary to meet the goals for sea lice and escapes as outlined above and agreed by Canada in order to mitigate the likely impacts of its operations on wild salmon populations in the area.

Summary

In our expert opinion, the proposed expansion at AQ#1205 as well as new sites at Mersey Point (AQ# 1432) and Brooklyn (AQ#1433) will elevate existing pressures on critically endangered local wild Atlantic salmon populations and significantly impair their survival and recovery, likely leading to their extirpation in rivers such as the Medway, Petite and Lahave found closest to Liverpool Bay. These likely impacts have also been recognized by the DFO (DFO 2021), and the information provided by the applicant is not sufficient to establish otherwise. Application of all relevant federal and provincial regulations will not likely mitigate these impacts in the absence of additional conditions designed specifically to protect wild salmon. If the expansion is approved the applicant should be required to take additional steps to reduce its negative impacts to wild salmon such as the use of sterile fish, monitoring of local rivers for escapes and genetic introgression, monitoring of wild salmon for increased sea lice and disease loads, and triggers for responses to sea lice and disease outbreaks that are specifically designed to protect wild salmon, as outlined in detailed in the NASCO/ISFA Best Management Practices and ASC standards. It is important to emphasize that implementing those best practices is crucial to improve protection of wild salmon within the existing regulatory framework, however no commercial-scale open net-pen aquaculture operation can be approved in Liverpool Bay without significantly threatening the survival and recovery of the critically endangered wild Atlantic salmon with which the operations directly or indirectly interact.

Appendix 1

Overview of the Impacts of Salmon Farms on Wild Salmon Populations

Growing domesticated salmon in sea cages in areas where there are wild Atlantic salmon invariably has negative impacts on local wild populations. These negative impacts have been well established by scientific studies (ICES 2016, Tab 18; Hutchinson 2006; Ford and Myers 2008, Tab 13; DFO 2013a, Tab 10). Salmon farms have been shown to impact wild Atlantic salmon populations in several ways which are briefly summarized here:

- **Farmed salmon escape and interbreed with wild populations**. Farmed Atlantic salmon have been selectively bred to improve commercially important traits (i.e. growth, feed utilization, filet quality) which results in them being poorly adapted to the natural environment (Solberg et al. 2013; Wacker et al. 2021, Tab 31). When farmed salmon escape and interbreed with wild salmon, the resulting offspring are genetically inferior to wild salmon and are therefore less fit for life in the wild (Fleming et al. 2000; McGinnity et al. 2003; Bourrett et al. 2011, Tab 4; DFO 2013b, Crowley et al., 2022).

  Escaped farmed salmon have been observed in rivers in all regions where salmon farming occurs (Morris et al. 2008; Thorstad et al. 2008) and maturity rates for those escapees can be over 50% (Madhun et al., 2023). Some estimates suggest the annual number of escapes from salmon farms in the North Atlantic may outnumber the total population of adult wild Atlantic salmon (Glover et al. 2017, Tab 16). Large-scale studies in Norway (Glover et al. 2013; Karlsson et al. 2016, Tab 19) and Canada (Wringe et al. 2018, Tab 32; Bradbury et al. 2020a, Tab 6) have demonstrated the significant extent to which interbreeding can occur when salmon farming overlaps with wild populations. Introgression occurs and results not only in the F1 generation, that is the offspring from wild x farmed salmon parents, but in subsequent generations where backcrossing occurs, where those F1 offspring contribute again to the next generation (Holborn et al., 2022).

  The viability and recovery of wild Atlantic salmon populations is threatened by the introduction of genetic material (i.e., genetic introgression) from farmed fish (Glover et al. 2020; Wacker et al. 2021, Tab 31). Smaller wild salmon populations in areas of intense aquaculture are particularly at risk due to the higher proportion of escapee farmed fish (Diserud et al. 2022, Tab 11) and introgression is likely to continue in the future under current practices (Glover et al., 2020). Long-term population level consequences of introgression include erosion of genetic diversity, reduced productivity, decreased resilience, and declining abundance (Hindar et al. 2006; Glover et al. 2017, Tab 7; Skaala et al. 2012, 2019; Sylvester et al. 2019, Tab 28). Several studies have demonstrated a decrease in the total productivity of wild salmon following introgression of farmed salmon genes (Fleming et al. 2000; McGinnity et al. 1997; McGinnity et al. 2003; Wacker et al. 2021, Tab 31).

- **Sea lice proliferate in salmon farms and are transmitted to wild fish.** Sea lice are a naturally occurring parasite on wild Atlantic salmon. When farmed salmon are stocked into open net pens they pick up sea lice from the environment which leads to frequent infestations and outbreaks within the farm. Farmed salmon then act as a reservoir for the majority portion of the louse population (Dempster et al., 2021, Tab 9). This increases the abundance of sea lice in the local area which has been demonstrated to increase the abundance of lice on wild salmon (Frazer 2009) and to increase mortality (especially of smolts) in wild populations (Bohn et al., 2020, Krkosek et al., 2007; Thorstad et al. 2015, Tab 29; ICES 2016, Tab 18). Smolt mortality due to farmed salmon originating lice has been estimated at over 30% in some Norwegian rivers (Johnsen et al., 2021)

  Numerous studies have demonstrated a link between salmon aquaculture and sea lice infestations on wild salmonids (Helland et al. 2012, 2015; Middlemas et al., 2010, 2013; Serra- Llinares et al. 2014). Elevated levels of sea lice on wild salmonids have been found up to 30km from salmon

farms (Thorstad et al. 2015, Tab 29). Smolt mortality attributable to salmon lice has been demonstrated to result in a significant reduction in adult returns (Shepherd and Gargan 2017, Tab 27) and to influence the achievement of conservation requirements for affected stocks (Gargan et al. 2012, Krkošek et al. 2013; Shepherd and Gargan 2017, Tab 27). Sea lice infestation also imposes sub-lethal physiological impacts, including reduced swimming speed (Wagner et al., 2003), osmoregulatory failure (Grimnes and Jakobsen, 1996;), increased sensitivity to ocean warming (Shepard and Gragan, 2020), and slower post-smolt growth (Skilbrei and Wennevik 2006; Skilbrei et al. 2013)

- **Salmon farms and escaped fish have negative ecological interactions with wild salmon.** These interactions include interfering with mating and competition for food and space (Naylor et al. 2005) and escapees spreading parasites and diseases to wild fish (Naylor et al. 2005; Krkosek et al., 2006; Krkosek et al., 2007). These interactions can lead to changes in productivity of native salmon populations through processes affecting growth and survival (Lacroix and Fleming, 1998; Hindar and Fleming, 2007). These interactions and the frequency of interactions will depend on characteristics specific to individual rivers, such as wild population numbers and discharge (Mahlum et al., 2020)

- **Diseases and pathogens proliferate in salmon farms and are transmitted to wild fish.** The Atlantic salmon farming industry has the capacity to play a central role in transportation and transmission of pathogens to wild salmon (Garseth et al. 2013). Transmission of pathogens and diseases from aquaculture to wild fish can occur through populations that are infected at the hatchery source, through infected escapees, and through wild fish migrating or moving within plumes of an infected pen or disease outbreak (Bateman et al., 2022, Madhun et al. 2015; Naylor et al. 2005; Johnsen and Jensen 1994). There is a continual emergence of viruses in net-pen salmon aquaculture (Kibenge 2019, Tab 21; Teffer et al. 2020) prompting increasing concern about the impacts of these diseases on wild Atlantic salmon populations and other marine wildlife (Bouwmeester et al. 2021, Tab 5).

- **Salmon farms alter the local environment thereby changing the selective pressures to which locally-adapted wild populations are subject.** Changes in selective pressures can lead to decreased survival, reductions in population size, increased genetic drift, and a lowering of long-term adaptive capacity in wild populations (Ferguson et al. 2007; Verspoor et al. 2015; DFO 2013b). Bradbury et al. (2020b, Tab 7) identified several examples of altered selective landscapes and genetic changes in wild salmon resulting from ecological processes associated with salmon farming, predominately through pathogen or parasite transmission leading to reductions in wild population abundance.

Collectively, these impacts have been correlated with significant declines in wild salmon populations. A global study by scientists at Dalhousie University found a reduction in survival or abundance of wild populations (of both salmon and sea trout) of more than 50% per generation on average, associated with salmon farming (Ford and Myers 2008, Tab 13). Such declines have significant social and economic impacts as recreational, commercial, and First Nations fisheries are reduced or eliminated (Wiber 2012; Naylor et al. 2005). Naylor et al. (2005) conclude that risks to wild populations, ecosystems, and society are highest where salmon are farmed in their native range, when large numbers of salmon are farmed near small natural populations, and when exotic pathogens are introduced with farmed fish.

Appendix 2

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From: Jon Carr
To: "Amanda.Ellis@maine.gov"; "Andrew.Sullivan@gnb.ca"; "Andrew.Taylor@dfo-mpo.gc.ca"; "Anthony.Snyder@novascotia.ca"; "Bruce.Hancock@novascotia.ca"; "C.purcell@ns.sympatico.ca"; "Carla.buchan@novascotia.ca"; "Cheryl.Brooking@dfo-mpo.gc.ca"; "Danielle.StLouis@novascotia.ca"; "David.bean@noaa.gov"; "Derek.hogan@dfo-mpo.gc.ca"; "Ernie.atkinson@maine.gov"; "Jason.leblanc@novascotia.ca"; "jnickerson@cookeaqua.com"; "jesse.jenkins@dfo-mpo.gc.ca"; "kohl.kanwit@maine.gov"; "kurt.samways@unb.ca"; "leah.castel@dfo-mpo.gc.ca"; "Leighanne.hawkins@gnb.ca"; [redacted]; "matt.abbott@conservationcouncil.ca"; "SherisseMMcWilliam@dfo-mpo.gc.ca"; "Nathaniel.feindel@novascotia.ca"; "s.farquharson@atlanticfishfarmers.com"; "Suzanne.dobson@dfo-mpo.gc.ca"; "Wende_Mahaney@fws.gov"; "Trudel_Marc"; "Bliss_Doug"; "jesse.jenkins@dfo-mpo.gc.ca"; "Clarke_Corey (PC)"; "Lenentine_Beth"; Bradbury_Ian R; "gideon.pringle@mowi.com"
Cc: Ellen Mansfield; Graham Chafe; Heather Perry; Jason Daniels; Andrew Clarke; Neville Crabbe; Nathan Wilbur; John Burrows; Robert Otto
Bcc: # Management Team; # Operations - Leadership; Stephen Sutton; "chilibeck.john@brunswicknews.com"; "Dory Shipley[redacted]"; "gburk"
Subject: RE: Magaguadavic River escaped farmed salmon update
Date: Monday, September 11, 2023 3:15:00 PM
Attachments: image001.png

Hi All,
Below is the latest update on escapee salmon collected at the head of tide fish ladder on the Magaguadavic River.

The escapee total as of September 11 is 54. The blank cells (sex and maturity) are for fish that we did not perform fish health collections. Those (whole and unopened) carcasses are in a freezer in case there is interest to run fish health or other type of data collections.

I wanted to bring everyone up to speed on a few things that have been falsely claimed:

1. 'ASF is killing wild salmon'. These salmon are not wild. We do a visual inspection, backed up and confirmed by scale analysis. I have been doing this for 30 years (10s of 1000s of salmon scales inspected) and have followed methods and protocol used by international community.

2. 'ASF is killing iBoF salmon from the recovery program'. Scales would look different on iBoF fish compared to a pure wild salmon (egg hatch in river; growth from fry to smolt in river; out to sea and returning as an adult salmon 1-2 years later) or a pure aquaculture salmon (grown in captivity from egg to adult).). IBoF staff collect wild smolts and presmolts from rivers (grown from egg to presmolt/smolt in the river), and then transport those fish to sea cages for captive rearing. The iBoF scale patterns would be somewhere in-between a pure wild or pure aquaculture salmon (as referenced above) but still distinguishable. Growth patterns on the scales we have inspected do not match what we would expect for iBoF recovery fish. IBoF recovery salmon are also pit tagged. We have been scanning the salmon we collect in the Magaguadavic with a PIT Tag reader. No pit tags have been detected which further confirms that these are not from the iBoF recovery program.

3. 'ASF has been stocking European salmon genes into the wild'. This is a false statement. The salmon in question are indeed **farmed escapes, not wild (as explained above).** DFO scientists have shown convincing evidence that the open net pen (OPN) industry has been illegally using European genetic strains (detected in oBoF, iBoF, and southern NL rivers). The question of genetics can easily be resolved and unequivocally confirmed as to origin of the salmon escapes that we are encountering at the Magaguadavic fish ladder. Here is how:

    1. These escapes are either from Cooke or MOWI. ASF has tissue samples available for industry to take and examine for genetic origin. This would determine what company these fish originated from and likely pinpoint the cage site where they were lost. This would be a much more productive and proactive way of understanding where the leaks have occurred and for industry to take mitigative measures to minimise future escape. ASF has offered ACFFA the farmed escapee tissue samples (from the Magaguadavic) for several years without uptake from the industry. The offer is still there – we are more than happy to share if there is interest.

    2. ASF continues to share escapee salmon tissue samples with DFO Scientists. What would be extremely productive and proactive is for DFO Management to audit the ONP industry and access their broodstock database (similar to what happens in the state of Maine). This action would rule out or confirm the use of European strains, and pinpoint what company is leaking fish and from where.

Please let me know if you have any questions, or if any of you can provide information on the escape(s).

Best regards,
Jon

Magaguadavic Escapee Salmon Information

# of Fish	Date	Fork Length (cm)	Weight (Kg)	Sex	Sexually Mature	Sea Lice	Sex Determined by Dissection
1	1-Aug-23	76	4.7	Male	Yes	0	Yes
2	2-Aug-23	63.3	2.5	Female	No	0	Yes
3	8-Aug-23	62	2.4	Male	No	1	Yes
4	10-Aug-23	68.2	3.4	Female	No	0	Yes
5	21-Aug-23	78.3	6.2	Female	Yes	0	Yes
6	21-Aug-23	67	4.4	Female	Yes	0	Yes
	21-Aug-						

7	23	65	3.8	Female	Yes	0	Yes
8	23-Aug-23	74	5.6	Male	Yes	0	Yes
9	28-Aug-23	72.5	5.9	Male	Yes	1	Yes
10	28-Aug-23	72.1	5.4	Male	Yes	0	Yes
11	30-Aug-23	69	5.5	Female	Yes	0	Yes
12	30-Aug-23	74	5.9	Female	Yes	0	Yes
13	30-Aug-23	77	6.2	Female	Yes	0	Yes
14	30-Aug-23	71.7	5.7	Male	Yes	0	Yes
15	31-Aug-23	81.8	6.8	Male	Yes	0	Yes
16	31-Aug-23	83	7.3	Female	Yes	0	Yes
17	31-Aug-23	83	7.9	Female	Yes	0	Yes
18	31-Aug-23	77.9	6.4	Male	Yes	3	Yes
19	31-Aug-23	81.2	6.5	Female	Yes	0	Yes
20	31-Aug-23	85	9.2	Male	Yes	0	Yes
21	1-Sep-23	78.9	5.5	Female	Yes	1	Yes
22	1-Sep-23	75.6	6.6	Female	Yes	0	Yes
23	1-Sep-23	79.6	6.9	Male	Yes	0	Yes
24	1-Sep-23	77.4	6.9	Female	Yes	0	Yes
25	1-Sep-23	82.9	5.4	Female	Yes	0	Yes
26	1-Sep-23	79	3.3	Male	Yes	0	Yes
27	2-Sep-23	82.4	7.10	Female	Yes	0	Yes
28	2-Sep-23	81.4	7.20	Female	Yes	0	Yes
29	2-Sep-23	72.7	4.80	Female	Yes	0	Yes
30	2-Sep-23	72.9	6.40	Female	Yes	0	Yes
31	3-Sep-23	79.8	6.30			0	
32	3-Sep-23	77.8	7.00			0	
33	3-Sep-23	82.0	7.80	Female	Yes	0	Yes
34	3-Sep-23	72.5	5.60	Female		0	
35	3-Sep-23	83.0	8.30	Female	Yes	0	Yes
36	3-Sep-23	75.5	5.60	Female		0	
37	5-Sep-23	82.8	7.50	Female	Yes	0	Yes
38	5-Sep-23	83.0	6.80	Male	Yes	0	Yes
39	5-Sep-23	74.5	6.90			0	
40	5-Sep-23	75.5	5.90			0	
41	5-Sep-23	82.0	8.10			0	
42	5-Sep-23	81.0	7.60			0	
43	5-Sep-23	63.0	3.90			0	
44	5-Sep-23	69.0	3.90			0	
45	5-Sep-23	61.5	3.90			0	
46	5-Sep-23	80.5	7.10	Female	Yes	0	Yes
47	6-Sep-23	74.2	5.80	Female	Yes	3	Yes
48	7-Sep-23	74.0	5.80	Female	Yes	0	Yes
49	7-Sep-23	74.8	6.20	Male	Yes	0	Yes
50	8-Sep-23	65.9	5.00	Female	Yes	3	Yes
51	10-Sep-23	64.0	3.40				
52	10-Sep-23	71.0	4.40				
53	10-Sep-23	54.7	1.70				
54	11-Sep-23	75.0	6.20	Male	Yes	0	Yes

From: Jon Carr
Sent: Tuesday, September 5, 2023 4:26 PM
To: Amanda.Ellis@maine.gov; Andrew.Sullivan@gnb.ca; Andrew.Taylor@dfo-mpo.gc.ca; Anthony.Snyder@novascotia.ca; Bruce.Hancock@novascotia.ca; C.purcell@ns.sympatico.ca; Carla.buchan@novascotia.ca; Cheryl.Brooking@dfo-mpo.gc.ca; Danielle.StLouis@novascotia.ca; David.bean@noaa.gov; Derek.hogan@dfo-mpo.gc.ca; Ernie.atkinson@maine.gov; Ian.Badbury@dfo-mpo.gc.ca; Jason.leblanc@novascotia.ca; jnickerson@cookeaqua.com; jesse.jenkins@dfo-mpo.gc.ca; kohl.kanwit@maine.gov; kurt.samways@unb.ca; leah.castel@dfo-mpo.gc.ca; Leighanne.hawkins@gnb.ca; lloydrobicheau@outlook.com; matt.abbott@conservationcouncil.ca; SherisseMMcWilliam@dfo-mpo.gc.ca; Nathaniel.feindel@novascotia.ca; s.farquharson@atlanticfishfarmers.com; Suzanne.dobson@dfo-mpo.gc.ca; Wende_Mahaney@fws.gov; Trudel, Marc <Marc.Trudel@dfo-mpo.gc.ca>; Bliss, Doug <Doug.Bliss@dfo-mpo.gc.ca>; jesse.jenkins@dfo-mpo.gc.ca; Clarke, Corey (PC) <corey.clarke@canada.ca>; Lenentine, Beth <Beth.Lenentine@dfo-mpo.gc.ca>
Cc: Ellen Mansfield <emansfield@asf.ca>; Graham Chafe <gchafe@asf.ca>; Heather Perry <hperry@asf.ca>; Jason Daniels <JDaniels@asf.ca>; Andrew Clarke <aclarke@asf.ca>; Neville Crabbe <ncrabbe@asf.ca>; Nathan Wilbur <NWilbur@asf.ca>; John Burrows <jburrows@asfmaine.org>; Robert Otto <rotto@asf.ca>
Subject: RE: Magaguadavic River escaped farmed salmon update

Hi All,
Here is the latest update on escapee salmon collected at the head of tide fish ladder on the Magaguadavic River.

The escapee total as of today is 46. The Sept 5 total (N=10) includes salmon that entered the trap between 11am Sept 3 to 8am on Sept 5. The blank cells (sex and maturity) are for fish that we did not perform fish health collections. Those (whole and unopened) carcasses are in a freezer in case there is interest to run fish health or other type of data collections.

Please let me know if you have any questions, or if any of you can provide information on the escape(s).

Jon

# of Fish	Date	Fork Length (cm)	Weight (Kg)	Sex	Sexually Mature	Sea Lice	Sex Determined by Dissection
1	1-Aug-23	76	4.7	Male	Yes	0	Yes
2	2-Aug-23	63.3	2.5	Female	No	0	Yes
3	8-Aug-23	62	2.4	Male	No	1	Yes
4	10-Aug-23	68.2	3.4	Female	No	0	Yes
5	21-Aug-23	78.3	6.2	Female	Yes	0	Yes
6	21-Aug-23	67	4.4	Female	Yes	0	Yes
7	21-Aug-23	65	3.8	Female	Yes	0	Yes
8	23-Aug-23	74	5.6	Male	Yes	0	Yes
9	28-Aug-23	72.5	5.9	Male	Yes	1	Yes
10	28-Aug-23	72.1	5.4	Male	Yes	0	Yes
11	30-Aug-23	69	5.5	Female	Yes	0	Yes
12	30-Aug-23	74	5.9	Female	Yes	0	Yes
13	30-Aug-23	77	6.2	Female	Yes	0	Yes
14	30-Aug-23	71.7	5.7	Male	Yes	0	Yes
15	31-Aug-23	81.8	6.8	Male	Yes	0	Yes
16	31-Aug-23	83	7.3	Female	Yes	0	Yes
17	31-Aug-23	83	7.9	Female	Yes	0	Yes
18	31-Aug-23	77.9	6.4	Male	Yes	3	Yes
19	31-Aug-23	81.2	6.5	Female	Yes	0	Yes
20	31-Aug-23	85	9.2	Male	Yes	0	Yes
21	1-Sep-23	78.9	5.5	Female	Yes	1	Yes
22	1-Sep-23	75.6	6.6	Female	Yes	0	Yes
23	1-Sep-23	79.6	6.9	Male	Yes	0	Yes
24	1-Sep-23	77.4	6.9	Female	Yes	0	Yes
25	1-Sep-23	82.9	5.4	Female	Yes	0	Yes
26	1-Sep-23	79	3.3	Male	Yes	0	Yes
27	2-Sep-23	82.4	7.10	Female	Yes	0	Yes
28	2-Sep-23	81.4	7.20	Female	Yes	0	Yes
29	2-Sep-23	72.7	4.80	Female	Yes	0	Yes
30	2-Sep-23	72.9	6.40	Female	Yes	0	Yes
31	3-Sep-23	79.8	6.30			0	
32	3-Sep-23	77.8	7.00			0	
33	3-Sep-23	82.0	7.80	Female	Yes	0	Yes

34	3-Sep-23	72.5	5.60	Female		0	
35	3-Sep-23	83.0	8.30	Female	Yes	0	Yes
36	3-Sep-23	75.5	5.60	Female		0	
37	5-Sep-23	82.8	7.50	Female	Yes	0	Yes
38	5-Sep-23	83.0	6.80	Male	Yes	0	Yes
39	5-Sep-23	74.5	6.90			0	
40	5-Sep-23	75.5	5.90			0	
41	5-Sep-23	82.0	8.10			0	
42	5-Sep-23	81.0	7.60			0	
43	5-Sep-23	63.0	3.90			0	
44	5-Sep-23	69.0	3.90			0	
45	5-Sep-23	61.5	3.90			0	
46	5-Sep-23	80.5	7.10	Female	Yes	0	Yes

From: Jon Carr
Sent: Thursday, August 31, 2023 10:00 PM
To: Amanda.Ellis@maine.gov; Andrew.Sullivan@gnb.ca; Andrew.Taylor@dfo-mpo.gc.ca; Anthony.Snyder@novascotia.ca; Bruce.Hancock@novascotia.ca; C.purcell@ns.sympatico.ca; Carla.buchan@novascotia.ca; Cheryl.Brooking@dfo-mpo.gc.ca; Danielle.StLouis@novascotia.ca; David.bean@noaa.gov; Derek.hogan@dfo-mpo.gc.ca; Ernie.atkinson@maine.gov; Ian.Badbury@dfo-mpo.gc.ca; Jason.leblanc@novascotia.ca; jnickerson@cookeaqua.com; jesse.jenkins@dfo-mpo.gc.ca; kohl.kanwit@maine.gov; kurt.samways@unb.ca; leah.castel@dfo-mpo.gc.ca; Leighanne.hawkins@gnb.ca; lloydrobicheau@outlook.com; matt.abbott@conservationcouncil.ca; SherisseMMcWilliam@dfo-mpo.gc.ca; Nathaniel.feindel@novascotia.ca; s.farquharson@atlanticfishfarmers.com; Suzanne.dobson@dfo-mpo.gc.ca; Wende_Mahaney@fws.gov; Trudel, Marc <Marc.Trudel@dfo-mpo.gc.ca>
Cc: Ellen Mansfield <emansfield@asf.ca>; Graham Chafe <gchafe@asf.ca>; Heather Perry <hperry@asf.ca>; Jason Daniels <JDaniels@asf.ca>; Andrew Clarke <aclarke@asf.ca>; Neville Crabbe <ncrabbe@asf.ca>; Nathan Wilbur <NWilbur@asf.ca>; John Burrows <jburrows@asfmaine.org>; Robert Otto <rotto@asf.ca>
Subject: Magaguadavic River escaped farmed salmon update

Hi Everyone,

The purpose of this email is to alert industry, managers, various stakeholders (i.e. groups and agencies that monitor rivers) on salmon escapee occurrence on the Magaguadavic with he hopes that source of escape can be identified and mitigated, and perhaps lead to monitoring other rivers for escapee occurrence and removals.

We are going to start providing weekly updates on escaped farmed salmon recorded at the Magaguadavic head of tide fish ladder. If we experience high numbers during a given period, then an alert will be sent out sooner.

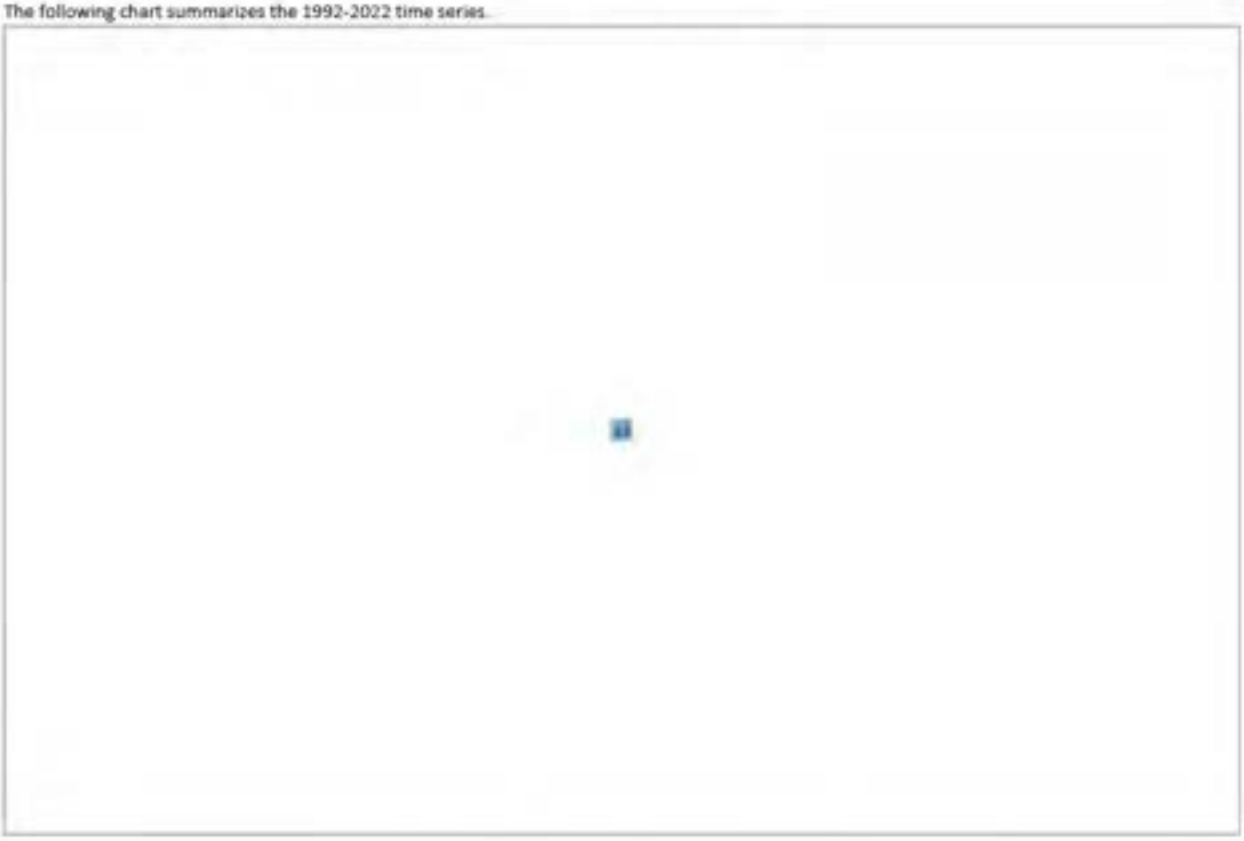
In 2023, there have been 21 aquaculture salmon escapes recorded at the Magaguadavic head of tide fish ladder as of August 31 (see below for more details below). There has likely been a significant breach of containment, possibly from more than one site judging from the variety of fish sizes. The ASF has not received any reports from industry or regulators in Atlantic Canada about any recent escapes. The sizes of the escapees recorded in the Magaguadavic do not match any fish from the reported escape in Maine in early August at Cross Island, in Cutler, Machias Bay (about 50,000 salmon escaped, estimated to be 200-400 gram weight).

The Magaguadavic River monitoring program has been ongoing since 1992, and is recognized globally as a North American index river for monitoring escaped farmed salmon. All farmed salmon have been removed from the fish ladder since 1996. We collect scales (for aging and identification purposes), fin conditions (clips, erosion), fin tissue (for DNA), sealice, date of capture, sex, maturity status, and relevant tissue/organs fir fish health evaluation. The chart below provides details pertaining to the escapes recorded at the Magaguadavic so far in 2023.

Date	**Fork Length (cm)**	**Weight (Kg)**	**Sex**	**Sexually Mature**	**Sea Lice**	
01-Aug-23	76.0	4.7	Male	Yes	0	
02-Aug-23	63.3	2.5	Female	No	0	
08-Aug-23	60.0	2.4	Male	No	1	
10-Aug-23	48.7	1.0	Female	No	1	
10-Aug-23	68.2	3.4	Female	No	0	
21-Aug-23	78.3	6.2	Female	Yes	0	
21-Aug-23	67.0	4.4	Female	Yes	0	
21-Aug-23	65.0	3.8	Female	Yes	0	
23-Aug-23	74.0	5.6	Male	Yes	0	
28-Aug-23	72.5	5.9	Male	Yes	1	
28-Aug-23	72.1	5.4	Male	Yes	0	
30-Aug-23	69.0	5.5	Female	Yes	0	
30-Aug-23	74.0	5.9	Female	Yes	0	

30-Aug-23	77.0	6.2	Female	Yes	0	
30-Aug-23	71.7	5.7	Male	Yes	0	
31-Aug-23	81.8	6.8	Male	Yes	0	
31-Aug-23	83.0	7.3	Female	Yes	0	
31-Aug-23	83.0	7.9	Female	Yes	0	
31-Aug-23	77.9	6.4	Male	Yes	3	
31-Aug-23	81.2	6.5	Female	Yes	0	
31-Aug-23	85.0	9.2	Male	Yes	0	

The following chart summarizes the 1992-2022 time series.



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Sincerely,
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ASC Salmon Standard

Version 1.3

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VERSION CONTROL, AVAILABLE LANGUAGE(S) AND COPYRIGHT NOTICE

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Version control

Document version history:

Version:	Release date:	Effective date:	Remarks/changes:
V1.3	July 11 h, 2019	December 26, 2019	Based on the PTI & Smolt review/revision cycle, the following indicators have been updated/modified: • Criteria 5.2 ('PTI review'): Rationale amended; 5.2.5 & 5.2.6 (reference to PTI removed, WNMT & parasiticide requirements added); 5.2.7 (new indicator, WNMT-related), 5.2.8 (new indicator: Integrated Pest Management /IPM), 5.2.9 (new indicator: IMP measures transparency), 5.2.10 (new indicator: monitoring of parasiticide residue levels outside AZE), 5.2.11 (indicator # changed: was 5.2.7 in v1.2); 5.2.12 (indicator # changed: 5.2.8 in v1.2); 5.2.13 (indicator # changed: 5.2.9 in v1.2); 5.2.14 (indicator # changed: 5.2.10 in v1.2); 5.2.15 (indicator # changed: 5.2.11 in v1.2). • Section 8 ('Smolt review'): "Additional requirements for open (net-pen) production of smolt': 8.24 (indicators deleted), 8.25 (requirement changed), former (in v1.2) indicators [8.26-8.31] deleted and replaced by 8.25. New Rationale for 8.25. Indicators 8.26, 8.27, 8.28 & 8.29 correspond to 'old' (i.e. in v1.2) indicators 8.32, 8.33, 8.34 & 8.35 (requirements unchanged). • Appendix VI (content changed for items #30, 31, 32). • Appendix VII (content changed: 'Parasiticide Treatment Methodology', instead of PTI). • Other updates include layout & UK English-consistent spell-check.
v1.2	March 7th, 2019	March 15th, 2019	Update of the standard to meet ASC style requirements (e.g. inclusion of structure of the standards, formatting and wording). Align the scope, 'about the ASC' and 'overview of the ASC system'. The content of the actual Standard, as defined by criteria/indicators/requirements under Principles [1-7], remains unchanged.

v1.1	April 26th, 2017	October 31st, 2017	Based on the first review/revision cycle: • following has been updated (in v1.1): 2.2.4 (requirement changed); 3.1.5 (updated footnote 43); 3.2.2 (updated footnote 50; requirement change); 4.2.1 (requirement changed); 4.2.2 (requirement changed); 4.3.1 (requirement changed); 4.3.2 (requirement updated); 4.3.4 (indicator expanded); 4.4.2 (requirement updated); 4.6.3 (requirement updated); 5.1.1 (indicator expanded); 5.2.6 (requirement updated); 5.4.4 (updated footnote 119); 6.11.1 (indicator expanded); 8.4 (requirement updated). • following is added (in v1.1): 2.2.6, 4.3.5, footnote 162. • following is removed (from v1.0): 2.5.2.
v1.0	June, 2012	July, 2012	Original version developed and approved by the Salmon Aquaculture Dialogue Steering Committee under the original title "Salmon Aquaculture Dialogue" and handed over to the Aquaculture Stewardship Council.

It is the responsibility of the user of the document to use the latest version as published on the ASC-website.

Available language(s)

The Salmon Standard document is available in the following language(s):

Versions:	Available languages
v1.3 v1.2 v1.1 v1.0	English (official language)
v1.0	Japanese

In case of any inconsistencies and/or discrepancies between available translation(s) and the English version, the online English version (pdf-format) will prevail.

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ABOUT THE AQUACULTURE STEWARDSHIP COUNCIL (ASC)

The Aquaculture Stewardship Council (ASC) is an independent, not-for-profit organisation that operates a voluntary, independent third-party certification and labelling programme based on a scientifically robust set of standards.

The ASC standards define criteria designed to help transform the aquaculture[1] sector[2] towards environmental sustainability and social responsibility, as per the ASC Mission.

ASC Vision

A world where aquaculture plays a major role in supplying food and social benefits for mankind whilst minimising negative impacts on the environment.

ASC Mission

To transform aquaculture towards environmental sustainability and social responsibility using efficient market mechanisms that create value across the chain.

ASC Theory of Change

A Theory of Change (ToC) is an articulation, description and mapping out of the building blocks required to achieve the organisation's vision**.**

ASC has defined a ToC which explains how the ASC certification and labelling programme promotes and rewards responsible fish farming practices through incentivising the choices people make when buying seafood.

ASC's Theory of Change can be found on the ASC website.

[1] **Aquaculture**: Aquaculture is the farming of aquatic organisms, including fish, molluscs, crustaceans and aquatic plants. Farming implies some form of intervention in the rearing process to enhance production, such as regular stocking, feeding, protection from predators, etc. Farming also implies individual or corporate ownership of the stock being cultivated (FAO).

[2] **Aquaculture sector**: Represents a group of industries (e.g. feed, farming, processing, etc.) and their markets that share common attributes (i.e. aquaculture products).

THE ASC DOCUMENT AND CERTIFICATION SYSTEM

ASC is a full member of the ISEAL Alliance and implements a voluntary, independent third-party certification system[3] consisting of three independent actors:

I.	Scheme Owner	i.e. Aquaculture Stewardship Council
II.	Accreditation Body	i.e. Assurance Services International (ASI)
III.	Conformity Assessment Body (CAB)	i.e. Accredited CAB's

Scheme Owner

ASC, as scheme owner:

- sets and maintains standards according to the ASC Standard Setting Protocol which is in compliance with the "ISEAL Code of Good Practice - Setting Social and Environmental Standards". The ASC standards are normative documents;

- sets and maintains Implementation Guidance which provides guidance to the Unit of certification (UoC) on how to interpret and best implement the indicators within the Standard;

- sets and maintains the Auditor Guidance which gives guidance to the auditor how to best assess a UoC against the indicators within the Standard;

- sets and maintains the Certification and Accreditation Requirements (CAR) which adheres at a minimum to the "ISEAL Code of Good Practice - Assuring compliance with Social and Environmental Standards". The CAR describes the accreditation requirements, assessment requirements and certification requirements. The CAR is a normative document.

These above listed documents are publicly available on the ASC-website.

Accreditation Body

Accreditation is the assurance process of assessing the Conformity Assessment Body (CAB) against accreditation requirements and is carried out by an Accreditation Body (AB). The appointed AB of ASC is Assurance Services International (ASI, "Accreditation Services International" prior to January 2019) which uses the CAR as normative document for the accreditation process.

Assessment findings of ASI-accreditation audits and an overview of current accredited CABs is publicly available via the ASI-website (http://www.accreditation-services.com).

[3] **Third-party Certification System**: Conformity assessment activity that is performed by a person or body that is independent of the person or organisation that provides the object, and of the user interests in that object (ISO 17000).

Conformity Assessment Body

The UoC contracts the CAB which employs auditor(s) that conduct a conformity assessment (hereafter 'audit') of the UoC against the relevant standard. The management requirements for CABs as well as auditor competency requirements are described in the CAR and assured through ASI accreditation.

ASC Audit and Certification Process

The UoC is audited at Indicator-level.

An ASC audit follows strict process requirements. These requirements are detailed in the CAR. Only ASI-accredited CABs are allowed to audit and certify a UoC against ASC standards. As scheme owner, ASC itself is not - and cannot be - involved in the actual audit and/or certification decision of a UoC. Granted certificates are the property of the CAB. ASC does not manage certificate validity.

Audit findings of all ASC audits, including granted certificates, are made publicly available on the ASC-website. These include the audit findings that result in a negative certification decision.

Note: in addition to the Standard's, there are certification requirements that apply to UoCs seeking certification; these requirements are detailed in the CAR.

ASC Logo use

ASC-certified entities shall only sell their product carrying the ASC Logo if a Logo Licence Agreement (LLA) has been signed. On behalf of the ASC, the Marine Stewardship Council (MSC) Licensing Team will issue logo license agreements and approve logo use on products. For more information see: ASC Logo.

Unauthorised logo display is prohibited and will be treated as a trademark infringement.

STRUCTURE OF ASC STANDARDS

A Standard is "*a document that provides, for common and repeated use, rules, guidelines or characteristics for products or related processes and production methods, with which compliance is not mandatory*".

ASC Standards are as follows designed:

- ASC Standards consist of multiple Principles – a Principle is a set of thematically related Criteria which contribute to the broader outcome defined in the Principle title;

- Each Principle consists of multiple Criteria – each Criterion defines an outcome that contributes to achieving the outcome of the Principle;

- Each Criterion consists of one or several Indicators – each Indicator defines an auditable state that contributes to achieving the Criterion outcome.

Both Principles and Criteria include Rationale statements providing a set of reasons (backed by reference notes if needed) as to why the Principle or Criterion is needed.

SCOPE AND UNIT OF CERTIFICATION

Linked to the ASC Vision, the Scope of the ASC Salmon Standard (hereafter "the Standard") addresses the key negative environmental and social impacts associated with the salmon aquaculture industry. An ASC-certified salmon farm contributes to the ASC Vision by reducing, mitigating or eliminating these negative impacts.

The Scope of the Standard is translated into seven Principles that apply to every UoC:

- Principle 1 – Comply with all applicable national laws and local regulations
- Principle 2 – Conserve natural habitat, local biodiversity and ecosystem function
- Principle 3 – Protect the health and genetic integrity of wild populations
- Principle 4 – Use resources in an environmentally efficient and responsible manner
- Principle 5 – Manage disease and parasites in an environmentally responsible manner
- Principle 6 – Develop and operate farms in a socially responsible manner
- Principle 7 – Be a good neighbour and conscientious citizen
- Section 8 – Requirements for suppliers of smolt

The Criteria within the Principles apply to every UoC.

Unit of Certification (UoC)

The applicable UoC is determined by the CAB/ auditor and adheres to the Standard's Criteria UoC-requirements as outlined in the CAR.

Biological and geographic scope to which the Standard applies

The ASC Salmon Standard v1.3 is applicable to salmonid (i.e. salmon and trout) species belonging to the genus *Salmo* and *Oncorhynchus,* farmed in all marine locations [with the current exclusion/exception of smolt produced or held in net pens and/or [in future/soon] Smolt having to be certified under the FW Trout Standard] and types of aquaculture production systems.

How to read this document?

In the following pages, tables with indicators and their corresponding requirements are included. Within each criterion, requirements tables are followed by a rationale section that provides a brief overview of why the issues are important and how the proposed requirements address them.

Definitions are provided in footnotes.

The ASC Salmon Standard will be supplemented by an auditor guidance document detailing the methodologies used to determine if the ASC Salmon Standard is being met, as well as guidance for producers to achieve compliance to the ASC Salmon Standard.

Metric Performance Levels

Several Indicators in the Standard require a Metric Performance Level (MPL). The applicable MPL is directly listed after the Indicator ("Requirement" section).

PRINCIPLE 1: COMPLY WITH ALL APPLICABLE NATIONAL LAWS AND LOCAL REGULATIONS

Principle 1 is intended to ensure that all farms aiming to be certified against the ASC Salmon Standard standards meet their legal obligations as a baseline requirement. Adhering to the law will ensure that producers meet the basic environmental and social requirements and the minimal structures, such as legitimate land tenure rights, on which the effectiveness of the requirements will stand.

Criterion 1.1 Compliance with all applicable local and national legal requirements and regulations

INDICATOR	REQUIREMENT
1.1.1 Presence of documents demonstrating compliance with local and national regulations and requirements on land and water use	Yes
1.1.2 Presence of documents demonstrating compliance with all tax laws	Yes
1.1.3 Presence of documents demonstrating compliance with all relevant national and local labour laws and regulations	Yes
1.1.4 Presence of documents demonstrating compliance with regulations and permits concerning water quality impacts	Yes

Rationale - Salmon aquaculture operations must, as a baseline, adhere to the national and local laws of the regions where production is taking place. Farm operations that, intentionally or unintentionally, break the law violate a fundamental benchmark of performance for certified farms. It is important that aquaculture operations demonstrate a pattern of legal and responsible behaviour, including the implementation of corrective actions for any legal violations.

PRINCIPLE 2: CONSERVE NATURAL HABITAT, LOCAL BIODIVERSITY AND ECOSYSTEM FUNCTION

Principle 2 is intended to address potential impacts from salmon farms on natural habitat, local biodiversity and ecosystem function. Specifically, the key impact areas of benthic impacts, siting, effects of chemical inputs and effects of nutrient loading are addressed within this principle.

Criterion 2.1 Benthic biodiversity and benthic effects[4]

INDICATOR	REQUIREMENT
2.1.1 Redox potential or[5] sulphide levels in sediment outside of the Allowable Zone of Effect (AZE),[6] following the sampling methodology outlined in Appendix I-1	Redox potential > 0 mV, or, Sulphide ≤ 1,500 µMol /L
2.1.2 Faunal index score indicating good[7] to high ecological quality in sediment outside the AZE, following the sampling methodology outlined in Appendix I-1	AZTI Marine Biotic Index (AMBI[8]) score ≤ 3.3, or, Shannon-Wiener Index score > 3, or, Benthic Quality Index (BQI) score ≥ 15, or, Infaunal Trophic Index (ITI) score ≥ 25
2.1.3 Number of macrofaunal taxa in the sediment within the AZE, following the sampling methodology outlined in Appendix I-1	≥ 2 highly abundant[9] taxa that are not pollution indicator species
2.1.4 Definition of a site-specific AZE based on a robust and credible[10] modelling system[11]	Yes

[4] Closed production systems that can demonstrate that they collect and responsibly dispose of > 75% of solid nutrients from the production system are exempt from standards under Criterion 2.1. See Appendix VI for requirements on transparency for 2.1.1, 2.1.2 and 2.1.3.

[5] Farm sites can choose whether to use redox or sulphide. Farms do not have to demonstrate that they meet both.

[6] **Allowable Zone of Effect** (AZE) is defined under this standard as 30 metres. For farm sites where a site-specific AZE has been defined using a robust and credible modelling system such as the SEPA AUTODEPOMOD and verified through monitoring, the site-specific AZE shall be used.

[7] ***"Good"*** Ecological Quality Classification: The level of diversity and abundance of invertebrate taxa is slightly outside the range associated with the type-specific conditions. Most of the sensitive taxa of the type-specific communities are present.

[8] http://ambi.azti.es/ambi/.

[9] **Highly abundant**: Greater than 100 organisms per square metre (or equally high to reference site(s) if natural abundance is lower than this level).

Rationale - This suite of indicators provides multiple layers of security related to benthic impacts, using a chemical proxy for health combined with biodiversity measurements both below and a distance from the cages. Technical experts suggest the chemical proxy of redox potential and sulphide levels, which are good chemical indicators for benthic health. Given that both methods are valid, audited farms can choose their preference for one or the other. Requirements have been set for both. Through the consultation of technical experts and review of Hargrave et al.[12] (2008), a level of µMol /L sulphide levels and equivalent redox potential of > 0 mV was set to ensure acceptable and transitory benthic conditions. As a precautionary approach, these requirements are applicable regardless of the depth of the site.

When considering benthic effects, experts recommended measuring effects below the cages and away from the cages, within and outside the AZE. Though an AZE is difficult to identify as a constant, experts discuss this in terms of 25 metres to 125 metres depending on a range of factors, including currents. In an effort to take a precautionary approach to permissible zone of benthic impact, the ASC Salmon Standard defines the AZE as a distance of 30 metres from cages. For sites where a site-specific AZE has been determined using a valid modelling and video surveillance system, farms will use the site-specific AZE and sampling stations based on actual depositional patterns. Within three years of the publication of the ASC Salmon Standard, all certified farms must have undertaken the appropriate analysis to determine the site-specific AZE and depositional patterns. This will help ensure that sampling is taking place in areas most appropriate to protect benthic health around farms.

Potential negative impacts on benthic biodiversity are addressed in the ASC Salmon Standard through the incorporation of an analysis using a benthic faunal index and minimum score at multiple monitoring stations outside the AZE, including a reference site (see Appendix I-1). Farms can use their choice of these four faunal indices to further establish the environmental quality of the soft-bottom benthos. The indices are calculated using the same dataset. Equivalencies for these indices were set using Hargraves et al. (2008) and Zettler et al. (2007)[13] and through consultation with experts. The scores were set to relate to an environmental quality status of good or better according to the definitions of the EU Water Framework Directive.[14] Within the AZE, a demonstration that two or

[10] **Robust and credible**: The SEPA AUTODEPOMOD modelling system is considered to be an example of a credible and robust system. The model must include a multi-parameter approach. Monitoring must be used to ground-truth the AZE proposed through the model.

[11] The CAB shall confirm that the AZE is correct and then to default to the social principles (P6 and P7) to ensure the farm is responding to stakeholder comments with the intention that the AZE is not arbitrary and meets stakeholder expectations.

[12] Hargrave, B.T., Holmer, M. and Newcombe, C.P. 2008. Towards a classification of organic enrichment in marine sediments based on biogeochemical indicators. Marine Pollution Bulletin 56, 810–824. https://www.researchgate.net/publication/5509807_Towards_a_classification_of_organic_enrichment_in_marine_sediments_based_on_biogeochemical_indicators

[13] Zettler, M.L., Schiedek, D. and Bobertz, B. 2007. Benthic biodiversity indices versus salinity gradient in the southern Baltic Sea. Marine Pollution Bulletin 55, 258–270. https://www.io-warnemuende.de/tl_files/bio/ag-benthische-organismen/pdf/zettler_et_al-2007-mpb.pdf

[14] Additional references for index equivalencies:

- Borja, A., Franco, J. and Perez, V. 2000. A marine biotic index to establish the ecological quality of soft-bottom benthos within European estuarine and coastal environments. Mar. Poll. Bull. 40, 1100–1114. http://www.ecasa.org.uk/Documents/AMBI-MarineBioticIndex.pdf
- Muxika, I., Borja, A. and Bonne, W. 2005. The suitability of the marine biotic index (AMBI) to new impact sources along European coasts. Ecological Indicators 5, 19–31. http://agris.fao.org/agris-search/search.do?recordID=AV20120155174

more benthic macrofaunal species, such as sessile macrophytes and worms, are present in high abundance is required to ensure that impacts fall within an acceptable level.

Criterion 2.2 Water quality in and near the site of operation [15]

INDICATOR	REQUIREMENT
2.2.1 Weekly average percent saturation[16] of dissolved oxygen (DO)[17] on farm, calculated following methodology in Appendix I-4	≥ 70%[18]
2.2.2 Maximum percentage of weekly samples from 2.2.1 that fall under 2 mg/L DO	5%
2.2.3 For jurisdictions that have national or regional coastal water quality targets[19], demonstration through third-party analysis that the farm is in an area recently[20] classified as having "good" or "very good" water quality[21]	Yes[22]

≠ Muniz, P. et al. 2005. Testing the applicability of a Marine Biotic Index (AMBI) to assessing the ecological quality of soft-bottom benthic communities in the South America Atlantic region. Marine Pollution Bulletin 50, 624–637. http://www.basqueresearch.com/uploads/fitxategiak/2769_1AMBI.pdf

[15] See Appendix VI for transparency requirements for 2.2.1, 2.2.2, 2.2.3 and 2.2.5.

[16] **Percent saturation**: Percent saturation is the amount of oxygen dissolved in the water sample compared to the maximum amount that could be present at the same temperature and salinity.

[17] Averaged weekly from two daily measurements (proposed at 6 am and 3 pm).

[18] An exception to this standard shall be made for farms that can demonstrate consistency with a reference site in the same water body.

[19] Related to nutrients (e.g. N, P, chlorophyll A).

[20] Within the two years prior to the audit.

[21] Classifications of "***good***" and "***very good***" are used in the EU Water Framework Directive. Equivalent classification from other water quality monitoring systems in other jurisdictions are acceptable, it is acceptable to use a benchmark level of water quality from farm monitoring data as defined in Appendix I-5.

[22] Closed production systems that can demonstrate the collection and responsible disposal of > 75% of solid nutrients as well as > 50% of dissolved nutrients (through biofiltration, settling and/or other technologies) are exempt from standards 2.2.3 and 2.2.4.

2.2.4	For jurisdictions without national or regional coastal water quality targets, evidence of monitoring of nitrogen and phosphorous[23] levels on farm and at a reference site, following methodology in Appendix I-5	Consistency with reference site
2.2.5	Demonstration of calculation of biochemical oxygen demand (BOD[24]) of the farm on a production cycle basis	Yes
2.2.6	Appropriate controls are in place that maintains good culture and hygienic conditions on the farm which extends to all chemicals, including veterinary drugs, thereby ensuring that adverse impacts on environmental quality are minimised.	Yes

Rationale - Water quality is essential for the health of farmed salmon and wild species surrounding a farm. One component of water quality, dissolved oxygen (DO), is particularly critical for the survival and good performance of farmed salmon. As a result, most farms regularly measure DO. DO levels (in mg/l) naturally fluctuate in the environment. This is due to a range of factors, including temperature, time of day and upwelling of oxygen-poor waters from deep in the ocean. Low DO levels can also be a sign of excessive nutrient loading. DO provides a useful overall proxy for a water body's ability to support healthy biodiversity and supplements the benthic indicators that will also pick up excessive nutrient loading.

Salmon ideally need a level of dissolved oxygen over 5 mg/L to avoid any possible stress, although they are able to live under lower oxygen concentrations, particularly if only for short periods. Under routine production, the average minimum percent saturation of DO in the water column should be above 70 per cent. Measuring DO as a percent saturation takes into account salinity and temperature at the farm site. Additionally, compliance with the requirement will limit the number of low DO readings in the water column below 2 mg/L to less than 5 per cent incidence rate, which will allow for periodic physical phenomena, such as upwelling. The requirement also addresses natural fluctuations in DO levels and percent saturation through allowing comparison to a reference site as a means to meet requirement 2.2.1. This will ensure that if the percent saturation is lower than ideal, it is the result of natural conditions in the water body and not due to nutrient release from the salmon farm.

[23] Farms shall monitor total N, NH_4, NO_3, total P and Ortho-P in the water column. Results shall be submitted to the ASC database. Methods such as a Hach kit are acceptable.

[24] **BOD** calculated as: ((total N in feed – total N in fish)*4.57) + ((total C in feed – total C in fish)*2.67). A farm may deduct N or C that is captured, filtered or absorbed through approaches such as IMTA or through direct collection of nutrient wasted. In this equation, "fish" refers to harvested fish. Reference for calculation methodology: Boyd C. 2009. Estimating mechanical aeration requirement in shrimp ponds from the oxygen demand of feed. In: Proceedings of the World Aquaculture Society Meeting; Sept 25-29, 2009; VeraCruz, Mexico. And: Global Aquaculture Performance Index BOD calculation methodology available at http://web.uvic.ca/~gapi/explore-gapi/bod.html.

The requirements also require that farms demonstrate they are located in areas of "good" or "very good" water quality, in jurisdictions such as the European Union that have coastal targets. Not all salmon-producing regions have such targets, however. In these situations, farms must collect data on nutrient levels near the farm and at a reference site and make that data available under Appendix VI. No threshold is placed on this requirement whilst the key factor, as with oxygen in Indicator 2.2.1, is that the requirement should address natural fluctuations in N and P levels through allowing comparison to a reference site as a means to meet requirement 2.2.3.

Lastly, the requirements require farms to calculate the BOD associated with their production cycle in order to better understand the input of nutrients from the farm to the water body. There is no performance threshold associated with this requirement, and the data from this requirement will provide data to better understand nutrient loads, ranges of performance, the degree to which different systems reduce BOD, and the relationship between calculated BOD and the other water quality indicators in the ASC Salmon Standard.

The SAD technical working group on nutrient loading identified the potential link between nutrients around salmon farms and harmful algal blooms as one that had yet to be established but around which there remained some uncertainty and for which there was an intuitive concern around the effect of the cumulative anthropogenic nutrient load into coastal waters. The group noted a shortage of field studies to validate hypotheses from lab-based work. The data collected under this criterion can be used to help better understand potential linkages around salmon farming, ambient nutrient levels and environmental phenomena such as harmful algal blooms. Farm operators may also find this data useful in management decisions, and it can be useful in ensuring that nutrient inputs from salmon farms and other sources fall within the carrying capacity of the water body. Data collected with regard to BOD and nutrient levels shall be reviewed, and the setting of a threshold related to nutrient loads should be seriously considered when the ASC Salmon Standard is updated. The ASC intend to develop a metric for indicator 2.2.6 good culture and hygienic conditions. Until which time the standard will include this best management practice type measure.

Criterion 2.3 Nutrient release from production

INDICATOR	REQUIREMENT
2.3.1 Percentage of fines[25] in the feed at point of entry to the farm[26] (calculated following methodology in Appendix I-2)	< 1% by weight of the feed

[25] **Fines**: Dust and fragments in the feed. Particles that separate from feed with a diameter of 5 mm or less when sieved through a 1 mm sieve, or particles that separate from feed with a diameter greater than 5 mm when sieved through a 2.36 mm sieve. To be measured at farm gate (e.g. from feed bags after they are delivered to farm).

[26] To be measured every quarter or every three months. Samples that are measured shall be chosen randomly. Feed may be sampled immediately prior to delivery to farm for sites with no feed storage where it is not possible to sample on farm. Closed production systems that can demonstrate the collection and responsible disposal of > 75% of solid nutrients and > 50% of dissolved nutrients (through biofiltration, settling and/or other technologies) are exempt.

Rationale - The release of nutrients into the environment from salmon farms was identified by SAD participants as a key impact of production. The impact is addressed throughout the requirements with a range of water quality and benthic performance metrics. Requirement 2.3.1 complements these other requirements by addressing the direct release of uneaten feed in the form of fines into the environment. By setting a maximum percentage of fines in the feed, it addresses the efficient and proper transport, storage and physical delivery of feed pellets to the farm site. Poor performance in any of the above phases of feed handling will result in a higher percentage of fines (fine particles of feed) and potentially increased environmental impacts, due to an increase in suspended organic particles and nutrients released into the environment.

Criterion 2.4 Interaction with critical or sensitive habitats and species

INDICATOR	REQUIREMENT
2.4.1 Evidence of an assessment of the farm's potential impacts on biodiversity and nearby ecosystems that contains at a minimum the components outlined in Appendix I-3	Yes
2.4.2 Allowance for the farm to be sited in a protected area[27] or High Conservation Value Areas[28] (HCVAs)	None[29]

[27] **Protected area**: "A clearly defined geographical space, recognised, dedicated and managed through legal or other effective means, to achieve the long-term conservation of nature with associated ecosystem services and cultural values." Source: Dudley, N. (Editor) (2008). Guidelines for Applying Protected Area Management Categories, Gland, Switzerland: IUCN. x + 86pp. http://cmsdata.iucn.org/downloads/guidelines_for_applying_protected_area_management_categories.pdf

[28] **High Conservation Value Areas** (HCVA): Natural habitats where conservation values are considered to be of outstanding significance or critical importance. HCVA are designated through a multi-stakeholder approach that provides a systematic basis for identifying critical conservation values—both social and environmental—and for planning ecosystem management in order to ensure that these high conservation values are maintained or enhanced (http://www.hcvnetwork.org/).

[29] The following exceptions shall be made for Standard 2.4.2:

- ≠ For protected areas classified by the International Union for the Conservation of Nature (IUCN) as Category V or VI (these are areas preserved primarily for their landscapes or for sustainable resource management).
- ≠ For HCVAs if the farm can demonstrate that its environmental impacts are compatible with the conservation objectives of the HCVA designation. The burden of proof would be placed on the farm to demonstrate that it is not negatively impacting the core reason an area has been identified as a HCVA.
- ≠ For farms located in a protected area if it was designated as such after the farm was already in operation and provided the farm can demonstrate that its environmental impacts are compatible with the conservation objectives of the protected area and it is in compliance with any relevant conditions or regulations placed on the farm as a result of the formation/designation of the protected area. The burden of proof would be placed on the farm to demonstrate that it is not negatively impacting the core reason an area has been protected.

Rationale - The intent of the requirements under criterion 2.4 is to minimise the effects of a salmon farm on critical or sensitive habitats and species. The habitats and species to consider include marine-protected areas or national parks, established migratory routes for marine mammals, threatened or endangered species, the habitat needed for endangered and threatened species to recover, eelgrass beds and HCVAs, where these have been defined. These requirements are consistent with the Global Reporting Index indicators EN12, EN14 and EN15, which relate to the identification and description of significant impacts of activities on biodiversity, protected habitats and threatened species, and the communication of strategies to manage these impacts and restore sensitive habitats (as defined by the assessment carried out for indicator 2.4.1).[30]

The requirements under Criteria 2.4 ensure that a farm is aware of any nearby critical, sensitive or protected areas, understands the impacts it might have on those areas, and has a functioning plan in place to address those potential impacts. They also ensure that extra care is taken in areas that are recognised for ecological importance either through designation as a protected area or through designation as being an area of high conservation value, by not allowing production in these areas to be eligible for certification, with some exceptions made if extra conditions are met to ensure that the farms are compatible with the conservation goals of the areas.

Criterion 2.5 Interaction with wildlife, including predators[31]

INDICATOR	REQUIREMENT
2.5.1 Number of days in the production cycle when acoustic deterrent devices (ADDs) or acoustic harassment devices (AHDs) were used	0
2.5.2 Number of mortalities[32] of endangered or red-listed[33] marine mammals or birds on the farm	0
2.5.3 Evidence that the following steps were taken prior to lethal action[34] against a predator: 1. All other avenues were pursued prior to using	Yes[35]

[30] Verification at the aquaculture facility shall include whether restoration is necessary, to what degree (evidence could include maps, aerial photos, satellite images, government certification etc.) and whether that the active restoration is suitable (i.e., will it be successful and restore a suitable area of sensitive habitat).

[31] See Appendix VI for transparency requirements for 2.5.2, 2.5.5 and 2.5.6.

[32] **Mortalities**: Includes animals intentionally killed through lethal action as well as accidental deaths through entanglement or other means.

[33] Species listed as endangered or critically endangered by the IUCN or on a national endangered species list.

[34] **Lethal action**: Action taken to deliberately kill an animal, including marine mammals and birds.

[35] Exception to these conditions may be made for a rare situation where human safety is endangered. Should this be required, post-incident approval from a senior manager should be made and relevant authorities must be informed.

	lethal action 2. Approval was given from a senior manager above the farm manager 3. Explicit permission was granted to take lethal action against the specific animal from the relevant regulatory authority	
2.5.4	Evidence that information about any lethal incidents on the farm has been made easily publicly available[36]	Yes
2.5.5	Maximum number of lethal incidents[37] on the farm over the prior two years	< 9 lethal incidents,[38] with no more than two of the incidents being marine mammals
2.5.6	In the event of a lethal incident, evidence that an assessment of the risk of lethal incident(s) has been undertaken and demonstration of concrete steps taken by the farm to reduce the risk of future incidences	Yes

Rationale - The suite of requirements related to mortalities and lethal incidents of predators or other wildlife is intended to ensure that certified farms have minimal impact on populations of wildlife, placing limits on both accidental and intentional mortalities of these species. The requirements ensure that endangered species have not died as a result of interaction with the farm and require transparency of farms on any lethal incidents and wildlife mortalities for non-threatened species. Good management practices with regards to when to take action and how to reduce risk of future incidents are also required.

A large variety of acoustic deterrent (and harassment) devices is used in salmon aquaculture. Based on available research[39] it appears that the effectiveness of these devices in reducing farmed salmon

[36] Posting results on a public website is an example of "easily publicly available." Shall be made available within 30 days of the incident and see Appendix VI for transparency requirements.

[37] **Lethal incident**: Includes all lethal actions as well as entanglements or other accidental mortalities of non-salmonids.

[38] Standard 2.5.6 applicable to incidents related to non-endangered and non-red-listed species. This standard complements, and does not contradict, 2.5.3.

[39] References for the section of the rationale related to ADDs/AHDs:

- Northridge, S.P., Gordon, J.G., Booth, C., Calderan, S., Cargill, A., Coram, A., Gillespie, D., Lonergan, M. and Webb, A. 2010. Assessment of the impacts and utility of acoustic deterrent devices. Final Report to the Scottish Aquaculture Research Forum, Project Code SARF044. 34pp. http://www.sarf.org.uk/cms-assets/documents/28820-18834.sarf044---final-report.pdf
- Morton, A. B., and Symonds, H. K. 2002. Displacement of *Orcinus orca* (L.) by high amplitude sound in British Columbia, Canada. ICES Journal of Marine Science, 59: 71–80. https://oup.silverchair-cdn.com/oup/backfile/Content_public/Journal/icesjms/59/1/10.1006_jmsc.2001.1136/3/59-1-71.pdf?Expires=1499859194&Signature=URpngb2fKVR8B2kFgMguget42wf4uSn3nDVMqD6C-nymcyQlow3frZfVe4l9aLUpkGsJ5H0M4y3h2S6WVJJKOBa0~qFI5fuVjJ2lQhobfCbLu3JkiexGslvDncRW498rq6-06oV8Qsk2Y-Up3QBNujCKBN-

predation by marine mammals can vary widely including by location, marine mammal species, period of use, etc. Available research suggests that noise and high-pitched sounds resulting from currently available acoustic devices can cause pain to dolphins, porpoises and whales. As intended, acoustic devices can cause marine mammals including seals, porpoises and whales to avoid areas that may be important for feeding, breeding and travel/migration. While the devices may be initially effective in deterring marine mammals in certain scenarios, research studies suggest that they lose their effectiveness over several years. Additionally, evidence suggests that alternative measures such as promptly removing dead fish, reducing stocking densities, net tensioning and use of seal blinds are important in reducing depredation on salmon farms.

Given the impacts associated with ADDs/AHDs and the availability of other, potentially less impactful and more effective deterrence practices, the requirements encourage farms not to use ADDs/AHDs, requires that they not be used on a continuous basis and that they are actively used less than 40 percent of the days in the production cycle. The requirement additionally requires that their use be phased out on certified farms within three years of the publication of the ASC Salmon Standard. Starting three years from the date of publication, no farm meeting the requirement shall use ADDs/AHDs. An exception to this requirement for new technologies may be granted by the Technical Advisory Group of the ASC if there is clear scientific evidence that future ADD/AHD technology presents significantly reduced risk to marine mammals and cetaceans.

07SWDpXdX3GvFsJTvxeEecDNojXRgLrYV7z6~iWsFHiVW4CiFO4arHhveN8tpu0yhYte~-byBwFih0BNCPpwQnRbIOCuwcIq6cVIsifQSDbMNSdkYUT72t3KJyocHMvMhvfPYBbAwvoZFYC3Bpvf~3pD4U0NjIkI9YnHQoY6zwShaORjbkq0CfRvc6w__&Key-Pair-Id=APKAIUCZBIA4LVPAVW3Q

- Scottish Association for Marine Science and Napier University (SAMS)2002. Review and synthesis of the environmental impacts of aquaculture. Scottish Executive Research Unit. www.scotland.gov.uk/cru/kd01/green/reia-00.asp.
- Milewski, I. 2001. Impacts of salmon aquaculture on the coastal environment: a review. https://www.iatp.org/sites/default/files/Impacts_of_Salmon_Aquaculture_on_the_Coastal_E.pdf
- Young, S. 2001. Potential adverse effects of aquaculture on marine mammals: in Tlusty, M.F., Bengston, D.A., Halvorson, H.O., Oktay, S.D., Pearce, J.B., Rheault, Jr., R.B. (eds.). Marine Aquaculture and the Environment: A Meeting for Stakeholders in the Northeast. Cape Cod Press, Falmouth, Massachusetts.

PRINCIPLE 3: PROTECT THE HEALTH AND GENETIC INTEGRITY OF WILD POPULATIONS

The primary aim of Principle 3, in combination with Principle 5, is to ensure that salmon farms do not harm the health of wild fish populations. This principle addresses impacts associated with disease and parasites, escapes and siting.

Criterion 3.1 Introduced or amplified parasites and pathogens[40, 41]

INDICATOR	REQUIREMENT
3.1.1 Participation in an Area-Based Management (ABM) scheme for managing disease and resistance to treatments that includes coordination of stocking, fallowing, therapeutic treatments and information sharing. Detailed requirements are in Appendix II-1.	Yes
3.1.2 A demonstrated commitment[42] to collaborate with NGOs, academics and governments on areas of mutually agreed research to measure possible impacts on wild stocks	Yes
3.1.3 Establishment and annual review of a maximum sea lice load for the entire ABM and for the individual farm as outlined in Appendix II-2	Yes

[40] Farm sites for which there is no release of water that may contain pathogens into the natural (freshwater or marine) environment are exempt from the standards under Criterion 3.1.

[41] See Appendix VI for transparency requirements for 3.1.1, 3.1.3, 3.1.4, 3.1.6 and 3.1.7.

[42] **Commitment**: At a minimum, a farm and/or its operating company must demonstrate this commitment through providing farm-level data to researchers, granting researchers access to sites, or other similar non-financial support for research activities.

3.1.4 Frequent[43] on-farm testing for sea lice, with test results made easily publicly available[44] within seven days of testing	Yes
3.1.5 In areas with wild salmonids,[45] evidence of data[46] and the farm's understanding of that data, around salmonid migration routes, migration timing and stock productivity in major waterways within 50 kilometres of the farm	Yes
3.1.6 In areas of wild salmonids, monitoring of sea lice levels on wild out-migrating salmon juveniles or on coastal sea trout or Arctic char, with results made publicly available. See requirements in Appendix III-1	Yes
3.1.7 In areas of wild salmonids, maximum on-farm lice levels during sensitive periods for wild fish.[47] See detailed requirements in Appendix II, subsection 2	0.1 mature female lice per farmed fish

Rationale - Salmon farms interact with wild fish populations that live or migrate near the open net pens. A particular concern is the interaction with wild salmon and sea trout with regard to pathogens and parasites. There is significant debate in the scientific literature about the extent of the interaction and impact. The Disease Report[48] commissioned by the SAD concluded that there is "shared benefit

[43] Testing must be weekly during and immediately prior to sensitive periods for wild salmonids, such as outmigration of wild juvenile salmon. Testing must be at least monthly during the rest of the year, unless water temperature is so cold that it would jeopardise farmed fish health to test for lice (below 4 degrees C). Within closed production systems, alternative methods for monitoring sea lice, such as video monitoring, may be used.

[44] Posting results on a public website is an example of "easily publicly available."

[45] For purposes of these standards, "**areas with wild salmonids**" are defined as areas within 75 kilometres of a wild salmonid migration route or habitat. This definition is expected to encompass all, or nearly all, of salmon-growing areas in the northern hemisphere.

[46] Farms do not need to conduct research on migration routes, timing and the health of wild stocks under this standard if general information is already available. Farms must demonstrate an understanding of this information at the general level for salmonid populations in their region, as such information is needed to make management decisions related to minimizing potential impact on those stocks. Such "evidence" would consist of, for example, peer review studies; publicly available government monitoring and reporting.

[47] Sensitive periods for migrating salmonids is during juvenile outmigration and approximately one month before.

[48] This report and other reports on State of Information of key impacts commissioned by the Salmon Aquaculture Dialogue are available at http://www.worldwildlife.org/pages/creating-standards-for-responsibly-farmed-salmon

to farm productivity and to minimising impacts on wild fish by continually seeking to reduce disease on salmon farms."

Sea lice have emerged as a pressing challenge for the salmon industry and its potential impacts on wild populations. The SAD's Sea Lice Technical Report concluded that the "weight of evidence is that sea lice of farm origin can present, in some locations and for some host species populations, a significant threat." The report called for a "concerted precautionary approach" in managing the issue.

Requirements under Criterion 3.1, in combination with requirements under Criterion 5.4, seek to address these concerns by establishing best practice in managing potential disease and parasite risks to wild populations. The requirements recognise that the cumulative impacts from a group of farms in an area can become harmful even when an individual farm is operating its own production in a responsible way. Farms located in areas of wild salmonids, defined as farms situated within 75 km of a migration route or sea trout habitat, have additional requirements because of the transmission of disease between farms and wild salmonids.

Area-based management (ABM) is a requirement. Some salmon-growing jurisdictions have begun to require ABM or are considering it because neighbouring farms can achieve significantly improved results when coordinating management of diseases and biosecurity measures. Conversely, a lack of coordination can lead to negative outcomes, such as resistance to treatments. Farms that don't have ABM schemes already established in their jurisdiction will need to show leadership in working with neighbouring farms to establish such a scheme, even if the regulatory structure doesn't require it.

The commitment to research required under 3.1.2 intends to ensure that farms are working with researchers and regulators to address the many gaps in understanding around a farm's interaction with wild populations. A demonstrated commitment means that the farm is participating in joint research efforts. Although funding of research is encouraged, transparency around site-level data and/or access to sites is seen as an extremely valuable contribution to scientific research and is, therefore, the requirement.

The requirements address the challenge of sea lice in several ways. Firstly, farms seeking certification must be able to demonstrate that the ABM scheme has set a maximum lice load for the entire area that reflects regulatory requirements. In areas of wild salmonids, the ABM must also show how this maximum load reflects the results of monitoring of wild populations (more below on monitoring).

The requirements also call for an enhanced level of transparency around sea lice monitoring data. Secondly, farms must conduct frequent testing of on-farm lice levels and make those results publicly available. This transparency reflects the goal of building credibility among the interested public around the actual experience of sea lice levels on the farm and in the wild.

Farms located in areas of wild salmonids must participate in monitoring of lice levels on wild out-migrating juvenile salmon or other important salmonids in the area, such as coastal sea trout or arctic char. The requirements assume this monitoring will be conducted in collaboration with researchers and/or regulatory bodies. Area-based management schemes must demonstrate how the scheme has incorporated the results of wild monitoring into maximum lice loads permitted across the area. These requirements require farms to show leadership in managing the interaction with wild populations. This leadership will mean that some farms seeking certification will need to take on roles and responsibilities that they previously didn't view to be inside the scope of responsibility for an individual farm. Enhanced leadership is an essential part of showing best practice in this high-priority issue of farm interaction with wild populations.

Under 3.1.7, the requirements also require farms located in areas of wild salmonids to demonstrate precautionary low lice levels near zero during sensitive periods for wild fish, such as during juvenile out-migration and immediately prior.

The monitoring and disease management presuppose that farmers are aware of salmon migration routes, the timing of out migration and basic information around stock status. This information, along with sea lice monitoring results, should be compiled by ASC in an effort to consolidate data and promote future research.

If national or local regulations prohibit the handling of wild salmonids then it should be clear that wild populations are being monitored and protected in another way. Cooperation from the farm is necessary so it must be able to provide the data, but the farm is not expected to catch the salmon themselves. The farm could, for example, provide existing evidence to the CAB on how control agents are impacting wild populations.

Criterion 3.2 Introduction of non-native species

INDICATOR	REQUIREMENT
3.2.1 If a non-native species is being produced, demonstration that the species was widely commercially produced in the area by the date of publication of the ASC Salmon Standard	Yes[49]
3.2.2 If a non-native species is being produced, evidence of scientific research[50] completed within the past five years that investigates the risk of establishment of the species within the farm's jurisdiction and these results submitted to ASC for review[51]	Yes [52]

[49] Exceptions shall be made for production systems that use 100 percent sterile fish or systems that demonstrate separation from the wild by effective physical barriers that are in place and well-maintained to ensure no escapes of reared specimens or biological material that might survive and subsequently reproduce.

[50] The research must at a minimum include multi-year monitoring for non-native farmed species, use credible methodologies and analysis, and undergo peer review.

[51] If the review demonstrates there is increased risk, the ASC will consider prohibiting the certification of farming of non-native salmon in that jurisdiction under this standard. In the event that the risk tools demonstrate "high" risks, the SAD expects that the ASC will prohibit the certification of farming of non-native salmon in that jurisdiction. The ASC intends to bring this evidence into future revision of the standard and those results taken forward into the revision process.

[52] Farms are exempt from this standard if they are in a jurisdiction where the non-native species became established prior to farming activities in the area and the following three conditions are met: eradication would be impossible or have detrimental environmental effects; the introduction took place prior to 1993 (when the Convention on Biological Diversity (CBD) was ratified); the species is fully self-sustaining.

3.2.3 Use of non-native species for sea lice control or on-farm management purposes	None

Rationale - Accidental or intentional introductions of non-native species are significant global environmental problems.[53] Aquaculture is considered one of the major pathways for introducing non-native aquatic plants and animals that may become harmful invasive species. The ASC believes these standards are in line with FAO guidelines that permit the culture of non-native species only when they pose an acceptable level of risk to biodiversity. This requirement does not permit introductions of non-native salmonids, unless farming of the species already occurs in the area, or a completely closed production system is used, or all cultured fish are sterile.

Research to date, reviewed by the SAD Technical Working Group on Escapes, has not shown that the production of farmed salmon has led to the establishment of viable populations in the wild of non-native species. Given this research and existing analyses of the risks associated with the farming of salmonids as either a native or non-native species, this requirement permits the certification of farming of non-native species in locations where production already exists.

Nonetheless, the requirement also requires that farms producing non-native salmon demonstrate new research every five years that investigates the risks of establishment in that jurisdiction. The requirement is intended to create an incentive for continuing research.

The use of alternatives to chemical treatments for farm management, such as the use of cleaner fish for sea lice control, is permitted and encouraged under the ASC Salmon Standard. However, any wrasse, cleaner fish or other species used for management during production must be native species in order to prevent introduction of new species to an area.

Criterion 3.3 Introduction of transgenic species

INDICATOR	REQUIREMENT
3.3 Use of transgenic[54] salmon by the farm	None

Rationale - Transgenic fish are not permitted under this requirement because of concerns about their unknown impact on wild populations. The culture of genetically enhanced[55] salmon is acceptable

[53] Leung, K.M.Y. and Dudgeon, D. 2008. Ecological risk assessment and management of exotic organisms associated with aquaculture activities. In M.G. Bondad-Reantaso, J.R. Arthur and R.P. Subasinghe (eds.) Understanding and applying risk analysis in aquaculture. FAO Fisheries and Aquaculture Technical Paper. No. 519. Rome, FAO. pp. 67–100. http://www.fao.org/3/a-i0490e/i0490e01e.pdf

[54] **Transgenic**: An organism, with the exception of human beings, in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination. Source EFSA.

[55] **Genetic enhancement:** The process of genetic improvement via selective breeding that can result in better growth performance and domestication but does not involve the insertion of any foreign genes into the genome of the animal.

under the ASC Salmon Standard. This allows for further progress in feed conversion, which should increase the efficient use of local resources. Also allowed under the Standard is the cultivation of triploid or all female fish, as long as those fish are not transgenic.

Criterion 3.4 Escapes[56]

INDICATOR	REQUIREMENT
3.4.1 Maximum number of escapees[57] in the most recent production cycle	300[58]
3.4.2 Accuracy[59] of the counting technology or counting method used for calculating stocking and harvest numbers	≥ 98%
3.4.3 Estimated unexplained loss[60] of farmed salmon is made publicly available	Yes
3.4.4 Evidence of escape prevention planning and related employee training, including: net strength testing; appropriate net mesh size; net traceability; system robustness; predator management; record keeping and reporting of risk events (e.g. holes, infrastructure issues, handling errors, reporting and follow up of escape events); and worker training on escape prevention and counting technologies	Yes

[56] See Appendix VI for transparency requirements for 3.4.1, 3.4.2 and 3.4.3.

[57] Farms shall report all escapes; the total aggregate number of escapees per production cycle must be less than 300 fish. Data on date of escape episode(s), number of fish escaped and cause of escape episode shall be reported as outlined in Appendix VI.

[58] A rare exception to this standard may be made for an escape event that is clearly documented as being outside the farm's control. Only one such exceptional episode is allowed in a 10-year period for the purposes of this standard. The 10-year period starts at the beginning of the production cycle for which the farm is applying for certification. The farmer must demonstrate that there was no reasonable way to predict the events that caused the episode. See auditing guidance for additional details.

[59] Accuracy shall be determined by the spec sheet for counting machines and through common estimates of error for any hand-counts.

[60] Calculated at the end of the production cycle as: Unexplained loss = Stocking count – harvest count – mortalities – other known escapes. Where possible, use of the pre-smolt vaccination count as the stocking count is preferred.

Rationale - Escaped farmed salmon have the potential to disrupt ecosystems and alter the overall pool of genetic diversity through competition with wild fish and interbreeding with local wild stocks of the same population. It has been shown that interbreeding of farmed with wild salmon of the same species can result in reduced lifetime success, lowered individual fitness and decreases in production over at least two generations.[61] The most effective way to address these risks is to reduce the number of escapes of farmed salmon to zero or near zero.

Escapes can occur in large events that are immediately noticeable at a farm, smaller events that are still noticeable, and through slower, lower levels of losses of fish that might go unnoticed. These requirements place a cap on the total amount of escapees. The cap effectively prevents a farm that has had a significant escape event from being certified, except under extremely unusual circumstances in which the farm can demonstrate there was no reasonable way to predict the cause.

The requirements require transparency about unexplained loss of salmon to help the farm and the public understand trends related to the cumulative numbers of losses of fish that go unnoticed during production. The accuracy of these numbers is limited by the margin of error of fish counting machines and other counting techniques. The requirements seek to encourage farmers to use counting devices that are as accurate as possible, requiring a minimum 98 per cent accuracy of the counting method.

A number of other requirements throughout the document complement the requirements on escapes from grow-out sites in terms of minimising impact on wild salmon populations. The ASC Salmon Standard includes requirements related to escapes from smolt production facilities, and a move away from production of smolts in open systems to closed and semi-closed systems with lower risk of escapees. Requirements related to escapees from smolt systems are particularly important in minimising the potential for interbreeding, as some studies show comparatively high reproductive success rates in escaped precocious male parr.[62] The ASC Salmon Standard also includes requirements related to siting in protected or high conservation value areas, including areas that are designated as such in order to protect threatened wild salmonid populations.

[61] Thorstad, E.B., Fleming, I.A., McGinnity, P., Soto, D., Wennevik, V. and Whoriskey, F. 2008. Incidence and impacts of escaped farmed Atlantic salmon *Salmo salar* in nature. NINA Special Report 36. 110 pp. http://www.fao.org/3/a-aj272e.pdf

[62] Garant, D., Fleming I.A., Einum, S. and Bernatchez, L. Alternate male life-history tactics as potential vehicles for speeding introgression of farm salmon traits into wild populations. Ecology Letters 2003;6: 541-549.

PRINCIPLE 4: USE RESOURCES IN AN ENVIRONMENTALLY EFFICIENT AND RESPONSIBLE MANNER

Principle 4 is intended to address negative impacts that stem from resource use, including feed and non-therapeutic chemical inputs.

Criterion 4.1 Traceability of raw materials in feed

INDICATOR	REQUIREMENT
4.1.1 Evidence of traceability, demonstrated by the feed producer, of feed ingredients that make up more than 1% of the feed.[63]	Yes

Rationale - Raw material traceability is fundamental to many of the ASC Salmon Standard and, therefore, is required under this requirement. This requirement will make raw material sourcing more transparent. It must be demonstrated at the feed manufacturer or feed producer level. For some feed ingredients, this will be evidence of traceability with regard to country of origin, while for other feed ingredients that relate specifically to other requirements, this may be a finer level of detail, such as traceability back to the fishery as outlined in the following criteria 4.2 and 4.3.

Criterion 4.2 Use of wild fish for feed[64]

INDICATOR	REQUIREMENT
4.2.1 Fishmeal Forage Fish Dependency Ratio (FFDRm) for grow-out (calculated using formulas in Appendix IV- 1)	< 1.2

[63] Traceability shall be at a level of detail that permits the feed producer to demonstrate compliance with the standards in this document (i.e., marine raw ingredients must be traced back to the fishery, soy to the region grown, etc.). Feed manufacturers will need to supply the farm with third-party documentation of the ingredients covered under this standard.

[64] See Appendix VI for transparency requirements for 4.2.1 and 4.2.2.

4.2.2 Fish Oil Forage Fish Dependency Ratio (FFDRo) for grow-out (calculated using formulas in Appendix IV- 1), or, Maximum amount of EPA and DHA from direct marine sources[65] (calculated according to Appendix IV-2)	FFDRo < 2.52, or, (EPA + DHA) < 30 g/kg feed

Rationale - The salmon aquaculture industry has significantly reduced the inclusion rates of fishmeal and fish oil from forage fish in salmon feeds during the past two decades. The Forage Fish Dependency Ratios (FFDR) contained in these requirements aim to support the trend toward lower inclusion rates and increasingly efficient use of marine resources, which are expected to continue. Fishmeal and fish oil are both finite resources that are shared across a range of users with increasing demands, from direct human consumption to aquaculture to pig and poultry production. The ASC Salmon Standard intends to promote the efficient use of these resources, producing increasing amounts of farmed salmon from a given input of fishmeal and oil.

The ratios, one for fishmeal and another for fish oil, calculate the dependency on forage fisheries through an assessment of the quantity of live fish from small pelagic fisheries required to produce the amount of fishmeal or fish oil needed to produce a unit of farmed salmon. The ASC Salmon Standard offers the calculation of levels of EPA and DHA from wild fish in feeds as an alternate method of measuring dependency on forage fisheries. The requirement encourages producers who want to produce salmon with high levels of omega-3 fatty acids to do so by sourcing the EPA and DHA from sources other than fish oil derived from direct industrial fisheries. The ratios complement the requirements described in criterion 4.3, which will move farms toward using feed with marine ingredients from fisheries certified as responsibly managed. Producers will be able to improve their FFDR by using a greater percentage of fishmeal and fish oil from trimmings and offal, using other sources of meal and oil (e.g. vegetables) and improving their feeding efficiency.

[65] Calculation excludes DHA and EPA derived from fisheries by-products and trimmings. Trimmings are defined as by-products when fish are processed for human consumption or if whole fish is rejected for use of human consumption because the quality at the time of landing does not meet official regulations with regard to fish suitable for human consumption.

Fishmeal and fish oil that are produced from trimmings can be excluded from the calculation as long as the origin of the trimmings is not any species that are classified as critically endangered, endangered or vulnerable in the IUCN Red List of Threatened Species (http://www.iucnredlist.org).

Criterion 4.3 Source of marine raw materials

INDICATOR	REQUIREMENT
4.3.1 Timeframe for all fishmeal and fish oil used in feed to come from fisheries[66] certified under a scheme that is an ISEAL member[67] and has guidelines that specifically promote responsible environmental management of small pelagic fisheries	Not required
4.3.2 Prior to achieving 4.3.1, the FishSource score[65, 68] for the fishery(ies) from which all marine raw material in feed is derived	All individual scores ≥ 6, and biomass score ≥ 6
4.3.3 Prior to achieving 4.3.1, demonstration of third-party verified chain of custody and traceability for the batches of fishmeal and fish oil which are in compliance with 4.3.2.	Yes
4.3.4 Feed containing fishmeal and/or fish oil originating from: by-products[69] or trimmings from IUU[70] catch or from fish species that are categorized as vulnerable, endangered or critically endangered, according to the IUCN Red List of Threatened Species,[71] whole fish and fish meal from the same species and family as the species being	None[72]

[66] This standard and standard 4.3.2 apply to fishmeal and oil from forage fisheries, pelagic fisheries, or fisheries where the catch is directly reduced (including krill) and not to by-products or trimmings used in feed.

[67] Meets ISEAL guidelines as demonstrated through full membership in the ISEAL Alliance, or equivalent as determined by the Technical Advisory Group of the ASC.

[68] Or equivalent score using the same methodology. See Appendix IV-3 for explanation of FishSource scoring.

[69] **Trimmings** are defined as by-products when fish are processed for human consumption or if whole fish is rejected for use of human consumption because the quality at the time of landing does not meet official regulations with regard to fish suitable for human consumption.

[70] **IUU**: Illegal, Unregulated and Unreported.

[71] The International Union for the Conservation of Nature reference can be found at http://www.iucnredlist.org/.

[72] For species listed as "vulnerable" by IUCN, an exception is made if a regional population of the species has been assessed to be *not* vulnerable in a National Red List process that is managed explicitly in the same science-based way as IUCN. In cases where a National Red List doesn't exist or isn't managed in accordance with IUCN guidelines, an exception is allowed when an assessment is conducted using IUCN's methodology and demonstrates that the population is not vulnerable.

farmed.	
4.3.5 Presence and evidence of a responsible sourcing policy for the feed manufacturer for marine ingredients that includes a commitment to continuous improvement of source fisheries.[73]	Yes

Rationale - Wild fish harvested from the ocean and reduced into fishmeal and fish oil are an important component of salmon feeds. Many wild small pelagic fish resources are fished at capacity or overfished.[74] Demand for these resources is increasing as the aquaculture industry expands and as forage fish are increasingly consumed by humans or by other industries including other animal production. There is concern that higher demand could lead to the overfishing—and collapse—of small forage fish stocks. Wild small pelagic fish play a critical role in the ecosystem and the marine food chain. Some conservation groups and scientists are concerned that even fisheries that are not classified as overfished from a population perspective are, or could be, overfished from an ecological perspective.

These indicators strive to ensure that marine-based feed ingredients come from sustainable sources in the short- and long-term. The requirements aim to align industry incentives to support processes that will lead to improved fisheries management and ultimately the certification of forage fisheries as an independent measure of the ecological health of those fisheries.

In the medium term, the requirements will require marine ingredients in feed to be certified by a widely recognised authority. This recognised authority must be a member of the ISEAL Alliance, which promotes transparent, multi-stakeholder processes. The authority must also have a methodology that specifically addresses the ecological role of low trophic-level species. As of the date of publication of this ASC Salmon Standard, the Marine Stewardship Council (MSC) is the only fishery scheme that is a full member of ISEAL, and MSC is in the process of developing specific requirements for small pelagic fisheries. Additional schemes may emerge in the future that meet these requirements. This requirement begins to be applicable five years after the publication of the ASC Salmon Standard because there is a current lack of such certified sources of fishmeal and fish oil and the transformation of the industry will take some time. The ASC Salmon Standard encourages fisheries to begin immediately to make any needed management changes or regulatory reforms needed to achieve certification.

In the short term, the requirements restrict fisheries currently known to have the poorest status from being used for fishmeal and fish oil and places traceability requirements on the fishmeal and fish oil used in the feed. Requirement 4.3.2 requires the fishmeal and fish oil from forage fisheries to originate from fisheries meeting a minimum score using the FishSource scoring methodology, which is outlined in Appendix IV-3.

[73] The policy should be written and include an assessment of source fishery status and identification of improvement needs and work plan to deliver improvements. The policy must include a commitment and timeline to source aquaculture and fishery products from responsible/best practice sources, such as those certified a standard benchmarked at minimum consistent with relevant FAO's eco-labelling guidelines or by identified independent risk assessment.

[74] FAO, The State of World Fisheries and Aquaculture (SOFIA), 2010.

Rigorous traceability requirements are built into requirement 4.3.3. The traceability scheme must also incorporate baseline measures related to sustainability that serve as an additional measure to ensure that fish from unsustainable fisheries are not used in feed. The International Fishmeal and Fish Oil Organization's Global Standard for Responsible Supply[75] or a future equivalent that might emerge can be used to meet this requirement.

Last, requirement 4.3.4 prevents the use of by-products and trimmings that come from species categorized as vulnerable or worse on the IUCN Red List of Threatened Species. Using by-products from fisheries for human consumption in salmon feeds is a valuable use of products that may otherwise be wasted. However, a minimum level of sustainability of these fisheries is still required under the ASC Salmon Standard. For species classified globally as vulnerable by IUCN, the requirement offers the opportunity for feed suppliers to demonstrate through a scientific process that a regional population of a species is not actually vulnerable.

Criterion 4.4 Source of non-marine raw materials in feed

INDICATOR	REQUIREMENT
4.4.1 Presence and evidence of a responsible sourcing policy for the feed manufacturer for feed ingredients that comply with recognised crop moratoriums[76] and local laws[77]	Yes
4.4.2 Percentage of soya or soya-derived ingredients in the feed that are certified by the Roundtable for Responsible Soy (RTRS) or equivalent[78]	100%
4.4.3 Evidence of disclosure to the buyer[79] of the salmon of inclusion of transgenic[80] plant raw	Yes, for each individual raw material containing > 1% transgenic content[81]

[75] http://www.iffo.net/iffo-rs

[76] **Moratorium**: A period of time in which there is a suspension of a specific activity until future events warrant a removal of the suspension or issues regarding the activity have been resolved. In this context, moratoriums may refer to suspension of the growth of defined agricultural crops in defined geographical regions.

[77] Specifically, the policy shall include that vegetable ingredients, or products derived from vegetable ingredients, must not come from areas of the Amazon Biome that were deforested after July 24, 2006, as geographically defined by the Brazilian Soy Moratorium. Should the Brazilian Soy Moratorium be lifted, this specific requirement shall be reconsidered.

[78] Any alternate certification scheme would have to be approved as equivalent by the Technical Advisory Group of the ASC.

[79] The company or entity to which the farm or the producing company is directly selling its product. This standard requires disclosure by the feed company to the farm and by the farm to the buyer of their salmon.

[80] **Transgenic**: An organism, with the exception of human beings, in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination. Source EFSA.

[81] See Appendix VI for transparency requirement for 4.4.3.

material, or raw materials derived from transgenic plants, in the feed	

Rationale - The ASC Salmon Standard aims to promote responsible sourcing of all feed ingredients. Thus, the ASC Salmon Standard requires producers to provide evidence that they are sourcing feed products from feed manufacturers that have a sustainable sourcing policy for feed ingredients.

Feed ingredients sourced from areas where significant ecological damage has occurred was of concern to the ASC. Therefore, the requirement requires producers to source feed from feed producers who comply with any relevant, recognised crop moratoriums that, at the time of the writing of these requirements, includes only the Brazilian Soy Moratorium,[82]. Such moratoriums are temporary measures intended to protect defined geographic regions. Looking to the future, the ASC Salmon Standard incorporates a requirement for feed manufacturers to use soy certified by the RTRS, which the ASC Salmon Standard recognises as the most environmentally meaningful soy certification process today. Because the scheme is recently starting up, the requirements build in a five-year window for this requirement.

Transgenic plants are commonly used in aquaculture and animal feeds throughout the world. Some consumers and retailers want to be able to identify food products, including farmed salmon, that are genetically modified or that have been fed genetically modified ingredients. The ASC Salmon Standard ensure transparency (above one per cent) around any transgenic material used in the feed in order to support informed choices by retailers and consumers. The ASC Salmon Standard require that the producer disclose to the first-order buyer of their salmon the use of any genetically modified ingredients in feed, and publicly disclose whether transgenic ingredients are used under Appendix VI.

Criterion 4.5 Non-biological waste from production

INDICATOR	REQUIREMENT
4.5.1 Presence and evidence of a functioning policy for proper and responsible[83] treatment of non-biological waste from production (e.g. disposal and recycling)	Yes
4.5.2 Evidence that non-biological waste (including net pens) from grow-out site is either disposed of properly or recycled	Yes

[82] See http://www.ablove.org.br/site/index.php?page=soy-moratorium&area=MTEtMy0x for additional information on the soy moratorium.

[83] Proper and responsible disposal will vary based on facilities available in the region and remoteness of farm sites. Disposal of non-biological waste shall be done in a manner consistent with best practice in the area. Dumping of non-biological waste into the ocean does not represent "proper and responsible" disposal.

Rationale - The purpose of these indicators is to ensure that all non-biological waste produced by a farm is recycled, reused or disposed of properly and does not affect neighbouring communities. Proper handling and treatment of wastes may vary across farms depending on the remoteness of the farm site and the disposal and recycling options available in the region.

Initial Auditing Guidance

The ASC Salmon Standard recognises that some farms are located in extremely remote locations with no viable recycling systems nearby and where waste disposal presents challenges. Auditing guidelines will need to clarify what "proper" disposal means and be flexible enough to recognise that what is "proper" on one site is different from what is "proper" on another site. Regardless of the remoteness of a farm, these requirements would, for example, prohibit the dumping of non-biological waste (e.g. feedbags or nets) into the ocean.

Criterion 4.6 Energy consumption and greenhouse gas emissions on farms[84]

INDICATOR	REQUIREMENT
4.6.1 Presence of an energy use assessment verifying the energy consumption on the farm and representing the whole life cycle at sea, as outlined in Appendix V-1	Yes, measured in kilojoule/mt fish produced/production cycle
4.6.2 Records of greenhouse gas (GHG[85]) emissions[86] on farm and evidence of an annual GHG assessment, as outlined in Appendix V-1	Yes
4.6.3 Documentation of GHG emissions of the feed[87] used during the previous production cycle, as outlined in Appendix V, subsection 2	Yes

Rationale - Climate change represents perhaps the biggest environmental challenge facing current and future generations. Because of this, energy consumption used in food production has become a source of major public concern. The ASC Salmon Standard recognises the importance of efficient and sustainable energy use. Therefore, these indicators will require that energy consumption in the production of fish should be monitored on a continual basis and that growers should develop means to improve efficiency and reduce consumption of energy sources, particularly those that are limited or carbon-based. The data collected in this process will help the ASC Salmon Standard set a meaningful numerical requirement for energy use in the future. Energy assessments are a new area for

[84] See Appendix VI for transparency requirements for 4.6.1, 4.6.2 and 4.6.3.

[85] For the purposes of this standard, **GHGs** are defined as the six gases listed in the Kyoto Protocol: carbon dioxide (CO_2); methane (CH_4); nitrous oxide (N_2O); hydrofluorocarbons (HFCs); perfluorocarbons (PFCs); and sulphur hexafluoride (SF_6).

[86] GHG emissions must be recorded using recognised methods, standards and records as outlined in Appendix V.

[87] GHG emissions from feed can be given based on the average raw material composition used to produce the salmon (by weight) and not as documentation linked to each single product used during the production cycle. Feed manufacturer is responsible for calculating GHG emissions per unit feed. Farm site then shall use that information to calculate GHG emissions for the volume of feed they used in the prior production cycle.

producers. Requiring that farms do these assessments will likely raise awareness of the issues related to energy and build support for adding a requirement in the future related to the maximum energy of GHG emissions allowed.

Criterion 4.7 Non-therapeutic chemical inputs[88,89]

INDICATOR	REQUIREMENT
4.7.1 For farms that use copper-treated nets[90], evidence that nets are not cleaned[91] or treated in situ in the marine environment	Yes
4.7.2 For any farm that cleans nets at on-land sites, evidence that net-cleaning sites have effluent treatment[92]	Yes
4.7.3 For farms that use copper nets or copper-treated nets, evidence of testing for copper level in the sediment outside of the AZE, following methodology in Appendix I-1	Yes
4.7.4 Evidence that copper levels[93] are < 34 mg Cu/kg dry sediment weight, or, in instances where the Cu in the sediment exceeds 34 mg Cu/kg dry sediment weight, demonstration that the Cu concentration falls within the range of background concentrations as measured at three reference sites in the water body	Yes

[88] Closed production systems that do not use nets and do not use antifoulants shall be considered exempt from standards under Criterion 4.7.

[89] See Appendix VI for transparency requirements for 4.7.1, 4.7.3 and 4.7.4.

[90] Under the SAD, "**copper-treated net**" is defined as a net that has been treated with any copper-containing substance (such as a copper-based antifoulant) during the previous 18 months, or has not undergone thorough cleaning at a land-based facility since the last treatment. Farms that use nets that have, at some point prior in their lifespan, been treated with copper may still consider nets as untreated so long as sufficient time and cleaning has elapsed as in this definition. This will allow farms to move away from use of copper without immediately having to purchase all new nets.

[91] Light cleaning of nets is allowed. Intent of the standard is that, for example, the high-pressure underwater washers could not be used on copper treated nets under this standard because of the risk of copper flaking off during this type of heavy or more thorough cleaning.

[92] Treatment must have appropriate technologies in place to capture copper if the farm uses copper-treated nets.

[93] According to testing required under 4.7.3. The standards related to testing of copper are only applicable to farms that use copper-based nets or copper-treated nets.

4.7.5 Evidence that the type of biocides used in net antifouling are approved according to legislation in the European Union, or the United States, or Australia	Yes

Rationale - Copper (Cu) is an abundant trace element found in a variety of rocks and minerals. It is an essential micronutrient and is also necessary for a wide range of metabolic processes in animals and plants. At elevated levels, however, Cu becomes toxic. Collectively, the set of requirements related to copper encourage any sites that can do so to not use copper. Simultaneously, they recognise that in some situations phasing out copper usage may not yet be possible if, for example, alternate antifoulants or cleaning methods don't leave nets at a given site clean enough for the use of wrasse to manage sea lice to be feasible. In situations where copper is used, the requirements ensure precautionary healthy levels of copper in the benthos.

In order to minimise release of Cu from salmon farms into the environment, the requirement includes better management practices of not cleaning copper treated nets in the aquatic environment and requires that land-based cleaning facilities have the appropriate effluent treatment.

Additionally, a maximum level of Cu concentration in the sediment outside of the AZE is built into the requirement to ensure that any benthic effect that may occur from the use of copper on the net pens is minimal. The variability in environmental factors makes it very difficult to identify a generic threshold of copper in the environment that can be used to define the environmental risk. However, experts suggest that the threshold of 34mg/kg sediment adequately protects the benthos. The level of 34 mg is also consistent with the level at which Scottish regulation requires some action to ensure benthic health, and with levels recognised by other jurisdictions as the level at which there may be possible environmental effect. Under the ASC Salmon Standard, if Cu levels in the sediment just outside the AZE are higher than the threshold, as may be the case in areas with naturally high levels of Cu, the farm must demonstrate that the level just outside of the AZE is consistent with reference sites and the background levels in the area.

The ASC Salmon Standard is aware that other biocides are commercially applied to netting material. It is difficult to address all biocides used or to be used in the future. To address the high variability of biocides used, the ASC Salmon Standard elected to limit use to those chemicals approved for legal use by the European Union, the United States or Australia. The ASC Salmon Standard encourages the development and review of alternative antifoulants that are protective of the marine environment. The European Union, the United States and Australia were selected as a representation of jurisdictions that were viewed to be undertaking rigorous analyses of biocides.

PRINCIPLE 5: MANAGE DISEASE AND PARASITES IN AN ENVIRONMENTALLY RESPONSIBLE MANNER

Principle 5 aims to address negative impacts of salmon farming associated with disease, parasites and therapeutic chemical inputs. The ASC Salmon Standard recognises the role of proper fish handling and minimised levels of fish stress as an important element in good husbandry and in reducing levels of disease on farms, mortalities and therapeutic treatments. In addition to addressing environmental risks, compliance with requirements under Principle 5 helps ensure farmed fish health and welfare.

Criterion 5.1 Survival and health of farmed fish[94]

INDICATOR	REQUIREMENT
5.1.1. Evidence of a fish health management plan for the identification and monitoring of fish diseases, parasites and environmental conditions relevant for good fish health, including implementing corrective action when required	Yes
5.1.2 Site visits by a designated veterinarian[95] at least four times a year, and by a fish health manager[96] at least once a month	Yes
5.1.3 Percentage of dead fish removed and disposed of in a responsible manner	100%[97]
5.1.4 Percentage of mortalities that are recorded, classified and receive a post-mortem analysis	100%[98]

[94] See Appendix VI for transparency requirements for 5.1.4, 5.1.5 and 5.1.6.

[95] A **designated veterinarian** is the professional responsible for health management on the farm who has the legal authority to diagnose disease and prescribe medication. In some countries such as Norway, a fish health biologist or other professional has equivalent professional qualifications and is equivalent to a veterinarian for purposes of these standards. This definition applies to all references to a veterinarian throughout the standards document.

[96] A **fish health manager** is someone with professional expertise in managing fish health, who may work for a farming company or for a veterinarian, but who does not necessarily have the authority to prescribe medicine.

[97] The SAD recognises that not all mortality events will result in dead fish present for collection and removal. However, such situations are considered the exception rather than the norm.

[98] If on-site diagnosis is inconclusive, this standard requires off-site laboratory diagnosis. A qualified professional must conduct all diagnosis. One hundred percent of mortality events shall receive a post-mortem analysis, not necessarily every fish. A statistically relevant number of fish from the mortality event shall be analysed.

5.1.5 Maximum viral disease-related mortality[99] on farm during the most recent production cycle	≤ 10%
5.1.6 Maximum unexplained mortality rate from each of the previous two production cycles, for farms with total mortality > 6%	≤ 40% of total mortalities
5.1.7 A farm-specific mortalities reduction programme that includes defined annual targets for reductions in mortalities and reductions in unexplained mortalities	Yes

Rationale - Farmed salmon are susceptible to numerous diseases that have the potential to be amplified and transferred, thereby posing a risk to the health of fish and other marine organisms in adjacent ecosystems. One of the best ways to mitigate the risk of disease transfer to wild stocks is to reduce or eliminate the disease from happening initially.

These requirements seek to ensure proactive health management on the farm through a detailed health management plan and frequent visits by the designated veterinarian and other fish health professionals. The requirements under Criterion 5.1 are complemented by requirements related to the health of smolts, as outlined under Section 8 of this document. Requirements related to smolt seek to ensure that farmed salmon have all relevant vaccinations and enter the water as healthy as possible.

Healthy farms also must keep detailed records of all mortalities and cause of death. The post-mortem analysis required in this requirement is essential to provide an early warning against emerging diseases. Repeated high mortality rates, or a high rate of unexplained mortalities, may indicate poor management or poor siting. The mortality requirements in 5.1.5 and 5.1.6 are not intended as a goal, but rather a minimum required. The requirement focuses on mortalities from viral disease and unknown causes, as those categories were highlighted by experts as presenting a greater potential risk to wild fish populations and neighbouring farms. The requirement requires that mortalities from viral disease be kept at or below 10 per cent. Only farms with mortality rates greater than six per cent per production cycle must also then meet the requirement related to percentage of unexplained mortalities. The farm must be able to demonstrate that it is working seriously to reduce its mortalities, including tracking diseases and carrying out a farm-specific plan to reduce diseases and mortalities. The information collected on mortalities will be useful for future revisions of the requirements.

[99] Viral disease-related mortality count shall include unspecified and unexplained mortality as it could be related to viral disease.

Criterion 5.2 Therapeutic treatments[100]

INDICATOR	REQUIREMENT
5.2.1 On-farm documentation that includes, at a minimum, detailed information on all chemicals[101] and therapeutants used during the most recent production cycle, the amounts used (including grams per ton of fish produced), the dates used, which group of fish were treated and against which diseases, proof of proper dosing, and all disease and pathogens detected on the site	Yes
5.2.2 Allowance for use of therapeutic treatments that include antibiotics or chemicals that are banned[102] in any of the primary salmon producing or importing countries[103]	None
5.2.3 Percentage of medication events that are prescribed by a veterinarian	100%
5.2.4 Compliance with all withholding periods after treatments	Yes
5.2.5 The farm shall publicly report (via Appendix VI) the: 1. Weighted Number of Medicinal Treatments (see Appendix VII) for each production cycle 2. The parasiticide load for each agent over the production cycle 3. The benthic parasiticide residue levels	Yes
5.2.6 The Weighted Number of Medicinal Treatments	Yes

[100] See Appendix VI for transparency requirements for 5.2.1, 5.2.5, 5.2.6 and 5.2.10.

[101] Chemicals used for the treatment of fish.

[102] "**Banned**" means proactively prohibited by a government entity because of concerns around the substance. A substance banned in any of the primary salmon-producing or importing countries, as defined here, cannot be used in any salmon farm certified under the SAD, regardless of country of production or destination of the product. The SAD recommends that ASC maintain a list of a banned therapeutants.

[103] For purposes of this standard, those countries are Norway, the UK, Canada, Chile, the United States, Japan and France.

shall be at or below the country Entry Level (see Appendix VII)	
5.2.7 The farm shall reduce the Weighted Number of Medicinal Treatments, after achieving indicator 5.2.6, with 25% per 2 years until the WNMT is at or below the Global Level (see Appendix VII).	Yes
5.2.8 The farm shall implement Integrated Pest Management (IPM) according to the guidance in Appendix VII.	Yes
5.2.9 The farm shall public present (e.g. via company website) the IPM-measures that the company applies which need to be approved by a authorised veterinarian.	Yes
5.2.10 The farm shall monitor parasiticide residue levels annually in the benthic sediment directly outside the AZE.	Yes
5.2.11 Allowance for prophylactic use of antimicrobial treatments[104]	None
5.2.12 Allowance for use of antibiotics listed as critically important for human medicine by the World Health Organization (WHO[105])	None[106]
5.2.13 Number of treatments[107] of antibiotics over the most recent production cycle	≤ 3
5.2.14 If more than one antibiotic treatment is used in the most recent production cycle, demonstration that the antibiotic load[108] is at least 15% less that of the average of the two previous production cycles	Yes[109]

[104] The designated veterinarian must certify that a pathogen or disease is present before prescribing medication.

[105] The fifth edition of the WHO list of "Critically important antimicrobials for human medicine" was released in 2017 and is available at: http://apps.who.int/iris/bitstream/10665/255027/1/9789241512220-eng.pdf?ua=1 .

[106] If the antibiotic treatment is applied to only a portion of the pens on a farm site, fish from pens that did not receive treatment are still eligible for certification.

[107] A **treatment** is a single course medication given to address a specific disease issue and that may last a number of days.

[108] **Antibiotic load** = the sum of the total amount of active ingredient of antibiotics used (kg).

[109] Reduction in load required, regardless of whether production increases on the site. Farms that consolidate production across multiple sites within an ABM can calculate reduction based on the combined antibiotic load of the consolidated sites.

5.2.15 Presence of documents demonstrating that the farm has provided buyers[110] of its salmon a list of all therapeutants used in production	Yes

Rationale - When disease outbreaks occur on salmon farms, farmers often opt to treat using chemical therapeutants as a means of protecting on-farm fish and the health of wild populations near the farm. With any chemical introduction into a wild environment, there is a need to ensure that non-target organisms are not being negatively impacted by the use of that chemical. Accurate and detailed documentation of all treatments is the first step to ensure proper dosing and safe use of therapeutants. The data collected from this requirement will also help the ASC set more measurable requirements in the future.

To minimise the risk of treatments posing a risk to the environment, farms shall not use treatments that have been banned by any of the regulatory bodies in the world's largest salmon-producing or importing countries. The chemical must have been proactively prohibited or banned, versus being not approved. Part of a farm's responsibility to operate within the law involves taking appropriate measures to ensure that its product complies with import laws of the countries where the salmon is eventually sold. Requirement 5.2.11 above ensures that buyers and importers have the information they need to verify that the product complies with import regulations.

Prophylactic use of antimicrobial treatments, and treatments that aren't prescribed by a licensed professional, are unacceptable under the requirement because they open the door to overuse and abuse of therapeutants.

Stakeholders within the SAD shared a common interest and common goal of reducing the use of parasiticides and reducing the risk of needed chemical treatments to the environment. The ultimate goal would be that farms could meet the ASC Salmon Standard without using therapeutants or without the risk of those therapeutants negatively impacting the environment. Simultaneously, the SAD focused on protecting wild stocks of salmonids and thus sets low thresholds (requirement 3.1.7) for allowable lice on farmed fish in areas with wild salmonids. Taking into account current technology and knowledge, and balancing between the objectives of minimising impact on wild stocks and at the same time addressing threats to the environment related to unrestricted use of therapeutants, the SC allowed restricted use of parasiticides to treat sea lice under the requirement.

The purpose of the requirement of 5.2.5 is to place a limit on the number of treatments using parasiticides, while taking into account regional differences in ecosystems and epidemiology, including differences in lice species, wild host reservoirs and susceptibility to lice attack, together with differences in mandatory regulatory requirements in the different countries. The standard seeks to use a progressive indicator which encourages reductions in medicinal product use and the associated risks of resistance from overuse whilst incentivising an increasing shift to non-medicinal means of control through expansion of integrated pest management (IPM) strategies. To promote this, the entry to the process is relatively inclusive in order to promote the progressive changes sought. For this purpose, after the first audit, the farm should show improvement in management against a progress ladder based on the principles of IPM against a time bound plan (Appendix VII) and a shift towards low to zero medicinal product usage (Indicator 5.2.7).

[110] **Buyer**: The company or entity to which the farm or the producing company is directly selling its product.

Indicator 5.2.5 addresses the number of medicinal treatments used on certified farms. The total amount of active ingredient used for medicinal treatments will be provided by the parasiticide load, Indicator 5.2.9. In addition, some more direct assessment of the fate of the various agents in the environment, both in the sediment and the water, is to be encouraged (Indicator 5.2.8) by requiring some monitoring of the concentration of the various agents in water and sediments at the edge and outside the Allowable Zone of Effects (AZE) either by using tools such as direct assay or models that have been scientifically validated (e.g. by peer review and documented testing) and which are approved by national regulatory bodies

In order to monitor effective progress in reduction of medicinal treatments, Indicator 5.2.6 requires that at the end of the second certification cycle following the introduction of the new requirements, that is after 6 years, and of every subsequent cycle, the WMNT can be audited over the preceding 6 years for an overall downward trend indicative of a reduction in medicinal treatment frequency. By this means there should be at least 4 or 5 data points upon which to base judgment. Reductions can be demonstrated at the individual farm or Area Based Management (ABM) level.

These requirements are consistent with industry efforts to reduce both frequency and amount of parasiticide used, as well as with initiatives to develop treatment methods that do not release parasiticides into the environment. To encourage thinking about cumulative use across a broader area, tracking of total use of parasiticides is required under the ABM.

With regards to the use of antibiotics, there is a global effort led by the WHO to ensure that antibiotics important for human medicine are used in a way that doesn't jeopardise their effectiveness in treating human diseases. These requirements seek to be in line with that effort. The requirements set a cap on a maximum allowable number of treatments of antibiotics on certified farms that is intended to set a reasonable limit on what may be needed on a well-managed farm and excludes any farms that fail to follow industry guidelines for prudent use of antibiotics. Through 5.2.10, the ASC Salmon Standard addresses environmental risk from cumulative load of antibiotics entering the environment from certified farms. The requirement requires a reduction, within five years, of the actual load of antibiotics released from farms that use more than one treatment of antibiotics. This is in line with industry goals to reduce total antibiotic use and with trends in industry to use precise pen-by-pen treatments when appropriate.

Additionally, the SAD's technical working group on chemical inputs recommended that antibiotics important for human health only be used with extreme reluctance. These requirements are also intended to further raise awareness within the aquatic veterinary community on the use of medically important antimicrobial drugs in food-animal production, and the public health risks associated with antibiotic resistance. This issue is addressed in requirement 5.2.8 and through a coordination requirement within the ABM related to the use of antibiotics classified by the WHO as "highly important" for human health.

Criterion 5.3 Resistance of parasites, viruses and bacteria to medicinal treatments

INDICATOR	REQUIREMENT
5.3.1 Bio-assay analysis to determine resistance when two applications of a treatment have not produced the expected effect	Yes
5.3.2 When bio-assay tests determine resistance is forming, use of an alternative, permitted treatment, or an immediate harvest of all fish on the site	Yes
5.3.3 Specific rotation, providing that the farm has >1 effective medicinal treatment product available, every third treatment must belong to a different family of drugs.	Yes

Rationale - One of the more serious risks of overusing medicinal treatments is the development of parasite drug resistance, which lowers the overall effectiveness of treatments. In some salmon-growing regions, resistance to a number of drugs has become a growing problem, increasing the challenge for salmon farmers to control sea lice on farmed and wild fish.

Efforts to prevent and monitor resistance are made most effectively through an area-based approach. Timely, accurate sea lice counts on the farm can detect when sea lice treatment is no longer effective. Bioassays are important to confirm if resistance is developing and a limit has been set on the number of repeat treatments of the same family of drugs that can be applied. A single treatment is considered to have taken place when the majority of a site (more than half of all cages) is treated. No more than two such treatments should use the same family of drugs; that is, at least every third treatment should be with a drug of a different class.

Criterion 5.4 Biosecurity management[111]

INDICATOR	REQUIREMENT
5.4.1 Evidence that all salmon on the site are a single year class[112]	100%[113]
5.4.2 Evidence that if the farm suspects an unidentifiable transmissible agent, or if the farm experiences unexplained increased mortality,[114] the farm has: 1. Reported the issue to the ABM and to the appropriate regulatory authority 2. Increased monitoring and surveillance[115] on the farm and within the ABM 3. Promptly[116] made findings publicly available	Yes
5.4.3 Evidence of compliance[117] with the OIE *Aquatic Animal Health Code*[118]	Yes
5.4.4 If an OIE-notifiable disease[119] is confirmed on the farm, evidence that:	Yes

[111] See Appendix VI for transparency requirements for 5.4.2 and 5.4.4.

[112] Gaps of up to six months between inputs of smolts derived from the same stripping are acceptable as long as there remains a period of time when the site is fully fallow after harvest.

[113] Exception is allowed for: 1) farm sites that have closed, contained production units where there is complete separation of water between units and no sharing of filtration systems or other systems that could spread disease, or, 2) farm sites that have ≥95% water recirculation, a pre-entry disease screening protocol, dedicated quarantine capability and biosecurity measures for waste to ensure there is no discharge of live biological material to the natural environment (e.g. UV or other effective treatment of effluent) .

[114] **Increased mortality**: A statistically significant increase over background rate on a monthly basis.

[115] Primary aim of monitoring and surveillance is to investigate whether a new or adapted disease is present in the area.

[116] Within one month.

[117] Compliance is defined as farm practices consistent with the intentions of the Code, to be further outlined in auditing guidance. For purposes of this standard, this includes an aggressive response to detection of an exotic OIE-notifiable disease on the farm, which includes depopulating the infected site and implementation of quarantine zones in accordance with guidelines from OIE for the specific pathogen. Quarantine zones will likely incorporate mandatory depopulation of sites close to the infected site and affect some, though not necessarily all, of the ABM. Exotic signifies not previously found in the area or had been fully eradicated (area declared free of the pathogen).

[118] OIE 2017. Aquatic Animal Health Code. http://www.oie.int/en/international-standard-setting/aquatic-code/access-online/

[119] **OIE-notifiable diseases** relevant to salmon aquaculture were: Epizootic haematopoietic necrosis, Infectious haematopoietic necrosis (IHN), Infectious salmon anemia (ISA), Viral hemorrhagic septicemia (VHS) and Gyrodactylosis

1. the farm at a minimum, immediately culled the pen(s) in which the disease was detected 2. the farm immediately notified the other farms in the ABM[120] 3. the farm and the ABM enhanced monitoring and conducted rigorous testing for the disease 4. the farm promptly[121] made findings publicly available	

Rationale - Biosecurity measures reduce the risk of disease transmission to the wild and between farms. These requirements aim to ensure that farms don't harm the health of wild populations by amplifying or spreading disease. It is recognised that disease flow is bidirectional between farmed and wild fish, and these requirements aim to minimise effect of disease transmission and retransmission. The ASC recognises that broad-level response to disease, in particular aggressive response to OIE-notifiable disease, must be led by regulators in the jurisdiction. This is important both because of legal implications of actions and because a mandatory response required by government has greatest potential to be effective.

(Gyrodactylus salaris). The actions required are applicable to exotic OIE notifiable diseases. Actions taken need to comply with national regulations.

[120] This is in addition to any notifications to regulatory bodies required under law and the OIE Aquatic Animal Health Code.

[121] Within one month.

PRINCIPLE 6: DEVELOP AND OPERATE FARMS IN A SOCIALLY RESPONSIBLE MANNER

Principle 6 aims to address potential negative social impacts related to farm development and operation, including labour concerns.

Criterion 6.1 Freedom of association and collective bargaining[122]

INDICATOR		REQUIREMENT
6.1.1	Evidence that workers have access to trade unions (if they exist) and union representative(s) chosen by themselves without managerial interference	Yes
6.1.2	Evidence that workers are free to form organizations, including unions, to advocate for and protect their rights	Yes
6.1.3	Evidence that workers are free and able to bargain collectively for their rights	Yes

Rationale - Having the freedom to associate and bargain collectively is a critical right of workers because it enables them to engage in collective bargaining over issues such as wages and other working conditions. Freedom of Association and the effective recognition of the right to collective bargaining is one of the core principles of the International Labor Organization's (ILO) "Declaration on Fundamental Principles and Rights at Work." The declaration was adopted in 1998 by the 86th International Labor Conference and has since been ratified by the overwhelming majority of ILO's 183 member nation-states.

[122] **Bargain collectively**: A voluntary negotiation between employers and organizations of workers in order to establish the terms and conditions of employment by means of collective (written) agreements.

Criterion 6.2 Child labour

INDICATOR		REQUIREMENT
6.2.1	Number of incidences of child[123] labour[124]	None
6.2.2	Percentage of young workers[125] that are protected[126]	100%

Rationale - The effective abolition of child labour is one of the core principles of the ILO "Declaration on Fundamental Principles and Rights at Work." Adherence to the child labour codes and definitions included in this section indicates compliance with what the ILO and international conventions generally recognise as the key areas for the protection of child and young workers. Children are particularly vulnerable to economic exploitation, due to their inherent age-related limitations in physical development, knowledge and experience. Children and youth need adequate time for education, development and play. Therefore, they should not have to work or be exposed to working hours and conditions that are hazardous[127,128] to their physical or mental well-being. To this end, the requirements related to what constitutes child labour will protect the interests of children and young workers at salmon farms certified to these requirements.

[123] **Child**: Any person under 15 years of age. A higher age would apply if the minimum age law of an area stipulates a higher age for work or mandatory schooling. Minimum age may be 14 if the country allows it under the developing country exceptions in ILO convention 138.

[124] **Child Labour**: Any work by a child younger than the age specified in the definition of a child.

[125] **Young Worker**: Any worker between the age of a child, as defined above, and under the age of 18.

[126] **Protected**: Workers between 15 and 18 years of age will not be exposed to hazardous health and safety conditions; working hours shall not interfere with their education and the combined daily transportation time and school time, and work time shall not exceed 10 hours.

[127] **Hazard**: The inherent potential to cause injury or damage to a person's health (e.g. unequipped to handle heavy machinery safely, and unprotected exposure to harmful chemicals).

[128] **Hazardous work**: Work that, by its nature or the circumstances in which it is carried out, is likely to harm the health, safety or morals of workers (e.g. heavy lifting disproportionate to a person's body size, operating heavy machinery, exposure to toxic chemicals).

Criterion 6.3 Forced, bonded or compulsory labour

INDICATOR		REQUIREMENT
6.3.1	Number of incidences of forced,[129] bonded[130] or compulsory labour	None

Rationale - Forced labour - such as slavery, debt bondage and human trafficking - is a serious concern in many industries and regions of the world. The elimination of all forms of forced or compulsory labour is one of the core principles of the ILO "Declaration on Fundamental Principles and Rights at Work." Ensuring that contracts are clearly articulated and understood by workers is critical to determining that labour is not forced. The inability of a worker to freely leave the workplace and/or an employer withholding original identity documents of workers are indicators that employment may not be at-will. Adherence to these policies shall indicate that an aquaculture operation is not using forced, bonded or compulsory labour forces.

Criterion 6.4 Discrimination[131]

INDICATOR		REQUIREMENT
6.4.1	Evidence of comprehensive[132] and proactive anti-discrimination policies, procedures and practices	Yes
6.4.2	Number of incidences of discrimination	None

[129] **Forced (Compulsory) labour**: All work or service that is extracted from any person under the menace of any penalty for which a person has not offered himself/herself voluntarily or for which such work or service is demanded as a repayment of debt. "Penalty" can imply monetary sanctions, physical punishment, or the loss of rights and privileges or restriction of movement (e.g. withholding of identity documents).

[130] **Bonded labour**: When a person is forced by the employer or creditor to work to repay a financial debt to the crediting agency.

[131] **Discrimination**: Any distinction, exclusion or preference that has the effect of nullifying or impairing equality of opportunity or treatment. Not every distinction, exclusion or preference constitutes discrimination. For instance, a merit- or performance-based pay increase or bonus is not by itself discriminatory. Positive discrimination in favour of people from certain underrepresented groups may be legal in some countries.

[132] Employers shall have **written anti-discrimination policies** stating that the company does not engage in or support discrimination in hiring, remuneration, access to training, promotion, termination or retirement based on race, caste, national origin, religion, disability, gender, sexual orientation, union membership, political affiliation, age or any other condition that may give rise to discrimination.

Rationale - The elimination of discrimination in respect of employment and occupation is one of the core principles of the ILO "Declaration on Fundamental Principles and Rights at Work." Unequal treatment of workers based on certain characteristics (such as sex or race), is a violation of a workers' human rights. Additionally, widespread discrimination in the working environment can negatively affect overall poverty and economic development rates. Discrimination occurs in many work environments and takes many forms. A common form is discrimination against women workers.

In order to ensure that discrimination does not occur at salmon farms certified to this requirement, employers must demonstrate their commitment to equality with an official anti-discrimination policy, a policy of equal pay for equal work, and clearly outlined procedures to raise, file and respond to a discrimination complaint in an effective manner. Evidence, including worker testimony, of adherence to these policies and procedures will indicate minimisation of discrimination. "Positive" discrimination (i.e., special treatment to protect the rights and health of particular groups of workers, or to provide opportunities for groups which have historically been disadvantaged) is allowed, and often required by laws related to such issues as maternity and affirmative action.

Criterion 6.5 Work environment health and safety

	INDICATOR	REQUIREMENT
6.5.1	Percentage of workers trained in health and safety practices, procedures[133] and policies on a yearly basis	100%
6.5.2	Evidence that workers use Personal Protective Equipment (PPE) effectively	Yes
6.5.3	Presence of a health and safety risk assessment and evidence of preventive actions taken	Yes
6.5.4	Evidence that all health- and safety-related accidents and violations are recorded and corrective actions are taken when necessary	Yes
6.5.5	Evidence of employer responsibility and/or proof of insurance (accident or injury) for 100% of worker costs in a job-related accident or injury when not covered under national law	Yes
6.5.6	Evidence that all diving operations are conducted by divers who are certified	Yes

[133] **Health and safety training** shall include emergency response procedures and practices.

Rationale - A safe and healthy working environment is essential for protecting workers from harm. It is critical for a responsible aquaculture operation to minimise these risks. One of the key risks to workers is hazards resulting from accidents and injuries. Consistent, effective and regular worker training in health and safety practices is an important preventative measure. When an accident, injury or violation occurs, the company must record it and take corrective action to identify the root causes of the incident, remediate, and take steps to prevent future occurrences of similar incidents. This addresses violations and the long-term health and safety risks. Finally, while many national laws require that employers assume responsibility for job-related accidents and injuries, not all countries require this and not all workers (in some cases migrant and other workers) will be covered under such laws. When not covered under national law, employers must prove they are insured to cover 100 per cent of worker costs when a job-related accident or injury occurs.

Criterion 6.6 Wages

	INDICATOR	REQUIREMENT
6.6.1	The percentage of workers whose basic wage[134] (before overtime and bonuses) is below the minimum wage[135]	0 (None)
6.6.2	Evidence that the employer is working toward the payment of basic needs wage[136]	Yes
6.6.3	Evidence of transparency in wage-setting and rendering[137]	Yes

Rationale - Wages and the process for setting wages are important components of the ILO core principles. For this reason, it is important to highlight under these requirements the importance of workers' basic wages meeting the legal minimum wage and being rendered to workers in a convenient manner. Unfortunately, minimum wage in many countries does not always cover the basic needs of workers. Unfairly and insufficiently compensated workers can be subject to a life of sustained poverty. Therefore, it is important for socially responsible employers to pay or be working toward paying a basic needs wage. The calculation of a basic needs wage can be complex, and it is important for employers to consult with workers, their representatives and other credible sources when assessing what a basic needs wage would be.

[134] **Basic wage**: The wages paid for a standard working week (no more than 48 hours).

[135] If there is no legal minimum wage in a country, basic wages must meet the industry-standard minimum wage.

[136] **Basic needs wage**: A wage that covers the basic needs of an individual or family, including housing, food and transport. This concept differs from a minimum wage, which is set by law and may or may not cover the basic needs of workers.

[137] Payments shall be rendered to workers in a convenient manner.

Certified salmon farms shall also demonstrate their commitment to fair and equitable wages by having and sharing a clear and transparent mechanism for wage-setting and a labour conflict resolution policy[138] that tracks wage-related complaints and responses. Having these policies outlined in a clear and transparent manner will empower the workers to negotiate effectively for fair and equitable wages that shall, at a minimum, satisfy basic needs.

Criterion 6.7 Contracts (labour) including subcontracting

INDICATOR		REQUIREMENT
6.7.1	Percentage of workers who have contracts[139]	100%
6.7.2	Evidence of a policy to ensure social compliance of its suppliers and contractors	Yes

Rationale - Fair contracting is important to ensure transparency between the employer and employee and fairness in the employment relation. Short-term and temporary contracts are acceptable but cannot be used to avoid paying benefits or to deny other rights. The company shall also have policies and mechanisms to ensure that workers contracted from other companies for specific services (e.g. divers, cleaning or maintenance) and the companies providing them with primary inputs or supplies have socially responsible practices and policies.

Criterion 6.8 Conflict resolution

INDICATOR		REQUIREMENT
6.8.1	Evidence of worker access to effective, fair and confidential grievance procedures	Yes
6.8.2	Percentage of grievances handled that are addressed[140] within a 90-day timeframe	100%

[138] See Criterion 6.8.

[139] **Labor-only contracting relationships** or **false apprenticeship** schemes are not acceptable. This includes revolving/consecutive labor contracts to deny benefit accrual or equitable remuneration. False Apprenticeship Scheme: The practice of hiring workers under apprenticeship terms without stipulating terms of the apprenticeship or wages under contract. It is a "false" apprenticeship if its purpose is to underpay people, avoid legal obligations or employ underage workers. Labor-only contracting arrangement: The practice of hiring workers without establishing a formal employment relationship for the purpose of avoiding payment of regular wages or the provision of legally required benefits, such as health and safety protections.

[140] **Addressed**: Acknowledged and received, moving through the company's process for grievances, corrective action taken when necessary.

Rationale - Companies must have a clear labour conflict resolution policy in place for the presentation, treatment and resolution of worker grievances in a confidential manner. Workers shall be familiar with the policy and its effective use. Such a policy is necessary to track conflicts and complaints raised, and responses to conflicts and complaints.

Criterion 6.9 Disciplinary practices

INDICATOR		REQUIREMENT
6.9.1	Incidences of excessive or abusive disciplinary actions	None
6.9.2	Evidence of a functioning disciplinary action policy whose aim is to improve the worker[141]	Yes

Rationale - The rationale for discipline in the workplace is to correct improper actions and maintain effective levels of worker conduct and performance. However, abusive disciplinary actions can violate workers' human rights. The focus of disciplinary practices shall always be on the improvement of the worker. Fines or basic wage deductions shall not be acceptable as methods for disciplining workforce. A certified salmon farm shall never employ threatening, humiliating or punishing disciplinary practices that negatively impact a worker's physical and mental[142] health or dignity.

Criterion 6.10 Working hours and overtime

INDICATOR		REQUIREMENT
6.10.1	Incidences, violations or abuse of working hours[143] and overtime laws	None

[141] If disciplinary action is required, progressive verbal and written warnings shall be engaged. The aim shall always be to improve the worker; dismissal shall be the last resort. Policies for bonuses, incentives, access to training and promotions are clearly stated and understood, and not used arbitrarily. Fines or basic wage deductions shall not be acceptable disciplinary practices.

[142] **Mental Abuse**: Characterised by the intentional use of power, including verbal abuse, isolation, sexual or racial harassment, intimidation or threat of physical force.

[143] In cases where local legislation on working hours and overtime exceed internationally accepted recommendations (48 regular hours, 12 hours overtime), the international standards will apply.

6.10.2 Overtime is limited, voluntary,[144] paid at a premium rate and restricted to exceptional circumstances	Yes

Rationale - Abuse of overtime working hours is a widespread issue in many industries and regions. Workers subject to extensive overtime can suffer consequences in their work-life balance and are subject to higher fatigue-related accident rates. In accordance with better practices, workers in certified salmon farms are permitted to work—within defined guidelines—beyond normal work week hours but must be compensated at premium rates.[145] Requirements for time off, working hours and compensation rates as described should reduce the impacts of overtime.

Criterion 6.11 Education and training

INDICATOR	REQUIREMENT
6.11.1 Evidence that the company regularly performs training of staff in fish husbandry, general farm and fish escape management and health and safety procedures	Yes

Rationale - Education and training can be beneficial to companies and enable workers to improve their incomes. Such human capital development should be encouraged where it is in the interest of the company. Incentives, such as subsidies for tuition or textbooks and time off prior to exams, should be offered. The offer of training may be contingent on workers committing to stay with the company for a pre-arranged time. This should be made clear to participants before they start the training.

Workers employed in husbandry activities require specific and adequate training and are aware of their responsibilities in aquatic animal health management practices.

[144] Compulsory overtime is permitted if previously agreed to under a collective bargaining agreement.

[145] **Premium rate**: A rate of pay higher than the regular work week rate. Must comply with national laws/regulations and/or industry standards.

6.12 Corporate policies for social responsibility

INDICATOR	REQUIREMENT
6.12.1 Demonstration of company-level[146] policies in line with the requirements under 6.1 to 6.11 above	Yes

Rationale - Companies must be able to demonstrate that not only are the specific farm sites applying for certification able to meet this robust set of social and labour requirements, but that they also have company-wide policies related to these key issue areas that are in line with the ASC Salmon Standard requirements. Such policies must relate to all of the company's salmon operations in the region, whether they be smolt production facilities, grow-out facilities or processing plants.

[146] Applies to the headquarters of the company in a region or country where the site applying for certification is located. The policy shall relate to all of the company's operations in the region or country, including grow-out, smolt production and processing facilities.

PRINCIPLE 7: BE A GOOD NEIGHBOUR AND CONSCIENTIOUS CITIZEN

Principle 7 aims to address any broader off-site potential social impacts associated with salmon production, including interactions with local communities.

Criterion 7.1 Community engagement

INDICATOR	REQUIREMENT
7.1.1 Evidence of regular and meaningful[147] consultation and engagement with community representatives and organizations	Yes
7.1.2 Presence and evidence of an effective[148] policy and mechanism for the presentation, treatment and resolution of complaints by community stakeholders and organizations	Yes
7.1.3 Evidence that the farm has posted visible notice[149] at the farm during times of therapeutic treatments and has, as part of consultation with communities under 7.1.1, communicated about potential health risks from treatments	Yes

Rationale - A salmon farm must respond to human concerns that arise in communities located near the farm and to concerns related to the farm's overall operations. In particular, appropriate consultation must be undertaken within local communities so that risks, impacts and potential conflicts are properly identified, avoided, minimised and/or mitigated through open and transparent negotiations. Communities shall have the opportunity to be part of the assessment process (e.g. by including them in the discussion of any social investments and contributions by companies to neighbouring communities).

Channels of communication with community stakeholders are important. Regular consultation with community representatives and a transparent procedure for handling complaints are key components of this communication. Negative impacts may not always be avoidable. However, the process for addressing them must be open, fair and transparent and demonstrate due diligence. A company shall

[147] **Regular and meaningful**: Meetings shall be held at least bi-annually with elected representatives of affected communities. The agenda for the meetings should in part be set by the community representatives. Participatory Social Impact Assessment methods may be one option to consider here.

[148] **Effective**: In order to demonstrate that the mechanism is effective, evidence of resolutions of complaints can be given.

[149] Signage shall be visible to mariners and, for example, to fishermen passing by the farm.

share with neighbouring communities' information about any potential human health risks that may be associated with the use of therapeutic treatments and communicate about typical treatment patterns. They shall also post notices around the farm during times of treatment.

Criterion 7.2 Respect for indigenous and aboriginal cultures and traditional territories

	INDICATOR	REQUIREMENT
7.2.1	Evidence that indigenous groups were consulted as required by relevant local and/or national laws and regulations	Yes
7.2.2	Evidence that the farm has undertaken proactive consultation with indigenous communities	Yes[150]
7.2.3	Evidence of a protocol agreement, or an active process[151] to establish a protocol agreement, with indigenous communities	Yes

Rationale - Interactions with and evidence of due diligence to prevent and mitigate negative impacts on communities is important globally, and takes on an additional dimension in regions where indigenous or aboriginal people or traditional territories are involved. In some jurisdictions, aboriginal groups have legal rights related to their territories. These shall be respected, as in Principle 1. It is also expected that operations seeking to meet the ASC Salmon Standard have directly consulted with bodies functioning as territorial governments and have come to agreement with indigenous governments, or are working towards an agreement, for farms that are operating in indigenous territories. The requirements are designed to be consistent with the United Nations Declaration on the Rights of Indigenous Peoples.

[150] All standards related to indigenous rights only apply where relevant, based on proximity of indigenous territories.

[151] To demonstrate an **active process**, a farm must show ongoing efforts to communicate with indigenous communities, an understanding of key community concerns and responsiveness to key community concerns through adaptive farm management and other actions.

Criterion 7.3 Access to resources

	INDICATOR	REQUIREMENT
7.3.1	Changes undertaken restricting access to vital community resources[152] without community approval	None
7.3.2	Evidence of assessments of company's impact on access to resources	Yes

Rationale - Companies should make a maximum effort to not affect the surrounding community's access to vital resources as a result of its presence and activities. Some change in access is expected. What is to be prevented is an unacceptable degree of change.

[152] Vital community resources can include freshwater, land or other natural resources that communities rely on for their livelihood. If a farm site were to block, for example, a community's sole access point to a needed freshwater resource, this would be unacceptable under the ASC Salmon Standard.

INDICATORS AND REQUIREMENTS FOR SMOLT PRODUCTION

This section of the document contains the full suite of principles, criteria, indicators and requirements for responsible salmon farming at freshwater smolt sites.

SECTION 8: REQUIREMENTS FOR SUPPLIERS OF SMOLT

A farm seeking certification must have documentation from all of its smolt suppliers to demonstrate compliance with the following requirements.[153] *The requirements are, in general, a subset of the requirements in Principles 1 through 7, focusing on the impacts that are most relevant for smolt facilities. In addition, specific requirements are applied to open systems (net pens), and to closed and semi-closed systems (recirculation and flow-through).*

Requirements related to Principle 1

INDICATOR	REQUIREMENT
8.1 Compliance with local and national regulations on water use and discharge, specifically providing permits related to water quality	Yes
8.2 Compliance with labour laws and regulations	Yes

Rationale - Please see the relevant Rationale in Principle 1. The requirements do not require the smolt producer to provide confidential business documents such as tax documentation.

Requirements related to Principle 2

INDICATOR	REQUIREMENT
8.3 Evidence of an assessment of the farm's potential impacts on biodiversity and nearby ecosystems that contains the same components as the assessment for grow-out facilities under 2.4.1	Yes

[153] The SAD SC proposed this approach to addressing environmental and social performance during the smolt phase of production. In the medium term, the SC anticipates a system to audit smolt production facilities on site. In the meantime, farms will need to work with their smolt suppliers to generate the necessary documentation to demonstrate compliance with the standards. The documentation will be reviewed as part of the audit at the grow-out facility.

8.4 Maximum total amount of phosphorus released into the environment per metric ton (mt) of fish produced over a 12-month period (see Appendix VIII-1)	4 kg /mt of fish produced over a 12-month period

Rationale - Please see the relevant Rationale in Principle 2. See also the relevant Rationale related to Additional Requirements for both open net-pen smolt production and closed and semi-closed smolt production.

Requirements related to Principle 3

INDICATOR	REQUIREMENT
8.5 If a non-native species is being produced, the species shall have been widely commercially produced in the area prior to the publication[154] of the ASC Salmon Standard	Yes[155]
8.6 Maximum number of escapees[156] in the most recent production cycle	300[157] fish
8.7 Accuracy[158] of the counting technology or counting method used for calculating the number of fish	≥98%

Rationale - Please see the relevant Rationale in Principle 3.

[154] **Publication**: Refers to the date when the final standards and accompanying guidelines are completed and made publicly available. This definition of publication applies throughout this document.

[155] Exceptions shall be made for production systems that use 100 percent sterile fish or systems that demonstrate separation from the wild by effective physical barriers that are in place and well-maintained to ensure no escapes of reared specimens or biological material that might survive and subsequently reproduce.

[156] Farms shall report all escapes; the total aggregated number of escapees per production cycle must be less than 300 fish.

[157] A rare exception to this standard may be made for an escape event that is clearly documented as being outside of the farm's control. Only one such exceptional episode is allowed in a 10-year period for the purposes of this standard. The 10-year period starts at the beginning of the production cycle for which the farm is applying for certification. The farmer must demonstrate that there was no reasonable way to predict the events that caused the episode. Extreme weather (e.g. 100-year storms) or accidents caused by farms located near high-traffic waterways are not intended to be covered under this exception.

[158] **Accuracy** shall be determined by the spec sheet for counting machines and through common estimates of error for any hand counts.

Requirements related to Principle 4

INDICATOR	REQUIREMENT
8.8 Evidence of a functioning policy for proper and responsible treatment of non-biological waste from production (e.g. disposal and recycling)	Yes
8.9 Presence of an energy-use assessment verifying the energy consumption at the smolt production facility (see Appendix V subsection 1 for guidance and required components of the records and assessment)	Yes, measured in kilojoule / t fish produced /production cycle
8.10 Records of greenhouse gas (GHG[159]) emissions[160] at the smolt production facility and evidence of an annual GHG assessment (See Appendix V, subsection 1)	Yes

Rationale - Please see the relevant Rationale in Principle 4.

Requirements related to Principle 5

INDICATOR	REQUIREMENT
8.11 Evidence of a fish health management plan, approved by the designated veterinarian, for the identification and monitoring of fish diseases and parasites	Yes
8.12 Percentage of fish that are vaccinated for selected diseases that are known to present a significant	100%

[159] For the purposes of this standard, **GHGs** are defined as the six gases listed in the Kyoto Protocol: carbon dioxide (CO_2); methane (CH_4); nitrous oxide (N_2O); hydrofluorocarbons (HFCs); perfluorocarbons (PFCs); and sulphur hexafluoride (SF_6).

[160] GHG emissions must be recorded using recognised methods, standards and records as outlined in Appendix V.

risk in the region and for which an effective vaccine exists[161]	
8.13 Percentage of smolt groups[162] tested for select diseases of regional concern prior to entering the grow-out phase on farm[163]	100%
8.14 Detailed information, provided by the designated veterinarian, of all chemicals and therapeutants used during the smolt production cycle, the amounts used (including grams per ton of fish produced), the dates used, which group of fish were treated and against which diseases, proof of proper dosing and all disease and pathogens detected on the site	Yes
8.15 Allowance for use of therapeutic treatments that include antibiotics or chemicals that are banned[164] in any of the primary salmon producing or importing countries[165]	None
8.16 Number of treatments of antibiotics over the most recent production cycle	≤ 3

[161] The farm's designated veterinarian is responsible for undertaking and providing written documentation of the analysis of the diseases that pose a risk in the region and the vaccines that are effective. The veterinarian shall determine which vaccinations to use and demonstrate to the auditor that this decision is consistent with the analysis.

[162] A **smolt group** is any population that shares disease risk, including environment, husbandry and host factors that might contribute to sharing disease agents for each group. Only diseases that are proven, or suspected, as occurring in seawater (and for which seawater fish-to-fish transmission is a concern) but originating in freshwater should be on the list of diseases tested. The designated veterinarian to the smolt farm is required to evaluate, based on scientific criteria and publicly available information, which diseases should be tested for. This analysis shall include an evaluation of whether clinical disease or a pathogen carrier state in fresh water is deemed to have a negative impact on the grow-out phase, thereby disqualifying a smolt group from being transferred. A written analysis must be available to the certifier on demand.

[163] Suitable measures must be in place to ensure that hatchery-raised seed are free from relevant/important pathogens before stocking for grow-out. This includes addressing on farm disease and parasite transfer (such as the ability to quarantine diseased stocks, separating equipment) as well as between the facility and natural fauna (such as disinfection of effluents for diseased stocks, fallowing). The approach should be relevant to the species, production system, scale of production, and legal requirements. Appropriate procedures or systems should include specific requirements or actions defined by the aquaculture facility through a suitable risk assessment or other evidence such as local or national regulations. Appropriate management measures in these cases could include treatment trigger levels of parasite numbers on the farm-facility or siting requirements that require that the aquaculture facility is located at suitable distances from wild populations. The CAB should verify that the management measures are suitable and employed.

[164] "**Banned**" means proactively prohibited by a government entity because of concerns around the substance.

[165] For purposes of this standard, those countries are Norway, the UK, Canada, Chile, the United States, Japan and France.

8.17 Allowance for use of antibiotics listed as critically important for human medicine by the WHO[166]	None[167]
8.18 Evidence of compliance[168] with the OIE *Aquatic Animal Health Code*[169]	Yes

Rationale - Please see the relevant Rationale in Principle 5.

Requirements related to Principle 6

INDICATOR	REQUIREMENT
8.19 Evidence of company-level policies and procedures in line with the labour standards under 6.1 to 6.11	Yes

Rationale - Please see the relevant Rationale in Principle 6.

Requirements related to Principle 7

INDICATOR	REQUIREMENT
8.20 Evidence of regular consultation and engagement with community representatives and organizations	Yes
8.21 Evidence of a policy for the presentation, treatment and resolution of complaints by community stakeholders and organizations	Yes

[166] The fifth edition of the WHO list of "Critically important antimicrobials for human medicine" was released in 2017 and is available at: http://apps.who.int/iris/bitstream/10665/255027/1/9789241512220-eng.pdf?ua=1 .

[167] If the antibiotic treatment is applied to only a portion of the pens on a farm site, fish from pens that did not receive treatment are still eligible for certification.

[168] **Compliance** is defined as farm practices consistent with the intentions of the Code, to be further outlined in auditing guidance. For purposes of this standard, this includes an aggressive response to detection of an exotic OIE-notifiable disease on the farm, which includes depopulating the infected site and implementation of quarantine zones in accordance with guidelines from OIE for the specific pathogen. Exotic signifies not previously found in the area or had been fully eradicated (area declared free of the pathogen).

[169] OIE 2017. Aquatic Animal Health Code. http://www.oie.int/en/international-standard-setting/aquatic-code/access-online/

8.22 Where relevant, evidence that indigenous groups were consulted as required by relevant local and/or national laws and regulations	Yes
8.23 Where relevant, evidence that the farm has undertaken proactive consultation with indigenous communities	Yes

Rationale - Please see the relevant Rationale in Principle 7.

Additional requirements for open (net-pen) production of smolt

In addition to the requirements above, if the smolt is produced in an open system, evidence shall be provided that the following is met:

INDICATOR	REQUIREMENT
8.25 Allowance for stocking smolts produced in cage-culture	Permitted only if supplying farms are 1) operated in a region where indigenous salmonids are present of the same species being cultivated and 2) the farm is certified to the ASC Freshwater trout Standard

Rationale - Due to the broader range of impacts associated with cage-culture smolt production in non-native regions, the ASC Salmon Standard prohibits the use of smolts produced in cage-culture in regions without indigenous salmonid species.

Using smolts produced from cage-culture is only allowed if they are produced in regions where indigenous salmonids are present of the same species being cultivated, and, if the farm is certified to the ASC Freshwater Trout Standard.

Additional requirements for semi-closed and closed production of smolts

Additionally, if the smolt is produced in a closed or semi-closed system (flow through or recirculation) that discharges into freshwater, evidence shall be provided that the following are met:[170]

	INDICATOR	REQUIREMENT
8.26	Water quality monitoring matrix completed and submitted to ASC (see Appendix VIII-2)	Yes[171]
8.27	Minimum oxygen saturation in the outflow (methodology in Appendix VIII-2)	60%[172,173]
8.28	Macro-invertebrate surveys downstream from the farm's effluent discharge demonstrate benthic health that is similar or better than surveys upstream from the discharge (methodology in Appendix VIII-3)	Yes
8.29	Evidence of implementation of biosolids (sludge) Best Management Practices (BMPs) (Appendix VIII-4)	Yes

Rationale - Effluent from semi-closed and closed smolt facilities can have an environmental effect on rivers, streams and other bodies of water that receive the discharge. Phosphorus is the key limiting nutrient in most temperate and cool freshwater systems. It is a stable nutrient in that it does not volatilize like nitrogen compounds. It is also added to feeds in proportions that can allow estimations of other waste constituents (organic matter and nitrogen). Thus, phosphorus is an ideal variable to set load limits for freshwater aquaculture. The SAD developed the phosphorus load requirement (8.4) based on a unit of production, making it an indicator of how well a farm is minimising nutrient discharges per ton of fish produced. From an environmental standpoint, farms should aim for as low an annual load of phosphorus per ton of fish as possible. Farms can lower their phosphorus load on the environment by using a better feeding strategy (ratio and feed distribution), improving feed conversion efficiency through the improvement of the environmental conditions in the farm, utilizing feed that is more digestible and has lower phosphorus content, and by employing cleaning technologies such as settling ponds and filters. Smolt production facilities are encouraged to develop

[170] Production systems that don't discharge into fresh water are exempt from these standards.

[171] See Appendix VI for transparency requirements for 8.32.

[172] A single oxygen reading below 60 per cent would require daily continuous monitoring with an electronic probe and recorder for at least a week demonstrating a minimum 60 per cent saturation at all times.

[173] See Appendix VI for transparency requirements for 8.33.

methodologies to reduce their phosphorus burdens over time, while ensuring farmed fish are getting the appropriate nutrients to protect the health of the smolt.

In an attempt to limit the oxygen burden on natural water bodies from the release of nutrients, these requirements include a minimum saturation level of dissolved oxygen at discharge. Benthic biodiversity is often a measure of aquatic ecosystem health. These requirements use faunal surveys as a reference for a farm's actual impact on the environment. By comparing surveys downstream and upstream from the farm's effluent discharge, the requirement aims to isolate the impact of the production facility and ensure that no significant impact is occurring.

Biosolids are a mixture of organic waste and sediment produced or accumulated through the farming activity. Biosolids discharged into natural water bodies are of concern because solids can restrict light penetration in water bodies, accumulate downstream, cover plants and habitat, and cause general shallowing of water bodies. Additionally, the organic component of biosolids will exert an oxygen demand as the organic matter decays. The simplest and best way to minimise these impacts is to remove sediments from the water column and allow organic matter to decay prior to discharge. Functionally, this infers the use of settling basins or ponds to let solids settle out of the water column, and for bacterial decomposition and oxygen depletion to occur at the same time prior to disposal of biosolids. To provide assurance of appropriate disposal of biosolids, these requirements include a small number of BMPs. These requirements do not require a specific effluent monitoring regime beyond the dissolved oxygen requirement and benthic analyses. However, the requirements do require farms to submit to the ASC the results of the effluent monitoring they conduct as part of their regulatory requirements. In particular, the requirement requires data on any sampling of phosphorus, nitrogen, total suspended solids (TSS) and biological oxygen demand (BOD). This data will help to distinguish the performance of farms certified by this requirement over time and assist in revisions to the requirement.

Appendix I: Methodologies Related to Principle 2 and Benthic Testing

Subsections

1. Sampling methodology for calculation of faunal index, macrofaunal taxa, sulphide and redox, and copper
2. Calculation methodology for the percent fines in feed
3. Biodiversity-focused impact assessment
4. Methodology for sampling dissolved oxygen
5. Methodology for sampling nitrogen and phosphorous

Appendix I-1. Sampling methodology for calculation of faunal index, macrofaunal taxa, sulphide and redox, and copper[174]

Grab sampling for the faunal index, macrofaunal taxa measurements, and sulphide and redox should be conducted at nine stations in duplicate during peak cage biomass for the production cycle.

1. Two stations should be from the cage edge, one at each end of the long axis of the farm.
2. Three should be from within the Allowable Zone of Effect (AZE), 25 metres from the edge of the array of cages at slack tide measured with a marked line and recorded using GPS. Of these three, one should be upstream and one downstream with respect to the direction of the residual current, and the other should be to one side of the farm in a direction orthogonal to the residual current.
3. Three should be 25 metres outside the AZE, or 55 metres from the edge of the array of cages measured with a marked line and recorded using GPS. Of these, one should be upstream and one downstream with respect to the direction of the residual current, and the other should be to one side of the farm in a direction orthogonal to the residual current.
4. One from a reference site 500-1000 metres from the farm (edge of the array of cages), in similar water depth and substratum type (where this exists), and recorded using GPS.
5. For farm sites using a site-specific AZE, sampling locations shall be determined based on that AZE, at distances consistent from the boundary of the AZE as for other farms (e.g. five metres inside of AZE and 25 metres outside of the AZE, recorded using GPS, and in multiple directions as determined appropriate through the modelling.
6. Values for requirements in Criterion 2.1 must be calculated using the results of samples from the edge of the AZE and the reference point. The CAB shall confirm that the AZE is correct and then to default to the social principles (P6 and P7) to ensure the farm is responding to stakeholder comments with the intention that the AZE is not arbitrary and meets stakeholder expectations.

[174] When biomass is estimated at ≥75% until harvest the audit can take place according to this guidance.

For farms using copper-based nets or copper-treated nets, copper sampling shall be conducted at the same locations outside the AZE as the other benthic sampling, at three stations outside the AZE, in duplicate. The reference site used shall also be the same, and two additional reference sites are needed. Timing shall also be the same, sampling at peak cage biomass during the production cycle.

Although the site visit should coincide with harvest period, it may be undertaken before end of harvest (at >75% peak biomass) and estimates of indicators requiring data from peak biomass / end of cycle provided in the draft report. The CAB shall review actual figures before the certification decision is made and include these figures in the final report.

Methodology for auditing indicators relating to peak biomass and end of cycle:

1) CABs shall carry out site visit audit at >75% peak biomass.

2) At the time of the audit the farm shall provide the CAB with estimates of values at that date for indicators that rely on information only available with the farm reaches peak biomass / end of cycle. The Farm shall provide the CAB with values of samples taken at peak biomass and end of cycle when they become available.

3) CAB shall raise a non-conformity for indicators where estimated values are used instead of actual values and note the estimated value in the draft audit report. It shall be explained in the draft audit report where figures are estimated and explain that these are to be updated in the final audit report.

4) CAB shall review the actual values and supporting evidence when they come back at peak biomass / end of cycle in order to make a certification decision.

5) CAB shall not make a certification decision and issue final report until actual values are provided for all indicators except biotic indicators 2.1.2 and 2.1.3.

6) In the case that biotic values are not available at the time of drafting the final report the CAB shall carry out a risk assessment to evaluate whether the biotic values are likely to meet the ASC standard. If the CAB finds evidence that the results of the biotic analyses are likely to meet the ASC standard then certification can be granted.

7) The CAB shall review biotic findings at the surveillance audit and raise non-conformities as appropriate when results have been found not meet the ASC standard.

Appendix I-2. Calculation methodology for the percentage of fines in feed

Introduction

This method determines the fines (dust and small fragments) in finished fish feed product, which has a diameter of 3 mm or more.

The amount of dust and fragments shall be determined when the feed is delivered to the farming site.[175]

Procedure

The test can be performed either by use of a sieving machine or by a manual test.

The sample of feed shall be put through a sieve with a maximum sieve opening of:

1. 1 mm when the particle diameter is equal to 5 mm or less
2. 2.36 mm when the particle diameter is more than 5 mm

Manual test

1. Put the accumulation box and the sieves on top of each other, with the accumulation box on the lowest part, then the smallest sieve and the biggest on top
2. Place the sieves on the balance and tare it
3. Weigh at least 300 g of the feed on the upper sieve, note the weight (**m0**)
4. Put on the lid
5. Sieve the feed smoothly and carefully for about 30 seconds
6. Remove the lid and weigh what is left in the accumulation box
7. Use a brush to remove all the particles from the sieves
8. The feed particles that have passed through all sieves are called dust (**md**)
9. If the feed is fatty, or if dust is unevenly distributed, two replicates must be taken

Sifting machine

1. Put the accumulation box and the sieves on top of each other, with the accumulation box at the bottom and the biggest sieve on top
2. Place the sieves on the balance and tare it
3. Weigh at least 300 g of feed on the upper sieve, note the weight (**m0**)
4. Place the sieves on the sifting machine and then close the cover properly

[175] Feed can be sampled prior to delivery to farm site for sites where there is no feed storage.

5. Press the "START" button by holding it for 2-3 seconds, and then run the machine twice (2 x 1 min)
6. Remove the sieves and weigh what is left in the accumulation box
7. The feed particles that have passed through all sieves are called dust (md)

Calculations

1. Weight of feed before sieving = **m0**
2. Weight of feed that has passed through all sieves = **md**

   Dust % = (md / m0) x 100

Feed Sampling Protocol

Sampling of feed lots—delivered as material in bulk, big bags or small bags—shall, at a minimum, be sampled as follows:

1. Cut a minimum of six increment samples from the lot, evenly distributed throughout the lot
2. Each increment sample should have a mass of approximately 500 grams
3. Make a pooled sample from all the increment samples and be sure to use all sampled material (i.e., around 6 kg)
4. Reduce the pooled sample to one analysis sample (for testing), each of approximately 500 grams

Appendix I-3. Biodiversity-focused impact assessment

Requirement 2.4.1 requires the farm to demonstrate that a biodiversity-focused environmental impact assessment has been undertaken for the farm.

The assessment shall include habitats and species that could reasonably be impacted by the farm. For example, cold-water corals near the farm could be impacted by nutrients, or whale populations in the region could be impacted by acoustic deterrent devices.

The assessment shall incorporate:

1. Identification of proximity to critical, sensitive or protected habitats and species:
   a. This includes key wild species within the marine environment around the farm
   b. Particular attention to be paid to species listed on International Union for the Conservation of Nature (IUCN) or national threatened/endangered lists and on any areas that have been identified as HCVAs, areas important for conservation/biodiversity or the equivalent
   c. Sensitive species may include non-threatened species of high economic value in the area that may be affected by the salmon farm (e.g. lobsters)
2. Identification and description of the potential impacts the farm might have on biodiversity, with a focus on those habitats or species

3. Description of strategies and current and future program(s) underway on the farm to eliminate or minimise any identified impacts the farm may have, and for the monitoring of outcomes of said programs and strategies
4. Where damage of sensitive habitats has been caused by the farm (as defined in the impact assessment) previously and where restoration is possible and effective; restoration efforts will or have resulted in a meaningful amount of restored habitat; either through direct on-farm restoration or by an off-farm offsetting approach. Grandfathering of historical losses is allowed.

Appendix I-4. Methodology for sampling dissolved oxygen

Requirements 2.2.1 and 2.2.2 require the sampling of dissolved oxygen on the farm site and the calculation of the percent saturation for those samples.

- DO, salinity and temperature shall be measured twice daily (proposed at 6 am and 3 pm, but with recognition that this will vary depending on region and operational practices). Percent saturation shall be calculated for each sample from the data and a weekly average percent saturation shall result.
    - A minimal amount of missed samples due to extreme weather conditions will be considered acceptable.
    - Sampling once daily shall also be considered acceptable, though not preferred.
- DO shall be measured at a depth of five metres at a location where the conditions of the water will be similar to those the fish experience. For example, measurements can be taken at the edge of the net-pen array, in the downstream direction of the current, or off a feed shed or housing structure on the site. Measurements shall be taken at the same location, recorded with GPS, at the same time to allow for comparison between days.
- Weekly averages shall be calculated and remain at or above 70 per cent saturation.
- Should a farm not meet the minimum 70 per cent weekly average saturation requirement, the farm must demonstrate the consistency of percent saturation with a reference site. The reference site shall be at least 500 metres from the edge of the net pen array, in a location that is understood to follow similar patterns in upwelling to the farm site and is not influenced by nutrient inputs from anthropogenic causes including aquaculture, agricultural runoff or nutrient releases from coastal communities.

Appendix I-5. Methodology for sampling nitrogen and phosphorous

Under requirement 2.2.4, some farms are required to monitor nitrogen and phosphorous levels on the farm and at reference sites. Farms shall monitor total N, NH_4NO_3, total P and Ortho-P in the water column. Monitoring of nitrogen and phosphorous shall follow the following methodology or an equivalent:

- This sampling regime should be carried out monthly for the first year to create the baseline against which long term changes can be assessed.

- The N and P sampling shall then be conducted four times a year (quarterly), once during each of the seasons, with three replicate samples at the edge of the AZE and three at the reference site 500m downstream on each occasion.
- Samples should be taken using a VanDorn or Kemmerer type water sampler. 500 ml samples should be placed in clear plastic bottles, placed on ice and in a cooler, and analysed within 48 hours. Ideally, analyses shall be done by a private (third-party) laboratory following standard methods. However, Hach field kits can be used. Clear and detailed records or the sampling frequency and analytical results must be kept. For best practice, the samples from Hach kits should be sent periodically (e.g. once a quarter and at minimum once a year) to an independent laboratory for analysis to ensure consistency of results and ensure/establish quality control.

Appendix II: Area-Based Management (ABM) Scheme

Subsections

1. Attributes and Required Components of the ABM
2. Setting and Revising ABM Lice Loads and On-farm Lice Levels

Appendix II-1. Attributes and required components of the ABM

Participation in an area-based scheme[176] for managing disease and parasites and resistance to treatments is required under the ASC Salmon Standard. This appendix outlines the main components of the area-based management scheme that the ASC Salmon Standard requires under Criteria 3.1 and 5.4.

The purpose of the area-based management scheme is to improve health and biosecurity management on the farm, with the ultimate goal of minimising potential negative impacts on wild populations.

II-1. A Definition of "area"

If area-based management is already a regulatory requirement of the farm's jurisdiction, then farms will use this definition of "area" for the purposes of these requirements. In jurisdictions where ABM is not a regulatory requirement, the area covered under the ABM must reflect a logical geographic scope such as a fjord or a collection of fjords that are ecologically connected. The boundaries of an area should be defined, taking into account the zone in which key cumulative impacts on wild populations may occur, water movement and other relevant aspects of ecosystem structure and function.

II-1. B Requirements related to participation in the scheme

Within the defined area, at least 80 per cent of farmed production (by weight) must participate in the area-based management scheme, even if not all farms are seeking certification under this requirement. Without the vast majority of farms participation, the scheme will likely be ineffective. All farms owned by the company applying for certification in the area must participate in the ABM, though not all must be applying for certification.

II-1. C ABM components and guidance

In order to be considered as applicable under the ASC Salmon Standard, the ABM scheme used by a farm must ensure that there is:

1. Clear documentation of the farms/companies included in the ABM, contact people (including contact information) and mechanisms for communication
2. Development and documentation of shared disease management goals and objectives for the ABM. Goals shall include components related to understanding and minimising risk of on-farm disease to wild fish. Objectives shall be updated regularly based on new information, including

[176] For more information on the principles of place-based or area-based management, see Young et al., 2007. Solving the Crisis in Ocean Governance: Place-Based Management of Marine Ecosystems. Environment: Volume 49, Number 4, pages 20–32.

concerns raised to the farms in the ABM from communities and wild fish interests are part of company engagement with stakeholders as outlined under 7.1.1.

3. Information and data-sharing among farms of any data needed to ensure coordination, including plans for stocking and fallowing; on-farm disease and parasite monitoring results including sea lice numbers; suspicion of an unidentifiable transmissible agent, information on therapeutic treatments; and data on resistance including information related to treatments not being as effective as expected.

The ABM scheme must include coordination among farms as relates to:

1. Application and rotation of treatments:
    a. Farmers must be able to demonstrate a coordinated treatment plan and evidence that the schedule and rotation of treatments are being implemented.
    b. Consideration of the cumulative use, and potential risks[177] of this use, of antibiotics classified as "highly important" by the WHO[178] is a required component of coordination and information-sharing about treatments.
    c. Where applicable, treatments and/or strategic harvesting of salmon is coordinated prior to outmigration of wild salmonids to ensure minimal on-farm lice levels at this sensitive time period for those species (as has been determined under 3.1.5).
    d. Tracking of cumulative use of parasiticides (by chemical, annually and by production cycle) within the ABM.
2. Stocking: Records must demonstrate that all stocked fish within the ABM are of the same year class and that stocking dates were coordinated with other farms.
3. Fallowing: Coordination of fallowing between each production cycle to help break disease cycles, with a clear period of time when there are no farmed salmon in the area in the water.
4. Monitoring schemes:
    a. On-farm disease and pathogen monitoring and information sharing among farms
    b. On-farm resistance monitoring and information sharing among farms
    c. For farms located in areas where there are wild salmonids, monitoring of wild salmonid populations that is relevant for the area must occur as specified under 3.1.6, either under the auspices of the ABM or under some other auspices
5. Setting and revising a maximum ABM lice load:
    a. The entire ABM scheme will set a maximum lice load, expressed as total mature female lice on all farms in the area. In areas of wild salmonids, the ABM scheme must demonstrate how the scheme incorporates the results of wild monitoring into revisions of this total lice load over time (see Section 2 below for additional details on this feedback loop).

[177] Assessment of risk shall take into account the cumulative use of these antibiotics from salmon production within the area in order to assess the potential risk to human health from the development of resistance in the environment. Prescribing antibiotics highly important for human health shall be considered as a last resort.

[178] The fifth edition of the WHO list of "Critically important antimicrobials for human medicine" was released in 2017 and is available at: http://apps.who.int/iris/bitstream/10665/255027/1/9789241512220-eng.pdf?ua=1 .

Appendix II-2. Setting and revising ABM lice loads and on-farm lice levels

Requirement 3.1.3 requires that the ABM scheme set a maximum lice load. A core purpose of this requirement is to be able to see the potential cumulative infection pressure from on-farm lice, expressed as the number of mature female lice on all farms in the scheme. This "total load" figure is a better reflection of the potential risks to wild populations than on-farm lice levels, measured as lice per farmed fish.

An ABM scheme shall initially set this total load figure based on the regulatory obligations of the jurisdiction in which it operates and the results of any wild monitoring done to date. In practice, this would mean that farms in most ABM schemes would take the on-farm lice levels they are required to achieve by regulators, and multiply them times the number of farmed fish in the area. This would be a starting place.

For farms located in areas of wild salmonids, the ABM scheme shall demonstrate how the scheme is using the results of wild monitoring to review and potentially revise the maximum lice load for the area each year and/or production cycle. Adjustments to the area's lice load would lead to corresponding limits on lice levels on individual farms. This feedback loop must be transparent and document how the ABM scheme is being protective of wild fish through the interpretation of wild monitoring data. Given the time lag in collecting and analysing data from wild monitoring, it is expected that the ABM scheme will look at data from previous periods, particularly sensitive periods such as outmigration of wild salmon juveniles.

Requirement 3.1.7 requires farms seeking certification to maintain on-farm lice levels at 0.1 mature female lice (leps) during and immediately prior to sensitive periods, particularly outmigration of wild juvenile salmon. The results of wild monitoring must inform this level over time, with a similar type of feedback loop as described for the ABM total lice level. If wild monitoring reveals that 0.1 mature female lice are not being protective of wild populations, the farm must set a lower level in subsequent sensitive periods. Conversely, data from wild monitoring that consistently demonstrates healthy wild populations would allow a farm to make the case for a level higher than 0.1. This case would need to be made for the ABM as a whole to the Technical Advisory Group of the ASC.

Appendix III: Methodologies and Thresholds Related to Monitoring Wild Salmonids

Appendix III-1. Methodologies for monitoring wild salmonids

The ASC Salmon Standard requires all farms located in areas of wild salmonids to participate in monitoring of sea lice on wild salmonids. The purpose of this monitoring is to assist in clarifying the link between the health of wild and farmed fish through objective information. These requirements do not demand a specific methodology for this monitoring. Nonetheless, the monitoring must comply with the following requirements:

- The methodology, the results and the analysis are made publicly available and demonstrate scientific rigor in the sampling size, location and method.
- Monitoring must be geographically relevant to the area where the farm/ABM is located, so it provides meaningful information for ABM management practices.
- The process must involve third parties beyond the farm, such as independent scientists. Government programs, in which the company may be contributing little or nothing are acceptable, given the programme is geographically relevant.
- Numbers of lice per wild fish, and prevalence of lice are both meaningful metrics that could be considered in the research.
- Species should be chosen based on importance to area (i.e., sea trout vs. salmon vs. arctic char).

Appendix IV: Feed Resource Calculations and Methodologies

Subsections

1. Forage Fish Dependency Ratio calculation
2. Calculation of EPA and DHA in feed
3. Explanation of FishSource scoring

Appendix IV-1. Forage Fish Dependency Ratio calculation

Feed Fish Dependency Ratio (FFDR) is the quantity of wild fish used per quantity of cultured fish produced. It is expected that the CABs raise major NCRs when FFDRs do not meet ASC requirements. This measure can be calculated based on fishmeal (FM) and/or fish oil (FO). In the case of salmon currently, in most cases the FFDR for fish oil will be higher than that for fishmeal. The dependency on wild forage fish resources shall be calculated for both FM and FO using the formulas noted below. This formula calculates the dependency of a single site on wild forage fish resources, independent of any other farm.

$$FFDR_m = \frac{(\%\ fishmeal\ in\ feed\ from\ forage\ fisheries) \times (eFCR)}{24}$$

$$FFDR_o = \frac{(\%\ Fish\ oil\ in\ feed\ from\ forage\ fisheries) \times (eFCR)}{5.0\ or\ 7.0,\ depending\ on\ source\ of\ fish}$$

Where:

1. Economic Feed Conversion Ratio (eFCR) is the quantity of feed used to produce the quantity of fish harvested (net production is the live weight).

$$eFCR = \frac{Feed,\ kg\ or\ mt}{Net\ aquacultural\ production,\ kg\ or\ mt\ (wet\ weight)}$$

i.

2. The percentage of fishmeal and fish oil excludes fishmeal and fish oil derived from fisheries' by-products.[179] Only fishmeal and fish oil that is derived directly from a pelagic fishery (e.g. anchoveta) or fisheries where the catch is directly reduced (such as krill or blue whiting) is to be included in the calculation of FFDR. Fishmeal and fish oil derived from fisheries' by-products (e.g. trimmings and offal) should not be included because the FFDR is intended to be a calculation of direct dependency on wild fisheries.

[179] Trimmings are defined as by-products when fish are processed for human consumption or if whole fish is rejected for use of human consumption because the quality at the time of landing do not meet official regulations with regard to fish suitable for human consumption. Restrictions on what trimmings are allowed for use under the standard are under 4.3.4.

3. The amount of fishmeal in the diet is calculated back to live fish weight by using a yield of 24%.[180] This is an assumed average yield.
4. The amount of fish oil in the diet is calculated back to live fish weight by using an average yield in accordance with this procedure:
    a. Group a - Fish oil originating from Peru and Chile and Gulf of Mexico, five per cent yield of fish oil
    b. Group b - Fish oil originating from the North Atlantic (Denmark, Norway, Iceland and the UK) seven per cent yield of fish oil
    c. If fish oil is used from other areas than mentioned above, they should be classified as belonging to group a if documentation shows a yield less than six per cent, and into group b if documentation shows a yield more than six per cent.
5. FFDR is calculated for the grow-out period in the sea as long as the smolt phase does not go past 200 grams per smolt. If the smolt phase goes past 200g then FFDR is calculated based on all feed used from 200 grams and onwards. If needed, the grow-out site shall collect this data from the smolt supplier.

Appendix IV-2. Calculation of EPA and DHA in feed

In order to demonstrate compliance with the requirement related to the maximum amount EPA and DHA from direct forage fisheries in the feed, the calculations shall be done according to the following formula:

$$\text{Grams of EPA and DHA in feed} = \frac{(\text{(grams of fish oil per kg feed)} \times \text{(\% of EPA and DHA in fish oil)})}{100}$$

Where:

1. If the fish oil content varies in different feeds used during the production cycle, a weighted average can be used. The grams of fish oil relate to fish oil originating from forage fisheries for industrial purposes.
2. The content of EPA and DHA of the fish oil shall be calculated using the average figures:
    a. group a - Fish oil originating from Peru and Chile and Gulf of Mexico, 30 per cent EPA and DHA in fish oil
    b. group b - Fish oil originating from the North Atlantic (Denmark, Norway, Iceland and UK) 20 per cent EPA and DHA in fish oil

[180] Reference for FM and FO yields: Péron, G., et al. 2010. Where do fishmeal and fish oil products come from? An analysis of the conversion ratios in the global fishmeal industry. Marine Policy, doi:10.1016/j.marpol.2010.01.027.

c. If fish oil is used from other areas than mentioned above, they should be classified as belonging to group a if analyses of EPA and DHA is above 25 per cent, and into group b if analyses of EPA and DHA is below 25 per cent.

Analyses of EPA and DHA are the percentage of fatty acids in the oil that are EPA and DHA. In the calculation above, we make the simplification that 100 per cent of the oil consists of fatty acids. EPA and DHA originating from fish oil originating from by-products and trimmings are not included in the calculation above. The feed producer can justify and demonstrate the amount of fish oil coming from trimmings and by-products by using a percentage of fish oil originating from trimmings based on information from purchases in an annual year, either using information related to the current year when the feed is produced or the previous year.

Appendix IV-3. Explanation of FishSource scoring

FishSource scores provide a rough guide to how a fishery stacks up against existing definitions and measures of sustainability. The FishSource scores currently only cover five criteria of sustainability, whereas a full assessment—such as that by the Marine Stewardship Council (MSC)—will typically cover more than 60. As such, the FishSource scores are not a firm guide to how a fishery will perform overall. Nonetheless, the FishSource scores do capture the main outcome-based measures of sustainability.

FishSource scores are based on common measures of sustainability, as used by the International Council for the Exploration of the Seas, the National Marine Fisheries Service and the MSC, among others (e.g. current fishing mortality relative to the fishing mortality target reference point, or current adult fish biomass relative to its maximum sustainable yield (B_{msy})).

Components of the FishSource score

Issue	Measure	Underlying Ratio
Is the management strategy precautionary?	Determine whether harvest rates are reduced at low stock levels	$F_{advised}/F_{target\ reference\ point}$ or $F_{actual}/F_{target\ reference\ point}$
Do managers follow scientific advice?	Determine whether the catch limits set by managers are in line with the advice in the stock assessment	Set TAC / Advised TAC
Do fishers comply?	Determine whether the actual catches are in line with the catch limits set by managers	Actual Catch / Set TAC
Is the fish stock healthy?	Determine if current biomass is at long-term target levels	SSB/B_{40} (or equivalent)
Will the fish stock be healthy in future?	Determine if current fishing mortality is at the long-term target level	$F/F_{target\ reference\ point}$

If existing measures of sustainability consider a fishery to be relatively well-managed, then it will typically score eight or more out of 10 on FishSource. If the fishery is judged to be doing okay, but

requires improvement, then it will typically score between six and eight on FishSource. A fishery falling short of minimum requirements of existing measures of sustainability is scored six or below, with the score declining as the condition of the fishery deteriorates.

The key relation between the MSC scoring system and FishSource scores is “80 <-> 8”. For example, a FishSource score of eight or above would mean an unconditioned passing for that particular aspect on the MSC system. Sustainable Fisheries Partnership devised scores in a way that, departing from eight, a score of six relates to a score of 60, and below six, an MSC “below 60”, “no-pass” condition. Please note, however, that the MSC criteria have been interpreted through time with a substantial degree of variability among fisheries.

More information on FishSource is available at www.fishsource.com, and an overview of the FishSource indices is available at http://www.fishsource.org/indices_overview.pdf.

About scoring and availability of product meeting a minimum score

A typical full assessment of a fishery through the MSC will include significantly more areas/criteria assessed than through FishSource, typically including more than 60 sustainability criteria. A fishery is deemed sustainable by the MSC if it scores 60 or more in every performance indicator, and an average of 80 or more at the principle level. The MSC requires certified fisheries to take corrective actions to improve any areas of the fishery that scored between 60 and 80, with the intention of achieving a score of 80 or above in every area of the fishery.

As of May 2011, FishSource released updated information on the ratings of the 25 principal forage fisheries around the Atlantic and South America in their “Reduction Fisheries League Table 2011.” Ten of the 25 fisheries met a minimum FishSource score of six in all categories with a minimum score of eight in the biomass category. These ten fisheries had a total combined 2009 catch of 9157 thousand mt, accounting for just over 66 per cent of the total catch of those 25 forage fisheries.

The ratings of fisheries under the FishSource methodology will change over time based on the performance of those fisheries. Farms undergoing certification and feed companies should be attuned to updates of the “Reduction Fisheries League Table” and use the latest version publicly available. Auditing guidelines will be developed around the timing of purchasing of fishmeal and fish oil and the updates of the ratings to ensure reasonable interpretation of the requirement and timing of shifts in purchasing if a fishery’s performance declines to a point where it fails to meet the minimum score needed under the requirement.

Appendix V: Energy Records and Assessment

Subsections

1. Energy use assessment and greenhouse gas (GHG) accounting for farms
2. GHG accounting for feed

Appendix V-1. Energy use assessment and GHG accounting for farms

The ASC encourages companies to integrate energy use assessments and GHG accounting into their policies and procedures across the board in the company. However, this requirement only requires that operational energy use and GHG assessments have been done for the farm sites that are applying for certification.

Assessments shall follow either the GHG Protocol Corporate Standard or ISO 14064-1 (references below). These are the commonly accepted international requirements, and they are largely consistent with one another. Both are also high level enough not to be prescriptive and they allow companies some flexibility in determining the best approach for calculating emissions for their operations.

If a company wants to go beyond the requirement of the ASC Salmon Standard and conduct this assessment for their entire company, then the full protocols are applicable. If the assessment is being done only on sites that are being certified, the farms shall follow the GHG Protocol Corporate Standard and/or ISO 14064-1 requirements pertaining to:

- Accounting principles of relevance, completeness, transparency, consistency and accuracy
- Setting operational boundaries
- Tracking emissions over time
- Reporting GHG emissions

Regarding the operational boundaries, farm sites shall include in the assessment:

- Scope 1 emissions, which are emissions that come directly from a source that is either owned or controlled by the farm/facility.
    - For example, if the farm has a diesel generator, this will generate Scope 1 emissions. So will a farm-owned/-operated truck.
- Scope 2 emissions, which are emissions resulting from the generation of purchased electricity, heating, or cooling.

Quantification of emissions is done by multiplying activity data (e.g. quantity of fuel or kwh consumed) by an emission factor (e.g. CO2/kwh). For non-CO2 gases, you then need to multiply by a Global Warming Potential (GWP) to convert non-CO2 gases into the CO2-equivalent. Neither the GHG Protocol nor the ISO require specific approaches to quantifying emissions, so the ASC Salmon Standard provides the following additional information on the quantification of emissions:

- Farms shall clearly document the emission factors they use and the source of the emission factors. Recommended sources include the Intergovernmental Panel on Climate Change (IPCC) or factors provided by national government agencies such as the United States Environmental Protection Agency (USEPA). Companies shall survey available emission factors and select the one that is most accurate for their situation, and transparently report their selection.

- Farms shall clearly document the GWPs that they use and the source of those GWPs. Recommended sources include the IPCC 2nd Assessment Report, on which the Kyoto Protocol and related policies are based, or more recent Assessment Reports.

References:

- GHG Protocol Corporate Standard Website: http://www.ghgprotocol.org/standards/corporate-standard
- ISO 14064-1 available for download (with fee) at http://www.iso.org/iso/catalogue_detail?csnumber=38381
- Some information on ISO 14064-1 is at http://www.iso.org/iso/pressrelease.htm?refid=Ref994
- IPCC 2nd Assessment Report: http://www.ipcc.ch/pdf/climate-changes-1995/ipcc-2nd-assessment/2nd-assessment-en.pdf
- All IPCC Assessment Reports: http://www.ipcc.ch/publications_and_data/publications_and_data_reports.shtml#1

Appendix V-2. GHG accounting for feed

The requirement requires the calculation of the GHG emissions for the feed used during the prior production cycle at the grow-out site undergoing certification. This calculation requires farms to multiply the GHG emissions per unit of feed, provided to them by the feed manufacturer, by the amount of feed used on the farm during the production cycle.

The feed manufacturer is responsible for calculating GHG emissions per unit feed. GHG emissions from feed can be calculated based on the average raw material composition used to produce the salmon (by weight) and not as documentation linked to each single product used during the production cycle.

The scope of the study to determine GHG emissions should include the growing, harvesting, processing and transportation of raw materials (vegetable and marine raw materials) to the feed mill and processing at feed mill. Vitamins and trace elements can be excluded from the analysis. The method of allocation of GHG emissions linked to by-products must be specified.

The study to determine GHG emissions can follow one of the following methodological approaches:

1. A cradle-to-gate assessment, taking into account upstream inputs and the feed manufacturing process, according to the GHG Product Standard
2. A Life Cycle Analysis following the ISO 14040 and 14044 requirements for life cycle assessments

Should the feed manufacturer choose to do a cradle-to-gate assessment:

1. It shall incorporate the first three phases from the methodology, covering materials acquisition and processing, production, and product distribution and storage (everything upstream and the feed manufacturing process itself).

Should the manufacturer follow the ISO 14040 and 14044 requirements for Life Cycle Assessment:

1. Feed manufacturers may follow either an ISO-compliant life cycle assessment methodology or the GHG Protocol product standard.

Regardless of which methodology is chosen, feed manufacturers shall include in the assessment:

- Scope 1 emissions, which are emissions that come directly from a source that is either owned or controlled by the farm/facility.
- Scope 2 emissions, which are emissions resulting from the generation of purchased electricity, heating or cooling.
- Scope 3 emissions, which are emissions resulting from upstream inputs and other indirect emissions, such as the extraction and production of purchased materials, following the Scope 3 standard.

Quantification of emissions is done by multiplying activity data (e.g. quantity of fuel or kwh consumed) by an emission factor (e.g. CO2/kwh). For non-CO2 gases, you then need to multiply by a Global Warming Potential (GWP) to convert non-CO2 gases into CO2-equivalent. The ASC Salmon Standard provides the following additional information on the quantification of emissions:

- Farms shall clearly document the emission factors they use and the source of the emission factors. Recommended sources include the IPCC or factors provided by national government agencies, such as the USEPA. Companies shall survey available emission factors and select the one that is most accurate for their situation, and transparently report their selection.
- Farms shall clearly document the GWPs that they use and the source of those GWPs. Recommended sources include the IPCC 2nd Assessment Report, on which the Kyoto Protocol and related policies are based, or more recent Assessment Reports.

References:

- GHG Product Standard: http://www.ghgprotocol.org/product-standard
- ISO 14044 available for download (with fee) at: http://www.iso.org/iso/iso_catalogue/catalogue_tc/catalogue_detail.htm?csnumber=38498
- Some information on ISO 14064-1 is at: http://www.iso.org/iso/pressrelease.htm?refid=Ref994
- IPCC 2nd Assessment Report: http://www.ipcc.ch/pdf/climate-changes-1995/ipcc-2nd-assessment/2nd-assessment-en.pdf
- All IPCC Assessment Reports: http://www.ipcc.ch/publications_and_data/publications_and_data_reports.shtml#1

Appendix VI: Transparency of Farm-Level Performance Data

The farm must provide evidence that it has submitted to ASC in the requested format the following information about its environmental and social performance.

Information pertaining to biomass and or stocking from which production volumes, timing and financial information can be extracted or inferred should be considered confidential in order to not put certified companies at a competitive disadvantage. Information related to production volumes or harvest timing may be made public with a time delay (e.g. if released post-harvest and sale).

Item	Option	Relevant Requirement	Measurement	Units	Measurement Frequency	Calculations and Sampling Methodologies, Additional Notes
1			Species in production	species		
2	a	2.1.1	Redox potential	mV	production cycle	Appendix I-1
	b		Sulfide levels	µMol/L	production cycle	Appendix I-1
3	a	2.1.2	AZTI Marine Biotic Index (AMBI)	AMBI score	production cycle	Appendix I-1
	b		Shannon-Wiener Index	S-WI score	production cycle	Appendix I-1
	c		Benthic Quality Index (BQI)	BQI score	production cycle	Appendix I-1
	d		Infaunal Trophic Index (ITI)	ITI score	production cycle	Appendix I-1
4		2.1.3	# of microfaunal taxa	#	production cycle	Appendix I-1
5		2.2.1	Average % DO saturation	%	weekly	Appendix I-4
6		2.2.2	Max % samples under 1.85 mg/L DO	%	weekly	Appendix I-4
7		2.2.4	Nitrogen monitoring	mg N/L	quarterly	Appendix I-5
8		2.2.4	Phosphorous monitoring	mg P/L	quarterly	Appendix I-5
9		2.2.5	Calculated BOD		production cycle	Footnote in 2.2.5
10		2.5.2	# days ADDs/AHDs	#	ongoing[181],	

[181] Ongoing: Logged as needed or as occurs. Data shall be logged such that it can be analysed on both an annual and a production cycle basis. This definition of "ongoing" applies throughout Appendix VI.

Item	Option	Relevant Requirement	Measurement	Units	Measurement Frequency	Calculations and Sampling Methodologies, Additional Notes
11		2.5.5 and 2.5.6	Lethal incidents of marine mammals and birds	#, species and cause per episode	ongoing	To be made publicly available (e.g. on web) by farming company shortly after incident
12		3.1.1	Fallowing period	dates		
13		3.1.3	Maximum sea lice load set for the ABM	number	annual	Appendix II and III
14		3.1.4 and 3.1.7	Weekly, on-farm sea lice levels		weekly	To be made directly publicly available by farming company within a week
15		3.1.6	In areas of wild salmonids, monitoring of sea lice on out-migrating salmon juveniles or costal sea trout			Appendix III, to be made publicly available within eight weeks of completion of monitoring
16		3.4.1-3.4.2	Escapes data	# episodes	production cycle	
				date of episode	ongoing	
				cause of episode	ongoing	
				# escapees per episode	ongoing	
				# total escapees	production cycle	
17		3.4.2	Counting technology accuracy	%	production cycle	Footnote 58
		3.4.3	Estimated unexplained loss	#	production cycle	Footnote 59
18		4.2.1	FFDR fishmeal (during grow-out)	FFDRm	production cycle	Appendix IV

Item	Option	Relevant Requirement	Measurement	Units	Measurement Frequency	Calculations and Sampling Methodologies, Additional Notes
19	a	4.2.2	FFDR fish oil (during grow-out)	FFDRo	production cycle	Appendix IV
	b		Max amount EPA and DHA	g/kg feed	production cycle	Appendix IV
20		4.4.3	Transgenic feed ingredients	Y/N	production cycle	
21		4.6.1	Energy use	kJ/mt fish	production cycle	Appendix V-1
22		4.6.2	GHG emissions on farm		annual	Appendix V-1
23		4.6.3	GHG emissions of feed		production cycle (not immediately applicable)	Appendix V-2
24		4.7.1	Copper-based antifoulants	Y/N	production cycle	
25		4.7.3 and 4.7.4	Results of copper sampling (outside AZE and at reference sites), if required	mg Cu/kg sediment	production cycle	Appendix I-1
26		5.1.5	Total mortality of farmed fish	%	ongoing	
27		5.1.4	Cause of mortalities (post-mortem analysis)	# mortalities per cause or disease	ongoing	
28		5.1.6	Maximum unexplained mortalities	% of total mortality	production cycle	
29		5.2.1	Amount of each chemical/therapeutant used for each (antibiotics, parasiticides, etc.)	product name	ongoing	Also 5.2.9
				active component name	ongoing	
				reason for use	ongoing	
				date	ongoing	
				kg	ongoing	
				t fish treated	ongoing	
				dosage	ongoing	

Item	Option	Relevant Requirement	Measurement	Units	Measurement Frequency	Calculations and Sampling Methodologies, Additional Notes
				# of treatments	ongoing	
				WHO classification (antibiotics only)	ongoing	
30		5.2.7	Reduction in WNMT	%	per 2 year after first audit after effective date	
			Amount of each parasiticide used	product name	ongoing	
				Active component name	ongoing	
				date	ongoing	
				kg	ongoing	
				t fish treated	ongoing	
				dosage	ongoing	
				Application method	ongoing	
				# of treatments	ongoing	
31		5.2.6	Weighted Number of Medicinal Treatments (WNMT)	No.	WNMT	Appendix VII
32		5.2.8	Results of environmental monitoring of benthic parasiticide levels	Name of active ingredient and/or residue found		Public disclosure of results within 30 days of findings
33		5.2.10	Antibiotic load compared to two previous production cycles, if required	kg	production cycle	Starting June 2017
34		5.4.2	Unidentifiable transmissible agent	Date(s) concern raised; disease detected from monitoring (if applicable)	ongoing	Public disclosure of results of surveillance within 30 days of findings

Item	Option	Relevant Requirement	Measurement	Units	Measurement Frequency	Calculations and Sampling Methodologies, Additional Notes
35		5.4.4	OIE-notifiable disease detected on farm	Disease(s), exotic or endemic, and detection date(s)	ongoing	Public disclosure of detection and results of surveillance within 30 days of findings
36		Section 8	Type of smolt production system	Open, semi or closed	production cycle	
37		8.32 and 8.33	Monitoring results from water quality analyses	See Appendix VIII-2		

Appendix VII: Parasiticide Treatment Methodology

Continuous reduction of applying medicinal parasiticide treatments

The ASC Salmon Standard requires farms to continuously reduce the number of medicinal treatments applied in treating sealice, a persistent marine ectoparasite. The ultimate vision is to no longer having to treat sealice with medicinal treatments. However, at the same time it is also recognised that this scenario is not yet achievable for the far majority of the industry at this moment in time.

In order to incentivise the development and implementation of non-medicinal measures (e.g. biological and mechanical control), the relevant indicators under Criteria 5.2 require farms to meet an Entry Level (EL) that expresses the Weighted Number of Medicinal Treatments (WNMT), after which a fixed rate of reduction needs to be achieved until the WNMT meets the defined Global Level (GL).

Parallel to the improvement process as described above, the Standard requires that farms apply Integrated Pest Management (IPM) in order to mitigate in an effective manner.

This Appendix gives more detail on the various concepts referenced above, as well as providing metric levels that relate to the EL, GL and rate of reduction.

Weighted Number of Medicinal Treatments (WNMT)[182]

The Weighted Number of Medicinal Treatment frequency is the total number medicinal parasiticide treatments applied over the production cycle, within the UoC. Partial treatments should be counted as a proportion of the cages treated.

Some examples are given on how to count the WNMT, e.g.

- treating an entire farm (all cages) once, counts as WNMT = 1;
- treating 1 cage, out of 10, once, will count as WNMT = 0.1;
- treating 1 cage, out of 10, twice (i.e. two unique treatments), will count as WNMT = 0.2;
- treating 5 cages, out of 20, once, will count as WNMT = 0.25.

Additional considerations:

1. Hydrogen peroxide (H_2O_2) must be considered as medicinal parasiticide treatment and thus be included in the WNMT-count;
2. If a *single* bath-treatment is prescribed to be applied as “coupled-treatment” (i.e. one treatment at t_1 and a follow-up treatment at t_2), then each treatment (t_1 and t_2) must be included in the WNMT-count.

Some more examples are given on how to count the WNMT, e.g.

- treating 1 cage, out of 10, once with hydrogen peroxide (H_2O_2), will count as WNMT = 0.1;

[182] Medicinal parasiticide includes hydrogen peroxide.

- treating 1 cage, out of 10, once with hydrogen peroxide (H_2O_2) as a coupled-treatment, will count as WNMT = 0.2;

Defining Entry Level (EL) and Global Level (GL)

A detailed statistical study was conducted and reviewed by a Technical Working Group in order to understand the regional characteristics of the number of sealice treatments applied per production cycle within the various production regions. The study, including the used data (in Excel) is publicly available on the ASC-website.

In summary, the study used 4 datasets, resulting in N = 896 data points. The data sets covered the following production regions: West Canada (BC), Chile, Faroe Islands, Ireland, Norway and Scotland. Subsequently, the study established distribution curves of the number of medicinal treatments applied per region and one global curve on the basis of N = 896.

On the basis of the 50th percentile for each of the regional curves, regional WNMT-numbers are set that form an Entry Level for farms in that region. Farms must be below, or at, EL for compliance. The results are presented in the table below:

Region	Entry Level (WNMT)	Global Level (WNMT)
Canada (BC)	1	3*
Chile	9	
Faeroes	6	
Ireland	3	
Norway	5	
Scotland	9	

Table: Regional Entry Level and Global Level (both in WNMT)

* GL is set at 3 WNMT, unless twice a "coupled-treatment" is applied (counted as 2*2 = 4 WNMT), then GL = 4 WNMT applies. In case of this exception, additional medicinal treatments applied will result in exceedance of GL=4

In addition to the defined regional Entry Levels, a Global Level (GL) was determined as well. It is required that farms progress from EL to GL according to a fixed timeframe. The GL is based on the 20-25th percentile of the used overall dataset. This resulted into GL = 3 WNMT. However, some bath-treatments are given as "coupled-treatment" (as per above), which with a GL = 3, could result into having a part of the treatment falling beyond GL = 3. In order to reflect the realities of applying these coupled-treatments, an exception is defined in case two times a coupled-treatment is applied. For this specific situation, GL = 4 WNMT applies. Situations that do not meet this exception, shall apply GL = 3 WNMT.

Reducing from EL to GL

It is required for farms to reduce from ∝EL to GL by means of a fixed rate of reduction. This rate is determined at 25% WNMT per 2-year.

Integrated Pest Management (IPM)

Integrated Pest Management (IPM) has long been recognised as being critical to effective and robust sea lice management. IPM is based upon the implementation of a number of proven techniques and approaches developed for pest management in terrestrial agriculture systems, often with the central aim of slowing the development of drug resistance in pest species.

The strategy of IPM generally involves coordinated application and integrated use of all available management practices, with surveillance, communication and cooperation between operators within a defined area. IPM seeks in particular to reduce reliance upon medicinal treatments, thus reducing scope for development of drug resistance and is therefore a process that ASC intends to promote.

The ASC Salmon Standard already contains several aspects of IPM through its current Criteria and Indicators, namely:

- Adherence to relevant thresholds/limits on sea lice levels and required action (Ind. 3.1.4)
- Regular counting and reported of sea lice levels (Ind. 3.1.7)
- Maintenance of treatment records (Appendix VI)
- Single year-class stocking (Ind. 5.4.1)
- Fallowing between cycles (Ind. 3.1.1)
- Health management / veterinary health plan (Ind. 5.1.1)
- Cleaning of nets to increase water flow
- Routine removal of moribund fish (Ind. 5.1.3)
- Monitoring of fish state (*e.g*. behaviour – 5.1.1)
- Monitoring and control of other fish diseases (Ind. 5.1.1)
- Strategic use of medicines *i.e.* the appropriate medicine used for the targeted stage/s of lice (Ind. 5.1.1)
- Medicine rotation, where possible (Crit. 5.3)
- Medicine resistance surveillance (site or area) (Crit. 5.3)
- Monitoring of treatment efficacy (Crit. 5.3)
- Area coordinated planning and management (Ind. 3.1.3)

In addition to the list above, the use of non-medicinal, mechanical and biological controls should be applied in order to reduce sea lice load and risk for resistance built-up. Some examples are given here: https://globalsalmoninitiative.org/en/what-is-the-gsi-working-on/biosecurity/non-medicinal-approaches-to-sea-lice-management/.

As applying these measures depends on various factors – including state of technological development, unintended health side-effects on fish, site-specific situations like strong currents – the standard requires farms to prepare a strategic plan that outlines which non-medicinal measures are (to be) applied at the farms. The plan must be made public and signed-off by an authorized veterinarian. It is required that the plan is reviewed and updated on a production cycle basis to reflect the effectiveness of applied methods and determine next approaches.

Appendix VIII: Methodologies Related to Water Quality and Smolt Systems

Appendix VIII-1. Calculation of Total Phosphorous discharged per tonne of smolt produced

Requirement 8.4 looks at how much phosphorus is discharged from the farm per unit of smolt produced. The requirement is set at 5 kg/mt for the first three years from date of publication of the ASC Salmon Standard, dropping to 4 kg/mt thereafter. Smolt facilities would calculate their discharge using a "mass balance" approach that calculates the discharge from the phosphorus in the feed and the phosphorus in the fish biomass. Farms would be able to subtract P that is physically removed in sludge (documented sludge removal with P levels tested).

To calculate P released to the environment, one must calculate the P used to produce one unit of fish and subtract the P taken up by the fish and P removed in sludge. The basic formula per time period, to be calculated for a maximum period of 12 months, is:

P released to the water body per unit of smolt produced = (P in – P out)/biomass produced

Where:

P in = Total P in feed

P out= (Total P in biomass produced) + (Total P in sludge removed)

Where the following definitions of the parameters apply in the basic formula:

1. *Total P in feed*
    a. ∑(Total amount of feed type (product) multiplied by content of phosphorus) $_{1.....X}$), where 1…….X represents the number of different feed types (products) used.
        i. The phosphorus content per feed type can be determined either by chemical analyses of the feed type, or based on declaration by the feed producer of phosphorus content in the feed type in jurisdictions where national legislation order phosphorus content of feed to be declared.
2. *Biomass produced*
    a. Biomass of fish produced over the specific time period is calculated as: (biomass harvested + biomass of mortalities + remaining standing biomass) – biomass at start of time period.
3. *P content in biomass produced*
    a. P content in biomass produced = (biomass produced)*(% of P in fish)
        i. For purposes of calculating this requirement, the following phosphorus percentages will be used for harvested fish or mortalities:
            1. Less than 1 kg: 0.43%
            2. More than 1 kg: 0.4%
4. *Total P in removed sludge*
    a. P content in sludge removed = (sludge removed) * (% of P in sludge)
        i. Phosphorus in sludge removed per unit shall be determined based on analytical values that are representative of the batch of sludge removed from the farm.
        ii. The smolt farm must demonstrate the sludge was physically removed from the farm site and that the sludge was deposed of according to the principles in requirement 8.35.

Appendix VIII-2: Water quality sampling methodology and data sharing for land-based systems

Land-based farms (flow-through and recirculation systems) must measure dissolved oxygen in the effluent. They also must submit to ASC the results from the effluent monitoring they conduct to comply with their local regulatory requirements. In particular, the requirement requires data on any sampling of phosphorus, nitrogen, TSS and BOD. This data will help to distinguish the performance of farms certified by this requirement over time, and assist in revisions to the ASC Salmon Standard.

Oxygen saturation must be measured at least monthly in the early morning and late afternoon. A single oxygen reading below 60 per cent would require daily continuous monitoring with an electronic probe and recorder for at least a week demonstrating a minimum 60 per cent saturation at all times.

Farms shall use the following table to submit the results of effluent monitoring to ASC. Please list each analysis separately over the previous 12-month period.

Date	Analysis *(TP, TN, BOD, TSS, etc.)*	Location *(Effluent, Inlet, etc.)*	Method *(Single grab, 24-hour bulk, etc.)*	Sampling by Third Party? *(Yes/No)*	Analysis by Third Party? *(Yes/No)*	Result *(including units)*

Appendix VIII-3: Sampling methodology for benthic macro-invertebrate surveys

Land-based smolt production systems must conduct sampling of the benthic macro-invertebrate habitats in the receiving body of water downstream and upstream of the effluent discharge point. The requirement requires that the downstream benthic status be similar or better than the upstream benthic status. To demonstrate this, the survey must demonstrate that the downstream location has the same or better benthic health classification as the upstream location.

Below are required components of the sampling methodology and classification scheme that a farm shall use. It is expected that a farm will use the faunal sampling regime in its own jurisdiction, as long as the regime includes the following minimum requirements.

This appendix also includes additional suggested ideas on conducting the surveys. The suggestions are intended as a guide only. The entity conducting the faunal survey should use its own discretion based on local knowledge, national fauna index systems, and expertise as to what specific sub-element or parameter will provide the best representation to document the status of the benthic macro invertebrates and the impact that the fish farm may have on this environment in the receiving water body.

Minimum requirements for faunal surveys:

Classification System

- The benthic health classification system must have at least five categories of benthic status.

Focus of the survey

- The survey must detect the composition, abundance diversity and presence of benthic invertebrate fauna in the receiving water body (upstream and downstream from farm outlet). The survey must focus on key sensitive indicator species for the region.

When and how often

- The samples must be collected once every year upstream and downstream from the farm outlet. In case the downstream survey drops a category according to the faunal index, two consecutive faunal surveys must be conducted during the following 12 months, using the same faunal index system, that demonstrate compliance with the requirement.
- After three years of demonstrating consistent results, a farm may reduce sampling to once every two years.

Where to sample

- The samples must be taken from both midstream and near the bank and must also include marginal areas with slacker water flow.
- All efforts must be made to isolate the impact of the farm, for example by seeking similar conditions, such as type of bottom, water flow and/or substrate types present along the bank, in the upstream and downstream locations.
- The location of sampling sites downstream from the farm must reflect a scientific assessment of the most likely area of potential impact from the farm, with consideration to the mixing of water and the minimum and maximum distance from the farm outlet.

Number of samples

- The survey must collect samples in at least three transects (10 metres apart), with at least four samples in each transect across the river. This must be conducted both upstream and downstream from the farm outlet.

Analysis of the samples and how to samples

- All collected samples must be analysed by an accredited laboratory and the sampling methodology must be approved by the laboratory conducting the analysis.

Further recommendations to sampling:

When and how

When collecting macro-invertebrates, consideration should be given to the seasonality of the presence of the macro-invertebrate species, namely insects in their larval stage of the life cycle. It is generally recommended that samples are conducted during summer and/or winter. In geographical regions like Scandinavia, spring and autumn are recommended as the best times for sampling.

Sampling gear

The sampling should be undertaken using standard equipment such as surber sampler, handnet and grab. More detailed sampling guidelines can also be found in ISO standards ISO 8265, 7828 and 9391.

References:

- Common Implementation Strategy for the Water Framework Directive (2000/60/EC) *Guidance document no. 7.* Monitoring under the Water Framework Directive.
- Biological assessment of running waters in Denmark: introduction to the Danish Stream Fauna Index (DSFI) Skriver et al.; 2000.
- The performance of a new biological water quality score system based on macro-invertebrates over a wide range of unpolluted running-water sites. Amitage, P.D. et al., 1982.
- Common Implementation Strategy for the Water Framework Directive (2000/60/EC) *Guidance document no. 13.* Overall approach to the classification of ecological status and ecological potential.
- UN/ECE Task Force on Monitoring & Assessment under the Convention on the Protection and Use of Transboundary Watercourses and International Lakes (Helsinki, 1992) Volume 3:Biological Assessment Methods for Watercourses.

Appendix VIII-4: Sludge BMPs for closed and semi-closed smolt systems

Methods to mitigate the impacts from fish metabolic wastes on water can range from the employment of simple settling ponds to the use of advanced technology filters and biological process. Dealing responsibly with the waste (sludge, liquid slurry, biosolids) from these processes is a critical element to responsible smolt facility management. The ASC acknowledges that BMPs related to other principles such as correct feed composition and texture as well as good feed management practices—such as not storing feed for too long—can also influence the effectiveness of biosolids capture, however this section deals with practices for cleaning, storage and disposal that will minimise the potential impacts of sludge/biosolids being released into the environment.

All closed and semi-closed smolt systems shall employ/undertake the following in relation to sludge/biosolids:

1. A process flow drawing that tracks/maps the water and waste flow of a farm including treatment of waste, transfer of wastes, waste storage and final waste utilisation options. Flow diagram should demonstrate the farm is dealing with biosolids responsibly.
2. Farm shall have a management plan for sludge/biosolids that details cleaning and maintenance procedures of the water treatment system. The plan must also identify and address the farm's specific risks such as—but not limited to—loss of power, fire and drought. The management can be evaluated in relation to maintenance records.
3. Farm must keep detailed records/log of sludge/bio-solid cleaning and maintenance including how sludge is discarded after being dug out of settlement ponds/basins.
4. Biosolids accumulated in settling ponds/basins shall not be discharged into natural water bodies.

Appendix VIII-5: Assimilative capacity assessment for cage (net-pen) smolt systems

Under 8.26, all open smolt farms in lake or reservoir settings must demonstrate that an assimilative capacity assessment has been conducted to determine if there is sufficient capacity from a water quality perspective to allow for the level of additional loading to the system.

Many suitable models exist that can help determine assimilative capacity, such as Dillon and Rigler (1975), Kirchener and Dillon (1975), Reckhow (1977), and Dillon and Molot (1996). The requirement does not favour one existing model over another but it is important to outline key elements of a credible assimilative capacity study.

At a minimum, the study must do the following:

- Undertake assessment as to allocation of capacity for the whole water body
- Undertake assessment as to land use, slope, sewage, other discharges, stream input
- Account for retention in lake and mixing
- Predict total phosphorus concentration
- Classify trophic status
- Undertake impact assessment of fish farm

The study must pay particular attention to the nature and morphology of the lake basin where the farm will be established. The study must analyse at a minimum:

1. Mixing of the surface and bottom waters
2. Whether bottom waters are isolated within the water body
3. The naturally occurring oxygen levels in the surface and bottom waters
4. Whether the water forms part of an enclosed basin, or an area with isolated bottom waters

Appendix VIII-6: Receiving water monitoring for open (net-pen) smolt systems

Sampling Regime for Receiving Water Quality Monitoring

Location of sampling stations: Stations will be established at the limit of the cage farm management zone on each side of the farm, roughly 50 metres from the edge of the cages and at reference stations located approximately 1-2 kilometres (km). All sampling locations will be identified with GPS coordinates on a schematic outline of the farm operations and on available satellite imagery.

Sampling methods: All water samples testing for total phosphorus shall be taken from a representative composite sample through the water column to a depth of the bottom of the cages. Samples will be submitted to an accredited laboratory for analysis of TP to a method detection limit of $\leq$ 0.002 mg/L. Dissolved oxygen measurements will be taken at 50 centimetres from the bottom sediment.

Frequency: At least once every three months during periods without ice, including at peak biomass.

**NOTE: Some flexibility on the exact location and method of sampling is allowed to avoid farms needing to duplicate similar sampling for their local regulatory regime.

	Boundary Stations (Note: if the farm is attached to land via a walkway, only three stations would be used)				Reference Stations	
	North	South	East	West	Upcurrent	Downcurrent
TP (mg/L)	X	X	X	X	X	X
DO profile (mg/L)	X	X	X	X	X	X

Appendix VIII-7: Trophic status classification and determining baseline trophic status

Requirement 8.30 requires a farm to determine a baseline trophic status for the water body and demonstrate through monitoring that the status is maintained. The ASC Salmon Standard use a modified version of the trophic status system developed by the Organization for Economic Cooperation Development (OECD) (Vollenweider and Kerekes, 1982). Trophic status is determined by the concentration of total phosphorus.

Trophic Status	Range of Total Phosphorus Concentration (≤ 20 πg/l)
Ultra-oligotrophic	< 4
Oligotrophic	4-10
Mesotrophic	10-20
Meso-eutrophic	20-35
Eutrophic	35-100
Hyper-eutrophic	> 100

(Note: these ranges are identical to ones described in an Environment Canada report titled "Canadian Guidance Framework for the Management of Phosphorus in Freshwater Systems, Science-based Solutions Report 1-8, February 2004")

Determining Baseline

Basic approach: Use the concentration in the most pristine area of the water body as possible, i.e., far from point sources of nutrients such as stream inflows, wastewater runoff, the farm or other fish farms. If the regulatory body has determined a historical baseline for the water body, that baseline shall be used.

Diseases and parasites in wild Atlantic salmon (*Salmo salar*) populations

Tor A. Bakke and Philip D. Harris

Abstract: The ecology of infectious diseases in wild and managed salmon populations is reviewed. Few pathogens have caused significant disease epidemics in the wild, and although parasites of returning adults are best documented, diseases among parr (e.g., *Gyrodactylus salaris*) are probably most important. The greatest diversity of parasites is known from the marine phase but few are likely to be significant pathogens, although conclusive evidence is lacking because diseased salmon cannot be tracked. The importance of stress as an immunosuppressant of fishes in degraded habitats is discussed. In addition, releases and restocking have probably also reduced the genetic disease resistance of wild fishes. We note that myxozoans, furunculosis, *G. salaris*, and sea lice are the pathogens most likely to threaten wild and managed salmon stocks in future. Despite abundant research on pathogens of farmed salmon, little is known of their impact on wild or managed stocks and an adequate theoretical framework for salmon disease epidemiology is urgently needed before disease becomes a limiting factor in salmon conservation.

Résumé : On examine l'écologie des maladies infectieuses chez les populations sauvages et issues du repeuplement. Peu de pathogènes ont causé d'importantes épizooties dans le milieu naturel, et, bien que les cas de parasitisme chez les adultes en montaison soient les mieux documentés, les maladies touchant les tacons (p. ex. *G. salaris*) sont probablement les plus importantes. C'est dans le milieu marin qu'on observe la plus grande diversité de parasites, mais peu de parasites risquent d'être d'importants pathogènes, même si nous manquons de preuves concluantes en raison du fait que les saumons malades ne peuvent être suivis. On traite aussi de l'importance du stress comme agent immunosuppressif chez les poissons qui vivent dans des habitats dégradés. De plus, les lâchers et le repeuplement ont probablement aussi réduit chez les poissons sauvages la résistance génétique aux maladies. Nous indiquons que les myxozoaires, la bactérie responsable de la furonculose, *G. salaris*, et les poux de poisson sont les pathogènes qui menaceront le plus les stocks de saumons sauvages et issus du repeuplement dans l'avenir. Malgré les nombreuses recherches sur les pathogènes des saumons d'élevage, on sait peu de choses de leur impact sur les stocks sauvages et issus du repeuplement, et il est urgent que nous disposions d'un cadre théorique adéquat pour l'épidémiologie des maladies du saumon avant que ces maladies deviennent un facteur limitant dans la conservation du saumon.

[Traduit par la Rédaction]

1. Introduction

Salmon are one of the most intensively studied of teleosts, with records of epidemic disease in wild populations dating back to furunculosis outbreaks in the late 19th century (Emmerich and Weibel 1894). Over the past 30 years many books have described the pathogens of farmed salmon (e.g., Post 1987; Bruno and Poppe 1996). This review however has a different purpose: to synthesise current knowledge of the ecology and epidemiology of infectious disease in wild Atlantic salmon (*Salmo salar*) populations. We are particularly concerned to identify those diseases that could influence population dynamics and growth in natural environments. The ecological and genetic consequences of salmon diseases and the impact of human influences (both direct management and indirect environmental factors) will also be discussed. Finally we review the impact of salmon farming on disease in wild fishes and speculate on future potential disease problems in the natural environment.

This review is written from an ecological perspective, introducing epidemiological concepts (e.g., Anderson 1982; Anderson and May 1978, 1979; Smith 1994; Grenfell and Dobson 1995), which have been slow to spread to the study of aquatic diseases (e.g., Dobson and May 1986). However, veterinarians, fishery managers, and ecologists differ significantly in their approach to infectious disease. For example, a paradigm of veterinary and human medicine has been that, for a particular disease, Koch's postulates can be satisfied (e.g., Hill 1965; Evans 1976). In aquatic wild organisms, this may simply not be possible: the pathogenicity of infectious agents may be so great that infected fishes die and disappear before they can be detected. This does not mean that their disease potential should be ignored. Indeed, Hill's (1965) criteria cannot be met for either the spread of furunculosis (Johnsen and Jensen 1994) or for the current epidemic of *Gyrodactylus salaris* (Halvorsen and Hartvigsen 1989; Johnsen and Jensen 1992), and in the latter case, this failure could arguably have contributed to the slow response of the Norwegian establishment to the emerging problem.

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We have therefore followed a precautionary principle, considering as potential pathogens in the natural environment those organisms that can cause disease in salmonid aquaculture or under experimental conditions and those organisms which are closely related to, or have a similar life history to, known pathogens.

Very few pathogens have had significant impacts on wild salmon populations and the spread of disease from farm to the wild remains a rare phenomenon, despite the current extent of salmonid aquaculture. Historically, only furunculosis (Inglis et al. 1993), a few other bacterial infections, ulcerative dermal necrosis (UDN; Roberts 1993), and *Gyrodactylus salaris* (Johnsen and Jensen 1991) have caused widespread conspicuous epidemics in wild populations. This does not necessarily mean that infectious disease is rare in natural populations (Kinne 1984; Munro et al. 1983; Lester 1984). It is often very difficult to quantify the effect of a pathogen on host population dynamics. For example, the aquatic fungus *Saprolegnia* is common on spawning adult salmon and can kill fishes (Pickering and Willoughby 1982). Nevertheless, it appears unable to infect healthy fishes, being a secondary pathogen exploiting weakened hosts. Similarly, when *G. salaris* was first described from Norwegian salmon (Johnsen 1978), it was not recognized as a primary pathogen because of its co-occurrence with *Saprolegnia*. The complex etiology of diseases in the wild is especially well demonstrated by UDN. Originally described as salmon disease and noted for its impact in the late 19th century (Roberts 1993), this disease then disappeared until the 1960s. The temporal and spatial spread of this disease (Roberts 1993) is strongly reminiscent of an infectious agent (Anderson 1982); however, despite intensive study, the causative agent remains unknown.

2. Parasite population ecology

2.1. Individual and population effects of parasitism

All parasites utilize energy otherwise available for host growth, survival, and reproduction and are important determinants of animal community structures (Minchella and Scott 1991). Many studies have shown sublethal effects on individual host survival, especially when it is advantageous to engineer the host's death to ensure transmission (see e.g., Lester 1977; Crowden and Broom 1980; Halvorsen and Andersen 1984; Milinski 1984; Rodger 1991; Bylund and Andersen 1994). Detecting the effects of parasitism in wild host populations, can however be very difficult (Anderson and Gordon 1982). To detect with confidence mortality due to selective predation of digenean-infected sticklebacks, Gordon and Rau (1982) sampled about 100 fishes per month; this would be quite impractical in most salmonid populations. Parasites and pathogens may therefore be overlooked as sources of mortality or sublethal morbidity, especially in juvenile fish populations (Gardiner and Geddes 1980; Clers 1993; Brabrand et al. 1994) from which dead and diseased individuals rapidly disappear. Disease is not thought to be important in salmonid population dynamics, except when conspicuous epidemics occur, and salmonid populations are instead regulated by density-dependent processes within the natal river (Elliott 1994; Elliott and Hurley 1998; Mills 1989). However, some evidence suggests that disease might regulate salmonid populations more generally. Clers (1993), for example, showed by modeling that *G. salaris*, infecting parr, could limit salmon population growth, while Levy and Wood (1992) felt that the cyclic abundance of sockeye salmon could best be explained by a pathogen. The life history of Atlantic salmon would blur the effects of disease epidemics and make them harder to detect; however, this lack of evidence does not mean that diseases play no part in wild salmon population dynamics.

Even small effects of parasitism may be critical in the wild, as reduction of parr growth by only a few percent may defer smolting by an entire year (see Marschall et al. 1998). The effect of a parasite on salmon reproductive rate might then be quite severe, although effects on population size and indeed the host itself may be undetectable. A further point to consider is the evolutionary cost of parasitism (Lehmann 1993; Grenfell and Dobson 1995). The production of xenobiotic chemicals is selected against when parasitism is relaxed (Behnke 1990) suggesting that excessively resistant hosts have their growth rate reduced as resources are diverted inappropriately to the immune system. The complexity and polymorphism of the immune system suggests that it is indispensable for survival (Price 1985; Wassom et al. 1986; Chevassus and Dorson 1990; Bakke et al. 1990; Gjedrem et al. 1991) and argues for the importance of parasitism as a selective force in natural salmon populations.

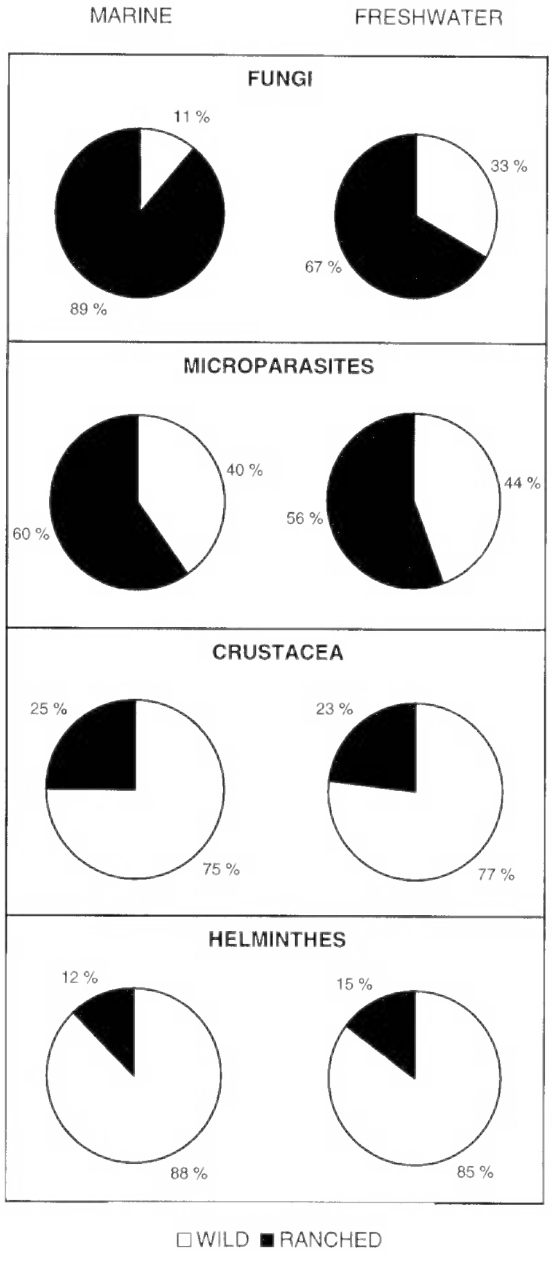
2.2. Microparasites versus macroparasites

An important distinction exists between microparasitic pathogens and macroparasites. Microparasites (e.g., viruses, bacteria, some protozoans, and *G. salaris*) reproduce in situ on the host, have small biomass, reproduce rapidly, and tend to induce lasting immunity (Anderson and May 1979). Macroparasites (including fish lice, tapeworms, nematodes, and some protozoans and fungal pathogens) have low reproductive rates, with long generation times and larger body size, and dispersal of young away from the host. The immune response may be less effective and infestations tend to be persistent (Dobson and May 1986). Figure 1 demonstrates that the proportion of macroparasites recorded from salmon in the literature is larger in wild populations, in contrast to microparasites, which dominate published diversity in cultured fishes. This can be explained by high host densities, simplified ecological conditions, and short residence time, favoring transmission of directly infecting microparasites, and by the greater ease of study of cultured fishes. These conditions seem also to favor the occurrence of fungi that are most frequently recorded in farms.

2.3. Parasites and pathogens have aggregated distributions

A few hosts carry the majority of the parasite population and most hosts are barely infected (Crofton 1971; Pennycuick 1971; Anderson and May 1978). Several factors are responsible for this. Microparasites reproduce in situ and therefore become highly aggregated. On the other hand, selective mortality, culling heavily infected fishes, reduces aggregation. Other factors may be environmental. Some parasites have extremely local distributions and spread slowly, so that spatially isolated metapopulations (e.g., parr in a riffle) may be heavily infected while neighboring meta-

Fig. 1. The proportion of species of infectious agents infecting ranched and wild Atlantic salmon under freshwater and marine conditions. The macroparasites are represented by the helminths and crustaceans, the microparasites by the virus, bacterians, and protoctistans. The results indicate that constrained conditions in farms favor the microparasitic life strategy for fungi. (The data are based on a literature survey of infectious pathogens reported from the Atlantic salmon.)



populations remain uninfected. Aggregation may also be related to the relative susceptibility of hosts, which is usually genetically controlled (Chevassus and Dorson 1990; Wakelin 1994), and at least as important as physiological and ecological factors in generating patchiness. Preliminary studies (Bakke et al. 1990; Bakke and MacKenzie 1993; Gjedrem et al. 1991) have shown genetic heterogeneity in disease resistance among salmon and other salmonids (Bakke et al. 1996), but the importance of this phenomenon in the field has not been considered and should be investigated. Heterogeneity of resistance is a paradigm of *G. salaris* epidemiology, with Norwegian salmon considered susceptible while Baltic salmon are resistant (Mo 1994). Our experimental studies have shown considerable heterogeneity between salmon stocks (see Figs. 2A, 2B), also Norwegian stocks, as well as within single stocks (see Figs. 2A, 2B) of both Norwegian and Baltic salmon. The figure demonstrates that both major stocks are susceptible to the Norwegian strain of *G. salaris* and acquire a response against the infection after the same time period, but with the Norwegian salmon most susceptible to parasite reproduction.

2.4. Stable versus unstable population dynamics

The microparasite–macroparasite demarcation profoundly affects the stability of host–pathogen interactions (Anderson and May 1979). Dispersal away from the host and long, complex life cycles favour stability, with parasite populations often limited by density dependent processes such as competition (Anderson 1982). Macroparasites, although sometimes causing conspicuous harm to heavily infected individuals, usually have negligible effects on wild host populations and often remain stable through time (e.g., Kennedy 1981; Amundsen et al. 1997). In contrast, microparasites lack stability, their populations oscillating between virtually extinct and dangerously large with alarming rapidity. Populations of *G. salaris,* for example, can vary in size by several orders of magnitude over periods of a few weeks, while the host population remains constant (Jansen and Bakke 1993*a*, 1993*b*; Bakke et al. 1990, 1996). Thus microparasites, which may be very difficult to detect, and which as individuals are negligible, are of overwhelmingly greater importance as pathogens, as potential regulators of host population size, and as selective agents.

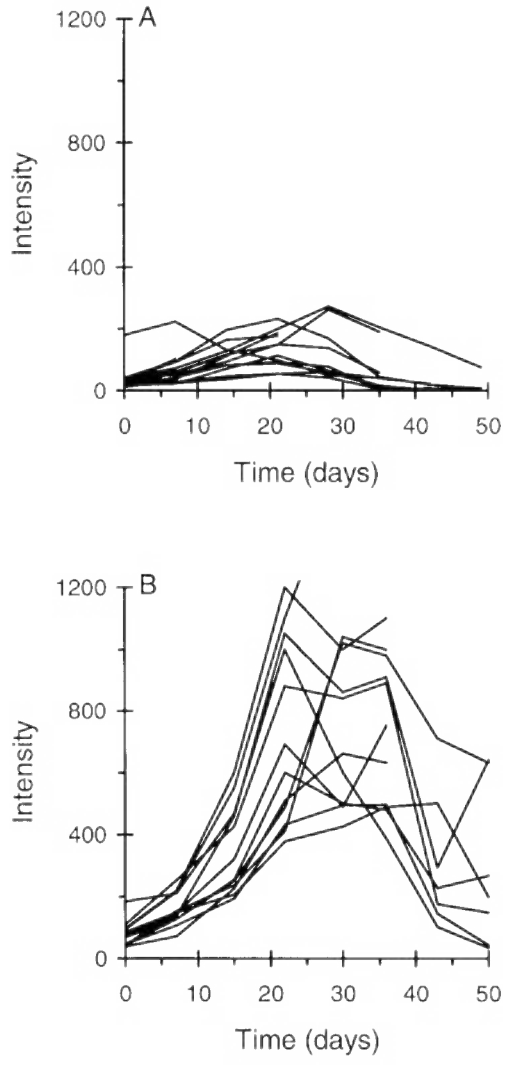
Within salmon farms a number of macroparasites can be pathogenic (Bristow and Berland 1991*a*; Rodger 1991). Fish lice are perhaps the best examples (cf. Boxshall and Defaye 1993; Heuch 1996), illustrating that ultimately the microparasite–macroparasite distinction is artificial. Within sea pens dispersal does not take place efficiently, allowing reinfection. Low juvenile mortality greatly shortens parasite generation time and the population dynamics become increasingly unstable and resemble those of a microparasite.

3. Cohorts and ecological groups of wild salmon

3.1. Salmon life cycles and parasites

Different stages of the salmon life cycle are both spatially and temporally separated in lotic and lentic freshwater systems and in marine coastal and pelagic areas, overlapping minimally. This has a prominent effect on the ecology of salmon–pathogen interactions (Zschokke 1880; Heitz 1920; Dogiel and Petroshevskij 1935; Margolis 1982*a*, 1982*b*; Soleng and Bakke 1997). Along a river system there are longitudinally restricted spawning redds; nonfeeding alevins (for which the oral route of infection is not available); fry, which disperse from the redd to individual territories and allow the early spread of infections through a riffle system; parr, which by their aggressive dominance behavior force

Fig. 2. Inter- and intra-populational heterogeneity is recorded in the susceptibility and resistance of Atlantic salmon to the Norwegian strain of *Gyrodactylus salaris.* (A) The course of infection of *G. salaris* on individually infected and isolated Baltic salmon from the River Neva, Russia (originals). (B) The course of infection of *G. salaris* on individually infected and isolated Norwegian salmon from the River Lierelva, south-east Norway (Bakke and MacKenzie 1993). (One line indicates one individually isolated fish, parasite numbers counted on anaesthetized fish; cf. Bakke et al. 1990).



the shoal to occupy larger and larger areas of the river, increasing opportunities for contraction and dissemination of disease; smoltifying parr, which undergo large physiological changes with consequences for their parasite burden; sea-running smolt, which move pathogens between parr patches and eventually carry them to the mouth of rivers; sea-migrating smolts, in which coastal parasites are aquired while freshwater parasites are lost; immature adults on the feeding grounds, able to ingest parasites in significant quantitities; returning adults, which disperse pathogens from the feeding grounds to the coastal areas; mature adults, which disperse pathogens upstream from the river mouth to the breeding sites; and kelts, which drifting seawards and heavily infected with opportunist pathogens may be particularly significant in the spread of disease (Soleng et al. 1997). Dwarf males may be continually present and, being immunosuppressed (Pickering 1987, 1989), may harbor a disproportionate total of the pathogens present in the population, while in landlocked salmon populations, only freshwater pathogens can survive. A literature survey of infectious agents reported from Atlantic salmon demonstrated a minimum of 225 species. Among those the digeneans and cestodes dominate the macroparasite groups, while bacteria and protoctistans dominate the microparasites (Table 1).

3.2 Juvenile stages in freshwater

The life span of each stage varies dramatically. That of eggs in redds, alevins, and fry is short, only a few months in total. Few pathogens, with the exception of some viruses and the opportunist hyphomycete *Saprolegnia* sp., are sufficiently mobile to infect spatially separated redds. Some vertically transmitted bacteria can overcome this problem by colonizing eggs during, or shortly after, laying and may cause early life stage mortality (Sauter et al. 1987). Lysozymes restrict the range of pathogens able to infect eggs, although some, for example *Renibacterium salmoninarum* (Yousif et al. 1994), and possibly *Piscirickettsia salmonis* (Larenas et al. 1996), can avoid neutralization by this defense mechanism.

Salmon parr have a variable lifespan, from 1 year in the south of the range (Nicieza et al. 1994) to 5 years in northern Norway and Canada. There may, therefore, be between 1 and 5 overlapping cohorts in a river, which greatly influences the possible dynamics of their pathogens. A parasite such as *G. salaris* might be expected to go extinct within small metapopulations of parr in the south of the host range, because of insufficient overlap between parr year-classes. Parr survivorship varies considerably with age (Rye et al. 1990; Clers 1994). In brown trout (*Salmo trutta*), a parasite infecting newly hatched fry can be 80% certain that its host will die within 20 days (Fig. 3A, recalculated from Elliott 1989*a*, 1989*b*, 1994) and must possess transmission and survival strategies to cope with this eventuality. Infection of 3-month-old parr, however (Fig. 3 B), gives a 70% chance that the host can be exploited for 3 months or more. This age-related difference is less dramatic for salmon (Figs. 3C, 3D; data recalculated from Mills 1989), possibly because detailed studies comparable to Elliott's (1989*a*, 1989*b*, 1994) studies of trout are not available. The basic reproductive rate, R_0, of a pathogen (Anderson 1982) infecting fry may need to be at least 20–100 to compensate for propagules infecting hosts which accidentally die of other causes. On the other hand, if infection is delayed the parr may already be unsuitable because of a preexisting infection. This trade-off, and its practical consequence when rivers are restocked, is poorly understood for any salmon pathogen.

3.3. The marine phase

Little is known about disease among marine salmon and the parasites/pathogens known from wild marine fishes are those larger macroparasites which probably cause least harm (e.g., Shulman and Shulman-Albova 1953; Polyanskii 1955; Pippy 1969*a*, 1969*b*, 1980; Hicks and Threlfall 1973;

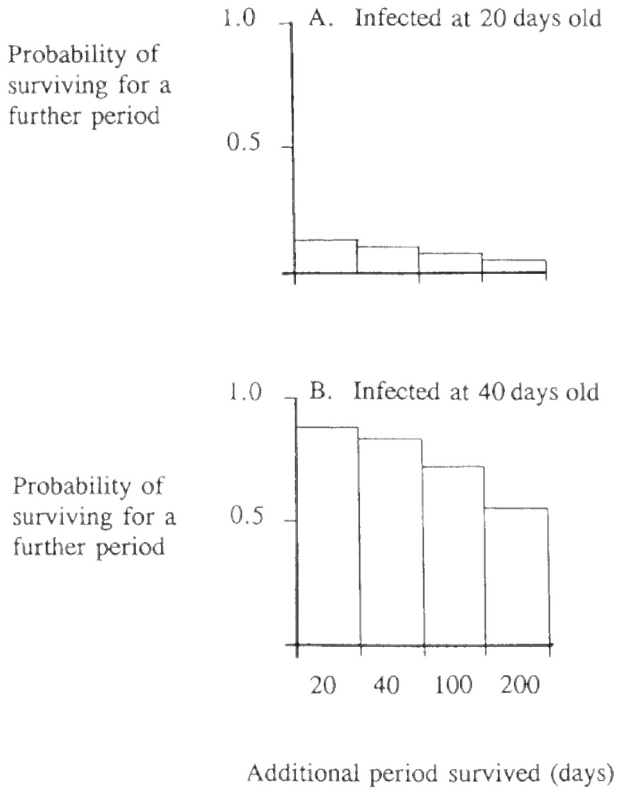
Table 1. The total number of species of infectious agents reported from wild and domesticated (ranched/hatchery) Atlantic salmon in both marine and freshwater habitats.

Group	Number of species
Virus	9
Monera	21
Protoctista	27
Animalia	
Hirudinea	3
Helminths	
Monogenea	11
Digenea	41
Cestoda	35
Nematoda	29
Acanthocephala	20
Crustacea	13
Mollusca	3
Acarina	2
Fungi	11
Total number	225

Note: Undetermined species are frequently reported but counted as one only if it represents the single record of a particular genus. The figures represent minimum numbers based on a literature survey.

Bristow and Berland 1991*b*; Holst et al. 1993; Mitenev 1993; Bristow et al. 1996). This reflects the more diverse environment, which juvenile salmon pass through on their way to the marine feeding grounds, offering opportunities for infection by parasites with complex multihost life cycles. It is also because the more virulent marine microparasites, if they exist, kill their hosts without trace. Sea pen culture has greatly increased our knowledge of marine pathogens, particularly revealing a diverse microparasite fauna. Viruses such as (ISA), known from ranched salmon in Norway (Thorud and Djupvik 1988), Nova Scotia (B. Dannevig, National Veterinary Institute, Oslo, personal communication) and most recently from Scotland, may occur naturally, but are sufficiently pathogenic to restrict transmission. Epidemics of ISA or other viral diseases among wild marine salmon are probably rare, because the density of salmon at sea is so low. However, this disease and others might impose an additional unseen mortality on smolts as they begin the marine phase (Uno 1990; Soleng et al. 1997). Olsen et al. (1997) describe rickettsial disease in smolts occurring only after seawater exposure in farms along the west coast of Norway; this may be a common pathogen of this life history stage but is only detected in net pens because of the difficulties of sampling migrating wild smolts. A similar situation may occur with the unidentified myxozoan associated with encephalitis of net pen reared salmon in Ireland (Frasca et al. 1998). Nothing is known of pathogens causing epidemic disease in wild marine salmon, but the fungus *Ichthyophonus hoferi* is almost ubiquitous among wild marine fishes and can cause major epidemics (Sindermann 1970; Møller and Anders 1986). At least one microparasite infecting marine salmon is therefore potentially capable of limiting host population size. The development of molecular (Mooney et al. 1995) and immunochemical probes will be very useful in determining the spectrum of pathogens to which marine fishes have been exposed.

Figs. 3A, 3B. Influence of host age on probability of successful completion of pathogen life cycle. (A) Probability of 20-day-old trout fry surviving for specified further periods following infection, to allow completion of the life cycle. (B) Probability of 40-day-old fry surviving for specified further periods following infection. Calculated from survival data for *Salmo trutta* in Black Brows Brook presented by Elliott (1989, 1994). Figure assumes that parasite does not adversely affect survival of fry.



4. Diseases of salmon (*Salmo salar*) in the wild — case studies

In this section we present an outline of the systematic groups to which salmon pathogens belong and identify aspects of their ecology which are of particular interest.

4.1. Viruses

Many viruses infect salmon within aquaculture facilities; viral infections of wild salmon are far harder to identify and to our knowledge there have been no reports of disease epidemics due to viruses in wild salmon populations. Viruses obviously exist within wild salmon populations, their frequency within aquaculture units reflects the importance of fish farms in sampling and amplifying pathogens in the natural environment. ISA, for example, presumably exists within the natural salmon population at low intensity but is not found because infected wild fishes die and disappear. Because salmon aquaculture is a relatively young industry,

new viral infections frequently appear and continue to be identified, including for example the togavirus reponsible for pancreas disease (Nelson et al. 1995; McLoughlin et al. 1996, 1998). There is however no evidence for transfer of viral infections from farm to wild salmon populations, although viruses such as infectious pancreatic necrosis (IPN) may be frequently isolated from escaped salmonids (Bucke 1993). Endemic viruses may have considerable impact on young fishes (fry and parr) but have not been looked for in studies of wild salmon population dynamics. The introduction of molecular and immunological probes, allowing the disease history of individual fishes to be recreated in the absence of obvious symptoms, will help greatly in understanding the pattern of microparasitic pathogens to which salmon are exposed.

4.2. Bacteria

Salmon are susceptible to numerous bacterial diseases, some of which have been studied intensively (Austin and Austin 1987; Inglis et al. 1993). In the wild, disease epidemics have been caused by several bacteria, including *Renibacterium salmoninarum,* which caused epidemic bacterial kidney disease (BKD) in the Aberdeenshire Dee in the 1930s (Smith 1964). BKD is a serious and fatal systemic infection of both farmed and wild salmonids (Bruno 1986; Bruno and Poppe 1996) but knowledge of its etiology is nevertheless fragmentary. The bacterium may be a normal member of the gut flora (Austin and Austin 1987), which at times of stress migrates to the kidney and causes overt disease. Bucke (1993) was unable to isolate *R. salmoninarum* from over 3000 wild salmonids in Britain, although full details of the sampling regime used were not given. Of the numerous other bacteria known from salmon, only furunculosis is a serious pathogen of wild fishes. Although bacteria such as *Vibrio* species (*V. anguillarum* and Hitra disease, *V. salmonicida*), *Yersinia ruckeri* (responsible for enteric redmouth), and *Flexibacter* species (e.g., *F. columnaris*, responsible for columnaris disease) are well known from farm situations, there appear to be no records of outbreaks of these diseases in wild salmon. This may be because their pathogenicity is so great that infected fishes die before they can be sampled, or, perhaps more likely, because there are no transmission cycles between farmed and wild fishes.

4.2.1 Furunculosis

Furunculosis, caused by *Aeromonas salmonicida*, is one of the best known and most important diseases of salmonids and has been extensively reviewed (Austin and Austin 1987; Inglis et al. 1993; Johnsen and Jensen 1994). The pathogen was first detected in Europe (Emmerich and Weibel 1894) and North America at the end of the 19th century, causing epidemics in wild trout. The British epidemic within wild trout and salmon at the start of the 20th century was particularly well documented (Masterman and Arkwright 1911; Lund 1967; Austin and Austin 1987). It began in the southwest peninsula in 1911, spreading steadily north and eastwards to reach the important Scottish salmon rivers by the late 1920s. A similar pattern was observed in Norway following introduction from Denmark in 1964 (Lunder and Håstein 1990). In this case, the pathogen was eventually exterminated in 1969 (Håstein 1989). In both cases, the pattern of spread was characteristic of an introduced pathogen, eventually slowing down and losing pathogenicity as the bacterium became endemic.

A second phase began with epidemics in farmed marine salmon from the mid-1980s onwards. The marine form of *A. salmonicida* originated in Scotland, spreading to Norway in 1985 (Egidius 1987; figs. 5, 6), and since then it has become entrenched in marine farms (Håstein et al. 1989). Furunculosis spread from coastal fish farms with the numerous escapes and wild fish in 74 rivers had been infected by the end of 1992 (Johnsen and Jensen 1994). The taxonomy of *A. salmonicida* is controversial (Austin and Austin 1987) and relationships between marine and freshwater strains are not clear. It is interesting that observations in the early part of the century suggested that this pathogen could not colonize salmon in seawater. Thus, 1339 smolts collected from British estuaries in the 1930s were all uninfected (cited in Austin and Austin 1987).

Several ecological factors provoke concern for the potential impact of furunculosis spreading from farms to wild salmonids. Nese and Enger (1993) recovered viable *A. salmonicida* from salmon lice collected from within net pens and Costelloe et al. (1998) have recorded *L. salmonis* larvae derived from farmed salmon dispersing within an estuary. It would therefore be theoretically possible for *A. salmonicida* to transfer to migrating smolts or returning adults passing through estuaries. Smolt infected in this way would be almost impossible to track, while adults migrating upstream could pass furunculosis on to immature salmon in their nursery rivers. Escaped farmed salmon may also be a significant source of furunculosis infections in natural salmon populations (Johnsen and Jensen 1994). *Aeromonas salmonicida* can also infect other fish species, including cleaner wrasse (Austin and Austin 1987), coalfish, cod, and turbot (Willumsen 1990; Hjeltnes et al. 1995), although infections are short-lived and nonpathogenic (Hjeltnes et al. 1995). Additional studies using techniques such as PCR capable of detecting very small *A. salmonicida* populations should be urgently applied to determine the status of this pathogen on salmon and sea trout transiently residing in estuaries (McArdle et al. 1993; Mooney et al. 1995).

A most worrying aspect of *A. salmonicida* is its propensity to transfer plasmids conferring drug resistance between strains (Austin and Austin 1987). Sakai (1987) has noted exchange of protease genes between pathogenic and nonpathogenic strains, under circumstances similar to those occurring in river sediments. Since protease phenotype is an important determinant of virulence in *A. salmonicida,* this is clearly a potential source of new, genetically and ecologically distinct strains of the pathogen which could possibly infect wild salmon populations.

4.3. Protoctista

Many of the approximately 2420 species of fish-infecting protoctistans are serious pathogens (Lom and Dykova 1992). Some cause significant mortalities of salmon, while others are not primary pathogens or do not significantly affect the fish. None are known to be problems in wild salmon populations, although several species could become pathogens.

4.3.1 Myxozoa

Myxozoans are well known for their distinctive propagules which are found within the fish's tissues, often in conspicuous cysts. Seven species infect Atlantic salmon.

Proliferative kidney disease (PKD), which affects most farmed freshwater salmonids, including *S. salar* (Ellis et al. 1985; Lom and Dykova 1992; Hedrick et al. 1993), is caused by an unidentified myxozoan usually referred to as "PKX." This pathogen has, in retrospect, been known for 75 years but was only recognized in the 1980s. The similarity between the observed sporogonic stages of PKX and corresponding stages in *Sphaerospora* species has been stressed and various *Sphaerospora* spp. infecting teleosts such as sticklebacks (Feist 1988; Lom and Dykova 1992) have been identified with the causative agent of PKD. Although Kent et al. (1993, 1994) implicated *Sphaerospora oncorhynchi*, a parasite of mature sockeye salmon in northwest North America, as the PKX organism, Marin de Mateo et al. (1996) have cautioned against accepting this hypothesis uncritically and the most recent molecular study (Kent et al. 1998) has suggested that PKX is not closely related to *Sphaerospora*.

The complex life cycle of myxozoans, which may involve a tubificid intermediate host, makes them unlikely to sustain persistent disease epidemics in the wild although infections from farmed to wild fishes may be possible. The epidemiology of marine myxozoans such as *Kudoa* (Harrell and Scott 1985; Whitaker and Kent 1991) and *Chloromyxum* (Wootten and Smith 1980; Holst et al. 1993; Bristow et al. 1996) species is so poorly understood that their effect on salmon survival or fitness is entirely unknown.

4.3.2. Other protoctistans

A range of protoctistans infect the outer surface of juvenile salmonids. *Ichthyophthirius multifiliis,* a highly pathogenic ciliate, causes greater economic losses worldwide than any other freshwater fish parasite (Cross 1994). This parasite has been recorded from salmon in freshwater hatcheries (Hare and Frantsi 1974; Wootten and Smith 1980; Valtonen and Keränen 1981), where its importance cannot be doubted; its role in wild salmon (Margolis and Arthur 1979) populations is less clear.

The other ciliophorans (e.g., *Capriniana, Chilodonella, Epistylis, Scyphidia, Trichodina*, and *Trichophrya*) are surface-dwelling opportunists, which are ubiquitous in freshwater and may form important components of the skin-surface community. Some may be pathogenic and trichodinids have been associated with the death of kelts (Khan 1991). The mastigophoran, *Ichthyobodo necator,* has a similar ecology (Roubal et al. 1994; Roubal and Bullock 1994) and is well known as a source of mortality in hatcheries. The role of these organisms in juvenile salmonid populations is unknown, but pathogenicity probably depends on unknown factors controlling the precise composition of the ectoparasitic community (Pickering and Richards 1980).

Flagellate species of the genus *Hexamita* cause significant disease problems in a range of fish species, including salmonids (Kent et al. 1992), and under subarctic conditions in Northern Norway a related flagellate, *Spironucleus barkhanus* (see Poppe et al. 1992; Poppe and Mo 1993; Sterud et al. 1997; Sterud 1998), has caused losses among net pen reared salmon. This eurythermic species was originally described from wild grayling (*Thymallus thymallus*) from freshwater river systems in southeastern Norway (Sterud 1998). Other strains infect anadromous Arctic char (*Salvelinus alpinus*) asymptomatically. Infection of Atlantic salmon is believed to have occurred either as juveniles in freshwater or through transmission in the sea from Arctic char. Uneaten surplus food often attracts wild fishes to fish farms, increasing the probability of disease transfer between the wild and farmed fishes (Sterud et al. 1998). Spironucleosis has not been reported since 1992 (Sterud et al. 1998) and there is a general lack of knowledge of the factors triggering spironucleid and hexamitid epidemics.

Other unusual protoctistans occur in aquaculture, for example *Paramoeba* sp., attached to the gills of farmed salmon in Tasmania (Roubal et al. 1989). Such infections are probably due to unnatural juxtapositions of pathogen and host when fishes are translocated outside their normal range, rather than being a normal feature of the host biology. Caged marine salmon may similarly be killed by algal blooms (Dahl et al. 1982; Jones et al. 1982; Bruno et al. 1989; Kent et al. 1995). However, although algae could settle on the gills of smolts or adult salmon moving through coastal waters, their high rate of movement probably prevents morbidity and the problem can be viewed as one of captivity.

4.4. Fungi

A range of fungi have been collected from Atlantic salmon but their impact in wild populations is poorly understood and probably small. The best known is the oomycete *Saprolegnia* sp, frequently recorded from adults and kelts, in association with other pathogens (Johnsen 1978; Roberts 1993), although it can initiate primary infections (Bruno and Stamps 1987). This fungus is also well known for its effects on eggs and immature salmonids in culture (Pickering and Willoughby 1982). It may preferentially infect stressed and hence immunosuppressed (see section 7.3) salmonids (Neish 1977). As an egg pathogen, *Saprolegnia* is also generally a secondary opportunist infecting moribund eggs and Austin and Austin (1987) record infections in the presence of pre-existing *Aeromonas salmonicida.*

Several fungi of the genera *Phialophora* and *Exophialia* also infect salmon; however, only *Ichthyophonus hoferi* is a significant pathogen of farmed salmon (McVicar 1977) and has a major impact on marine fish populations (principally herring) in the northwest Atlantic (Sindermann 1970; Møller and Anders 1986). There is no evidence that this fungus has a significant impact on marine salmon stocks, but further investigations on this point would be worthwhile.

5. Metazoan pathogens

5.1. Monogenea

Salmon are infected by one monogenean with the typical life cycle in which eggs are released into the environment to hatch to a swimming larva. This is the gill parasite *Discocotyle sagittata.* Although potentially pathogenic (see Gannicott and Tinsley 1997), there is no evidence that this parasite causes problems to either wild or farmed salmon.

One monogenean genus, *Gyrodactylus,* has an aberrant life cycle, retaining embryos in utero until fully grown and already containing a developing embryo. This microparasitic life cycle, with very rapid reproduction in situ upon the host, has resulted in some gyrodactylids being serious pathogens. Salmon are infected by several species, including *Gyrodactylus derjavini* (Mo 1993), *G. salmonis, G. colemanensis*, and *G. nerkae* in Canada (Cone and Cusack 1988; Cone et al. 1983, 1988; Malmberg 1993), *G. caledoniensis* in Scotland (Shinn et al. 1995), *G. truttae* in the Czech Republic (Ergens 1983), and *G. masu* in Japan (Ogawa 1986), all of which are nonpathogenic. In the Baltic Basin, salmon are infected by *G. salaris,* which in Finland is nonpathogenic (Bakke et al. 1990; Rintamaki-Kinnunen and Valtonen 1996). In Norway, however, and in some rivers of the Swedish west coast and the Russan White Sea coast, *G. salaris* is a serious pathogen of wild salmon parr and has been blamed for the significant decline in the fishery (Johnsen and Jensen 1991; Mo 1994, but see also Halvorsen and Hartvigsen 1989). Salmon are also infected by a marine gyrodactylid, *Gyrodactyloides bychowskyi* (Mo and Mackenzie 1991; Bristow et al. 1996), which as far as is known, is nonpathogenic.

5.2. Intestinal helminths

These comprise the Digenea and Cestoda (both Platyhelminthes), the Nematoda, and the Acanthocephala. All have complex life cycles involving at least one, and usually several, intermediate hosts which are links in the food web of the salmon (Køie 1993). This complexity, and the need to coordinate with at least two hosts, means low transmission rates (Kennedy 1985); epidemics rarely occur. Because of the short summers at high latitudes, many helminths become entrained to seasonal patterns of infection (Chubb 1977, 1979, 1980, 1982). Although the individual host may carry an impressive burden of large helminths, the impact on the host population remains small (Halvorsen and Andersen 1984). Some helminths do raise concerns for wild salmon populations (Hoole 1994); the tapeworm *Eubothrium crassum* has sublethal effects on the salmon in sea pens (Bristow and Berland 1991a) and is moderately common in wild salmonids (Kennedy 1978; Andersen and Kennedy 1983; Bristow and Berland 1989) and the nematode may also be pathogenic in freshwater (Larsen & Lund 1997) and may infect fry in large numbers (Hare and Burt 1975a, 1975b). These helminth groups are the most species-rich yet to be considered.

5.3. Extraintestinal helminths

Atlantic salmon is also host to species living extraintestinally including exotic marine species such as *Hepatoxylon squali*. The final host of this tapeworm is the shark, the salmon being an intermediate host which must be eaten for completion of the life cycle. Among the nematodes, the air bladder nematode *Cystidicola farionis* may be pathogenic in freshwater (Knudsen and Klemetsen 1994; Chappell and Owen 1969) and *Anisakis* may have public health significance, as infections of humans can result from consumption of under cooked salmon (Berland and Fagerholm 1994). As this pathogen is associated with pelagic existence (Smith and Wootton 1978), this is peculiarly a risk associated with wild salmon caught during or after their sojourn at sea.

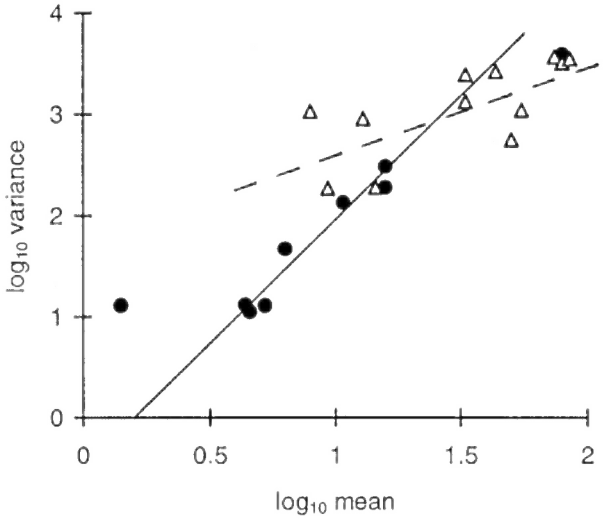
Over 40 species of digenean infect salmon and one is suggested to cause an effect upon the hosts' migratory behavior. The adult digenean *Phyllodistomum umblae* in the ureters of Arctic char and other salmonids may suppress anadromous migration (Nordeng 1983), although this hypothesis has been criticized by Bakke and Bailey (1987). One important digenean group in freshwater, are the eye flukes in the genera *Diplostomum* and *Tylodelphus*. The metacercariae stages of these flukes may blind the infected fish, making it vulnerable to predation, and are important in trout farms (Chappell et al., 1994). They are also important in natural non-salmonid populations (Lester 1977; Kennedy 1981), and, where especially bird predation is a problem they might be expected to have significant effects upon salmon parr survival.

5.4 Crustacea

Sea lice (*Lepeophtheirus salmonis* and *Caligus elongatus*) represent one of the greatest threats to the salmon marine ranching industry, with the possibility that they may become major pathogens of wild salmonids also. Although known to be occasionally pathogenic (White 1940), sea lice have generally been regarded as relatively harmless in wild populations. However, their importance within sea pens has been increasing since the mid-1960s (Pike 1989), while reports of disease outbreaks in wild salmonids have also increased (Johnson et al. 1996; Grimnes et al. 1996; Tully et al. 1993; Birkeland 1996). Sea lice are epithelial browsers (Boxshall 1974; Pike 1989) and do little damage to this constantly renewed tissue in light infections. Within sea pens however, heavy infections build up, with significant erosion of the epidermis, especially around the head (Jonsdottir et al. 1992). Death then follows, from osmotic shock or secondary infections. In heavy infections of wild fishes, death results from the same epidermal erosion (Grimnes et al. 1996; Tully et al. 1993) and both Calderwood (1905) and White (1940) noted the white head of infected fishes, as skin sloughs off leaving unpigmented tissue. In wild fishes the disease is associated with delays within river mouths (Calderwood 1905; White 1940; Johnson et al. 1996), waiting for water levels to rise, and there seems little doubt that sea louse transmission takes place most efficiently in warm shallow coastal waters (Johnson et al. 1996), although these parasites also survive in the open sea (Nagasawa et al. 1993). It is particularly unfortunate, therefore, that the estuarine environment is also that used for salmon ranching, with release of enormous numbers of sea louse larvae into the environment. Tully and Whelan (1993) recorded naupliar populations of up to 40 million in some Irish embayments and Costelloe et al. (1996, 1998) noted significant densities of nauplii (up to 5 m^{-3}) downstream of salmon farms.

It remains controversial whether sea lice from farmed salmon have a significant effect on wild salmonid populations. In Ireland, the decline in sea trout (*Salmo trutta*) populations from 1989 has been attributed to sea lice and is associated with early return to freshwater of post-smolts (Tully et al. 1993). Although the death of individual wild sea trout due to salmon lice cannot be doubted, the overall impact of this parasite and its involvement in the stock decline

Fig. 4. Taylor's power law applied to recalculated data from Sharp et al. (1994) and Tully et al. (1993) on the occurrence of *Lepeophtheirus salmonis* on Scottish (●) and Irish (Δ) sea trout, respectively. The lower value in Irish fishes suggests that their fish lice populations are less aggregated at high population densities, which indicates an impact on host mortality at the population level. Different population processes are affecting *L. salmonis* in the two areas.



has been questioned (Anonymous 1991; Sharp et al. 1994). In particular, Sharp et al. (1994) argue that the stock decline began before sea louse populations had peaked and that the decline in stocks also occurred in Scotland although sea lice were not a problem. It was noted earlier (section 2) that it was usually difficult to detect significant parasite-induced host mortality at the population level because of the large samples needed. Such samples are available for sea louse infections and it is strange that the methods of Anderson and Gordon (1982) have not been used to resolve the importance of sea lice in the decline of the Irish sea trout fishery. Taylor's Power Law (Taylor 1961; Anderson and Gordon 1982), which predicts a consistent relationship between mean intensity of infection and variance for any given species, shows a different relationship for Scottish and Irish sea trout infected with *Lepeophtheirus salmonis* (data recalculated from Tully et al. 1993 and Sharp et al. 1994). On Scottish fishes, mean intensity of infection is low and the gradient of the relationship between $\log_{10}$ mean and $\log_{10}$ variance is close to 2 (Fig. 4). For Irish fish, however, mean intensity of infection was somewhat higher and the slope of the relationship between $\log_{10}$ mean and $\log_{10}$ variance was close to 1. This lower value in Irish fishes suggests that their *L. salmonis* populations are less aggregated at high population densities. Anderson and Gordon (1982) list parasite mortality, density-dependent processes (e.g., reductions in parasite fecundity), and parasite-induced host mortality as reducing aggregation. The relatively low aggregation of *L. salmonis* on Irish sea trout may therefore suggest an impact on host mortality at the population level and it is clear that different population processes are affecting this parasite in the two areas. While Sharp et al. (1994) are justified in their claim that sea lice do not affect host mortality in Scotland, there can be no justification for the claim that similar processes are at work in Ireland. The relatively low correlation between log mean and log variance in Tully et al.'s (1993) data suggests that a number of disparate processes are occurring in different sampling sites and that further analysis should be undertaken. In particular, it would be interesting to analyze changes in variance:mean ratio within a single cohort through time (Anderson and Gordon 1982) as a sensitive indicator of parasite-induced host mortality. No comparable studies for sea lice on wild salmon exist, although Berland (1993) noted a sharp rise in infection levels between 1988 and 1992. Many published accounts provide only the range of parasite burdens encountered; this limits their value, as no objective measure of the distribution of parasites within the host population can be gained without estimates of both mean and variance. Another problem concerns methods of sampling. If sea lice do influence returning behavior, then sampling regime becomes critical. This has been shown to be the case for sea trout in Norway, where the prematurely returning post-smolts carry significantly larger infections than older migrants (Birkeland 1996). The work of Sharp et al. (1994), however, grouped both pre-smolts and older fishes within the same samples, while Tully et al. (1993) concentrated on pre-smolts. Most studies concentrate on returning fishes and sea trout are likely to be predisposed to sea louse mortalities because they do not migrate far off shore. For salmon the most interesting age group to study, where mortality is most likely to be significant, is the group of smolts passing out through the estuary and not returning. These are a much more difficult group of fishes to sample and as yet no accounts of their sea louse infections have been published.

6. The immune system

6.1. Salmonid immune system

Salmonids have the same sophisticated inducible immune system of other vertebrates (Woo 1992), producing antibodies (Wilson and Warr 1992; Secombes 1994) and having a well developed cellular response (Secombes and Fletcher 1992; Secombes 1994). Nonspecific, humoral responses, including complement, lysozyme, and nonspecific haemolysins (Alexander and Ingram 1992; Sakai 1992) are also well developed and may be a particularly important (Sakai 1992) first line of defense for wild fishes, especially when low temperatures delay antibody synthesis. The complement system may be activated via specific antibodies (the classical pathway) or, in early infections, via complex carbohydrates on the pathogen surface (the alternative pathway). The latter, being antibody independent, can initiate defenses immediately upon invasion. Complement is involved in determining the susceptibility of brook trout to the blood pathogen, *Cryptobia salmositica* (Forward and Woo 1996), and we have found *G. salaris* highly susceptible to salmon complement via the alternative pathway (Harris et al. 1998). Furthermore, variation in serum complement activity has been shown to be a useful marker of disease resistance in selective breeding programmes (Røed et al.1992; Hollebecq et al. 1995).

6.2. Age and the immune response

The immune system of newly hatched fry matures rapidly over the first few months of life (Ellis 1977; Manning and Mughal 1985; Tatner 1986; Zapata et al. 1997). It is not clear whether alevins can mount a specific immune response; parr can certainly do so and are routinely immunized against a variety of pathogens in aquaculture (Lillehaug 1997). Smolts (Maule et al. 1987; Muona and Soivio 1992) and spawning adults and kelts (Pickering and Christie 1980, 1981; Pickering 1989) may be immunosuppressed as a result of hormonal changes. Precocious dwarf males may also be immunosuppressed and may therefore be important carriers of disease.

The response to different pathogens may vary throughout life. Furunculosis, for example, although still dangerous, appears much less pathogenic to parr and smolt than to adult fishes (Austin and Austin 1987). An additional protective effect is due to herd immunity in very young fishes. An infectious disease introduced into an alevin population may kill many fishes and induce immunity in the remainder but many fishes will also die of unrelated causes and the reproductive output of the pathogen will be limited because of the poor life expectancy of the host (section 3.2). If the same pathogen is introduced into a parr population, on the other hand, the reproductive output will be much greater because of the longer life span of the host. This may have exacerbated the *G. salaris* epidemic in Norway where the response to large-scale parr mortality was to restock with naive parr.

6.3. Stress and disease

Stress raises blood cortisol concentrations (Pickering 1987; Barton and Iwama 1991), reducing immunocompetence and predisposing salmonids to disease (Pickering and Duston 1983; Pickering 1989; Pickering and Pottinger 1989). Sex hormones can also immunosuppress (Pickering and Christie 1980). Stress may therefore immunosuppress asymptomatic carrier hosts, allowing pathogens to multiply and kill the host (Sindermann 1983). The source of this stress is unimportant. Poorly fed fishes are also immunosuppressed (Blazer 1992) and fishes which are low in the dominance hierarchy are likely to be more susceptible to disease. Stock supplementation may therefore not substantially increase the number of smolts migrating to sea, but may provide stressed parr to act as a reservoir of infection and a potential focus for epidemic disease. It is noticeable that UDN (Roberts 1993), furunculosis (Lund 1967), and *G. salaris* (Halvorsen and Hartvigsen 1989) have all been most important when natural stock densities have been very high. Although density dependence in regulation of population size in juvenile salmonids is well documented (Elliott 1989*a*, 1989*b*, 1994), the role of epidemic and endemic disease in generating this phenomenon has not been investigated.

7. The influence of man

Within Europe, little of the range of the Atlantic salmon remains unmodified by man. Salmon have disappeared from much of their historical range and in many rivers can only breed with human intervention. Many populations are exposed to pollution or low and managed water flows. The influence of these factors on parasites and pathogens is particularly difficult to predict. In general, practices such as damming, by slowing river flow and allowing the development of shallow water habitats with emergent macrophytes, would increase habitat stability and complexity, allowing colonization by a wider range of invertebrates, which can act as intermediate hosts for parasites. Eutrophication may simplify invertebrate communities, reducing the range of potential intermediate hosts but also favoring organisms such as *Asellus* and tubificid oligochaetes, which may be important intermediate hosts (Khan and Thulin 1991). Periodic catastrophes, including drought–spate, episodic acidification, or even the deliberate rotenone treatment of rivers to eliminate *G. salaris*, probably have significant, but poorly studied effects on the salmon parasite fauna. A loss of parasites, particularly those with complex life cycles and fastidious ecological requirements, might be predicted. However, reductions in predatory invertebrates could increase the probability of survival of infective parasite larvae, while too little is known of the immunological and competitive interactions between parasites to predict the consequences for the salmonid population of eliminating a single species from the parasite community. The experiences of using rotenone to control *G. salaris* in Norway are instructive in this regard. The parasite was eliminated from fish farms in the early 1990s but continues to persist in many rivers. Episodic rotenone treatment has been employed to eradicate all salmon and hence *G. salaris* from individual rivers. All aerobically respiring invertebrates (most of the insect larvae) are also eliminated, along with all other fishes in addition to the salmonids. Salmonids then recolonize from the sea and by restocking, while invertebrates recolonize by drift from untreated upstream sources. Although recolonization occurs quickly, *G. salaris* has either not been exterminated or has recolonized very quickly three rotenone treated rivers. As far as the authors are aware no environmental impact assessments of the rotenone treatment have ever been published and data are not available to confirm the assertion that the post-rotenone invertebrate fauna is indistinguishable from that before treatment. The range of parasites infecting salmon parr before and after rotenone treatment has also not been compared.

A final nonspecific effect is the slow rise in river temperatures following global warming (Dobson and Carper 1992). Again, the effects on parasite fauna and disease of wild salmonids are impossible to predict. However, low environmental temperatures are immunosuppressive for ectothermic vertebrates, such as teleosts (Bly and Clem 1992), and higher temperatures may lead to enhanced immune responses but thermal stress and low water levels may immunosuppress (see section 7.3). The age at smoltification may be reduced, leading to changes in the age structure of salmonid populations, which will impact upon their disease profile but the longer transmission period may increase disease. It is too early to speculate on the consequences of these changes on salmon parasites; however, we must consider wild salmon as a highly managed resource throughout most of its range and consideration of patterns of disease should form part of the management strategy employed to conserve these stocks.

7.1. Pollution, stress, and the immune response

In recent years, interest in the interactions between the immune system, disease resistance, pollution, and stress has intensified (Ellis 1981; Bly et al. 1997). The pollution response is largely only a specific case of stress-induced immunosuppression (section 7.3). Stress affects wild fishes more than domesticated strains (Woodward and Strange 1987) and fish diseases are more common in polluted environments (Møller 1985). The interactions of parasites with pollutants are complex and sometimes synergistic (Møller 1986). Such interactions have been largely neglected and field studies linking parasites on salmon with river pollution, comparable with Pippy and Hare's (1969) microbiological investigations, are lacking.

Specific effects of pollutants do also occur. The organophosphates dichlorvos and trichlorfon, widely used in sea louse control, have adverse effects on immune function (Dunier and Siwicki 1993). In general, however, these specific effects remain secondary to the overall immunosuppression induced by the pollutant as a stressor. Similar comments would apply to the observed effects of polychlorinated biphenyls (PCBs) on B-cell function in salmonids (Arkoosh et al. 1994).

In some cases pollution may actually improve fish health when the parasite proves more susceptible than the host. *Gyrodactylus salaris* is particularly sensitive to aluminium in acidified water (Soleng et al. 1996) and this may offset harmful effects of aluminium to the host. Overall, parasites are an important factor in the complex interactions between fish, environment, and man, which can result in disease and mortalities.

7.2. Translocation and introductions of parasites

There is a clear link between introductions and disease, although few introduced pathogens are economically or ecologically important (Kennedy 1994). Two barriers naturally restrict the interchange of salmon parasites in the wild, the osmoti–physiological stress of the fresh-salt-freshwater migration and the homing response which returns spawning salmon to their natal river. These have been highly effective in restricting the dispersal of salmon parasites but have however been broken down by man's movements of salmon stocks (Hoffman 1970; Bauer and Hoffman 1975; Hudson and Hill 1993; Holcik 1991). Translocations have been significant at a number of levels. Locally, the movements of fishes between watersheds by anglers have been significant. In Norway, the watershed of west-flowing rivers inhabited by Atlantic race salmon is separated in some areas by only a few kilometres from watersheds of easterly flowing rivers inhabited by Baltic race salmon and there are fears that *G. salaris* could be spread between these rivers by anglers during the course of a day's fishing (Halvorsen and Hartvigsen 1989; Mo 1994). Additionally, farmed rainbow trout infected with *G. salaris* (which is nonpathogenic to this salmonid, see Bakke et al. 1991) in northern Finland (where *G. salaris* is not a problem) occur only a few kilometres from Norwegian rivers where the introduction of this pathogen into natural salmon populations would be disastrous.

A second level at which translocations take place is with escapees from farms. Estimations of escapes from Norwegian salmon farms run into millions and these fishes, which are less faithful to their natal river and enter later than wild salmon (Økland et al. 1991; Heggberget et al. 1993), are thought to have contributed significantly to the spread of furunculosis (Johnsen and Jensen 1994).

The third, potentially most significant risk of pathogen dispersal has been with large-scale movements of salmonids as part of the international trade in these organisms. This began during the 19th century, with large-scale shipments of salmonid juveniles in both directions across the Atlantic and within Europe. The original epidemic spread of furunculosis in both Europe and America at this time and particularly in the British Isles (Lund 1967) is strongly suggestive of an introduced pathogen. Furunculosis was reintroduced into Norway in this way (Egidius 1987). It should be noted that far fewer risks are associated with the transfer of ova (see section 3.2), which are the preferred form of transfer in modern aquaculture.

There is a very real danger that conservation and stock management arguments for exclusion of infectious diseases can lose out to the political and economic case for free world trade in salmonids and salmonid products. The complexity of this issue is perfectly illustrated by *G. salaris.* There is a ban on the export of live salmon from Norway to prevent dissemination. However, the parasite is not pathogenic in Finland, where it predominantly infects rainbow trout. This fish can be moved within the European Community and *G. salaris* has consequently been introduced into the White Sea coast of Russia from Finland (Mo 1994) and has been recorded from rainbow trout in Germany, Spain, Denmark, and Portugal (Malmberg 1993). These latter countries have no trade in salmon or reason to be especially vigilant against *G. salaris* but every reason to oppose attempts to restrict trade in rainbow trout. The introduction of *G. salaris* into Spain is worrying, given the small number of ecologically sensitive Atlantic salmon populations in that country and the presence of *G. salaris* in all German rainbow trout farms (Lux 1990) may have implications for attempts to reintroduce salmon into the Rhine. Despite vigilance, *G. salaris* will almost certainly eventually invade the U.K., probably with rainbow trout imported from a country far distant from Scandinavia. Fortunately, most parasite introductions fail (Kennedy 1994) and spectacular examples like furunculosis and *G. salaris* are rare.

The final concern is the potential for genetic interchange in parasites from previously isolated host populations. This has been clearly demonstrated in the case of plasmid exchange between *Vibrio* and *Aeromonas* species and could have major effects for genetic change in these organisms. Introduced fish may also form a highly susceptible resource for the multiplication of native parasites normally found on other fishes. For example, brown trout are relatively resistant to the monogenean *Discocotyle sagittata*. Introduction of susceptible rainbow trout to the Isle of Man has led to the development of much larger parasite populations among which genetic exchange can occur freely, with the potential to generate more pathogenic strains (Gannicott and Tinsley 1997).

7.3. Selective breeding and disease resistance

Selection of commercial stocks is generally based on high growth rate and late maturation, little attention being paid to

disease resistance. Selective breeding for disease resistance (furunculosis and ISA) has been incorporated into the Norwegian breeding programme for salmon only since the early 1990s (Fjalestad 1994) but has identified a genetic component to resistance to *Vibrio* species, BKD and IPN (T. Refsti, Institute of Aquaculture Research, Sunndalsøra, personal communication). Genetic variation in susceptibility to *Gyrodactylus* is also well known (Madhavi and Anderson 1985; Bakke et al. 1990; Leberg and Vriejenhoek 1994; personal observations). The evidence that hosts are heterogeneous in their capacity to develop and express protective responses and that this variation is genetically determined and therefore subject to evolutionary change has important implications concerning epidemiology and as a basis for the development of predictive models for practical disease control (Wakelin 1994). Because fishes with an inappropriate spectrum of disease responses will be rapidly eliminated from the population (see section 2.1), natural populations are optimally selected for resistance to the particular pathogens encountered in their environment. This results in particular allele frequencies for major histocompatibility complex (MHC) polymorphisms, conferring resistance to specific pathogens (Kronenburg et al. 1994). Reintroduction of parr into rivers usually involves fishes, which, while they may be of the same overall genetic background as their parents, have not been selected for disease resistance and their MHC alleles are not normally tested (Dixon et al. 1995). These introductions are usually to replace fishes lost to *G. salaris* or to rotenone treatment (Mo 1994) and may significantly alter the spectrum of MHC alleles present in the natural population. If no account is taken of MHC diversity when restocking, then at best the onset of evolved disease resistance in the managed population could be delayed, while at worst the disease problem could be greatly exacerbated. Similar considerations must apply to escapees and if the scale of escapes is as reported (Johnsen and Jensen 1994) then dilution of resistant MHC genotypes (especially if genetic drift in small breeding populations occurs) may be a major problem. Probes for salmon MHC genes are available (Grimholdt et al. 1993) and it would be of great interest to compare MHC diversity in salmon populations exposed to different patterns of disease, restocking, and interbreeding with escapees.

7.4. Management of wild populations

Stresses due to handling or transport elevate blood cortisol (Pickering 1987), predisposing fishes to disease (section 7.3). Restocking may also influence the establishment of the dominance hierarchy (Kalleberg 1958; Symons 1971), which could in turn modify disease patterns. Restocking programmes may therefore end as experiments in pathogen culture. The addition of smolts, rather than parr, has the additional disadvantage that exposure to pathogens at the start of the life cycle does not occur, preventing the development of immunity, which may be needed later in life.

7.5. Salmon farming and disease patterns in wild populations

Salmonid farms can have profound effects on the abundance of parasite dispersal stages around them. Poynton (1986) found that *Hexamita* were much more abundant downstream of farm outflows than upstream, with associated risks for the wild and managed salmonids of the river. Marine pens in fjords have the greatest potential for this interchange of parasites and pathogens with wild fishes, because they span the migration routes of fishes into and out of the freshwater environment. The complexities surrounding this problem are highlighted by Costelloe et al. (1998), mapping the distribution of sea louse larvae within an estuary utilized by wild salmonids. In this case, high larval densities downstream of the salmon farm could be clearly demonstrated, but other high densities of larvae also existed within the estuary, up to 10 km from the farm. These “hot spots” of larval distribution were probably not derived from farmed fishes but from heavily infected salmonids returning to the river to breed (Costelloe et al. 1998). The relative importance for wild fish of larvae from farmed fish will clearly depend greatly upon the particular hydrographic regime of the river concerned.

Marine pens also have other deleterious effects favoring disease outbreaks. The buildup of waste material (Ackefors and Enell 1994) can lead to sediment enrichment (Johnsen et al.1993) and anoxia (Black et al. 1996), possibly leading to changes in the underlying invertebrate communities. In particular, sediment enrichment and anoxia promote colonization by tubificid worms (Gray 1979; Brinkhurst 1982), which are now known to be intermediate hosts for myxozoans. The high density of farmed salmon in sea cages greatly increases the risk of traumatic skin injury and the spread of pathogens such as fish lice, *Trichodina* sp., *Ichthyobodo*, and *Spironucleus,* which may possibly disperse to passing wild fishes (Sterud et al. 1998). The exchange of plasmids by bacteria in the environment under marine pens has been mentioned previously (section 4.2.1) but should now become less of a problem as antibiotic use is reduced. In all cases, the concern should mainly be for the effect of pathogens from marine pens on smolts passing through the environment, since these fish live for several years and cannot be tracked adequately in the sea.

8. Important future pathogens

The epidemiology of salmonid diseases is still in its infancy. New pathogens are still being discovered and we speculate here on the groups most likely to cause problems in the future and those which may be particularly important for wild and managed salmon stocks.

Gyrodactylus salaris remains a significant concern for eastern Atlantic salmon stocks, particularly the possibility that it may be introduced to the U.K., Ireland, or Iceland. Population models (Clers 1993) suggest an effect on salmon population dynamics, a prediction borne out by Norwegian fishery statistics (Johnsen and Jensen 1988), although other parasites, particularly furunculosis (Johnsen and Jensen 1994), may complicate the Norwegian situation. Much remains enigmatic about *G. salaris*, in particular its patchy distribution as a pathogen (Johnsen and Jensen 1992), interspersed with rivers in which it is less pathogenic (Appleby 1996; Jansen 1994). The discovery of pathogenic populations in the Swedish west coast, where there is no history of introduction (Malmberg and Malmberg 1993), susceptible Baltic stocks (Bakke et al. unpublished data), and relatively resistant Norwegian stocks (Jansen and Bakke 1993*a*;

Appleby 1996) has confused the original suggestion that *G. salaris* was introduced to Norway recently (cf. Halvorsen and Hartvigsen 1989). Careful modeling studies are needed to assess the epidemiological constraints on pathogenicity in *G. salaris*, preferably integrating the considerable data on gyrodactylid epidemiology (Scott and Anderson 1984; Jansen 1994; Appleby 1996) with a sophisticated model of salmon population behavior.

A further group of pathogens that should be considered are the myxozoans. These parasites are easily disseminated before their pathogenicity has been noticed and eutrophication under salmon pens (Johnsen et al. 1993; Black et al. 1996) could favor their tubificid intermediate hosts (Gray 1979; Brinkhurst 1982). The emergence of PKX, and more recently of the unidentified myxozoan reported by Frasca et al. (1998), indicates the potential of myxozoans as disease organisms and with so little known of the life cycles of marine species their effects upon the survival and fitness of marine phase salmon should be investigated.

Finally we would stress again the position of marine rearing pens as pathogen culture facilities at the crossroads for migrating salmonids moving between fresh and saltwater. We know nothing of the epidemiology of disease of marine salmon, beyond the fact that highly pathogenic organisms such as ISA are unlikely to have a wide distribution in nature because they would kill salmon before they had dispersed. The possibility of organisms such as this colonizing smolts on migration and then having a significant impact on marine salmon stocks should be treated very seriously.

9. Conclusions

Preparing this review has made it very clear that the study of disease in wild salmon populations is in its infancy. In particular, while veterinary viewpoints are well represented in the literature, an ecological focus on salmonid disease is almost entirely lacking. Only two papers, both theoretical, deal with the demographic consequences of disease for salmonids: that of Clers (1993), exploring the potential impact of *G. salaris* on wild salmon, and the work of Levy and Wood (1992), who inferred the existence of a pathogen within pacific salmon populations from the observed population cycles. Detailed field observations, comparable to those of Costelloe and coworkers (Costelloe et al. 1996, 1998) on the dispersal of infective sea lice larvae within and between host metapopulations, are almost entirely lacking for the majority of pathogens, including the important *Gyrodactylus salaris*. Management decisions, for example restocking after depletion of a natural population by *G. salaris,* are not being informed by consideration of their likely epidemiological consequences, although an adequate theoretical framework for this has been published (Anderson 1982). There is a need for integration of general epidemiological theory (Anderson 1982; Grenfell and Dobson 1995; Swinton et al. 1998) into the sphere of salmonid biology and management. At the same time, much more research into the diseases of wild salmonid populations, not only during conspicuous and costly epidemics, is desirable. We are particularly lacking in data on the role of disease in early life cycle stages (fry and parr) and on events during the marine phase and positive efforts must be made to target these life cycle stages rather than the well-studied and demographically less important adult returners. Apart from field studies of the role of disease in natural salmon populations, there should also be attempts to integrate the findings of such studies into quantitative models of salmon population behavior (e.g., that of Korman et al. 1994) to gain an insight into the possible role of pathogens and parasites in controlling salmonid abundance and growth. Given the very large number of salmon pathogens and parasites known and given that spectacular epidemic diseases (e.g., furunculosis) occur, it is almost inconceivable that endemic disease does not play a part in salmon ecology. Our success in managing, conserving, and ultimately reintroducing Atlantic salmon throughout its former range will ultimately depend in large part on our success in understanding these disease processes.

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ORIGINAL ARTICLE

Temporal change in genetic integrity suggests loss of local adaptation in a wild Atlantic salmon (*Salmo salar*) population following introgression by farmed escapees

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In some wild Atlantic salmon populations, rapid declines in numbers of wild returning adults has been associated with an increase in the prevalence of farmed salmon. Studies of phenotypic variation have shown that interbreeding between farmed and wild salmon may lead to loss of local adaptation. Yet, few studies have attempted to assess the impact of interbreeding at the genome level, especially among North American populations. Here, we document temporal changes in the genetic makeup of the severely threatened Magaguadavic River salmon population (Bay of Fundy, Canada), a population that might have been impacted by interbreeding with farmed salmon for nearly 20 years. Wild and farmed individuals caught entering the river from 1980 to 2005 were genotyped at 112 single-nucleotide polymorphisms (SNPs), and/or eight microsatellite loci, to scan for potential shifts in adaptive genetic variation. No significant temporal change in microsatellite-based estimates of allele richness or gene diversity was detected in the wild population, despite its precipitous decline in numbers over the last two decades. This might reflect the effect of introgression from farmed salmon, which was corroborated by temporal change in linkage-disequilibrium. Moreover, SNP genome scans identified a temporal decrease in candidate loci potentially under directional selection. Of particular interest was a SNP previously shown to be strongly associated with an important quantitative trait locus for parr mark number, which retained its genetic distinctiveness between farmed and wild fish longer than other outliers. Overall, these results indicate that farmed escapees have introgressed with wild Magaguadavic salmon resulting in significant alteration of the genetic integrity of the native population, including possible loss of adaptation to wild conditions.
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Introduction

Farmed fish are reared in environments where selective pressures are directed toward improving commercially important traits, namely rapid growth and older age at maturity (Theodorou and Couvet, 2004). Consequently, farmed salmon are known to exhibit pronounced differences with wild counterparts at many phenotypic traits, such as growth rate, aggressiveness, predator avoidance behavior, acid tolerance (Jonsson and Jonsson 2006; Fraser *et al.*, 2008), which are likely associated with reduced reproductive success in the wild. Moreover, relaxed selection resulting from absence of predators, constant supply of food and medical treatment, as well as increased genetic drift caused by small effective population sizes may favor the accumulation of deleterious alleles. Furthermore, genetic changes unrelated to adaptation to captivity are bound to occur as a result of founder effects in the farmed populations (Roberge *et al.*, 2006). Consequently, change in the genetic composition of captive populations has been observed in many studies on farmed Atlantic salmon of northern Europe (Skaala *et al.*, 2004). Nevertheless, farmed salmon reproduce and hybridize with wild conspecifics, which raises major concerns for conservation programs aimed at protecting the genetic integrity of already diminished populations (Hutchings and Fraser, 2008).

Indeed, the impacts of farmed fish escaping and mixing with wild populations represent a serious threat to natural populations (Fleming *et al.*, 2000; McGinnity *et al.*, 2003; Theodorou and Couvet, 2004). Potential alteration of the genetic composition of the wild population impacted by escapees may have significant consequences in terms of reduction of potential to respond to changing environments. Moreover, studies of phenotypic variation have shown that interbreeding between farmed and wild salmon may lead to a reduction in fitness and local adaptation, ultimately

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contributing to the possible extinction of wild populations (McGinnity *et al*., 2003; Fraser *et al*., 2008). Introgressive hybridization between farm and wild salmon also lead to misregulation of gene expression in the latter (Roberge *et al*., 2008; Normandeau *et al*., 2009). Yet, despite the potential genetic consequences arising from the introduction of farmed salmon, few studies have attempted to assess the impact of gene flow from farmed Atlantic salmon to wild Atlantic salmon populations at the genetic level. Such studies require long-term monitoring of impacted populations, and are rare, especially in North America.

Many wild Atlantic salmon (*Salmo salar*) populations of the Bay of Fundy region (Canada) have collapsed since the 1980s and are now threatened with extirpation or have already been extirpated (DFO, 2009). Causes for this rapid decline may include changes in oceanographic conditions, freshwater habitat degradation as well as impacts from the Bay of Fundy's commercial Atlantic salmon aquaculture industry (Carr *et al*., 2004). The Magaguadavic River, located in the Bay of Fundy region (Canada) has been monitored by local authorities since the establishment of the aquaculture industry over 30 years ago. The production of farmed salmon increased dramatically over the last 20 years, resulting in large numbers of fish escaping into the wild. Indeed, in the mid-1990s, farmed salmon entering the Magaguadavic River far exceeded the number of wild salmon (Carr *et al*., 1997). Farmed females have also been shown to successfully spawn in the river. In 1993 alone, up to 55% of the redds were at least partially of farmed origin, despite a greater number of wild salmon entering the river compared with farmed salmon (Carr *et al*., 1997). At present, wild salmon represent a small proportion of salmon entering the river, whereas farmed fish are still abundant in the system. Clearly, the Magaguadavic River wild Atlantic salmon population may be facing two major challenges in regard to protecting its genetic integrity; a declining census population size and possible interbreeding with farmed salmon.

The main goal of this study was to document temporal change in the genetic composition of the Magaguadavic River wild Atlantic salmon population following the rapid increase in the number of farmed salmon entering the river from 1980 to the early 2000s. We used both microsatellite and single-nucleotide polymorphism (SNP) markers (comprising both putatively adaptive and neutral markers) to detect the possible effects of interbreeding between wild and farmed salmon at the genetic level. Under the assumption of admixture between the two groups, we first expected neutral markers to exhibit a lower diversity in the domesticated strain and consequently reduced diversity over time in wild introgressed individuals. As the domestic strain originated from the nearby Saint John River (approximately 60 km from the Magaguadavic River), we also expected the genetic markers to naturally reveal weak divergence at the majority of markers between farm and wild fish. However, we expected strong divergence at some markers as a result of the radically different selective pressures in captive and wild environments indicated by markers potentially affected (either directly or indirectly through linkage) by divergent or directional selection. Finally, since evidence of farmed salmon spawning in this system has been shown earlier, if farm salmon have indeed been interbreeding with wild salmon through the 1990s, we expected genetic divergence between these two groups of salmon to be reduced over time as a result of introgression.

Materials and methods

Sample collection

Samples were collected from wild and farmed salmon captured in the Magaguadavic River between 1980 and 2005. Wild adults were captured at the head of tide fishway trap after ascending the river (Figure 1). From mid-April to June 2000 smolts were either trapped as they descended the river using a fykenet in a small stream serving as the downstream fish bypass outlet for the head of tide hydroelectric dam, or they were collected 24 km upstream of the dam using a rotary screw trap (for example, Solutions Inc., Corvallis, OR, USA). Adult salmon used in this study were identified as wild, except in 1992 and 2000 when both wild and farmed individuals were sampled. Wild and farmed smolts were sampled in 2000. Adults and smolts were classified as wild or farmed origin using morphological features such as fin and gill cover erosion (associated with the farming environment), and by examining circuli patterns on scales as previously described by Carr *et al*. (1997) and Carr and Whoriskey (2006). Blood was collected and frozen for samples from 1992 and either air-dried scale

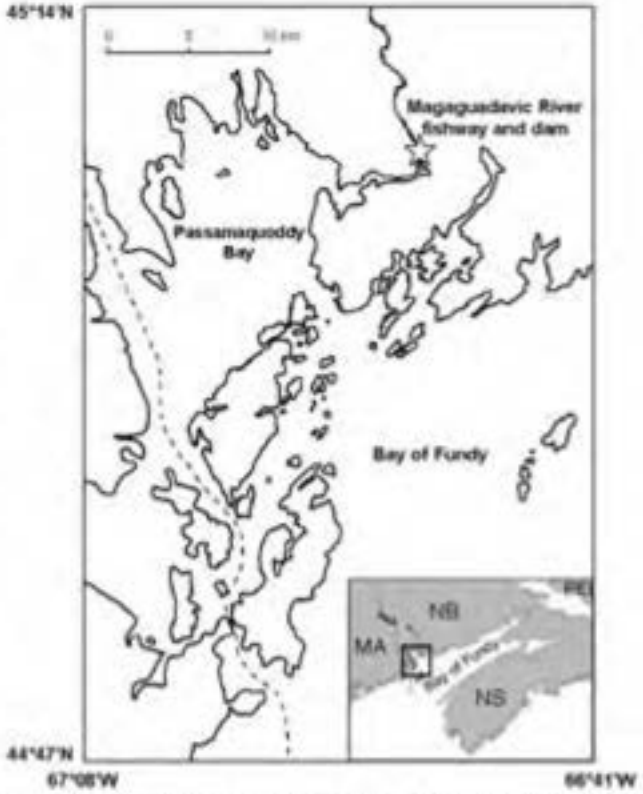

Figure 1 Map showing the Magaguadavic River mouth and the fishway located close to the Passamaquoddy Bay where most of New Brunswick commercial salmon sea cage sites are in the Bay of Fundy. Modified with permission from Carr *et al*. (1997) and Carr *et al*. (2004). (NB, New Brunswick; NS, Nova Scotia; PEI, Prince Edward Island; MA, Maine).

and/or fin clips conserved in 95% ethanol were collected from individuals captured in 1980, and from 1996 to 2005. In summary, the sample collections (also called populations hereafter) used were the following: (1) WILD-1980, (2) WILD-1992, (3) FARMED-1992, (4) WILD-1996, (5) WILD-1998_99, (6) WILD-2000_adults, (7) WILD-2000_smolts, (8) FARMED-2000_adults, (9) FARMED-2000_smolts and (10) WILD-2002+.

DNA genotyping

The microsatellite and SNP analyses carried out here involved two somewhat overlapping but different groups of samples, primarily because of the unsuitability of older samples for SNP analyses. Organization of samples for analyses is summarized in Table 1. A group composed exclusively of wild adults caught in 1980 (WILD-1980) ($n=31$), 1992 (WILD-1992) ($n=60$) and 2000 (WILD-2000_adults) ($n=34$), farmed adults from 2000 (FARMED-2000_adults) ($n=53$) as well as wild and farmed smolts sampled in 2000 (WILD-2000_smolts, FARMED-2000_smolts) ($n=48$ and $n=39$, respectively) was genotyped for microsatellites only (referred to as group A). A second group was genotyped for SNPs (referred to as group B). This group was organized into different clusters based on aquaculture history in the Bay of Fundy as follows: farm escapees from early-aquaculture 1992 (FARMED-1992) ($n=20$), wild adults from the early-aquaculture period in 1992 (WILD-1992) ($n=18$), wild adults from the mid-aquaculture period in 1995–1996 (WILD-1996) ($n=20$), wild adults from the late-aquaculture period in 1998–1999 (WILD-1998_99) ($n=22$) and wild adults from the very late-aquaculture period in 2002+ (WILD-2002+) ($n=15$). Note that all individuals analyzed for SNP variation were also genotyped at the same microsatellite loci as were the samples in group A and the group B WILD-1992 sample collection was a subset of the samples contained in the WILD-1992 collection.

DNA was extracted using Qiagen 96-well DNeasy plates, following the manufacturer's specifications (Qiagen, Valencia, CA, USA). We genotyped individuals at eight microsatellite loci. PCR amplifications were carried out in 10 µl volumes, containing between 1 and 100 ng of template DNA, 2 mM each dNTP, 0.5 µM labelled and unlabelled primers, 50 mM KCl, 0.5 units of Taq DNA polymerase supplied by MBI Fermentas (Burlington, ON, Canada) and 2.0 mM $MgCl_2$. Thermal cycling conditions were as follows: (94 °C for 3 min) × 1, (94 °C for 1 min, 58 °C for 30 s and 72 °C for 30 s) × 5 and (90 °C for 30 s, 58 °C for 30 s and 72 °C for 30 s) × 30, followed by a 15-min extension step at 72 °C. Primer sequences for loci *Ssa* 197 and *Ssa* 202 are given in O'Reilly *et al.* (1996), *SSsp* 2201, *SSsp* 2210, *SSsp* 2215, *SSsp* 2216, *SSsp* 1G7 and *SSsp* 1605 are given in Paterson *et al.* (2004). PCR products were combined, and salt, unincorporated dNTPs, unincorporated labelled and non-labelled primers were removed using Qiagen's 96 MinElute 96 UF PCR purification kits, following the manufacturer's procedures. Fragments were size fractionated and detected using an Applied Biosystems 3130 XL (Applied Biosystems, Carlsbad, CA, USA). One sample from each strip of eight tubes was duplicated to identify sample placement errors, strip inversions and plate inversions. In all, 2 of 10 laboratory samples were analyzed in each group of 96 samples both to identify individual batches, and to ensure allele calls were standardized across batches of samples analyzed over time.

SNP genotyping was done using the iPlex Gold assay on the MassARRAY platform (Sequenom, San Diego, CA) according to the manufacturer's instructions. In total, a panel of 388 Atlantic salmon SNPs were genotyped in 16 different multiplexes, with multiplexing levels ranging from 12 to 36. The 13 multiplexes with the highest number of SNPs from Moen *et al.* (2008) as well as the 3 multiplexes with the highest number of SNPs from Lorenz *et al.* (2010) were selected for genotyping. PCR and extension primers were designed with the software MassARRAY AssayDesign v3.1 (Sequenom). Allele

Table 1 Summary of sample collection organization, number of successfully genotyped markers and principal genetic diversity parameters per population: allelic richness ($\hat{A}$) for microsatellites (excluding *SSsp* 2201), Nei's gene diversity, observed heterozygosity (H_O), value of heterozygote deficit (F_{IS}), average SNP expected (SNPs H_E) and observed (SNPs H_O) heterozygosities

Group	*Population*	*Years sampled*	N *samples*	N *microsatellites*	N *SNPs*	$\hat{A}$	*Nei's gene diversity*	H_O	F_{IS}	*SNPs* H_E	*SNPs* H_O
A	WILD 1980	1980	31	8		9.019	0.862	0.791	0.071		
	WILD 1992	1992	60	8		8.865	0.864	0.842	0.016		
	WILD 2000 adults	2000	34	8		8.766	0.864	0.817	0.054		
	WILD 2000 smolts	2000	48	8		8.733	0.862	0.847	0.024		
	FARM 2000 adults	2000	53	8		9.130	0.867	0.827	0.049		
	FARM 2000 smolts	2000	39	8		8.960	0.865	0.836	0.038		
B	WILD 1992*	1992	18	8	112	9.183	0.871	0.872	0.002	0.246	0.263
	WILD 1996	1996	20	8	112	8.609	0.878	0.780	0.114	0.242	0.269
	WILD 1998 1999	1998	2	8							
		1999	20		112	8.764	0.858	0.820	0.038	0.252	0.247
	WILD 2002+	2002	7	8	112	8.677	0.831	0.841	0.011	0.249	0.257
		2003	4								
		2004	2								
		2005	2								
	FARM 1992	1992	20	8	112	8.847	0.878	0.874	0.009	0.279	0.247

Abbreviation: SNP, single nucleotide polymorphism.
*WILD 1992 of group B is a subsample within WILD 1992 of group A.

separations were performed using the Sequenom MassARRAY Analyzer. Genotypes were assigned in real time on the basis of the mass peaks (Tang *et al.*, 1999) using the MassARRAY SpectroTYPER RT v3.4 software (Sequenom). Manual inspection of all the results was carried out using the MassARRAY TyperAnalyzer v4.0 software (Sequenom). Individuals were genotyped only once for each multiplex since previous experiences with the SNPs revealed no discrepancies between replicate analyses (CIGENE, unpublished information). Moreover, the genotyping was followed by highly stringent quality control criteria to keep only high quality markers for further analyses (see results).

Genetic variation and differentiation

For comparative purposes, we considered separate temporal replicates as individual populations in subsequent analyses. For microsatellite analysis, populations of group B were considered with group A with the exception of WILD-1992. Neutral microsatellite genetic variation within each population was quantified using standard descriptive statistics for each locus individually and globally: observed and expected heterozygosities (H_O and H_E) as well as Weir and Cockerham (1984) F_{IS} values were estimated using GENETIX (Belkhir *et al.*, 2001), and the number of observed alleles as well as Nei's (1977) estimator of gene diversity, using FSTAT 2.9.3 (Goudet *et al.*, 2002). We calculated allelic richness ($\hat{A}$), which is the number of different alleles corrected for variation in sample size, using the rarefaction method employed by FSTAT 2.9.3 to the smallest sample ($n = 11$). Locus *SSsp* 2201 was excluded from the calculation of allelic richness to increase the power of detecting differences in $\hat{A}$ (Leberg, 2002) between populations because the sample size would have become too small had it included locus *SSsp* 2201 ($n = 3$). The distributions of within population gene diversity did not all conform to normality after Shapiro–Wilk tests (four tests with $P < 0.01$). On the other hand, allelic richness distribution all conformed to normality after Shapiro–Wilk tests (all $P \gg 0.01$) and homogeneity of variance among populations was verified using a Bartlett test ($K^2 = 0.759$, df = 9, $P = 0.999$). Therefore, respectively, a Kruskal–Wallis analysis of variance (non-parametric) and an analysis of variance (parametric) were used to determine if gene diversity and allelic richness changed among samples. Conformity of individual loci to Hardy–Weinberg equilibrium (HWE) expectations was also tested using FSTAT 2.9.3 and Fisher's combined probabilities across loci for each population was used to determine a global P-value for the set of HWE tests (Mosteller and Fisher, 1948). Genic differentiation (G; Guo and Thompson, 1992) at individual loci between all pairs of populations and significance values over all loci were obtained using Fisher's method (Ryman and Jorde, 2001) as implemented using GENEPOP 3.4 (Raymond and Rousset, 1995). The sequential Bonferroni correction for multiple tests was applied to maintain the table-wide significance level at $\alpha = 0.05$ (Rice, 1989) while testing for HWE and genic differentiation. After removal of putatively non-neutral loci identified in FDIST2 analyses (Beaumont and Nichols, 1996) (see below), microsatellites and SNP markers were used separately for calculations of pairwise genetic differentiation between populations using the F_{ST} of Weir and Cockerham (1984) (θ) as calculated with GENETIX after 1000 permutations for significance.

Linkage disequilibrium

We do not present any admixture analysis, for instance based on clustering methods (for example, Structure, Pritchard *et al.*, 2000). Although such attempts were made (results not shown), the low level of differentiation observed for the vast majority of markers used (see Results section) resulted in insufficient power to apply such methods adequately (Vaha and Primmer, 2006). On the other hand, as natural outbred populations are expected to be in near genome-wide linkage equilibrium relative to a situation of interbreeding with an invasive population, which is expected to generate linkage disequilibrium (LD) in a recently introgressed population (Gaut and Long, 2003; Allendorf and Luikart, 2007), we used ARLEQUIN 3.5 (Excoffier and Lischer, 2010) to test for pair-wise LD within populations genotyped for SNPs. More specifically, we tested for the presence of significant associations between pairs of loci, based on a likelihood ratio test, wherein the likelihood of observing the sample evaluated under the hypothesis of no association between loci (linkage equilibrium) is compared with the likelihood of observing the sample when association is allowed (Excoffier and Lischer, 2010). When the likelihood of an association between two loci is significantly greater than no association, they are considered to be linked or under LD. The distributions of the numbers of linked loci within populations deviated significantly from normality (Shapiro–Wilk tests, all $P < 0.01$). Therefore, the median number of loci under LD per locus within each population was compared among populations with a Kruskal–Wallis analysis of variance to determine if LD changed across temporal samples of wild Magaguadavic Atlantic salmon. When significant differences among groups were observed, Wilcoxon signed-rank tests were used to compare median values between populations and determine which samples differed. Furthermore, we used MULTILOCUS (Agapow and Burt, 2001) to calculate r_d, the multilocus LD within population, as a measure of genome-wide LD over all SNP markers.

Genome scans

Numerous methods to detect loci potentially under the effects of natural selection have been developed over the last decade, most based on principles reported by Lewontin and Krakauer (1973). Some of these methods proposed to compare levels of genetic diversity and differentiation between populations as loci under directional selection should show larger differences, and loci under balancing selection should present less divergence between populations than neutrally evolving loci. FDIST, (Beaumont and Nichols, 1996), was one of the first programs widely available to test for departures from neutrality, and the analyses carried out have become widely known as 'genome scans'. In the latest version of FDIST2, simulations under a finite island-model are performed to obtain a null distribution of F_{ST} values across loci as a function of heterozygosity, and loci with an unusually high or low F_{ST} value ('outliers') are generally considered to be potentially under the effect of natural selection (either directly or indirectly through

linkage). Here, we scanned genetic variation at SNP markers by comparing aquaculture samples to all wild temporal samples to detect changes in loci potentially under divergent selection. We used a confidence interval of 0.95 for the expected null differentiation meaning that loci over this interval had to be in the upper 0.025 tail of the distribution to be considered as potentially under directional or divergent selection or in the lower 0.025 tail of the distribution to be considered as potentially under balancing selection.

Results

Genetic variation

Over all samples, all microsatellite loci were highly polymorphic, with the total number of alleles per locus ranging from 11 to 36 (mean = 24.38), and observed heterozygosity per locus across populations ranging from 0.5 to 1.0 (mean = 0.831) (Supplementary Table S1). Mean gene diversity per locus per population ranged from 0.831 to 0.878 and allelic richness from 1 to 20 alleles per locus per population. Neither gene diversity nor allelic richness were significantly different among samples (Kruskal–Wallis $K^2 = 2.759$, df = 9 and $P = 0.973$; analysis of variance $F = 0.019$, df = 1 and $P = 0.891$, respectively). The null hypothesis of HWE was not rejected for any locus and for any population after correcting for multiple tests ($\alpha = 0.000625$, $k = 80$). Fisher's combined probabilities across loci was not significant for seven out of eight populations ($P > 0.05$) with only FARM-2000_adults presenting significant departure from HWE ($P = 0.001$) and with small but positive F_{IS} indicative of a slight heterozygote deficiency (Supplementary Table S1).

Out of the 388 SNP (Supplementary Table S2) assayed, 348 markers yielded at least one genotype but only 267 markers were polymorphic. Consequently, 81 were discarded for further analyses. Markers with call rate inferior to 95% (54) and minor allele frequencies <0.05 (101) were also excluded to ensure only high-quality informative markers were utilized. Thus, the final group of SNPs used in all subsequent analyses numbered 112, and average expected and observed heterozygosities are given in Table 1.

Differentiation

Pair-wise genic tests of population differentiation at individual microsatellite loci yielded only 26 significant comparisons out of 360 after correction for multiple comparisons ($\alpha = 0.000139$, $k = 360$). This translated into a nonsignificant ($P = 0.999$) global F_{ST} value of 0.00096, reflecting an overall very weak level of differentiation among populations. Furthermore, microsatellite pair-wise F_{ST} values (θ) were significant in only 20 out of 45 comparisons (Table 2) and ranged from 0.005 between WILD-2000_smolts and FARM-2000_adults to 0.019 between WILD-1980 and FARM-2000_adults and between WILD-1996 and WILD-2002+ when significant. The WILD-1980 population was associated with the greatest number of significant pair-wise F_{ST} estimates (eight of nine), and the largest F_{ST} values, ranging from 0.009 to 0.018 when compared with other wild population temporal replicates, and from 0.011 to 0.019 when compared with aquaculture sample collections. The sample collection WILD-2002+ was associated with the next largest F_{ST} values, ranging from 0.010 to 0.019 when compared against other wild temporal replicates, of which four of five were significant (WILD-1992, WILD-1996, WILD-2000_adults and WILD-2000_smolts). The lowest significant F_{ST} values based on microsatellite data were observed for pair-wise comparisons between wild replicates post-1980 and aquaculture samples (range between 0.005 and 0.009). For SNP markers, pair-wise F_{ST} values varied between 0.000 (in 5/10 comparisons) and 0.006 (between WILD-1996 and FARM-1992) (results not shown). None of these pair-wise F_{ST} values were significant after removal of six SNPs identified by FDIST2 as potentially under divergent selection in a global test over all samples at a significant level of 0.025 (results not shown).

Linkage disequilibrium

SNP markers yielded variable patterns of LD in wild temporal replicates and the 1992 farm salmon samples (Figure 2). The Kruskal–Wallis test of equality of medians among populations was significant ($K^2 = 139.470$, df = 4 and $P << 0.001$). Pair-wise comparisons showed that FARM-1992 and WILD-1996 as well as WILD-1998_99 and WILD-2002+ exhibited similar numbers of linked loci per locus (W = 6686 and 6753 with $P = 0.394$ and 0.321, respectively) while all eight other comparisons were significantly different after corrections for multiple comparisons ($P << 0.001$). Multilocus LD (r_d) values within each population are given in Figure 2 and corroborate the detailed locus-by-locus LD analyzes within populations in the sense that all post-1992 wild temporal samples had higher r_d values than the 1992

Table 2 Pair wise measures of genetic differentiation based on allelic identity (θ_{ST}) at microsatellites

	WILD 1992	*WILD 1996*	*WILD 1998 1999*	*WILD 2000 adults*	*WILD 2000 smolts*	*WILD 2002+*	*FARM 1992*	*FARM 2000 adults*	*FARM 2000 smolts*
WILD 1980	0.009*	0.018*	0.015*	0.011*	0.012*	0.008	0.014*	0.019*	0.011*
WILD 1992		0.011*	0.002	0.004	0.001	0.012*	0.003	0.008*	0.008*
WILD 1996			0.000	0.005	0.004	0.019*	0.003	0.003	0.009*
WILD 1998 1999				0.000	0.000	0.011	0.000	0.000	0.005
WILD 2000 adults					0.000	0.012*	0.000	0.002	0.003
WILD 2000 smolts						0.010*	0.003	0.005*	0.008*
WILD 2002+							0.007	0.008*	0.010
FARM 1992								0.000	0.001
FARM 2000 adults									0.006*

*Significant comparison ($P < 0.05$).

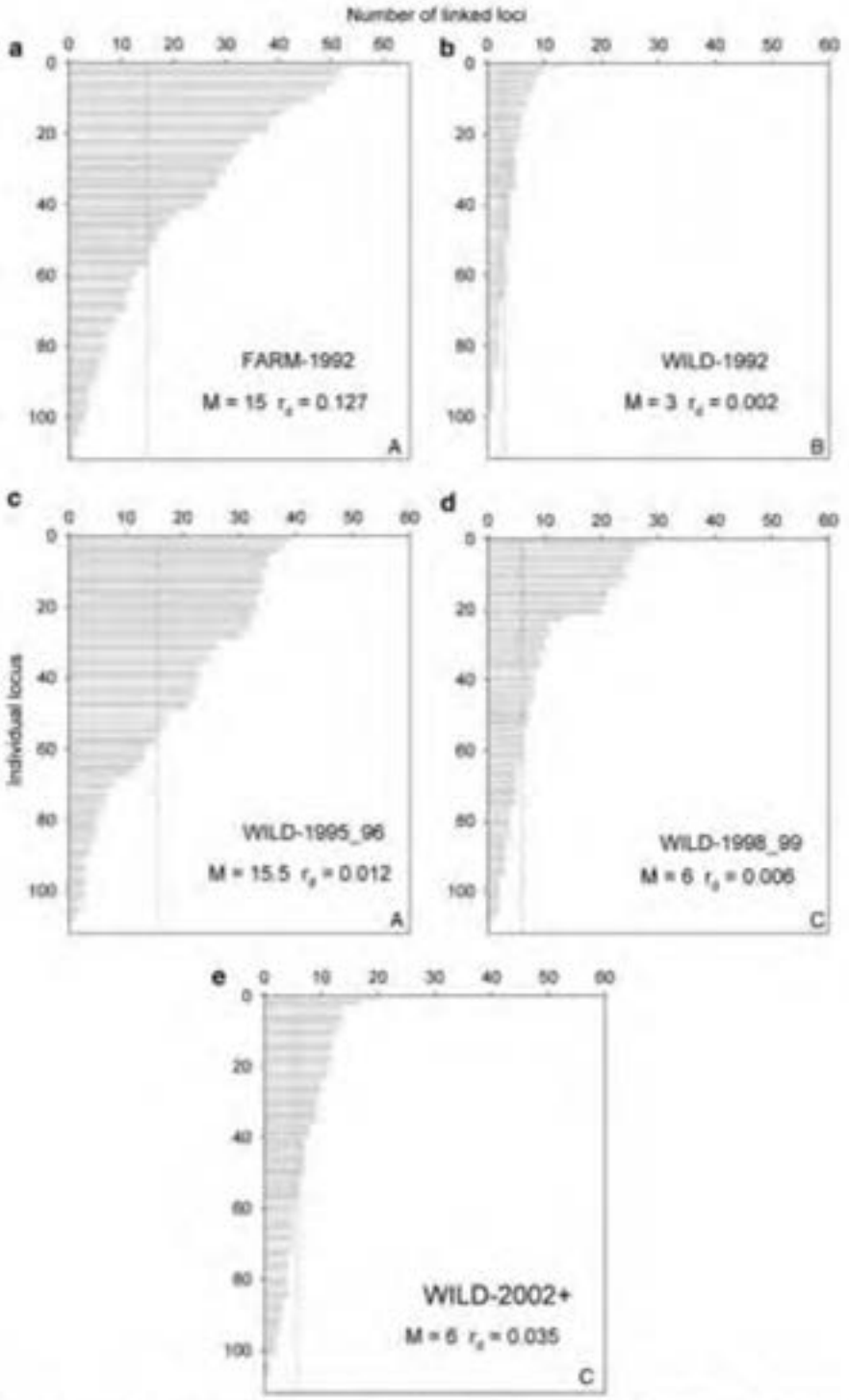

Figure 2 Number of SNP linked loci per individual SNP locus within population for: (a) FARM 1992, (b) WILD 1992, (c) WILD 1996 (d) WILD 1998 99 and (e) WILD 2002+. Loci are not arranged in the same order among populations but by decreasing order according to the *x* axis. Median number of linked loci per locus per population (*M*) and multilocus r_d per population are indicated on each panel. Within population *M* are not different for populations sharing the same capital letter in the bottom right corner of panels after Wilcoxon tests (see Results section).

wild sample, and the aquaculture sample (FARM-1992) presented the highest multilocus LD.

FDIST outlier detection test

Genome scan plots of marker F_{ST} as a function of heterozygosity comparing each of the four wild samples with the farmed salmon (FARM-1992) are shown in Figure 3, with outliers potentially under divergent selection over the 95% confidence level. Single locus F_{ST} values for pair-wise comparisons were approximately 0.01, varying slightly from locus to locus (between 0.006 for WILD-1998_99 and 0.011 for WILD-1996) whereas outlier F_{ST} values were all above 0.10.

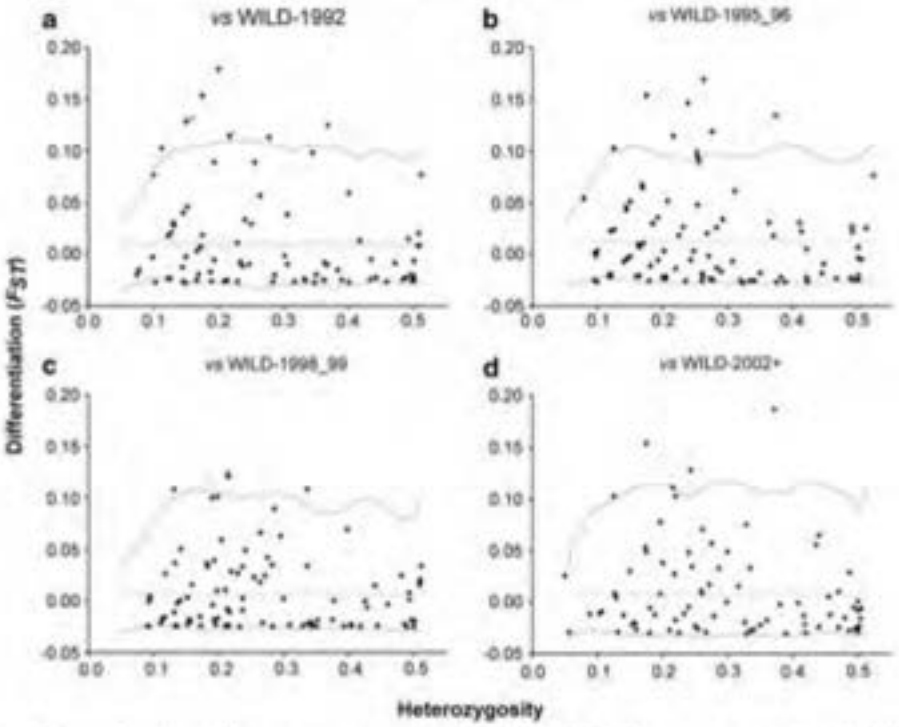

Figure 3 Differentiation (F_{ST}) as a function of heterozygosity as calculated by FDIST2 when comparing FARM 1992 with: (a) WILD 1992, (b) WILD 1996 (c) WILD 1998 99 and (d) WILD 2002+. On each panel, solid line represent upper and lower 95% confidence level and dotted line indicates the average F_{ST} across loci. Asterisk (*) on outliers indicate two values represented by the same dot on the graphic (double asterisks (**) three values). Only SNP markers were used.

Overall, we found a twofold reduction in the number of observed markers potentially under the effect of divergent selection after 1996, with the observed number of outliers, in chronological sequence, of 10 (WILD-1992), 9 (WILD-1996), 4 (WILD-1998_99) and 5 (WILD-2002+). Table 3 presents a summary of these markers, including their names, known linkage group used from Boulding *et al.* (2008) and Lorenz *et al.* (2010), as well as gene annotation when available. Three outliers (Contig14899_0107, Contig15610_550 and Contig16686_0431) identified in the genome scan involving WILD-1992, were also identified in comparisons with WILD-1995_96 and/or WILD-1998_99. Of these markers, Contig14899_0107 presented a nearly significant reduction in F_{ST} through time ($r^2 = 0.88$, $P = 0.062$), and was no longer an outlier in the WILD-2002+ genome scan (Figure 4). Loci showing less differentiation than that represented by the lower 95% confidence interval would normally be considered as potentially under balancing selection, but as this confidence level always fell below the $F_{ST} = 0$, we rejected the hypothesis of these markers being under the effect of balancing selection.

Discussion

This study is the first to examine the genetic impacts of introgression from farmed Atlantic salmon into a wild population using a wide panel of SNP, in addition to known neutral microsatellite markers. The long-term monitoring of the Magaguadavic River of the Bay of Fundy in Eastern Canada permitted a comparison of the genetic composition of wild samples before and after the establishment and growth of the salmon farming industry in the area. More specifically, we assessed whether introgression affected (i) levels of neutral genetic diversity in wild Magaguadavic River Atlantic Salmon over time, (ii) the extent of differentiation between wild and farmed replicates at both types of markers, (iii) the signature of divergent selection possibly operating at some SNP loci and (iv) the initial degree of distinctiveness or conversely, the homogenization of wild and farmed salmon. Our results strongly suggest that introgression did change the genetic signature of the wild population, by, for example, reducing the divergence at neutral and non-neutral markers. Although levels of within population genetic diversity in the wild population did not appear to change, levels of differentiation between wild and farmed salmon were reduced through time, likely in response to the increasing presence and influence of farmed salmon in the Magaguadavic River. Perhaps more importantly, the overall number of loci potentially under divergent selection was reduced when wild temporal samples were contrasted with a reference farmed sample. Furthermore, a specific marker putatively involved in local adaptation (see Discussion below) epitomized the homogenizing effect of introgression by showing a reduction of differentiation in wild populations compared with farmed samples over time, until patterns of locus variability and population differentiation at this locus were indistinguishable from that expected because of neutral evolutionary forces.

Neutral genetic changes

The unexpected stability of levels of gene diversity and allelic richness of wild temporal replicates might have

Table 3 Summary information on outliers detected in genome scans comparing FARM 1992 to wild temporal samples at 112 SNPs

FARM 1992 vs	*Outliers' ID*	*Linkage group*	*Gene annotation*
WILD 1992	BASS119 B7 A09 382SNP	AS15	UNKNOWN
	BASS19 B7 F09 342SNP	AS14	UNKNOWN
	Contig14899 0107	AS22	NADH dehydrogenase subunit 5
	Contig17364 0264	NA	Elongation factor
	Contig16686 0431	NA	Small ubiquitin related modifier
	Contig16628 1277	NA	Antithrombin III precursor
	Contig16686 0312	NA	Small ubiquitin related modifier
	Contig17081 268	AS04	Serine incorporator
	Contig15610 550	NA	Glutamyl aminopeptidase
	Contig13137 0137	AS18	Protein MON2 homolog
WILD 1996	Contig16856 0321	NA	Renin receptor precursor
	Contig16378 0529	ASA	Cell division protein kinase
	Contig16053 552	NA	Mitogen activated protein kinase
	Contig15610 504	NA	Glutamyl aminopeptidase
	Contig15610 550	NA	Glutamyl aminopeptidase
	Contig16207 0498	AS10	UNKNOWN
	Contig14579 490	AS02	Eukaryotic initiation factor
	Contig14782 0767	AS18	Very long chain acyl CoA synthetase
	Contig14899 0107	AS22	NADH dehydrogenase subunit 5
WILD 1998 99	Contig16221 0769	NA	26S proteasome non ATPase
	BASS133 B7 H09 429SNP	AS12	Retinoic acid receptor gamma b
	Contig16686 0431	NA	Small ubiquitin related modifier
	Contig14899 0107	AS22	NADH dehydrogenase subunit 5
WILD 2002+	BASS111 B7 D03 200SNP	AS05	MHC class I antigen
	Contig14638 214	AS22	Mitochondrial 28S ribosomal protein
	BASS113 B6A F03 685SNP	AS27	UNKNOWN
	Contig15118 153	AS17	Mitochondrial 28S ribosomal protein
	Contig16207 0498	AS10	UNKNOWN

Abbreviations: MHC, major histocompatibility complex; NA, non available; SNP, single nucleotide polymorphism.
For each comparison, outlier's ID is given with its known linkage group and gene annotation. Nomenclature used for linkage group corresponds to that used in the ASalBASE (powered by cGRASP) Atlantic salmon linkage map (http://www.asalbase.org/sal bin/map/index).

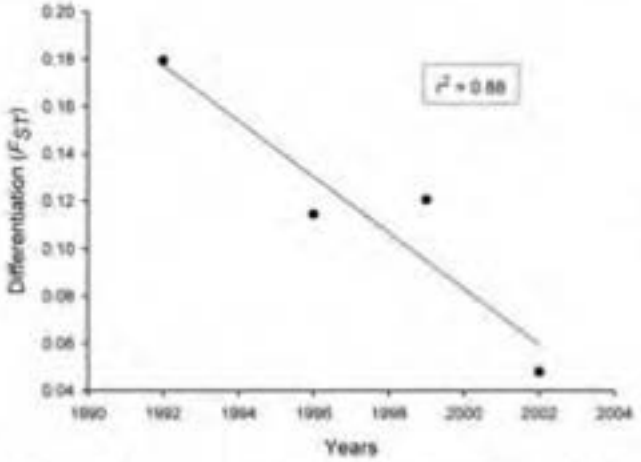

Figure 4 Differentiation (F_{ST}) of Contig14899 0107 across time as estimated by FDIST2 in genome scans (Figure 3). Wild temporal samples were considered as single year sample based on the sample year predominant in the population (WILD 1996 1996, WILD 1998 99 1999 and WILD 2002 + 2002). Regression value is indicated in the top right corner (P 0.062).

been foreseen in the light of introgression by a similarly diverse group of individuals and consistent input from this group. Previous studies of European domesticated fish strains showed reduced allelic variation compared with their European wild relatives (Mjølnerød *et al*., 1997; Norris *et al*., 1999; Skaala *et al*., 2004). Furthermore, as farmed salmon typically involve a restricted number of effective breeder, selection for performance traits and low or no gene flow, we expected farmed Atlantic salmon to show this kind of reduced genetic variation. However, we found that farmed salmon caught entering or descending the river as smolts did not exhibit lower levels of allelic richness or gene diversity compared with wild salmon. Although this remains to be rigorously tested, it is possible that some of the numerous freshwater aquaculture facilities each has lost and retained different alleles over time, and that collectively, they contain a large standing pool of genetic variation. These stocks would have acted partly as a metapopulation in neutralizing any possible effect of an isolated group of founders or of genetic drift.

Admixture from farm escapees in the wild may threaten natural populations through 'maladaptive' genetic changes (Skaala *et al*., 2006). Starting between 1992 and 1996, there were indications that introgression occurred in the wild Magaguadavic population. This was illustrated by the number of linked loci per locus in the wild replicate sample collections that went from a relatively stable state corresponding to genome-wide equilibrium to numerous LD strongly suggestive of a blending between two somewhat different populations. Indeed, the number of linked loci per locus in the wild sample from 1996 was similar to that observed in the farmed sample, which was possibly attributable to some variability in the origins or allele frequency distributions among the farmed population within the Magaguadavic River system. Moreover, this time period corresponded with the expansion of the farming industry in the

Magaguadavic River and the observed turning point in relative abundance of wild versus farmed Atlantic salmon returning to the river as adults (Carr *et al.*, 2004). Population bottlenecks also have the potential to increase LD for a short period of time (Gaut and Long, 2003) but we would argue that in this study, although the rapid decline of the wild population might have contributed to the increase LD and cannot be ruled out, the sudden genome-wide increase in LD is more consistent with a scenario involving recombination imposed by admixture. Consequently, while after 1994 the number of farmed fish returning to the river outnumbered the rapidly declining wild Atlantic salmon population, our results suggest that the reduction of gene diversity expected in this declining population was counteracted by the outbreeding effect of introgression from a slightly divergent and genetically variable population of aquaculture salmon.

To our knowledge, the most comparable study to ours involving wild Atlantic salmon was carried out by Skaala *et al*. (2006) and showed a reduction of differentiation and genetic distances among wild populations affected by farm escapees for 20–30 years despite no genetic diversity change in wild populations. Using microsatellites, we found a modest albeit significant genetic difference between pre-aquaculture wild individuals from 1980 and all other wild replicates except for the very late-aquaculture period samples. Moreover, comparisons of wild temporal samples also yielded significant levels of differentiation. Of the five of seven significant comparisons involving pre- or early-aquaculture period samples and farmed fish, the highest value was reached between the oldest wild samples (WILD-1980 and WILD-1992) and most recent aquaculture adults (FARM-2000_adults). These results, along with the finding that the majority of nonsignificant genetic differences observed among the most recent (mid-aquaculture period and later) wild samples and aquaculture samples are consistent with a homogenization effect occurring between the farmed and wild population of the Magaguadavic River because of introgression. In similar studies, Koskinen *et al*. (2002) and Hansen *et al*. (2010) reached similar conclusions when they contrasted historical and contemporary replicates of, respectively, wild grayling and wild brown trout populations admixed with hatchery fishes; both observed a significant reduction in levels of differentiation between wild and hatchery strains reflecting recent admixture. In a recent study on brook charr (*Salvelinus fontinalis*), Marie *et al*. (2010) also documented a homogenization of populations structure among wild populations following stocking events with domestic fish, accompanied with a greater genetic similarity between wild and domestic fish. Our study yielded similar findings that admixture did reduce genetic differentiation between the wild and farm salmon despite no overall effect detected on the level of genetic diversity in the wild population.

Possible effects on local adaptation

Although the genome scans performed here can be criticized for the lack of fit to reality with respect to the underlying models leading to false positives (for example, inclusion of populations that have experienced recent and marked population bottlenecks (Foll and Gaggiotti, 2008), we nevertheless consider the use of this approach as useful in at least obtaining a qualitative measure of the number of loci highly divergent between farmed salmon and wild temporal replicates as a good indication of the proportions of genomic regions potentially under the effect of divergent selection in each comparison. Here, outliers were approximately one order of magnitude more differentiated than the average SNP marker analyzed in the two sample groups compared. Moreover, our results suggest a 50% reduction in the number of loci potentially under divergent selection between wild and farmed salmon after only 3–5 generations of significant admixture occurring in the wild population. Also, the locus with the most pronounced differentiation ($F_{ST}=0.18$) in the comparison between farmed and wild salmon in 1992 (early aquaculture period) was still identified as an outlier when compared in the mid- and late-aquaculture periods with a reduction of its differentiation value until it faded away into the neutral distribution in the most recent comparison. Admittedly, the finding of a single locus, Contig14899_0107, exhibiting this pattern over four time points is not unlikely to occur by chance, even given the high regression coefficient observed. However, this marker was associated with a quantitative trait locus for parr mark number in a previous study (Boulding *et al*., 2008). Parr marks have been suggested to be a variable trait important for predator avoidance in the wild, allowing juvenile Atlantic salmon to blend in with the local substrate of the river (for example, Donnelly and Dill, 1984). In summary, the large decrease in F_{ST} over four time points, the association of the locus with a previously identified quantitative trait locus, and the fact that it presented the highest differentiation in the original genome scan represents compelling evidence that the trend observed is biologically meaningful and did not occur purely by chance. Therefore, this particular case of homogenization between wild and aquaculture fish in addition to the previously demonstrated reduction for markers potentially under divergent selection, suggests that the wild population of Atlantic salmon in the Magaguadavic River likely suffer from a loss of local adaptation exacerbated by introgression from farmed salmon.

On the other hand, this reduced number of outliers might also reflect the fading of selected traits in farmed Atlantic salmon as there is no indication of the directionality for the divergent selection pressure underlying the high differentiation of these markers. However, why selection (both intentional and unintentional) in a captive environment would have caused an increase in genetic similarity with a given wild population is unclear. In any case, it could still be argued that regardless of whether the selected trait(s) represent both local adaptations to native river conditions in the wild population and the hatchery environment in the captive population, the homogenization most probably resulted in reduced adaptation to both environments. The admixture between both groups probably resulted in a breakdown of LD around divergently selected regions of the genome (Via and West, 2008) and incidentally caused the observed reduction of detected outliers regardless to the actual selection occurring in the wild, which would have to be very strong to counteract the recombination

occurring following introgression. Of course, further studies (such as that carried out by Fraser *et al*., 2008), comparing fitness of wild and farmed–wild hybrid salmon, should be undertaken to measure fitness-related traits of differently introgressed individuals in association with their actual allelic composition. However, in an obligate non-invasive field study such as this one, we argue that until more important genomic resources are available, or another significant decrease in the cost of whole genome sequencing is realized, our results make a strong case for reduced local adaptation in a wild population impacted by farm escapees.

Perspectives for aquaculture management

Hutchings and Fraser (2008) recently debated the pros and cons of using aquaculture strains derived from local versus non-local wild populations for salmon farming for a given region. Adding to this debate, our study suggests that although we observed introgression from farm escapees in the wild population with significant loss of neutral differentiation and a reduction of the potential number of loci under divergent selection, neutral genetic variability still prevailed in a rapidly declining population. As there is evidence of captive-reared salmon establishing themselves outside of their native ranges (Volpe *et al*., 2000; Soto *et al*., 2001), despite the inferior fitness of farmed Atlantic salmon in natural environments (McGinnity *et al*., 2003), one could argue that a foreign strain might have had stronger impacts on the wild salmon in terms of changing the genetic composition of such a vulnerable population, impacting both neutral and selective divergence. Nevertheless, given the geographic and likely phylogenetic proximity of the wild and putative source population of the aquaculture strain analyzed here, detecting outlier loci was already evidence of differential selective pressures acting on farmed and wild salmon. In addition, their fading through time, especially when associated with an important quantitative trait locus for juvenile Atlantic salmon, certainly should be regarded as a warning for the potential loss of local adaptation in any kind of farming industries unintentionally releasing individuals in the natural environment

In conclusion, despite its relatively limited genomic coverage, this study emphasizes the potential importance of long-term monitoring of wild populations impacted by farmed escapees. Furthermore, in this context and with increasingly important genomic resources soon to become available for non-model species such as Atlantic salmon, more in depth studies could further disentangle the relative impacts of farmed escapees on the neutral and adaptive diversity of affected populations on a genome-wide basis.

Conflict of interest

The authors declare no conflict of interest.

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REVIEW

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Collateral diseases: Aquaculture impacts on wildlife infections

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Abstract

1. Aquaculture is a promising source of fish and other aquatic organisms to ensure human food security but it comes at the price of diverse environmental impacts. Among others, these include diseases which often thrive under the conditions in aquaculture settings and can cause high economic losses. These diseases may also affect wildlife, however, the impacts of aquaculture on disease dynamics in wild species in surrounding ecosystems are poorly understood.
2. In this Review, we provide a conceptual framework for studying the effects of aquaculture on wildlife diseases, and illustrate the different mechanisms identified with examples from the literature. In addition, we highlight further research needs and provide recommendations for management and policy.
3. We identified five potential means by which farmed populations may alter wildlife disease dynamics: (a) farmed species may co-introduce parasites to the new environment, which infect wild conspecifics without infecting other species (intraspecific parasite spillover); (b) these co-introduced parasites from farmed species may infect other wild host species potentially leading to emerging diseases (interspecific parasite spillover); (c) parasites from other wild host species may infect farmed species, amplifying parasite numbers and increasing parasite infections when spilling back to wild hosts (interspecific parasite spillback); (d) farmed species may acquire parasites from wild conspecifics, increasing parasite population size and subsequently raising infection loads in the wild host population (intraspecific parasite spillback); and (e) farmed species may be neither hosts nor parasites, but affect the transmission of parasites between wild host species (transmission interference). Although these mechanisms can alter wildlife disease dynamics, we found large knowledge gaps regarding collateral disease impacts and strong biases in terms of production countries, aquaculture practices and host taxa.
4. *Synthesis and applications*. The strong potential for aquaculture to affect the dynamics of diseases in wildlife populations calls for the consideration of collateral disease impacts in risk assessments and biosecurity protocols regarding aquaculture. In particular, comprehensive parasite inventories of both farmed and wild hosts as well as disease monitoring in wildlife surrounding farms will be necessary to increase our knowledge on aquaculture impacts on wildlife disease and to develop adequate prevention and mitigation measures.

KEYWORDS
aquaculture, biosecurity, disease ecology, environmental impact, risk assessment, wildlife diseases

1 | INTRODUCTION

The per capita consumption of fish and other aquatic animals such as crustaceans and molluscs has considerably increased over the previous decades, reaching a record-high of 20.3 kg per capita per year in 2016 (FAO, 2018). Meeting the global demand for fish and other aquatic food products and ensuring human food security are therefore becoming increasingly challenging (Béné et al., 2015; Jennings et al., 2016). While capture fisheries are unable to keep up with the demand for aquatic food products, aquaculture, i.e. the farming of aquatic organisms, has been responsible for the ever increasing supply for human consumption, with 53 percent of global aquatic food production coming from aquaculture in 2016 (FAO, 2018; Figure 1). Aquaculture is practiced inland, in coastal and in marine environments in a variety of aquaculture systems, ranging from ponds and cages to highly sophisticated water reuse systems (Boyd & McNevin, 2005; Lucas et al., 2019). Like the variety of culture systems, the range of different species produced in these facilities varies extensively. While the bulk of species produced in aquaculture is comprised of fish, many species of other taxa are also farmed, such as crustaceans and molluscs, and their production is increasing as well (Metian et al., 2020).

Although promising from the point of human food security, the rapid growth of aquaculture has also raised concerns about its ecological impacts; ensuring the environmental sustainability of future growth constitutes one of the main challenges for aquaculture (Barrett et al., 2019; Beveridge et al., 1994; Campbell et al., 2019; Costello et al., 2019; Diana, 2009; Hall et al., 2011; Subasinghe et al., 2019; Subasinghe et al., 2009). Among the ecological impacts of aquaculture activities are the widespread use of wild fish as feed for aquaculture stocks (Naylor et al., 2000, 2009; Tacon & Metian, 2009, 2015), the genetic pollution of wild stocks (Cross et al., 2008; Glover et al., 2012; Jørstad et al., 2008; McGinnity et al., 1997), water quality issues such as local eutrophication (Pitta et al., 2009; Price et al., 2015) as well as the introduction of non-native species through escapees from farms or the co-introduction of other species with the translocation of aquaculture stocks (Diana, 2009; Naylor et al., 2001; Peeler et al., 2011; Savini et al., 2010).

Another ecological impact that affects aquaculture itself is related to diseases. The specific nature of aquaculture practices makes farmed aquatic organisms particularly prone to disease outbreaks: (a) the translocation and introduction of aquaculture stocks can lead to the co-introduction of pathogens and parasites (Peeler et al., 2011), (b) the often low genetic diversity of aquaculture stocks can increase the susceptibility of hosts and increase the virulence of pathogens (Kennedy et al., 2016) and (c) stocking densities in aquaculture settings are often much higher than would be found in natural environments which provides excellent conditions for pathogens and parasites to thrive (Krkošek, 2010; Salama & Murray, 2011). Accordingly, disease outbreaks frequently occur in aquaculture settings (Lafferty et al., 2015; Leung & Bates, 2013; Sweet & Bateman, 2015) and there are numerous examples of diseases ravaging farmed salmon (e.g. salmon lice *Lepeophtheirus salmonis* and *Caligus elongatus* (Revie et al., 2002), infectious salmon anaemia (Mullins et al., 1998) and infectious haematopoietic necrosis (Saksida, 2006)), shrimp (e.g. white spot syndrome (Chou et al., 1995) and acute hepatopancreatic necrosis disease (Soto-Rodriguez et al., 2015)) and other cultured organisms (Lafferty et al., 2015). The economic losses associated with such disease outbreaks in aquaculture, including the costs of disease control measures, are enormous. For example, sea lice infections of salmon in Norway generate economic costs equivalent to 9% of farm revenues and have led to damages estimated at >US$ 400 million in 2011 alone (Abolofia et al., 2017). On a global scale, economic losses in aquaculture due to diseases are estimated to amount to at least several billion US$ per year (World Bank, 2014). Due to these considerable economic risks, disease outbreaks represent one of the main obstacles for the sustainable growth of aquaculture (Stentiford et al., 2012; Subasinghe et al., 2019) and the problem has been termed the 'global aquaculture disease crisis' (Stentiford et al., 2017).

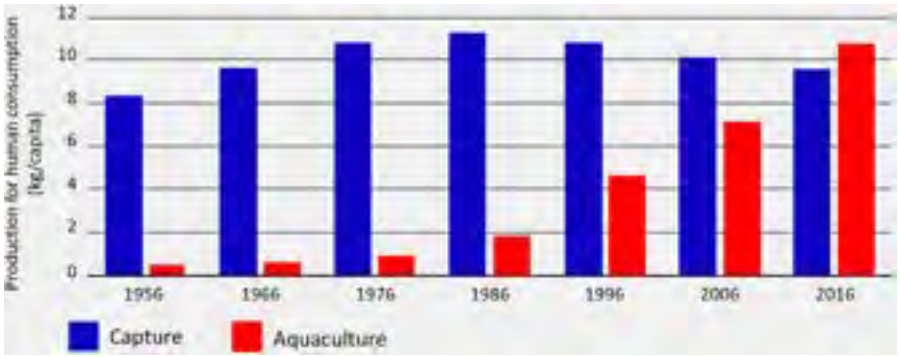

FIGURE 1 Origin of aquatic food production for human consumption over the past five decades, showing the increasing share of aquatic food products originating from aquaculture and capture of wild fish (for commercial, industrial, recreational and subsistence purposes). Data retrieved from FAO (2018)

Given the tremendous economic risks associated with disease outbreaks in farms, it comes as no surprise that diseases in aquaculture have been extensively studied, in particular with respect to the identification and treatment of responsible agents and the prevention of disease outbreaks based on risk assessments and biosecurity protocols (Hine et al., 2012; Subasinghe et al., 2019). However, diseases in aquaculture settings are not necessarily confined to farms themselves but can affect and interact with wild hosts in the vicinity of farms as well, with aquaculture held responsible for several reported cases of wildlife diseases (Diana, 2009; Lafferty et al., 2015). For example, salmon lice originating from farmed salmon in North America have been shown to infect wild juvenile pink salmon *Oncorhynchus gorbuscha* when passing salmon farms during their migration, leading to strong population declines and local risk of extinction of the wild host species (Krkošek et al., 2007). However, studies into the effects of aquaculture on wildlife disease ecology have been few, and the diversity and magnitude of impacts of aquaculture activities on disease dynamics in wild hosts in surrounding ecosystems are generally poorly understood.

This review examines the possible effects of aquaculture on wildlife disease dynamics and provides a conceptual framework for studying the effects of aquaculture on parasite–host interactions, borrowing from mechanisms and conceptual frameworks developed for biological invasions (e.g. Dunn & Hatcher, 2015; Goedknegt et al., 2016; Kelly et al., 2009; Young et al., 2016). As discussed above, aquaculture introduces host or parasite species to environments where they had been absent before. Therefore, many of the mechanisms of parasite and disease exchange between farmed and wild hosts may be similar to interactions between introduced and native hosts and parasites. In the following, we first review the most common methods used in aquaculture to pinpoint possible means of parasite exchange between farmed organisms and wildlife. We then identify the various ways in which these exchanges can affect parasite–host interactions, and illustrate the different mechanisms with examples from the literature. Finally, we highlight further research needs and recommendations for management and policy.

2 | THE MANY FORMS OF AQUACULTURE

Aquaculture is practised in many different ways. Species are cultured in freshwater, brackish and marine environments, with the majority of production coming from inland freshwater facilities (FAO, 2018). According to FAO (2018), based on known and documented practices, there are 598 different species of organisms used in aquaculture, and these include 369 fishes, 109 molluscs, 64 crustaceans, nine other invertebrates, seven amphibians and 40 algae (FAO, 2018). A variety of distinct methods are used for cultivating such a wide range of species. In the following, we describe some of the most commonly used methods, and identify the possible routes of parasite exchange with the environment surrounding the facilities.

2.1 | Ponds

Ponds are the most commonly used system for fish and crustacean aquaculture, with an estimated 11×10^6 ha of global aquaculture pond surface area (Verdegem & Bosma, 2009). Ponds can be constructed in several ways. Watershed ponds are created by building a dam to confine runoff, either from overland flow of rainfall or from an existing stream (Boyd & McNevin, 2005). Ponds may also be excavated or constructed by building an earthen embankment, a so called embankment pond, which is the main type of system used in shrimp farming (Boyd & Clay, 1998; Boyd & McNevin, 2005; Figure 2a). These types of ponds usually require a water supply from an external source such as a stream, well or irrigation system (Boyd & McNevin, 2005). This external water supply offers a potential vector by which parasites from the wild are able to enter the pond system. Additionally, ponds are usually equipped with drainage structures to discharge excess water or to drain them entirely, which is common practice during harvest (Boyd & McNevin, 2005; Verdegem & Bosma, 2009). When inadequate action is taken to disinfect this effluent, drainage of culture ponds has the potential to release parasites of cultured species in the environment, thus offering a mechanism for parasite exchange from farmed to wild organisms (Kurath & Winton, 2011).

2.2 | Cages and net pens

Another frequently used aquaculture system is the use of enclosures situated in natural bodies of water, usually cages or net pens (Figure 2b). These enclosures can be as small as 1 m^3 or as large as 1,000 m^3 and are stocked with fish densities ranging from <20 to over 200 kg/m^3 (Schmittou, 1993). Atlantic salmon *Salmo salar*, the most common marine aquaculture species, are usually grown out in enclosures at sea, but the method can also be applied to other species such as marine shrimps (FAO, 2018; Paquotte et al., 1998). Because cages and net pens are placed directly in the natural environment and allow for free water exchange with the surrounding environment, the chance of parasite exchange between wild and farmed fish stocks is particularly high for these types of systems (Johansen et al., 2011). Furthermore, the likelihood of fish escaping from net pens is high, and escapes are known to occur on a regular basis (Diana, 2009; Johansen et al., 2011). In addition, cages and net pens attract aggregations of wild fish seeking food or shelter, further increasing the risk of parasite exchange between farmed and wild fish and between neighbouring farms (Dempster et al., 2009; Johansen et al., 2011).

2.3 | Flow through raceways

A system often used for farming rainbow trout is a raceway supplied with water originating from a natural water source such as a

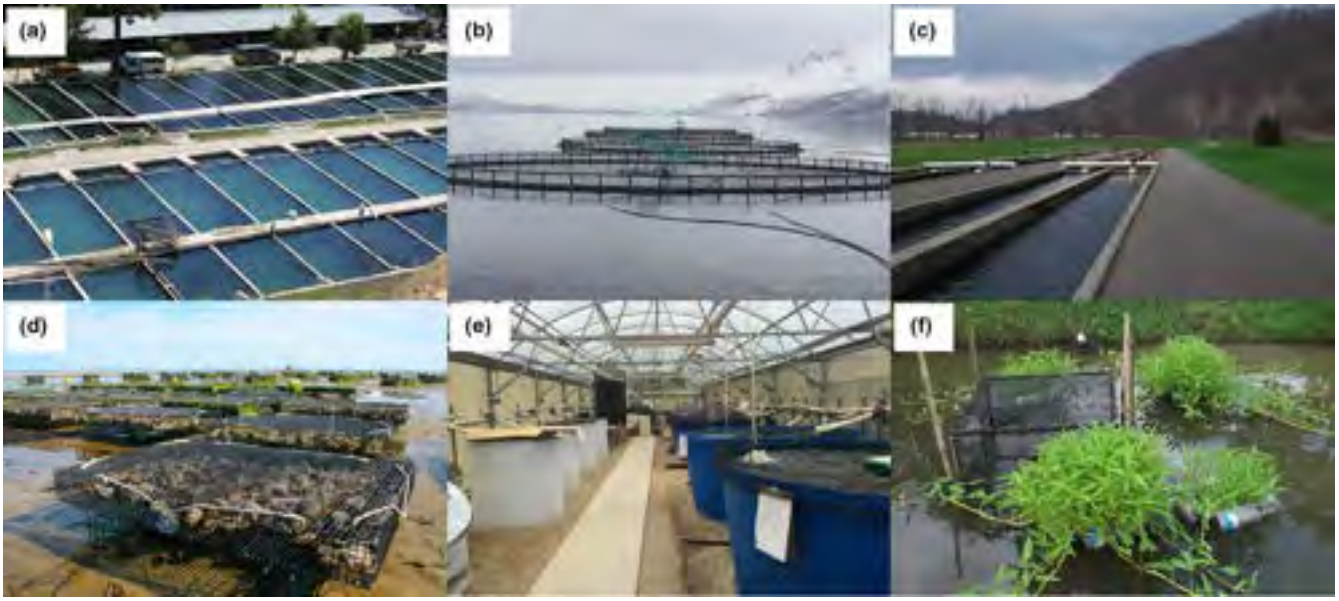

FIGURE 2 Examples of the various methods used for aquaculture: (a) fish farming in ponds, (b) marine cage aquaculture facility, (c) freshwater flow-through raceway system, (d) off-bottom oyster cages, (e) indoor recirculating aquaculture system (RAS), and (f) small scale integrated multi-trophic aquaculture (IMTA) system in a freshwater pond. Photo credits: (a) Vera Kratochvil, Wikimedia Commons, Public Domain, (b) Thomas Bjørkan, Wikimedia Commons, CC BY-SA 3.0, (c) Brian M. Powell, Wikimedia Commons, CC BY-SA 3.0, (d) Pixabay, Public Domain, (e) Narek Avetisyan, Wikimedia Commons, CC BY-SA 4.0, (f) Saifullahrony, Wikimedia Commons, CC BY-SA 3.0 [Colour figure can be viewed at wileyonlinelibrary.com]

spring, stream or lake (Boyd & McNevin, 2005). They are usually made of concrete and positioned in series, in which the water from the upper units flows into the units below (Figure 2c). Water exchange occurs via gravity flow at a rate of approximately two or three times the volume of a culture unit per hour and from the lowermost unit it is discharged into a natural body of water (Boyd & McNevin, 2005). These raceways generally harbour higher stock densities than ponds, ranging from 80 to 160 kg/m^3 for rainbow trout (Soderberg, 1994). High stocking densities along with the release of effluent into natural waterbodies provide risks of parasite exchange with wild populations, and could be cause for concern.

2.4 | Mollusc and seaweed culture

Bivalve molluscs and seaweeds are generally produced in coastal waters, although there are a few species which are cultured in ponds. Bivalves and seaweed are either grown out on the bottom (on-bottom culture), or by so called off-bottom culture in which spat or seaweed propagules are fixed to longlines, rafts or racks for grow-out (Boyd & McNevin, 2005; Figure 2d). The latter method is deemed more efficient as it eliminates the limiting effects of benthic predators and impaired sediment quality while permitting three-dimensional use of the water column (Boyd & McNevin, 2005). Because culture occurs directly in natural coastal waters, parasites can be exchanged between farmed and wild populations, seemingly without any restriction.

2.5 | Recirculating aquaculture systems

Recirculating aquaculture systems (RAS) are closed culture systems in which waste water is treated and subsequently re-used to allow for a more efficient use of water and a greater fish production per volume of water (Figure 2e). Waste water from culture units usually passes into a sedimentation basin, where coarse solid waste is removed. Subsequently the water is purified naturally or through technologically more complex purification systems (Boyd & McNevin, 2005). As a result, waste water volume released into the environment is greatly reduced (Boyd & McNevin, 2005; Edwards, 2015), lowering the chances of parasites from culture organisms being released into the wild.

2.6 | Integrated multi-trophic aquaculture

In some cases, extractive species such as bivalve molluscs or seaweeds are used as a means of removing excess nutrients and other waste, both in closed RAS and open systems such as cages or net pens (Figure 2f). These extractive species are then harvested as well. This use of multiple species of different trophic levels in a single culture system is known as integrated multi-trophic aquaculture (IMTA). Although this relatively new approach has been the subject of ongoing research and many of these are positive about its potential, there is some debate regarding the efficiency of bivalves in capturing organic wastes from fish cultures, especially in open systems (Edwards, 2015). In IMTA systems, extractive species have the potential to change parasite–host interactions, as they have been shown

to be capable of reducing free-living parasite stages in the water, so called transmission interference (Burge, Closek, et al., 2016; Molloy et al., 2011). However, the addition of more species to a farm could also lead to the introduction of additional parasites along with these extractive species, with the potential to infect native hosts. In addition, there is a possibility for amplification of already pre-existing parasite populations (Burge, Closek, et al., 2016; Kelly et al., 2009).

3 | AQUACULTURE IMPACTS ON WILDLIFE DISEASES

Considering the aforementioned possibilities of parasite exchange between aquaculture farms and surrounding wildlife, and the numerous examples of cultured species escaping and becoming invasive, aquaculture has the potential to alter parasite–host interactions and diseases in wildlife inhabiting the environment surrounding farms. In the following, we identify the different mechanisms by which aquaculture affects wildlife parasite–host interactions and diseases and provide examples of their occurrence from the literature. By doing so, we provide a conceptual framework for studying the effects of aquaculture on wildlife diseases (Figure 3). The mechanisms presented are not mutually exclusive, it is possible that several or even all of the different mechanisms occur in a specific aquaculture setting. For our review, we extensively searched the literature for studies on aquaculture disease impacts on wildlife using Web of Science and Google Scholar, as well as by scanning existing reviews and books on aquatic diseases and aquaculture. Although we did not conduct a formal meta-analysis, we believe that we have found the majority of existing studies and we thus consider our overview of examples to be reasonably representative.

3.1 | Interspecific parasite spillover

Whenever a species is taken from its environment and transported to a new one, there is a possibility of transporting parasites along with them. In invasion ecology, the process of introducing a parasite along with its host is known as parasite co-introduction (Goedknegt et al., 2016; Lymbery et al., 2014). This principle can be applied to aquaculture as well. When a parasite is co-introduced with a host species to an environment which is inhabited by other naive potential host species, there is a possibility of the parasite switching hosts. The switch from the original host to naive wild host species is known in invasion ecology as parasite spillover (Kelly et al., 2009). When aquaculture species are farmed in systems that allow for water exchange with the environment, interspecific spillover events to wild species are known to occur (Peeler et al., 2011). A similar phenomenon can be observed in domestic animals when parasites spill over from domestic animals to wildlife populations living in proximity (Daszak et al., 2000). There are numerous examples of diseases from aquaculture farms affecting

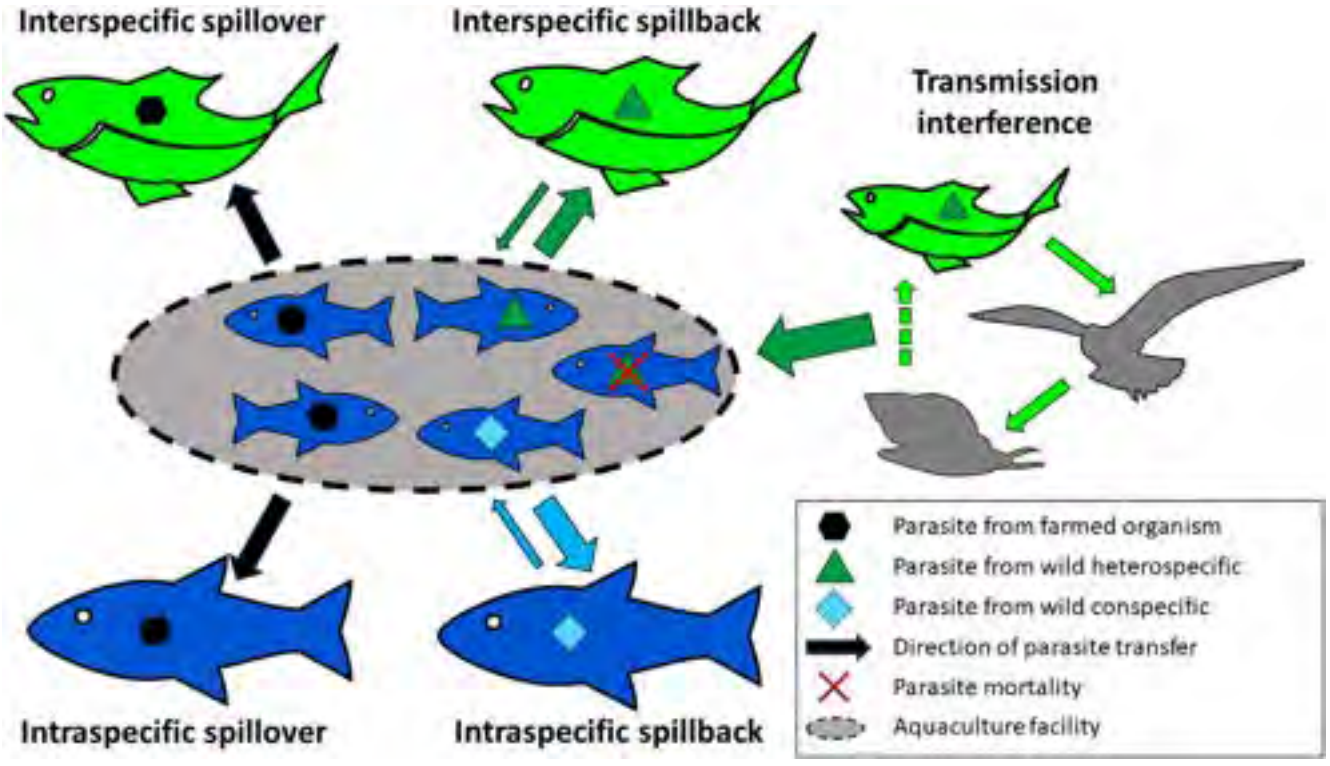

FIGURE 3 Conceptual framework showing the five different mechanisms through which aquaculture activities can affect diseases in wildlife in the environment surrounding aquaculture facilities. See main text for further details and examples of each of these mechanisms [Colour figure can be viewed at wileyonlinelibrary.com]

wild populations. Out of 35 interspecific spillover events of invasive parasites to native species in marine ecosystems listed in a review by Goedknegt et al. (2016), aquaculture was named as the most likely vector for 20, and five more were caused by stocking for fisheries. One example of such an interspecific spillover event involves the parasitic copepod *Mytilicola orientalis*, co-introduced to Europe with the Pacific oyster *Crassostrea gigas* imported for aquaculture. This parasite has been found in wild populations of several native bivalve species such as blue mussels *Mytilus edulis*, common cockles *Cerastoderma edule* and Baltic tellins *Macoma balthica*, indicating an interspecific spillover effect (Goedknegt et al., 2017). Another example involves infectious hypodermal and haematopoietic necrosis virus (IHHNV) in penaeid shrimps in the Gulf of California. This disease probably did not occur in wild shrimp populations in this region prior to 1987, but has become established in wild populations of Pacific blue shrimp *Penaeus stylirostris* and possibly other native shrimp species, following importation of *Penaeus vannamei* postlarvae to local shrimp farms (Pantoja et al., 1999).

Although many of the aforementioned interspecific spillover events of aquaculture parasites are the result of escaping culture species or close contact between farmed and wild populations in open farm systems, direct contact between species might not always be necessary for parasite spillover to occur. The parasitic swimbladder nematode *Anguillicoloides crassus* which affects eels (*Anguilla* spp.) was co-introduced in Europe with Japanese eel *Anguilla japonica* in the 1980s and spilled over to native European eel *Anguilla anguilla*, spreading rapidly across the continent (Kennedy & Fitch, 1990; Kirk, 2003; Koops & Hartmann, 1989). The spread of *A. crassus* was mainly due to the transport of live eels, which may have escaped (Kennedy & Fitch, 1990; Koops & Hartmann, 1989). However, infective stages of this parasite are capable of surviving and remaining infective for up to 2 weeks in the water column and introductions in Britain occurred mainly along the routes of lorries transporting eels, which exchange water several times during transport (Kennedy & Fitch, 1990). Therefore it is possible that at certain locations *A. crassus* interspecific spillover into European eels occurred via infective stages that were flushed out with waste water (infecting freshwater copepod intermediate hosts), rather than direct contact between eels (Kennedy & Fitch, 1990; Kirk, 2003; Peeler et al., 2011). Furthermore, *Anguilla japonica* has also been responsible for the interspecific spillover of two monogeneans *Pseudodactylogyrus anguillae* and *P. bini* to European eel *Anguilla anguilla* and American eel *Anguilla rostrata* in Europe and the US, respectively (Hayward et al., 2001; Morozińska-Gogol, 2009).

Diseases that occur in a novel species after an interspecific spillover event are known as emerging diseases, and can have devastating consequences (Daszak et al., 2000). Due to the fact that naive hosts do not have a co-evolutionary history with the novel parasite, they can be particularly vulnerable, leading to negative effects on the new host species, communities and even entire ecosystems (Goedknegt et al., 2016). This can be especially dangerous if the parasite does not cause high mortality rates in its original host, but does so in the novel host, while the original host remains present as a reservoir of the disease. For instance, the crayfish plague, a fungal disease caused by *Aphanomyces astaci*, spilled over from American signal crayfish *Pacifastacus leniusculus* to European crayfish *Astacus astacus*. While *P. leniusculus* rarely succumbs to the disease, it causes extremely high mortality rates in *A. astacus*, threatening the latter species with extinction (Alderman, 1996; Peeler et al., 2011).

3.2 | Intraspecific parasite spillover

Many cultured species are not bred in captivity, but larvae or juveniles are caught from the wild and transported to aquaculture facilities for grow-out (Boyd & McNevin, 2005). If these juveniles are infected, parasites are co-introduced to the farm environment, potentially leading to disease outbreaks within the farmed stock. In invasions, co-introduced parasites do not always lead to infections in wild native hosts by switching hosts, but affect only the invader (Goedknegt et al., 2016). In the same way, outbreaks of co-introduced parasites in aquaculture species do not have to lead to interspecific spillover in other wild species. However, a co-introduced parasite is likely to spread to neighbouring wild populations of the same species, as it does not need to cross the species barrier. For example, ostreid herpesvirus OsHV-1 µVar has recently been co-introduced to European oyster aquaculture with imports of Pacific oysters *C. gigas* from East-Asia, causing up to 90% mortality in farmed oyster, but has so far only affected this species in Europe (Goedknegt et al., 2016; Mineur et al., 2015). However, this virus has been found in wild (invasive) populations of *C. gigas* in the Dutch Wadden Sea (Gittenberger et al., 2016), although mortalities in wild populations are unknown. Similarly, intraspecific spillover was the source of bonamiasis outbreaks in European flat oysters *Ostrea edule*, caused by the parasitic protozoan *Bonamia ostreae*. The parasite is invasive and reached Europe via oyster transports from Europe to North America and back to France, bringing the parasite with them and spilling over to wild oyster populations (Chew, 1990; Engelsma et al., 2014). Intraspecific parasite spillover has also been observed in fish aquaculture. The monogenean parasite *Gyrodactylus salaris* which infects Atlantic salmon *S. salar* has been introduced to Norwegian waters with translocated salmon from hatcheries in the Baltic Sea, where salmon populations are tolerant or resistant to infections. In contrast, Norwegian salmon populations proved to be highly susceptible to the parasite and high mortalities in wild salmon populations have occurred (Bakke et al., 2007; Johansen et al., 2011; Johnsen & Jensen, 1992). This example shows that intraspecific spillover events can have important ecological implications as they can have an intense regulatory effect on the population dynamics of affected wild populations, which in turn may alter competitive interactions between affected hosts and other wild species (Goedknegt et al., 2016).

3.3 | Interspecific parasite spillback

In addition to wild species acquiring parasites from cultured species, parasites from wild species in the proximity of aquaculture farms may also spillover into cultured species, a phenomenon similar to the 'reverse spill-over' of parasites from wild populations to susceptible domesticated animals (Daszak et al., 2000). When aquaculture species are competent hosts for wild parasites, they could amplify parasite populations, which can subsequently spill back into wild hosts, increasing the number of parasite infections in wild host species (Goedknegt et al., 2016; Kelly et al., 2009; Leung & Bates, 2013). This is because the high stocking densities used in aquaculture can increase local host densities and thus boost parasite propagule production, which in turn can increase the risk for wild hosts to become infected. For example, the shell boring polychaete *Polydora ciliata* which infects the shells of wild molluscs in European seas has been acquired by the Pacific oyster *C. gigas* which is cultured in oyster farms and has also spread outside farms. In the wild, the parasite is more prevalent in Pacific oysters than in blue mussels *M. edulis* (Goedknegt et al., 2019), potentially leading to an interspecific spillback effect for wild mussels (Goedknegt et al., 2019). Another example comes from Atlantic salmon *S. salar* which is cultured along the Chilean Pacific coast and has become infected with copepods *Caligus rogercresseyi* and nematodes *Hysterothylacium aduncum* originating from a wide range of wild host species (Sepúlveda et al., 2004). Due to the high infection levels, it is likely that these parasites spill back to wild hosts, leading to increased infection levels in wild host populations. Likewise, American brine shrimp *Artemia franciscana* have been commercially imported from North America to the southern Iberian Peninsula where they escaped aquaculture farms and entered habitats with wild native *Artemia* populations (Green et al., 2005). Here, the invasive brine shrimp became infected with a variety of native cestodes that cause high infection prevalences in wild brine shrimp (*A. parthenogenetica* and *A. salina*; Georgiev et al., 2007). These examples indicate that interspecific parasite spillback can have large consequences for wild species and that the effects may not only originate from the aquaculture farms themselves but also from populations that escaped from these facilities.

3.4 | Intraspecific parasite spillback

Aquaculture species are not always newly introduced to an area, wild species are also commonly farmed locally. This leads to unnaturally high local densities of wild species within, for example, cages or net pens, while wild conspecifics live at much lower densities in the surrounding waters. This is for instance the case in the farming of salmon species, where the species farmed also naturally occur in the wild. Many disease outbreaks in salmon farms may have been acquired through exchanges with wild salmon populations, although it is often not clear whether disease originated from farmed or wild stocks. However, when a parasite is transferred from wild to farmed salmon stock it could be amplified during an outbreak in the farm, due to the high stocking densities, and subsequently spill back high numbers of infective stages to the wild population, similar to the interspecific spillback previously described, except without the need for a shift in host species. Such intraspecific spillback events are known for salmon lice *L. salmonis* and sea lice *Caligus* spp., which are naturally occurring parasites of salmonids. They can be exchanged between wild salmonids, such as the pink salmon *Oncorhynchus gorbuscha*, and farmed conspecifics along the Pacific coast of North America. Juvenile pink salmon in close proximity to salmon farms have been shown to have high rates of lice infestation, higher than those in areas without salmon aquaculture, leading to high juvenile mortality (Krkošek et al., 2007). Similar effects occur in salmon lice in farmed Atlantic salmon *S. salar* in Europe where these parasites are naturally present in wild Atlantic salmon populations. They are known to cause massive outbreaks in salmon farms and there is evidence that they subsequently cause elevated infection levels in wild salmon populations (Costello, 2009; Thorstad & Finstad, 2018; Torrissen et al., 2013). Likewise, intraspecific spillover may also affect the oyster *Ostrea chilensis*, native to New Zealand, which is cultured in Foveaux Strait between the South Island and Stewart Island in New Zealand, where wild populations also exist. Cultured oysters have experienced epizootics of the parasite *Bonamia exitiosa*, which have been catastrophic for the industry and will most likely have affected wild populations as well (Cranfield et al., 2005). Although the evidence for intraspecific spillover events is limited, spillback effects from farmed to wild conspecifics are very likely as there is no threshold for host switching that needs to be overcome, and this may be a highly underestimated effect of aquaculture on parasite–host dynamics in wildlife. Like interspecific parasite spillback between different species, intraspecific parasite spillback has the potential to induce high mortalities in wild populations, and in doing so, negatively affect wild ecosystem functioning.

3.5 | Transmission interference

One subtle effect of cultivated species on wild parasite–host interactions does not involve acting as a host or a parasite. Instead they might disturb wild parasite transmission from one host to the next, so called transmission interference (Burge, Closek, et al., 2016; Goedknegt et al., 2016; Thieltges et al., 2009). In general, many farmed and wild species that do not act as a host for a particular parasite can be so called dead-end hosts, predate on infective stages or interfere in other ways (see review by Thieltges et al., 2008). An aquaculture species which has been shown to interfere with the transmission of wild parasites is the Pacific oyster *C. gigas*, which can remove the free-living infective larval stages of wild trematode parasites affecting blue mussels *M. edulis* by filter feeding, without being infected itself (Goedknegt et al., 2015; Thieltges et al., 2009; Welsh et al., 2014). Pacific oysters are also extensively cultured in open systems in coastal waters. It is possible that oysters in farm

cultures filter infective larval stages of parasites in the same way their escaped counterparts have been shown to do. This could lead to lower infection levels in wild blue mussels in close vicinity of the farm. The extent to which filter feeding organisms can remove infective stages of parasites depends on a number of factors such as the prey size range of the filter-feeder, the transmission mode and host specificity of a particular parasite (Burge, Closek, et al., 2016). Whether such transmission interference by aquaculture farms truly occurs remains unknown, as it is yet to be studied. If it is the case, it could lead to substantial increases in the wild host population, especially if a heavy parasite burden is lifted due to the interference. This way, transmission interference has the potential to change the local communities surrounding the aquaculture facility and affect both the farm and wild ecosystem. In a similar way, certain aquaculture practises themselves, such as parasite control treatments or effluents dispersing from farms into ecosystems, may affect parasite transmission in wild hosts. However, such indirect effects of parasite control treatments on wildlife diseases are beyond this review.

4 | COLLATERAL DISEASE RISK, RESEARCH NEEDS AND RECOMMENDATIONS FOR MANAGEMENT AND POLICY

The chances of the above mechanisms occurring in a specific aquaculture facility and causing collateral disease risk for wildlife depend on the interactions between farmed and wild populations. In closed systems, where effluent water is kept to a minimum, parasite exchange between farm and wild populations is unlikely to play a major role. In pond systems, interactions are more likely, as pond water is often released in the environment during harvest or heavy rainfall. Aquaculture systems that are partially or entirely open such as raceways, cages, net pens and coastal mollusc cultures pose the highest risk for parasite exchange between farmed and wild populations, through any of the five mechanisms in our conceptual framework. These systems allow for free flow of water potentially containing infective stages and have a high risk of escapes that may establish wild populations.

Although the various aquaculture practices probably have different impacts on the collateral disease risk for wildlife, there is very limited research on this issue to date. A recent global meta-analysis of the wider impacts of aquaculture activities on the environment included only 22 studies regarding potential disease transmission between farmed and wild populations, most of which were about sea lice (Barrett et al., 2019). Only 11 of those studies actually investigated changes in infection levels in wild fish associated with farms, all of which found higher infection levels in the presence of active fish farms (Barrett et al., 2019). There are most likely more diseases in wildlife that can be affected by aquaculture practices but the extent of these collateral disease effects remains elusive, mainly due to the lack of baseline information on background prevalence of parasites and diseases in wildlife (Lafferty et al., 2015). An important step will thus be to identify the parasite communities in wildlife surrounding aquaculture facilities prior to stocking. In addition to parasite screenings of aquaculture stocks to be introduced, such comprehensive inventories could (a) indicate potential candidates for spillover and spillback scenarios for which further experimental work on transmission and host specificity could evaluate the risk of disease exchange, and (b) establish baselines to monitor ensuing changes in disease prevalence in the course of aquaculture activities. Unfortunately, parasites and diseases are generally difficult to detect in natural ecosystems but emerging technologies such as environmental DNA (eDNA) are promising tools in addition to traditional methods of parasite detection, such as histology (Bass et al., 2015; Burge, Friedman, et al., 2016; Gomesa et al., 2017). Given the likelihood of farm–wildlife disease exchanges and the potentially dramatic effects of collateral diseases on wildlife, we propose to implement wide-scale parasite and disease screenings of wildlife surrounding proposed farm sites prior to aquaculture activities in risk assessments and biosecurity protocols. Biosecurity measures are already generally in place for aquaculture activities (Arthur et al., 2009; Hine et al., 2012; Subasinghe & Bondad-Reantaso, 2006; Subasinghe et al., 2019) but they currently mainly focus on the health of stocks and specific parasites relevant for the farmed species. Adding a stronger wildlife perspective to aquaculture biosecurity and identifying the potential for farm–wildlife disease exchange prior to stocking activities would strongly help to reduce the risk for parasite spillover and spillback scenarios and associated collateral disease impacts.

The establishment of reliable baseline information on background prevalence of parasites and diseases in wildlife in the vicinity of farms would also allow to monitor changes in wildlife diseases once aquaculture activities have started. If implemented in biosecurity protocols, wildlife disease monitoring would make the early detection of collateral disease impacts possible and thus help to initiate containment and eradication or mitigation measures to reduce further impact. Disease monitoring should include both farmed and wild hosts so that the exchange between farmed stocks and surrounding wildlife can be quantified. Any disease monitoring should ideally be further supplemented by monitoring of the population dynamics of wildlife potentially at risk of collateral disease impacts so that any effects on host populations can be detected. This in turn may then initiate further experimental research into the underlying mechanisms.

A general implementation of collateral disease impacts in aquaculture biosecurity protocols would also help to redress the current knowledge gaps in regard to the pervasiveness and magnitude of collateral disease impacts and the biases in existing information in regard to producing nations and culture systems. This bias also exists for aquaculture impacts in general. The global meta-analysis by Barrett et al. (2019) noted that research effort on interactions between wildlife and aquaculture is not equally distributed among producing countries and significantly correlated with a country's developmental index and the size of its aquaculture industry. However, several major producing countries did not follow this trend. China, by far the largest aquaculture producer in the world, was not represented in the

relevant English-language studies found in the analysis, as were other major Asian producers. This is in line with our experience, as we did not find a single English-language study on diseases in wildlife related to aquaculture activities from China. According to the analysis of Barrett et al. (2019), research effort into the general environmental effects of aquaculture was also biased regarding production systems, with sea cages being overrepresented and freshwater systems being clearly underrepresented. The high representation of sea cages is not surprising, however, considering the open nature of those systems, allowing for interactions between farm and wildlife populations. The same pattern is also true for disease related studies as we could only trace very few studies regarding inland freshwater aquaculture. Finally, our current knowledge on the collateral disease effect of aquaculture activities is also biased with respect to the host taxa covered by existing studies. Most studies to date have focused on fish (mainly on salmon species) and to a lesser extent on crustaceans and molluscs as sources of farm-wildlife disease transfers. Hence, studies are needed that widen the taxonomic scope of aquaculture impacts on wildlife diseases.

5 | CONCLUSIONS

This review demonstrates that aquaculture activities can have an array of effects on wildlife diseases in the surrounding environment. The conceptual framework developed here provides a basis for further studies on the impacts of aquaculture on wildlife disease ecology and we propose to integrate collateral disease impacts in risk assessments and biosecurity protocols regarding aquaculture.

The risk of disease transfers related to aquaculture activities echoes similar risks in other food production environments such as agriculture and livestock management. There is a wealth of information on disease exchanges between natural ecosystems and crops or livestock (Blitzer et al., 2012; Daszak et al., 2000; Power & Mitchell, 2004). For example, many natural populations of animals serve as reservoirs for livestock diseases, such as badgers for tuberculosis in cattle in the UK (Donnelly et al., 2003) and bison that may transmit brucellosis to livestock in the US (Dobson & Meagher, 1996), creating conditions for spill back into wild host populations. Similarly, plant pathogens might transfer to cultivated crops and spill back when their wild hosts spread into cultivated areas, such as the transfer of crown rust and stem rust from wild to cultivated oats in Australia (Burdon et al., 1983; Oates et al., 1983). Examples of parasite spillover from cultivated to natural systems have also been documented (reviewed by Blitzer et al., 2012). For instance, foot-and-mouth-disease in domestic cattle in Mongolia caused an outbreak in wild gazelles (Nyamsuren et al., 2006). Parasite spillover from agriculture settings can also cause problems for nature conservation when co-introduced parasites infect vulnerable and rare species (Blitzer et al., 2012), e.g. when parasites spillover from commercial pollinators to infect wild bees (Lipa & Triggiani, 1988; Otterstatter & Thomson, 2007). These examples from terrestrial ecosystems demonstrate that more research on similar interactions between aquaculture activities and aquatic wildlife is warranted. Given that the impact of aquaculture is expected to rapidly intensify with the expanding global aquaculture production, increased research efforts into the risks of collateral diseases are urgently needed.

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AUTHORS' CONTRIBUTIONS

All authors designed the study and contributed to the writing led by M.M.B.; all authors revised the manuscript and gave final approval for publication.

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For this review article no new data have been used.

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Model-based evaluation of the genetic impacts of farm-escaped Atlantic salmon on wild populations

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ABSTRACT: Genetic interactions (i.e. hybridization) between wild and escaped Atlantic salmon *Salmo salar* from aquaculture operations have been widely documented, yet the ability to incorporate predictions of risk into aquaculture siting advice has been limited. Here we demonstrate a model-based approach to assessing these potential genetic interactions using a salmon aquaculture expansion scenario in southern Newfoundland as an example. We use an eco-genetic individual-based Atlantic salmon model (IBSEM) parameterized for southern Newfoundland populations, with regional environmental data and field-based estimates of survival, to explore how the proportion of escapees relative to the size of wild populations could potentially influence genetic and demographic changes in wild populations. Our simulations suggest that both demographic decline and genetic change are predicted when the percentage of escapees in a river relative to wild population size is equal to or exceeds 10% annually. The occurrence of escapees in southern Newfoundland rivers under a proposed expansion scenario was predicted using river and site locations and models of dispersal for early and late escapees. Model predictions of escapee dispersal suggest that under the proposed expansion scenario, the number of escapees is expected to increase by 49% and the highest escapee concentrations will shift westward, consistent with the location of proposed expansion (20 rivers total >10% escapees, max 24%). Our results identify susceptible rivers and potential impacts predicted under the proposed aquaculture expansion scenario and illustrate how model-based predictions of both escapee dispersal and genetic impacts can be used to inform both aquaculture management decisions and wild salmon conservation.

KEY WORDS: Hybridization · Atlantic salmon · Aquaculture · Management · Newfoundland

1. INTRODUCTION

Genetic interactions (i.e. hybridization) between wild and escaped Atlantic salmon *Salmo salar* from aquaculture operations have been documented across the natural range of the species where the 2 co-occur (Glover et al. 2017, Keyser et al. 2018). Escaped farmed Atlantic salmon regularly occur in both Europe and Atlantic Canada (Keyser et al. 2018, Diserud et al. 2019, Glover et al. 2019) and have been commonly found in rivers at distances of up to 200 km from the nearest aquaculture site, although distant occurrences at sea have also been reported (Hansen et al. 1993, 1997, Hansen & Jacobsen 2003, Jensen et al. 2013). As a consequence, hybridization between wild and domestic salmon can be both spatially extensive and represent a significant proportion of a population's annual production (Glover et al. 2013,

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2017, Karlsson et al. 2016, Sylvester et al. 2018, Wringe et al. 2018). Both experimental and field studies have demonstrated decreased survival of hybrids in the wild (Fleming et al. 2000, McGinnity et al. 2003, Sylvester et al. 2019), and suggest that wild population decline and genetic change are the likely outcomes of hybridization and introgression (Hindar et al. 2006, Castellani et al. 2015, 2018, Sylvester et al. 2019). As a result, genetic interactions with escaped farmed salmon have been identified as a significant threat to the persistence and stability of wild Atlantic salmon populations (Forseth et al. 2017).

In Atlantic Canada, Atlantic salmon aquaculture escapees (Morris et al. 2008, Keyser et al. 2018) and hybridization with wild individuals have been observed throughout the region (O'Reilly et al. 2006, DFO 2018a, Sylvester et al. 2018, Wringe et al. 2018). In particular, recent studies have documented widespread hybridization between wild salmon and aquaculture escapees following a single escape event that occurred in 2013 in southern Newfoundland (Wringe et al. 2018). Model-based projections following this escape event using cohort-based estimates of survival suggest negative impacts on population productivity and genetic integrity (Sylvester et al. 2019). These results are consistent with evidence of genetic changes in wild Norwegian salmon populations, which show levels of introgression as high as 47% (Karlsson et al. 2016), reductions in productivity (Fleming et al. 2000, Skaala et al. 2019), and changes in key life history traits (Bolstad et al. 2017). In Atlantic Canada, Atlantic salmon aquaculture expansion has been proposed for several regions, including those with threatened or at-risk wild salmon populations. Salmon populations in the Bay of Fundy, eastern Nova Scotia, and southern Newfoundland have been classified as threatened or endangered by the Committee on The Status of Endangered Wildlife In Canada (COSEWIC 2010), with many populations at record lows of abundance (DFO 2018a, b, 2019). Accordingly, there is a pressing need to develop approaches to predict the genetic impacts of salmon net-pen aquaculture on wild populations for use in aquaculture management and spatial planning.

Model-based approaches to explore escape events from net-pens and their impacts on wild populations allow the opportunity to evaluate escape scenarios and management decisions and are currently under development for salmonids as well as other marine species (e.g. Baskett et al. 2013). For Atlantic salmon, several models of genetic and demographic interactions among wild and farm escapees have been developed and applied, including OMEGA (ICF International 2012), IBSEM (Castellani et al. 2015), and that of Hindar et al. (2006). Of these, IBSEM, an individual-based eco-genetic Atlantic salmon life history model, has been most extensively used. Applications include understanding how the proportion of escapees scales with demographic and genetic impacts in Norway (Castellani et al. 2015, 2018), how natural straying may mitigate these impacts (Castellani et al. 2018), and how varying the strength of selection against offspring of aquaculture escapees in the wild influences population outcomes (Sylvester et al. 2019). In addition to these modeling efforts, a recent study has modeled the escape, dispersal, and survival of escapees from release sites to wild rivers in Iceland (e.g. Johannsson et al. 2017).

The combination of model-based estimates of impact with empirical data provides an unprecedented opportunity to inform management and policy decisions related to genetic outcomes for populations affected by escaped farmed Atlantic salmon. Consequently, the goal of this study was to illustrate the potential for model-based approaches to (1) predict genetic and demographic change as a result of escapees under a proposed Atlantic salmon aquaculture expansion scenario and (2) to contribute to aquaculture siting and management decisions. Specifically, the population impacts (i.e. demographic and genetic) of farm escapees were examined using IBSEM, parameterized for southern Newfoundland populations (Castellani et al. 2015, 2018). To further illustrate potential applications to siting and risk assessment, we modeled the distribution of escapees in the wild prior to and following an aquaculture expansion scenario in southern Newfoundland using a spatial model of dispersal and survival recently implemented in Iceland (Johannsson et al. 2017). This study builds directly on modeling and empirical studies from across Canada and Europe (Castellani et al. 2015, 2018, Johannsson et al. 2017, Sylvester et al. 2019) and demonstrates how consideration of genetic impacts of escapees on wild salmon populations may be incorporated into management decisions.

2. METHODS

2.1. Individual-based modeling of direct genetic impacts

Detailed modeling methods using IBSEM are described in Castellani et al. (2015, 2018) and Sylvester et al. (2019). IBSEM models wild population changes in abundance, genotype, and individual size in

response to the introduction of domesticated individuals. The model considers the duration of invasion, wild population size, number of invaders, environmental conditions, individual size, and genotypic and phenotypic differences between individuals of farm and wild origin. Growth and survival are simulated by stochastic processes that are influenced by genotype, fish size and age, water temperature, and population density at 3 life stages: embryo, juvenile, and adult. Simulated loci are unlinked with possible gamete recombination and random inheritance, and have a range of influences on phenotype and therefore performance in the environment. The sum of the genetic effects is linearly related to phenotype, such that genotypic values approaching 1 are associated with growth and survival rates typical of wild salmon, and values approaching 0 are associated with rates observed in farm escapees. Reproductive success of farm escapees is reduced relative to wild salmon, and the success of both is sex-specific, with female fertility dependent upon weight and male reproductive success dependent upon length, with the possibility of precocial sexual maturation as parr. A full list of parameters representative of Newfoundland salmon and environmental conditions in the region can be found in Sylvester et al. (2019).

Simulations utilized estimates of feral fry and parr survival calculated from genetic analysis of individual cohorts following an escape event in southern Newfoundland in 2013 (Wringe et al. 2018, Sylvester et al. 2019). These estimates of survival are lower than most previous estimates of relative survival of feral parr (McGinnity et al. 1997, Fleming et al. 2000, McGinnity et al. 2003, Skaala et al. 2019), and increasing survival in freshwater has been shown to increase both genetic and demographic impacts (Sylvester et al. 2019). We simulated the population consequences of invasion over a 50 yr period in a wild population of 500 individuals with the proportion of invaders varying from 0 to 100% of that of the wild population annually. The model simulates the accumulation of changes (i.e. allele frequency) over this 50-yr period resulting from both the continual influx of escapees and any successfully returning hybrid or escapee progeny. All models were run for 100 yr prior to invasion to ensure model stability and for 100 yr after the 50-yr invasion period ceased. We compared the change in combined adult population abundance (both wild and escaped farmed fish) and the sum of the genetic effects across the adult set of genes included in the simulation to observe changes in the genetic fitness of the population. For each iteration, we calculated the adult population abundance or allele frequency at the end of the invasion period and compared this to the mean value (10 replicates) for the no invasion scenario at the same time point. We used the mean value for the zero-invasion scenario instead of the initial starting value for the respective scenarios because at this initial time point (start of invasion period), farmed individuals are introduced into the population and thus it does not represent a baseline value.

2.2. Propagule pressure

To explore the potential changes in genetic interactions between wild and domestic salmon associated with the proposed expansion scenario in Newfoundland, we calculated propagule pressure following Keyser et al. (2018) for both the existing and proposed production regimes. Propagule pressure was calculated for each river using maximum stocking allowable at an aquaculture site (number of individuals, see below), divided by the distance from the river to that site (km), and summed across all aquaculture sites. That is:

$$\text{Propagule pressure for a given river } (R) = \sum_{i,y=1}^{S} \frac{F_{i,y}}{\text{LCD}\left(S_{i,y} \text{ to } R\right)} \quad (1)$$

where $S_{i,y}$ represents an aquaculture site (i) in a given year (y), R represents a given river, $F_{i,y}$ is the number of fish at site S_i, and LCD represents the least-cost distance function. This metric has been shown to correlate with both the occurrence of escapees and genetic interactions between wild and farm escapees in Atlantic Canada (Keyser et al. 2018).

2.3. Dispersal modeling of escapees

To model the distribution of farm escapees and to allow scenario testing, we applied a simple dispersal model that incorporates the best information on local levels of production, rates of escape, survival, behavior, environment, and size of wild populations. Details on the dispersal model can be found in Johannsson et al. (2017), but a summary is included below. Three main categories of data were considered. First, the production data were considered and included locations, biomass, size, age, and average proportion of escapees per unit harvest. Second, geographic factors considered include distribution of rivers along the coast, and any directionality of local currents. Finally, the model included any existing life history

data and behavioral differences between wild and farmed salmon. Two independent models were used, one for early escapees (i.e. smolts), and one for late escapees (i.e. adults) to allow for differences in behavior and survival among life stages. The model was implemented in R (R Development Core Team 2016) with a web-based interface.

For this analysis, we focused on 76 rivers known to have wild Atlantic salmon populations along the south coast of Newfoundland, spanning the region from Bear Cove Brook to Renews River (Fig. 1). This region has been demonstrated previously to encompass genetic impacts following escape events in the region (Keyser et al. 2018, Wringe et al. 2018). As information is generally lacking on the size of wild populations in the majority of these rivers (Porter et al. 1974, DFO 2013, 2018b), such estimates of population size were derived using an established relationship between river size and wild population size for Newfoundland following Wringe et al. (2018). River size was calculated as axial length to complete obstruction using data from Porter et al. (1974). However, as the relationship derived by Wringe et al. (2018) is based on habitat, the estimates may not reflect population declines experienced over recent decades (COSEWIC 2010, DFO 2018b) and therefore may overestimate the current population size and underestimate the proportion of escapees. In the event of any error in our initial parameters, the estimates of the proportion of escapees would be more conservative than would likely be the case in the field. Nonetheless, they represent the only available estimates of population size for most of these systems.

Reported stocking, harvest information, and licensed maximum stocking allowable from 2013 to 2017 were obtained for all existing aquaculture locations in southern Newfoundland from Aquaculture Management of Fisheries and Oceans (C. Hendry pers. comm.). For consistency among existing and proposed sites, we used the maximum licensed stocking numbers. Numbers of fish were converted to harvest biomass using an individual fish weight of 3 kg, reducing by 25% to account for fallow periods and the production/fallow cycle, and finally multiplying by 0.65, a ratio estimated from a comparison of stocking and harvest that excludes sites with catastrophic losses. The expected number of escapees per unit production is required to estimate escapees in the environment. In the absence of an extensive escapee monitoring program in southern Newfoundland, we rely on Norwegian statistics of annual production and escape events for the period 2009–2016 to estimate the expected number of escapees per ton of fish production (www.fiskeridir.no/English/Aquaculture/Statistics). However, these estimates of escapees have been shown to be an underestimate (Skilbrei et al. 2015, Glover et al. 2017); therefore, they were adjusted following Skilbrei et al. (2015) as per Johannsson et al. (2017). As a result, the estimate is ~0.8 fish per ton of production, but, given uncertainty in this value for Newfoundland, extensive sensitivity analyses were conducted to explore the effect of other values from 0.2 to 1.2.

The proportion of escapees that enter estuaries and could ascend rivers was estimated to be 17% based on Hamoutene et al. (2018), with correction for estuaries without receivers. This calculation assumes all escapees detected in estuaries will enter adjacent rivers, and although it is actually unknown what proportion of escapees in estuaries will enter rivers, es-

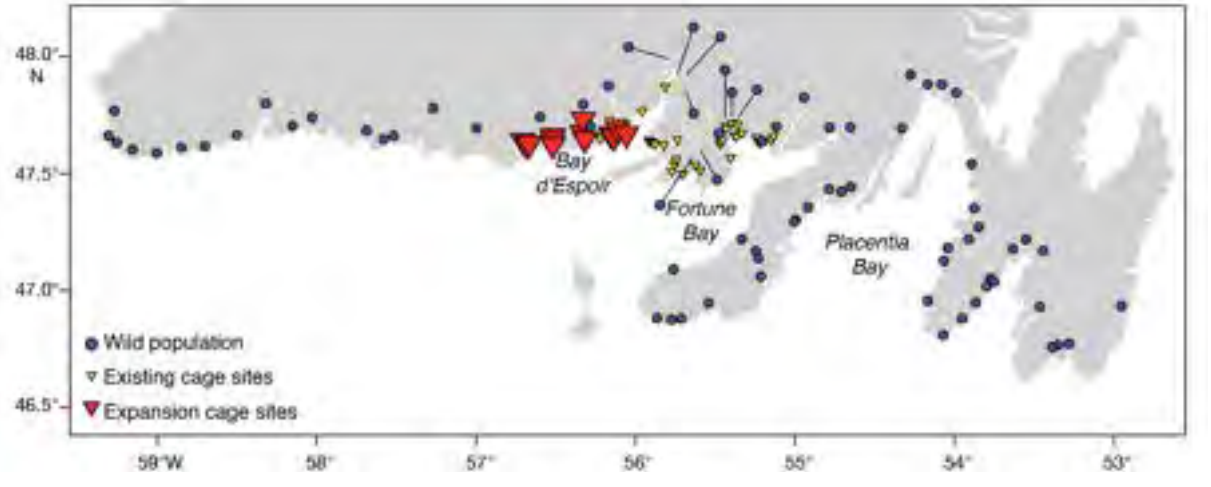

Fig. 1. Southern Newfoundland rivers known to contain wild Atlantic salmon, existing aquaculture sites, and proposed expansion sites

capees have been detected in rivers throughout the region (Hamoutene et al. 2018, Keyser et al. 2018), and 17% represents the best information at present. The proportion of escapees that are reproductively mature during freshwater entry has been estimated for the Garnish system in southern Newfoundland (located on the east side of Fortune Bay) as 63%, calculated using counting fence data for 2015–2017. This is, however, based only on individuals phenotypically identified as escapees (i.e. late escapees) at the counting fence, and, as early escapees could be undetected, this is likely an underestimate. Overall, based on the best available data, we estimate that the proportion of escapees that enter freshwater and mature is ~11%. This is comparable to a value of 15% currently in use in similar modeling exercises in Iceland (Johannsson et al. 2017).

Two models of dispersal were calculated, one for early escapees (i.e. smolts) and one for late escapees (i.e. adults), and we assumed an equal split between the 2 in absence of data on early escapees. The number of late escapees from a single site that arrive at rivers (E_G) was calculated using Eq. (2), where P is aquaculture production, S_G is the escapees per ton of production, and M is the likelihood that an escapee becomes sexually mature and enters freshwater. $\frac{R}{T}$ represents the time period (R) relative to the total time (T) in the cages that an individual could escape, survive, and sexually mature. We estimated this ratio at 0.66 as it is unlikely an escapee would survive beyond this time (i.e. 1 yr) in the wild (Hansen & Youngson 2010, Hamoutene et al. 2018).

$$E_G = PS_G \frac{R}{T} M \quad (2)$$

The total number of early escapees from a single site that make it to local rivers was calculated using Eq. (3), where S_s is the escapees per ton of production, L represents the proportion of smolts that survive at sea in the wild, and $\frac{L_f}{L_w}$ is the ratio of farmed to wild smolt survival.

$$E_s = PS_s L\left(\frac{L_f}{L_w}\right) \quad (3)$$

At present, the marine survival (smolt to adult) of Atlantic salmon in monitored rivers of Newfoundland varies from ~4 to 8% (DFO 2018b); therefore, we set a value of 6% for this exercise. The relative survival of farm to wild smolts was set at 0.37 following Hindar et al. (2006).

To simulate the dispersal of escapees from cage sites to rivers, we used a Weibull distribution shaped by 2 parameters, representing both the width and the shape or skewness of the distribution. To estimate the width of the distribution, or the distance escapees may disperse along the coast, we used a combination of experimental release data (Hamoutene et al. 2018), escapee recaptures (Keyser et al. 2018), and genetic estimates of hybridization for Newfoundland (Sylvester et al. 2018, Wringe et al. 2018). Similarly, Morris et al. (2008) reported escaped farmed salmon occurring in 56 of 62 Canadian rivers within 300 km of aquaculture operations. We set a maximum distance at 200 km, which is smaller than used elsewhere (i.e. Johannsson et al. 2017), but still larger than both tagging and genetic indications of escapee dispersal in southern Newfoundland. Modifying the shape or skewness of the distribution can allow projections to account for the influence of ocean currents, which can influence distribution patterns (Hansen & Youngson 2010). Ocean currents in the region are largely wind-driven and predominately from the northeast in winter and spring and southwest in summer and fall. Recent tagging work (Hamoutene et al. 2018) suggests no obvious east or west bias in movements along the coast. Therefore, we used a symmetrical distribution for the dispersal of both early and late escapees. See Johannsson et al. (2017) for further details regarding the spatial dispersal model.

Sensitivity analyses were conducted by varying several parameters separately and examining the resultant number and distribution of escapees in rivers under the proposed expansion scenario. First, the number of escapees per unit harvest was varied from 0.2 to 1.2 fish per ton. Second, we varied the proportion of early to late escapees from all early, equal proportions of both, and all late escapees. Finally, we varied the proportion of late escapees that mature and enter rivers from 0.06, and 0.11, and 0.16.

3. RESULTS

3.1. Individual-based modeling of direct genetic impacts

Individual-based model simulations allowed trends in population abundance and allele frequency to be examined in response to varying levels of invasion by escaped farmed salmon. The annual levels of invasion were varied from 0 to 100% of the size of the wild population (500 individuals). All runs stabilized near a wild population size of 500 individuals pre-invasion and all levels of invasion ranging from 10 to

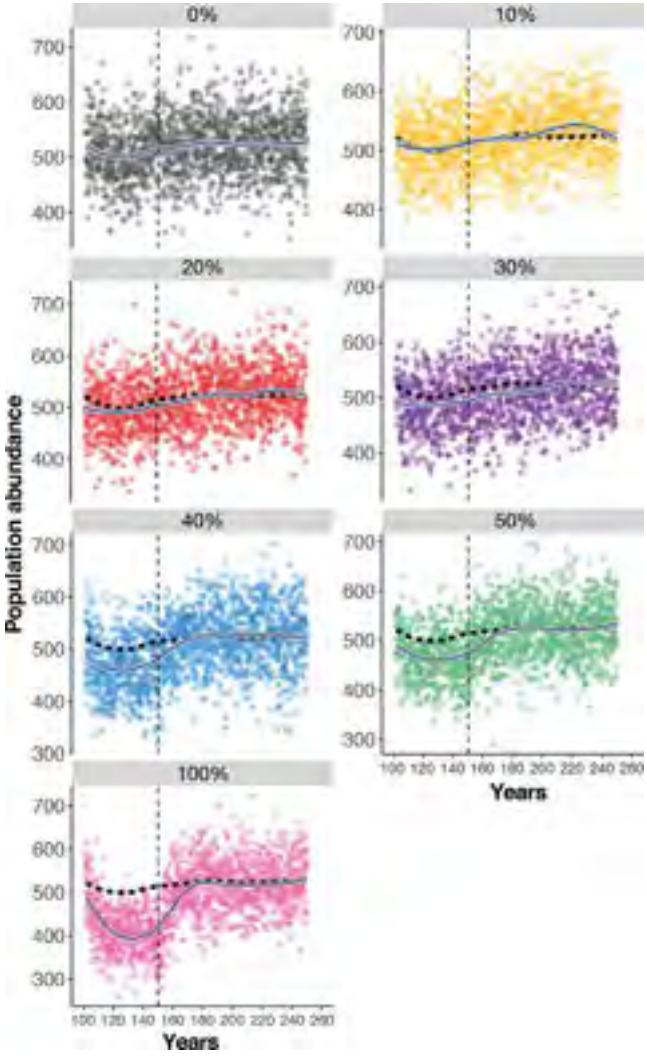

Fig. 2. Demographic changes over time during and following 50 yr of invasion by escaped farmed salmon in southern Newfoundland. All simulations were conducted using IBSEM; see Section 2 and Castellani et al. (2015, 2018), Sylvester et al. (2019) for details. Horizontal dashed line represents the smoothed line of the zero-invasion simulation with 90% CI (grey shading); vertical dashed line represents the end of simulated invasion of escaped farmed salmon. Solid blue lines represent the smoothed line of 10 replicates shown by the points. Smoothed lines were generated using the geom_smooth function in the R package gg plot2 with the loess regression and a span of 0.5

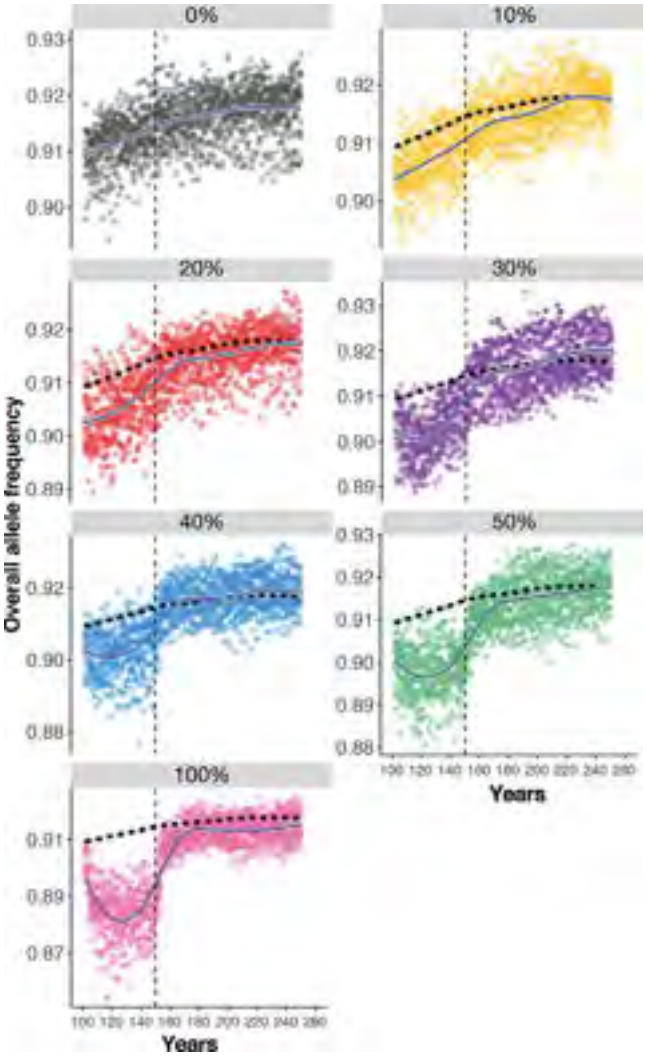

Fig. 3. Changes in overall allele frequency over time during and following 50 yr of invasion by escaped farmed salmon in southern Newfoundland. Wild populations characterized by an allele frequency of 1 and aquaculture populations an allele frequency of 0. See Fig. 2 for further details

100% displayed evidence of demographic decline (Fig. 2) and genetic change (Fig. 3) in the wild population. Overall, the magnitude of demographic decline and genetic change increased with increasing proportions of farm escapees present when compared to the no invasion scenario (Fig. 4). The magnitude of demographic decline resulting from genetic changes ranged from ~0% under no invasion to ~25% decline under 100% annual invasion (Figs. 2 & 4). The amount of genetic change predicted varied from <1% to ~3% (Figs. 3 & 4). The time to recover both population size and allele frequency once invasion ceased increased with level of invasion and varied from a few yr to 50+ yr (Figs. 3 & 4). Overall, the simulations suggest that both demographic decline and genetic change are predicted when the proportion of escapees relative to wild population size equals or exceeds 10% annually (Fig. 4). As such, a threshold of 10% escapees relative to the wild population of a given river was used as a threshold for subsequent simulations, see below. Levels of invasion between 1 and 9% were also examined but were

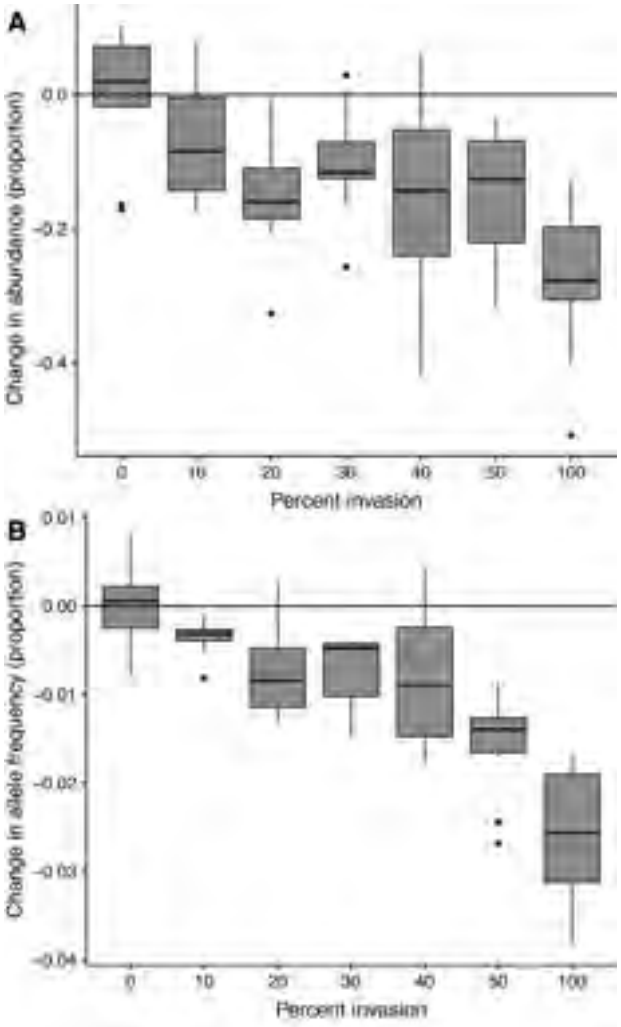

Fig. 4. (A) Magnitude of demographic decline and (B) genetic change observed after 50 yr of invasion by escaped farmed salmon into a wild population. Annual levels of invasion vary from 0 to 100% of the wild population. Changes were calculated by comparing each scenario (and iteration) against the mean of the zero-invasion scenario at the end of the invasion period. The box limits represent the third (75th percentile) and first (25th percentile) quartile, with whiskers showing the 1.5× interquartile range. The centre line within boxes represents the median and the points outside the boxes represent outliers. Each boxplot represents results based on 10 iterations for the scenario

highly variable, displayed no consistent trend, and largely did not differ from the zero-invasion scenario.

3.2. Propagule pressure

Our calculation of propagule pressure under the current magnitude and distribution of production (Fig. 1) indicates that the areas of highest expected propagule pressure are located at the head of Fortune Bay (Fig. 5). Under the proposed expansion scenario (Fig. 1), the areas of highest propagule pressure are predicted to expand to the west and include the Bay d'Espoir area (Fig. 5), where the propagule pressure is expected to at least double in 7 rivers.

3.3. Dispersal modeling of escapees

Under the existing level and distribution of production, the total number of escapees predicted to reach rivers in southern Newfoundland is estimated at 1278 individuals annually. Under this regime, 19 rivers are predicted to meet or exceed the 10% threshold, with a maximum value of 15.6% (Fig. 6). Escapees are predicted to occur in all but 11 rivers in Fortune Bay and westwards, with numbers ranging from 1 to 150 escapees per river. Rivers characterized by the largest percentage of escapees are concentrated in Fortune Bay, as well as a few Bay d'Espoir rivers (Fig. 6). Model predictions for the Garnish River suggested 13 escapees annually, which is comparable to the average of 6 escapees detected at the counting fence during the summer months annually.

Under the proposed expansion scenario, the total number of escapees predicted to reach rivers was estimated at 1915 individuals annually, which represented a 49% increase in the number of escapees predicted in rivers along the coast (Fig. 6). Twenty rivers were predicted to meet or exceed the 10% threshold, with 8 rivers exceeding 20% escapees and a maximum value of 24% (Fig. 6). Escapees were predicted to occur in all but 8 rivers in Fortune Bay and west, with numbers ranging from 1 to 275 escapees per river. Under the proposed expansion, the rivers characterized by the largest number of escapees shift to the head of Bay d'Espoir and to the west (Fig. 6).

We explored the sensitivity of the model predictions to changes in several key parameters. Research using simulated escape events in Norway suggests the actual number of escapees per ton is likely between 0.4 and 0.8 (Skilbrei et al. 2015). We thus varied the number of escapees per ton of harvest from 0.2 to 1.2. The total number of escapees doubled with each doubling of the number of escapees per harvest (Fig. 7A). We also examined how varying the proportion of late or early escapees per ton influenced model predictions (Fig. 7B). Interestingly, we observed a 2.75-fold increase in the percentage of escapees predicted to occur when only late escapees are considered versus early escapees, with estimates ranging from 2860

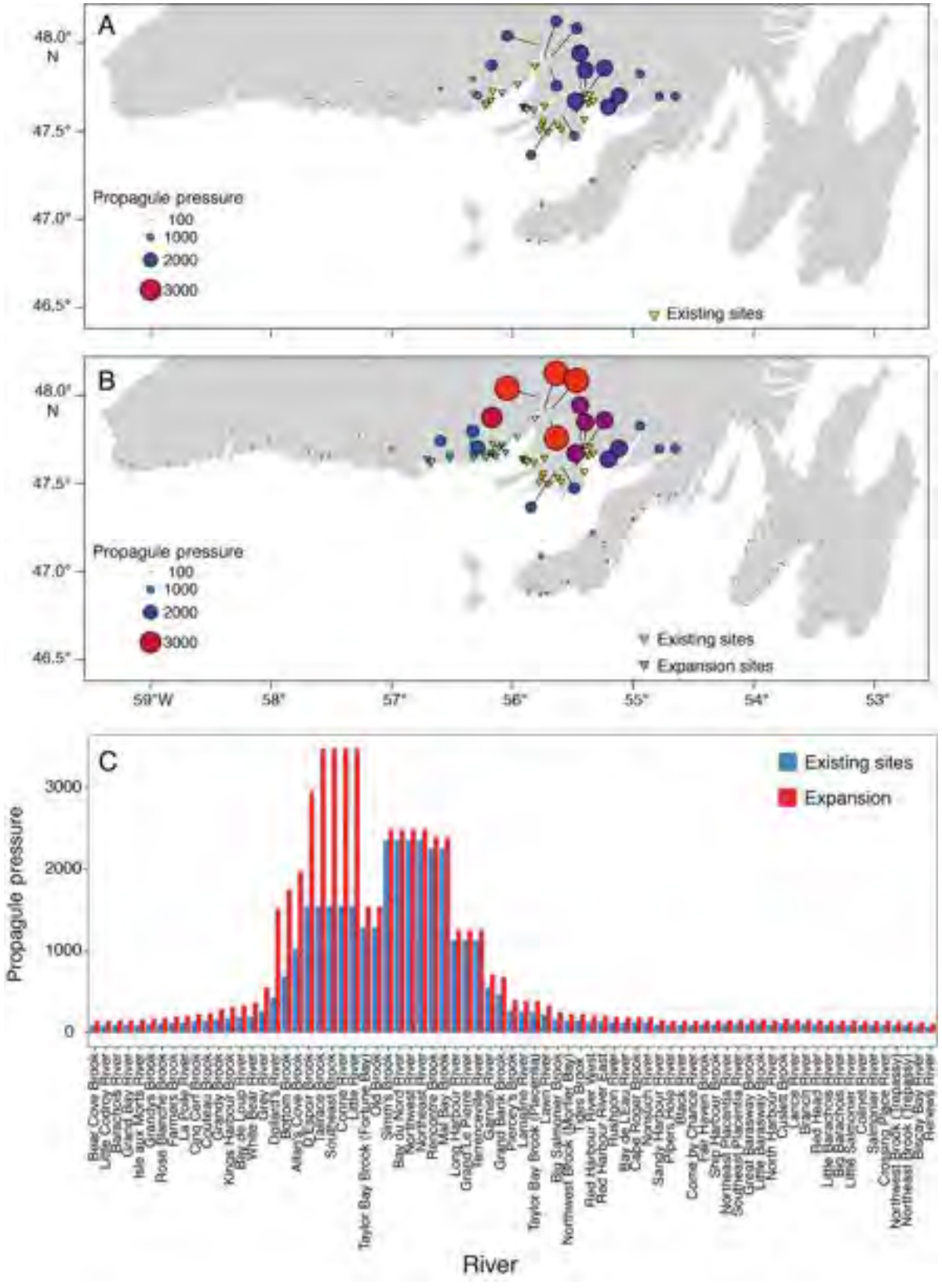

Fig. 5. Propagule pressure calculated following Keyser et al. (2018) for southern Newfoundland under (A,C) the existing production regime and (B,C) the proposed expansion scenario. See Section 2 for details. (C) Rivers are arranged west to east along the *x*-axis

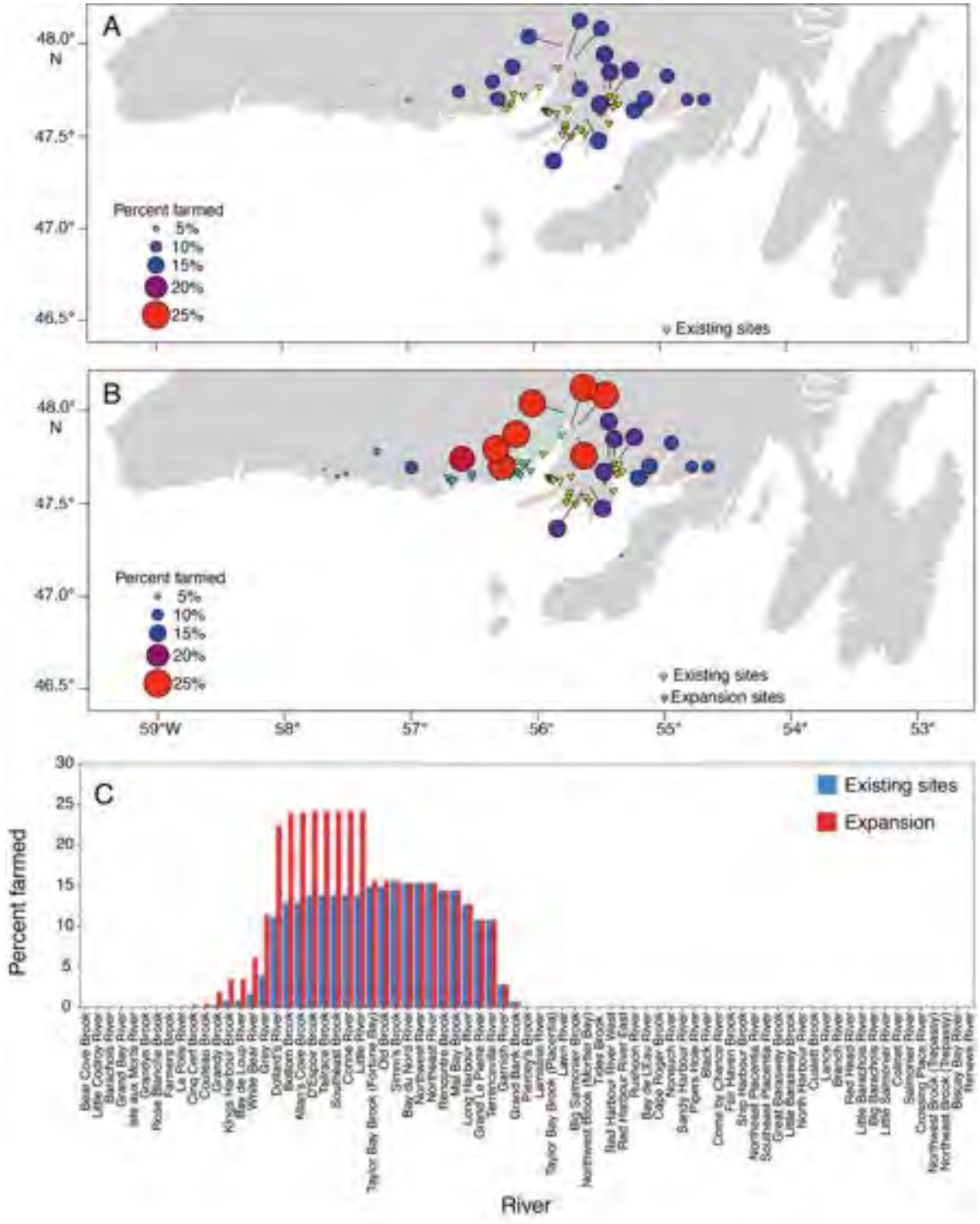

Fig. 6. Predicted spatial distribution and relative percentage of escaped farmed salmon to wild salmon for southern Newfoundland under (A,C) the existing production regime and (B,C) the proposed expansion scenario. See Section 2 for details

(late only) to 970 escapees (early only). For the late escapees only scenario, escapees were also distributed across more locations with higher percentages of escapees compared with only early escapees (Fig. 7B). Varying the proportion of late escapees resulted in numbers of escapees in rivers ranging from 1265 to 2565 (Fig. 7C). However even under the lowest probability examined (e.g. 0.06), 14 rivers were

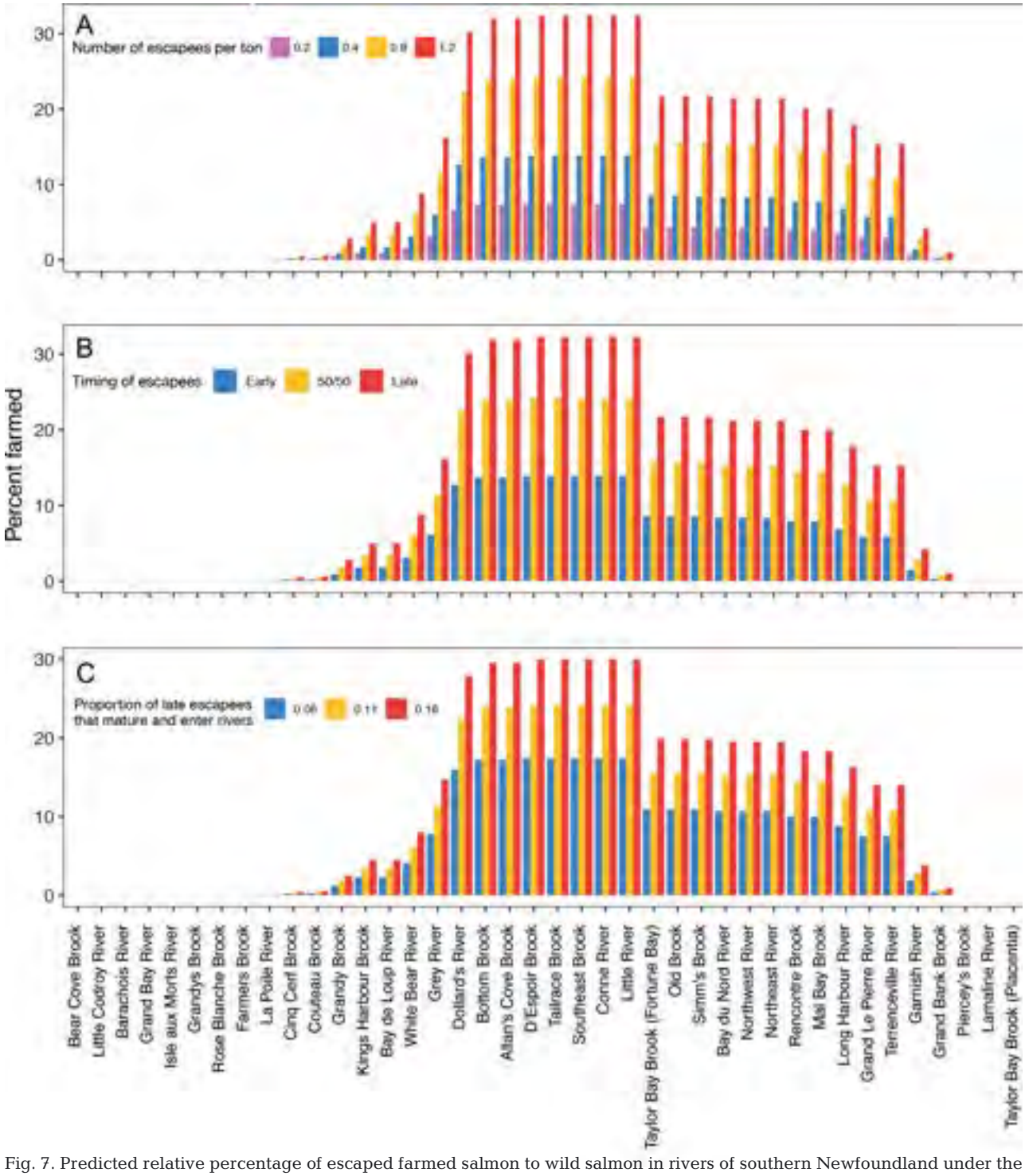

Fig. 7. Predicted relative percentage of escaped farmed salmon to wild salmon in rivers of southern Newfoundland under the proposed expansion scenario, varying (A) the number of escapees per unit harvest, (B) the proportion of early to late escapees, and (C) the proportion of late escapees that mature and enter rivers. See Section 2 for details regarding simulations. Rivers are arranged west to east along the *x*-axis

still predicted to exceed 10% escapees under the proposed expansion scenario (Fig. 7C).

Modifying the maximum dispersal distance did not significantly alter the number of escapees found in rivers overall; only the distribution of escapees across rivers (Fig. 8). At a maximum dispersal distance of 100 km, escapees were only predicted to occur in 21 rivers with a maximum percentage of 25.4% es-

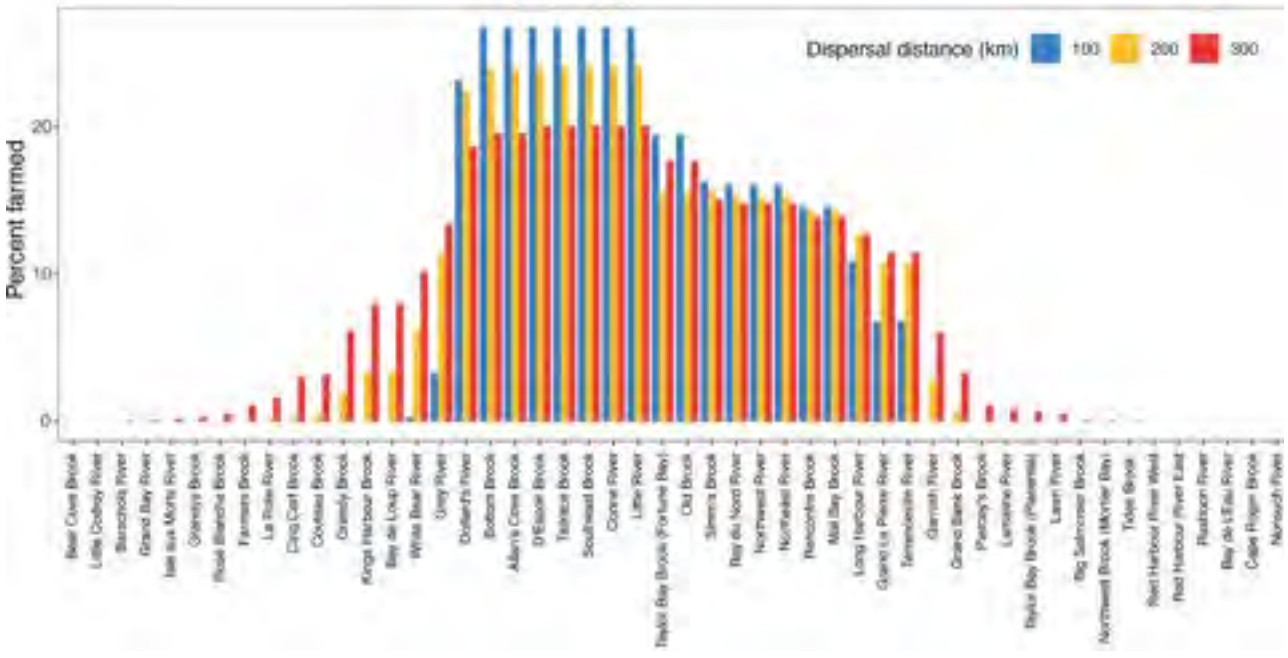

Fig. 8. Predicted relative percentage of escaped farmed salmon to wild salmon in rivers of southern Newfoundland under the proposed expansion scenario, varying the maximum dispersal distance for escapees. See Section 2 for details regarding simulations. Rivers are arranged west to east along the *x*-axis

capees. At a maximum dispersal distance of 200 km, escapees were predicted to occur in 29 rivers with a maximum percentage of 23.3% escapees (Fig. 8). Finally, at a maximum dispersal distance of 300 km, escapees were predicted to occur in 37 rivers with a maximum percentage of 19.6% escapees (Fig. 8).

4. DISCUSSION

Genetic interactions between wild and escaped Atlantic salmon have been documented both in Europe (Glover et al. 2017) and North America (Bourret et al. 2011, Sylvester et al. 2018, Wringe et al. 2018) and represent a significant threat to the persistence of wild salmon populations where they occur (Forseth et al. 2017). Nonetheless, the ability to incorporate predictions of risk into aquaculture siting advice and management decisions has been limited to date. Our goal was to demonstrate the utility of recently developed model-based approaches (e.g. Castellani et al. 2015, Johannsson et al. 2017) to predict potential genetic interactions resulting from escapees using a proposed site expansion scenario in southern Newfoundland as an example. Our individual-based simulations suggest that as the proportion of escapees within a population increases beyond 10%, both population decline and genetic change are expected, and thus allow an assessment of the risk various levels of escapees pose to wild populations. Our analysis of propagule pressure and simulations of escapee dispersal into southern Newfoundland rivers (estimated population size ~22 000 individuals, COSEWIC 2010) suggest increased numbers of escapees (49% or 1.5-fold increase) and westward shifts in the predicted distribution of escapees associated with the proposed expansion scenario. Our results directly build on previous modeling and empirical studies (Hindar et al. 2006, Glover et al. 2017, Castellani et al. 2018, Keyser et al. 2018, Sylvester et al. 2019) and directly illustrate how predictions of genetic impacts from aquaculture site expansion can be used to inform management decisions and salmon conservation.

4.1. Individual-based model predictions of impact

Population impacts of hybridization with escaped farmed salmon have been shown to vary (Glover et al. 2017, Sylvester et al. 2018) and, as such, predicting population responses to the presence of escaped farmed salmon remains a challenge. Our individual-based eco-genetic simulations suggest that demographic decline and genetic change are apparent once the percentage of escapees in rivers equals or exceeds 10%, and that the observed impacts in-

crease with the proportion of escapees. These predictions are consistent with empirical estimates of reduced aquaculture offspring survival (Fleming et al. 2000, McGinnity et al. 2003, Skaala et al. 2012, Sylvester et al. 2019) and reductions in wild population productivity resulting from hybridization with farm escapees (Fleming et al. 2000, Castellani et al. 2018, Sylvester et al. 2019). For example, Fleming et al. (2000) report a reduction of >30% in productivity of a wild population experiencing hybridization. The magnitude of the predicted demographic changes observed here varied with the proportion of escapees present in the river, but ranged from <10% to >50% decline and were generally less than 30% for most simulations over the modeled 50 yr period. The predicted genetic changes are consistent with both local evidence of hybridization and introgression in the region following escape events (Sylvester et al. 2018, Wringe et al. 2018) and recent studies suggesting significant changes to key life history traits due to introgression (Bolstad et al. 2017, Skaala et al. 2019). As these impacts scale with the proportion of escapees present, the ultimate impact to wild populations experiencing escapees may be significantly greater in small or depressed populations and existing empirical data support this hypothesis (Heino et al. 2015, Sylvester et al. 2018, Wringe et al. 2018).

A significant outcome of the individual-based modeling is the prediction that genetic and demographic impacts are likely when the proportion of escapees in a river equals or exceeds 10%. Estimates of the proportion of escapees occurring in rivers have been used as a management or conservation tool elsewhere and model predictions of population impacts of escapees can directly inform siting decisions and mitigation action. In Norway, extensive summer and autumn surveys for escapees are used to estimate an index of the proportion of escapees in rivers (Svenning et al. 2017, Diserud et al. 2019, Glover et al. 2019). Based on these surveys, the incidence of escapees in rivers is designated as clearly above or below 10% and used to prioritize rivers for mitigation action such as the active removal of escapees (Glover et al. 2019). Similarly, a recent risk assessment in Iceland opted for a 4% threshold for the proportion of escapees in rivers to provide a precautionary approach to siting as the industry develops (Johannsson et al. 2017). These values are consistent with both levels of straying in the wild (<10%, Stabell 1984, Thorstad et al. 2010) and our observations here that demographic and genetic change are likely when the percentage of escapees equals or exceeds 10%. This value of 10% escapees relative to wild salmon provides a useful metric against which to evaluate field detections of escapees and predictions of future impact.

Ultimately, although the best available regional data were used to parameterize the individual-based model, improved empirical estimates of several key parameters may improve these model predictions and any subsequent management advice. The population-specific life history and environmental data considered here were from the Conne River, which represents the best studied population/river in southern Newfoundland. Although these data are likely representative of the region, additional data from other populations would allow regional variation in demography, life history, and environmental features to be considered in model predictions. Similarly, potential key variables such as stage specific survival of aquaculture escapees and offspring have been shown to be both spatially and temporally variable (Skaala et al. 2019). Moreover, recent work suggests that population outcomes may be highly influenced by differences in the survival of escapees and hybrids (Sylvester et al. 2019) as well as rates of straying among wild populations (Castellani et al. 2018). As such, further refinement of empirical estimates of these interactions is needed to improve predictions of population outcomes. It is also worth noting that we did not vary the level of invasion annually during the invasion period, and although high annual rates of invasion (50–100%) may be unlikely for large populations, many of the populations under consideration here likely have small population sizes (<100 adults returning annually) for which these levels of invasion seem possible. Previous modeling studies have varied the levels of invasion annually and reported contrasting results, with either greater impacts from intermittent large escape events (Hindar et al. 2006) or from low level continual invasion (Baskett et al. 2013).

4.2. Predictions of escapee dispersal

Ultimately the magnitude and spatial extent of hybridization between wild salmon and domestic escapees will be dependent on the number of escapees, the scale of escapee dispersal in the wild, and the size of wild populations. Our use of a simplified dispersal kernel informed by all available data on escapee dispersal patterns suggests that under the existing distribution of production in the region, the head of Fortune Bay is likely to be characterized by the highest numbers of mature escapees entering rivers. Under the proposed expansion plan, the number of escapees is predicted to increase 1.5-fold (49%), and

the area with the highest number of mature escapees entering rivers will shift to the head of Bay d'Espoir. This shift is entirely consistent with our estimates of propagule pressure, the proposed increases in production (~50%), and the shift in location of dominant production to the area west of Fortune Bay. Although field detections of aquaculture salmon indicate regional as well as season- and size-specific dispersal patterns (Morris et al. 2008, Keyser et al. 2018, Glover et al. 2019), our model results are consistent with the emerging consensus for escapees in Atlantic Canada, suggesting they are usually found in rivers at moderate to small distances (i.e. 10s to 100s km) from escape locations (Morris et al. 2008, Keyser et al. 2018). These observations are supported by experimental releases conducted by Hamoutene et al. (2018) in southern Newfoundland indicating maximum dispersal distances of 80 km, with most salmon remaining in the embayment of release. Moreover, genetic identification of hybrids following a single escape event in southern Newfoundland detected first generation hybrids at distances of up to 100 km from the escape event (Sylvester et al. 2018, 2019, Wringe et al. 2018). Similarly, Morris et al. (2008) reported escaped farmed salmon occurring in 56 of 62 maritime rivers within 300 km of aquaculture operations.

When considering the predicted proportions of escapees to wild individuals, it is important to note that there is uncertainty in both the estimates of predicted escapees and the estimates of wild population size. The estimates of escapees per unit production used here are based on Norwegian statistics, and there is uncertainty as to their applicability to Newfoundland. Also, the estimates of wild population size used here are the best currently available for many of the rivers considered and are based on habitat-abundance associations identified using a larger geographic area. However, as stated above, these estimates may not adequately reflect recent declines in population size that have occurred in southern Newfoundland (COSEWIC 2010, DFO 2013, 2018b). As such, our predictions of the proportions of escapees in wild populations may be underestimated in some instances, particularly in small populations. Improved estimates of wild population size and the presence of escapees for rivers in the region would improve the assessment of genetic and demographic risk. It is also noteworthy that our predicted number of escapees at the Conne River (located at the head of the Bay d'Espoir) under the current production regime significantly exceed detections there to date based on the summer monitoring period. Although escapees and hybrids have been detected in Conne River (Dempson et al. 2004, Wringe et al. 2018), the proportions have generally been low even following significant escape events. The mechanism for this discrepancy is unknown at this time, but it is possible that escapees are entering the environment undetected, possibly at times outside the limited monitoring period, are being diverted to the adjacent rivers based on flow patterns in the area, or are not surviving.

The dispersal kernels used in our simulations were parameterized to provide predictions consistent with detections of escapees at the Garnish River counting fence, which is the only monitoring facility regularly detecting escapees in the region. Simulating the observed number of escapees at the Garnish River required using a maximum dispersal distance of 200 km. However, this value exceeds existing empirical estimates for the region, and therefore the model may overestimate dispersal potential in some instances. By comparison, our sensitivity analysis indicated that reducing the maximum dispersal distance to 100 km reduced the spatial scale of impact but increased the number of escapees predicted to occur in the Bay d'Espoir area under the proposed expansion, with 7 rivers predicted to experience 25% escapees. Also, we assume the influence of ocean currents in the region on the shape of the dispersal kernel is negligible. This assumption is consistent with the dominance of wind-driven flow in the area and existing tagging data of escapees in the region (Hamoutene et al. 2018).

Examinations of the sensitivity of the spatial model results were used to explore the influence of varying several parameters, including the life stage of escapees, the survival and maturity probability of escapees, and the magnitude of escapees per unit harvest produced. In all 3 cases, the number of predicted escapees increased with increased values for these parameters. It is notable that in most scenarios tested, the rivers in the Bay d'Espoir area were predicted to be characterized by >10% escapees under the proposed expansion. Overall, our use of sensitivity analyses provides invaluable insight into the scope for uncertainty in our chosen parameters to influence predictions of impact and ultimately demonstrates that our conclusions are generally robust to changes in key parameters.

5. CONCLUSIONS

Genetic impacts of escaped farmed salmon on wild populations have been demonstrated in both Canada and Europe (Glover et al. 2017), and escapees have

been identified as an ongoing threat to the persistence of wild salmon populations (Forseth et al. 2017). Our individual-based population simulations suggest that as the percentage of escapees within a population equals or exceeds 10%, both demographic decline and genetic change are expected, and the magnitude of these changes increases with increasing proportions of escapees present. Model predictions of escapee dispersal under the examined expansion scenario suggest increases and shifts in both the number and distribution of escapees in southern Newfoundland rivers, consistent with estimates of propagule pressure. In future, spatial predictions could be improved with data on escapees in the region, including the number and distribution of escapees in the wild, the proportion of early and late escapees that actually enter freshwater, and the temporal occurrence of escape events across the production cycle. Ultimately, the approaches applied here allow the identification of potential impacts predicted under aquaculture expansion and illustrate how model-based predictions of escapee dispersal and genetic impacts can be used to inform both aquaculture management decisions and wild salmon conservation.

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REVIEW

Beyond hybridization: the genetic impacts of non-reproductive ecological interactions of salmon aquaculture on wild populations

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ABSTRACT: Cultured Atlantic salmon *Salmo salar* are of international socioeconomic value, and the process of domestication has resulted in significant behavioural, morphological, and allelic differences from wild populations. Substantial evidence indicates that direct genetic interactions or interbreeding between wild and escaped farmed Atlantic salmon occurs, genetically altering wild salmon and reducing population viability. However, genetic interactions may also occur through ecological mechanisms (e.g. disease, parasites, predation, competition), both in conjunction with and in the absence of interbreeding. Here we examine existing evidence for ecological and non-reproductive genetic interactions between domestic Atlantic salmon and wild populations and the potential use of genetic and genomic tools to resolve these impacts. Our review identified examples of genetic changes resulting from ecological processes, predominately through pathogen or parasite transmission. In addition, many examples were identified where aquaculture activities have either altered the selective landscape experienced by wild populations or resulted in reductions in population abundance, both of which are consistent with the widespread occurrence of indirect genetic changes. We further identify opportunities for genetic or genomic methods to quantify these impacts, though careful experimental design and pre-impact comparisons are often needed to accurately attribute genetic change to aquaculture activities. Our review indicates that ecological and non-reproductive genetic interactions are important, and further study is urgently needed to support an integrated understanding of aquaculture ecosystem interactions, their implications for ecosystem stability, and the development of potential mitigation and management strategies.

KEY WORDS: Atlantic salmon · Aquaculture · Management · Genetic

1. INTRODUCTION

Atlantic salmon *Salmo salar* aquaculture is of international socioeconomic importance, and the process of domestication has resulted in significant phenotypic (i.e. physiological, Handeland et al. 2003; behavioural, Fleming et al. 1996; morphological, Fleming et al. 1994); and genetic (Cross & King 1983, Karlsson et al.

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2011, Wringe et al. 2019) differences from wild populations. Escape events from Atlantic salmon net pen aquaculture are a regular occurrence (Keyser et al. 2018), and the number of escapees can equate to an appreciable fraction of, or exceed, wild Atlantic salmon census size (Morris et al. 2008, Skilbrei et al. 2015, Wringe et al. 2018). There is substantial evidence that direct genetic interactions, defined as interbreeding, occurs between wild Atlantic salmon and escaped domestic individuals (Karlsson et al. 2016, Glover et al. 2017, Wringe et al. 2018) and can genetically alter wild salmon and reduce population viability (McGinnity et al. 2003, Bourret et al. 2011, Glover et al. 2013, Bolstad et al. 2017, Bradbury et al. 2020). Both in Canada and Norway, recent evidence suggests hybridization may be extensive following escape events (Karlsson et al. 2016, Wringe et al. 2018) and accounts for a substantial proportion of production in smaller rivers (Sylvester et al. 2018b). Accordingly, escaped farmed salmon and direct genetic interactions have been identified as a major threat to the persistence and stability of wild Atlantic salmon across the North Atlantic (Forseth et al. 2017, Bradbury et al. 2020).

However, genetic impacts may also occur, either in concert with or in the absence of hybridization (Verspoor et al. 2015), due to ecological interactions such as competition, predation, and disease or parasite transfer. These non-reproductive genetic changes in wild populations can result from ecological changes that either alter the selective landscape experienced by native fish, and thus change allele frequencies of loci linked to fitness, and/or reduce population abundance, resulting in a loss of genetic diversity (Fig. 1). As these effects do not involve hybridization, they can arise whether domestic animals escape or remain in containment and impact wild populations of any native species. Although practices to limit reproductive genetic interactions with wild Atlantic salmon have been implemented in many areas through the use of sterilization (Verspoor et al. 2015), exotic species, and improved containment strategies (Diserud et al. 2019), these efforts do not prevent non-reproductive genetic effects. In other species such as brown trout *Salmo trutta* or Pacific salmon species (*Oncorhynchus* spp.) where hybridization with escapees is not common or possible, ecologically induced genetic interactions with Atlantic salmon aquaculture remain an ongoing concern (e.g. Coughlan et al. 2006, Ford & Myers 2008). Moreover, given recent trends in industry expansion (e.g. DFO 2016, 2018) and growing concerns regarding the amplification of pests and pathogens such as sea lice through net pen aquaculture (e.g. Vollset et al. 2016, Karbowski et al. 2019), the potential for both ecological and non-reproductive genetic interactions is likely to increase. Nonetheless, despite the potentially broad reaching and significant impacts of non-reproductive genetic interactions on wild Atlantic salmon and other species, the evidence for their presence and our ability to quantify their magnitude has been limited to date (Verspoor et al. 2015).

The goal of this review is to highlight evidence pertaining to the potential for ecological and associated non-reproductive genetic impacts of Atlantic salmon aquaculture on wild populations. Specifically, our

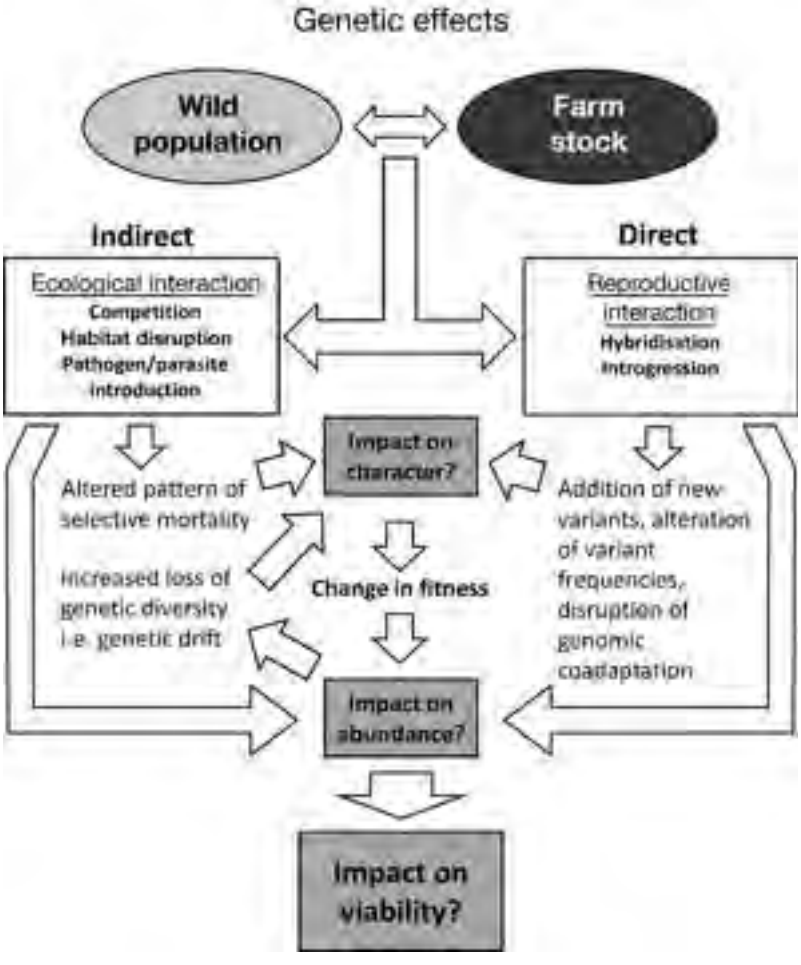

Fig. 1. Schematic of reproductive and non reproductive genetic interactions between wild and domestic Atlantic salmon *Salmo salar*

objectives are to (1) review examples of genetic changes in wild populations resulting from ecological interactions, or likely more common, evidence for changes in population abundance or the environment experienced by wild populations; and (2) discuss the opportunity recent advances in population genomic approaches present for the assessment of these genetic impacts. Through our review, we highlight opportunities for the further study of non-reproductive genetic impacts of Atlantic salmon aquaculture on wild populations. We directly build on previous reviews and empirical studies focusing on hybridization and introgression (e.g. Karlsson et al. 2016, Glover et al. 2017, Bradbury et al. 2020) and on risk assessments considering both reproductive and non-reproductive effects (e.g. Verspoor et al. 2015). Ultimately, we suggest that ecological and subsequent non-reproductive genetic impacts are likely ubiquitous wherever salmon farming occurs, and that further research is urgently required to better understand the magnitude of these interactions and provide advice regarding impact management and mitigation.

2. EVIDENCE FOR ECOLOGICAL AND NON-REPRODUCTIVE GENETIC IMPACTS

Atlantic salmon net pen aquaculture represents a substantial change to the natural environment and thus the adaptive landscape experienced by wild individuals (Garcia de Leaniz et al. 2007). As such, it can alter the stability and future evolutionary trajectories of wild populations. Furthermore, it might be expected that adjustments to a new adaptive landscape will result in reductions in productivity through increased maladaptation predicted by theoretical demographic-evolutionary models (Bürger & Lynch 1995, Gomulkiewicz & Holt 1995, Kirkpatrick & Barton 1997). Existing studies address genetic changes in naïve populations through disease and parasite transmission, the potential for recovery of disease or parasite resistance through natural selection, observations on genetic changes in co-occurring congener species, and impacts of the farming of non-native species or subspecies. Examples of the latter are the farming of European origin salmon on both the east and west coasts of North America as well as in western South America or Australia. Below we review the literature related to non-reproductive genetic interactions associated with disease and parasite transfer, increased predation pressure, and finally, increased competition (see Table 1). In each case, we first highlight examples of genetic change resulting from these interactions and then set out evidence of demographic decline or the potential for selection consistent with significant genetic impacts. In practice, it can be difficult to distinguish the impacts of reproductive and non-reproductive genetic interactions in examples related to wild Atlantic salmon. As such, here we focus on instances where mechanisms have been identified which are clearly non-reproductive in nature.

2.1. Ecological and non-reproductive genetic changes through disease transmission

Ecological and genetic interactions via disease transmission may result in both alterations to the selective landscape potentially impacting immune associated genetic variation as well as reductions in overall genetic diversity due to demographic decline. To date, few studies have examined the presence of genetic changes due to disease transfer (Table 1A). However, de Eyto et al. (2007, 2011) present evidence of genetic impacts due to novel disease exposure associated with aquaculture activities. In these studies, the progeny of Atlantic salmon from a river without previous exposure to aquaculture were transferred to a river with a long history of associated farming and captive breeding that was expected to have acquired novel micro- and macro-parasitic communities. This experimental design enabled the exposure of animals to novel disease challenges associated with escapes or inadvertent or deliberate introductions. Comparison of observed and expected genotype frequencies at a marker locus for the MHC class II alpha gene and control neutral microsatellite loci of parr and migrant Atlantic salmon stages in the wild demonstrated that genetic change had occurred, and that selection was likely a result of disease-mediated natural selection, rather than any demographic event.

A substantial and growing body of research supports the hypothesis that wild salmon populations are adapted to local pathogen communities both in space and time (Dionne et al. 2007, Tonteri et al. 2010, Consuegra et al. 2011, Kjærner-Semb et al. 2016, Pritchard et al. 2018, Zueva et al. 2018). This suggests a genetic basis for differences in population immunity and that the introduction of new pathogens into susceptible populations could both impose novel selection pressures and reduce genetic diversity through demographic decline. The possibility that pathogen transfer from domestic to wild salmon could drive genetic change in wild populations is supported by

Table 1. Summary of studies presenting evidence for or consistent with the potential for ecological and non-reproductive genetic interactions among Atlantic salmon *Salmo salar* aquaculture and wild salmonid populations. N/A: not applicable

Interaction	Primary observation	Evidence (direct or supportive)	Selection / demographic	Species impacted	Reference
(A) Disease transfer					
Common garden experiment (naïve non-local wild population introduced into different river system as eggs)	Evidence of allele frequency change at major histocompatibility (MH) genes during first 6 months in introduced population; no change in local population)	Supportive	Selection	Atlantic salmon *S. salar*	de Eyto et al. (2007)
Common garden experiment (naïve non-local wild population introduced into different river as eggs)	Evidence of different allele frequency change at major histocompatibility (MH) genes in introduced population from 6 months to 18 months; no change in local population)	Supportive	Selection	Atlantic salmon *S. salar*	de Eyto et al. (2011)
Genetic survey of natural populations (not associated with aquaculture)	Evidence of clinal geographical response in major histocompatibility (MH) genes in response to water temperature variation)	Supportive	Selection	Atlantic salmon *S. salar*	Dionne et al. (2007)
Genetic survey of natural populations potentially sensitive and tolerant of *Gyrodactylus salaris*	Evidence of clinal geographical response in major histocompatibility (MH) & other immune relevant genes in response to water temperature variation)	Supportive (possible direct link to *G. salaris* parasite)	Selection	Atlantic salmon *S. salar*	Tonteri et al. (2010)
Genetic survey of natural populations in areas with and without aquaculture activity	Evidence of spatial allele variation at major histocompatibility (MH) genes	Supportive (possible direct link to viral pathogens)	Selection	Atlantic salmon *S. salar*	Consuegra et al. (2011)
Genetic survey of natural populations in region of significant aquaculture activity	Evidence of SNP variation associated with selective sweeps of immune response genes	Supportive (source of selective agent unknown)	Selection	Atlantic salmon *S. salar*	Kjærner-Semb et al. (2016)
Genetic survey of natural populations within single large river complex (not associated with aquaculture)	Evidence of SNP variation associated with major histocompatibility (MH) genes	Supportive	Selection	Atlantic salmon *S. salar*	Pritchard et al. (2018)
Disease screening of escaped farmed Atlantic salmon in a wild river	Virus infected escaped farmed salmon entering rivers near cage sites	Supportive	Both	Atlantic salmon *S. salar*	Madhun et al. (2015)
Disease screening of returning wild Atlantic salmon in Norway at 6 sites	Evidence for the infection of wild salmon from escaped farmed salmon at marine feeding areas	Supportive	Both	Atlantic salmon *S. salar*	Madhun et al. (2018)
Genetic screening of PRV in wild and farmed Atlantic salmon	Evidence for long distance transmission of PRV likely associated with aquaculture industry	Supportive	Both	Atlantic salmon *S. salar*	Garseth et al. (2013)

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Table 1 (continued)

Interaction	Primary observation	Evidence (direct or supportive)	Selection / demgraphic	Species impacted	Reference
Review of studies documenting furunculosis prevalence in Norway from 1964–1992	Evidence for the transfer of furunculosis from fish farms to wild salmonids in Norway	Supportive	Demographic	Various salmonids	Johnsen & Jensen (1994)
Genetic screening of ISAV variants in wild and farmed salmon in Norway	Evidence for the horizontal transmission of ISAV variants seen in farmed salmon to wild populations	Supportive	Both	Atlantic salmon *S. salar*	Nylund et al. (2019)
(B) Parasite transfer					
Statistical modeling of the effect on return rates of sea lice levels (low/med/high) over a 26 year period for 1SW Erriff salmon	Wild salmon returns were strongly reduced (>50%) following years with high lice levels during smolt out-migration (farms located at the mouth of the estuary)	Supportive	Both	Atlantic salmon *S. salar*	Shephard & Gargan (2017)
Tag/recapture experiment of prophylactically treated smolts exposed to different farm-origin sea lice pressure	Recapture rate of untreated adult salmon following exposure to high sea lice density was 0.03% compared to treated salmon (1.86%)	Direct	Both	Atlantic salmon *S. salar*	Bøhn et al. (2020)
Association between sea lice counts on farmed Atlantic salmon and wild out-migrating chum salmon	Significant positive association between the sea lice abundance on farms and the likelihood that juvenile chum salmon would be infested. Increased abundance of lice on farms was not significantly associated with the levels of infestation observed on juvenile chum salmon	Supportive	Both	Chum salmon *Oncorhynchus keta*	Nekouei et al. (2018)
Experimental sea lice infection of wild brown trout post-smolts and examinations of marine migratory behavior	Experimental sea lice infection associated with increased mortality, and decreased migration distance, and marine residency	Supportive	Both	Sea trout *S. trutta*	Serra-Llinares et al. (2020)
Review paper: integrating laboratory and field observational studies of lice on out-migrating *S. salar* and *S. trutta*	Sea lice loads on out-migrating sea trout in areas with aquaculture commonly exceed threshold levels that are known to induce physiological compromise or mortality in laboratory experiments	Supportive	Both	Sea trout *S. trutta*	Thorstad & Finstad (2018)
Review paper: integrating laboratory and field observational studies of lice on out-migrating *S. salar* and *S. trutta*	Premature migratory return	Direct	Demographic	Sea trout *S. trutta*	Thorstad & Finstad (2018)

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Table 1 (continued)

Interaction	Primary observation	Evidence (direct or supportive)	Selection / demographic	Species impacted	Reference
Review paper: integrating laboratory and field observational studies of lice on out-migrating *S. salar* and *S. trutta*	Summary of meta-analysis and tagged treated smolt survival to returning adults experiment	Supportive	Both	Atlantic salmon *S. salar*	Thorstad & Finstad (2018)
Sea lice abundance on out-migrating pink salmon and chum salmon differences pre- and post-exposure to Atlantic salmon farms	Quantitative estimate of transmission rates from farm to out-migrating pink and chum salmon, including subsequent transmission dynamics of lice within the wild population	Supportive	Demographic	Pink salmon *O. gorbuscha* and chum salmon *O. keta*	Krkošek et al. (2005)
Hierarchical model of stock-recruit dynamics of coho salmon with differential sea lice infestation	Coho salmon population productivity in an area of intensive salmon aquaculture was depressed approximately sevenfold during a period of salmon louse infestations compared to unexposed populations.	Supportive	Demographic	Coho salmon *O. kisutch*	Connors et al. (2010)
Modeling effect of sea lice infections on population abundance of pink salmon	Pink salmon populations exposed to salmon farms; mortality rate caused by sea lice was estimated to range from 16 to 97%	Supportive	Demographic	Pink salmon *O. gorbuscha*	Krkošek et al. (2007)
Analysis of spawner-recruit data and sea lice abundance on farms	Sea lice counts on fish farms were negatively associated with adult returns of 2 species of Pacific salmon	Supportive	Demographic	Pink salmon *O. gorbuscha* and Coho salmon *O. kisutch*	Krkošek et al. (2011a)
Screening of pyrethroid resistance genotype in *Lepeophtheirus salmonis* over time	Widespread changes in the frequency of genotype associated with pyrethroid resistance in sea lice across the North Atlantic	Direct	Selection	Salmon louse *Lepeophtheirus salmonis*	Børretzen Fjørtoft et al. (2020)
G. salaris infection associated with wild salmon population decline	Wild stocks decreased in size by an average of 85% and smolt numbers decreased by as much as 98% following introduction of *G. salaris* into Norway	Supportive	Demographic	Atlantic salmon *S. salar*	Denholm et al. (2016)
Genomic basis of resistance to *G. salaris*	Identified 3 genomic regions associated with adaptation to parasite resistance in wild salmon	Supportive	N/A	Atlantic salmon *S. salar*	Zueva et al. (2014)
Genomic basis of resistance to *G. salaris*	Identified 57 candidate genes potentially under positive selection associated with *G. salaris* resistance and enriched for lymph node development, focal adhesion genes and anti-viral responses	Supportive	N/A	Atlantic salmon *S. salar*	Zueva et al. (2018)

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Table 1 (continued)

Interaction	Primary observation	Evidence (direct or supportive)	Selection / demographic	Species impacted	Reference
Growth and survival of sea lice infected Arctic Charr	Infection intensity correlated positively with mortality and negatively with growth in experimental trials	Supportive	Both	Arctic charr *Salvelinus alpinus*	Fjelldal et al. (2019)
(C) Predation					
Increased predation on wild species	Increased avian predation on wild salmon and brown trout following the release of captive bred smolts	Supportive	Demo-graphic / selective?	Brown trout *S. trutta*	Kennedy & Greer (1988)
Predation on released farmed escapes	High levels of predation on released farmed Atlantic salmon near cage sites	Supportive	Demo-graphic / selective?	Atlantic salmon *S. salar*	Hamoutene et al. (2018)
(D) Competition					
Competition between wild and farmed juvenile Atlantic salmon in freshwater	30% reduction in wild population productivity in the presence of farmed fish	Supportive	Demo-graphic	Atlantic salmon *S. salar*	Fleming et al. (2000)
Competition between wild and farmed juvenile Atlantic salmon in freshwater	Overlap in diet among types of crosses demonstrates competition	Supportive	Demo-graphic	Atlantic salmon *S. salar*	Skaala et al. (2012)
Metabolic rate and survival of farmed Atlantic salmon offspring	Presence of wild-farmed hybrids reduced survival of wild individuals	Supportive	Demo-graphic	Atlantic salmon *S. salar*	Robertsen et al. (2019)

several recent findings documenting the potential for exposure and supporting pathogen transfer as mechanisms for genetic impacts (Table 1A). First, Madhun et al. (2015) report the detection of virus infected escaped farmed salmon entering rivers near cage sites, suggesting clear evidence of exposure of freshwater rearing juvenile salmon populations to aquaculture associated pathogens. Second, Madhun et al. (2018) also document the presence of piscine orthoreovirus (PRV) in returning wild adult Atlantic salmon in Norway, and that the frequency of infection increased with body size and displayed no geographic signal, suggesting infection was occurring between escapees and wild salmon at marine feeding areas. Nylund et al. (2019) report that infectious salmon anemia virus (ISAV) variants in farmed salmon are increasing in prevalence in the wild consistent with horizontal transmission from farmed salmon to wild populations. Similarly, Garseth et al. (2013) examine pathogen transfer between wild and farmed salmon using analysis of protein coding sequences in PRV in Norway and suggest occurrence in the wild is due to long distance transmission likely associated with the aquaculture industry. Finally, several studies have documented the spread of furunculosis, a septicemic bacterial disease, from fish farms to wild salmonids in Norwegian rivers (Johnsen & Jensen 1994). Taken together, these findings indicate that ecologically induced genetic impacts on wild salmon populations associated with disease transmission from aquaculture populations are highly likely. However, both the magnitude of new selection pressures and demographic impacts are uncertain and likely case specific.

Diseases, introduced or increased in incidence by salmon aquaculture activities, could also have an impact on co-occurring wild species such as anadromous brown trout, as implied by the steep decline in anadromous trout numbers in many Irish, Scottish, and Nor-

wegian rivers since the late 1980s, which may be linked to sea lice infestations (see Section 2.2) associated with marine salmonid farming. A study by Coughlan et al. (2006) in some Irish rivers suggested that salmon farming and ocean ranching could indirectly affect, most likely mediated by disease, the genetics of cohabiting anadromous brown trout by reducing variability at major histocompatibility class I genes. A significant decline in allelic richness and gene diversity at the Satr-UBA marker locus, observed since aquaculture started, which may indicate a selective response, was not reflected by similar reductions at neutral loci. Subsequent recovery of variability at the Satr-UBA marker, seen among later samples, may reflect an increased contribution by resident brown trout to the remaining anadromous population. Similarly, Miller et al. (2011) link genomic profiles consistent with viral infection with increased likelihood of mortality prior to spawning in Fraser River sockeye salmon *Oncorhynchus nerka*. Morton et al. (2017) document piscine orthoreovirus (PRV) in 95% of farmed Atlantic salmon in British Columbia, Canada, and infection rates in wild Pacific salmon of 37–45% near salmon farms, and of 5% at sites distant to farms suggesting PRV transfer is occurring from salmon farms to wild salmon populations.

2.2. Ecological and non-reproductive genetic effects through parasites

Like disease transfer, the introduction of novel parasites could both impose new selection pressures and drive demographic decline. Although no examples of genetic change attributable to parasite transfer from salmon aquaculture were identified, substantial research has demonstrated the (1) transfer of parasites from aquaculture salmon to wild populations, (2) significant demographic impacts resulting, and (3) a genetic basis to resistance, all of which support the presence of genetic change occurring as a result. Examples to date have most notably been via infections of sea lice or the monogenetic trematode *Gyrodactylus salaris* (Table 1B). Declines in wild stocks attributed to sea lice outbreaks in farm-intensive areas have been documented in Ireland, Scotland and Norway. Thorstad & Finstad (2018) reviewed the literature related to sea lice impacts on wild stocks documenting 12–29% fewer returning adult spawners due to lice-induced mortality from fish farms. In one of the most extreme cases documented to date, Shephard & Gargan (2017) suggested that one-sea-winter (1SW) salmon returns on the River Erriff were more than 50% lower in years following high lice levels on nearby farms. This increased mortality was in addition to decreased returns due to poorer marine survival. Similarly, Bøhn et al. (2020) tagged and released Atlantic salmon smolts both with a prophylactic treatment against lice and without such treatment, and recaptured survivors returning to freshwater after spending 1–4 yr at sea. They report that the mortality of untreated smolts was as much as 50 times higher compared to treated smolts during sea lice outbreaks. It is worth noting that these estimates of lice-induced mortality among Atlantic salmon should be considered as minimum estimates for species such as anadromous brown trout, whose marine migrations are more coastal, thus increasing their exposure to net pen sites (Thorstad & Finstad 2018). Recent work by Serra-Llinares et al. (2020) reports increased mortality, reduced marine migrations, and reduced marine residency in brown trout experimentally infested with sea lice, consistent with significant demographic impacts of sea lice infection in brown trout. Similarly, for migratory Arctic char *Salvelinus alpinus* exposed to elevated sea lice burden due to fish farming activity (Bjørn et al. 2001), the negative impact on growth and survival may potentially lead to selection against anadromy (Fjelldal et al. 2019).

In addition to potential impacts on Atlantic salmonids, evidence also exists that the transfer of sea lice from farmed Atlantic salmon to Pacific salmon species occurs (e.g. Nekouei et al. 2018), again consistent with the potential non-reproductive genetic interactions. For example, out-migrating juvenile pink salmon *O. gorbuscha* and chum salmon *O. keta*, are estimated to experience 4 times greater sea lice infection pressure near Atlantic salmon farms compared to background infection levels (Krkošek et al. 2005), and in juvenile sockeye salmon *O. nerka*, infection rates were elevated after migration past these salmon farms (Krkošek et al. 2005, Price et al. 2011). For Coho salmon *O. kisutch*, ecological interactions with infected species, as well directly with Atlantic salmon farms, can result in higher infection levels (Connors et al. 2010). These lice infections in Pacific salmon species have also been associated with population declines. Krkošek et al. (2007) found that sea lice infestation from Atlantic salmon farms on out-migrating pink salmon smolts have led to declines in wild populations in the Broughton Archipelago, with forecasting models suggesting that local extinction was imminent. For these pink salmon populations exposed to salmon farms, mortality rate caused by sea lice was estimated to range from 16 to 97% (Krkošek et al. 2007), and population declines were also ob-

served in Coho salmon populations (Connors et al. 2010). Krkošek et al. (2011a) demonstrated that sea lice abundance on fish farms in British Columbia, Canada, were negatively associated with nearby returns of both pink salmon and Coho salmon. Furthermore, changes in parasite management on salmon farms have been shown to help reduce infection rates on wild salmon (Peacock et al. 2013), supporting this linkage and suggesting mitigation might be possible.

Given evidence of significant sea lice associated demographic declines, it seems likely that sea lice-induced mortality could drive reductions in genetic diversity. However, a large body of research suggests resistance to sea lice may have a genetic basis and be heritable (Tsai et al. 2016, Correa et al. 2017, Robledo et al. 2019), making it highly likely that wild populations would change in response to new selection pressures. In support of this hypothesis, Børretzen Fjørtoft et al. (2020) documented large-scale genetic changes in sea lice in response to chemotherapeutant usage across the North Atlantic. They observed significant temporal changes in wild sea lice populations in the frequency of a genotype associated with pyrethroid resistance due to strong selection pressure associated with its usage in Atlantic salmon aquaculture. Similarly, Dionne et al. (2009) reported significant changes in myxozoan resistance associated MHC alleles in Atlantic salmon, most likely linked with an infection-related mortality event, further supporting the potential for parasite-associated genetic impacts in wild populations.

The first appearance of *G. salaris* in Norway has been linked to the introduction of Atlantic salmon from Baltic catchments, resulting in high levels of mortality among wild populations (Johnsen & Jensen 1991). Admittedly, the spread of *G. salaris* in the wild does not seem primarily linked to salmon aquaculture. Instead, the transfer of individuals associated with stocking activities seems to have played a dominant role in transmission. Nonetheless, it is included here, as it clearly illustrates the potential for the introduction of non-native individuals to transfer parasites to local populations, the potential for subsequent significant demographic impacts, and a genetic basis to parasite resistance. In *G. salaris* infections, very high rates of mortality in naïve wild populations strongly supports the potential for significant demographic decline, losses of genetic diversity, and parasite driven selection, as has been recently concluded (Karlsson et al. 2020). For example, following several independent introductions of *G. salaris* into Norway, exposed wild populations decreased in abundance by an average of 85%, and smolt numbers decreased by as much as 98% (Denholm et al. 2016). Several studies suggest a genetic basis to *G. salaris* resistance among wild salmon populations in Europe. Gilbey et al. (2006) identified 10 genomic regions associated with heterogeneity in both innate and acquired resistance using crosses of resistant Baltic and susceptible Atlantic populations. Zueva et al. (2014) compared Baltic and Atlantic Atlantic salmon populations characterized by different levels of resistance to *G. salaris* and identified 3 genomic regions potentially experiencing parasite-associated adaptation in the wild. More recently, Zueva et al. (2018) compared salmon populations from northern Europe classified as extremely susceptible or resistant to *G. salaris*. They identify 57 candidate genes potentially under resistance-associated selection and this set of loci was shown to be enriched for genes associated with both innate and acquired immunity. These findings suggest that ecological and non-reproductive genetic impacts on wild populations associated with parasite transmission, such as sea lice from aquaculture installations, are highly likely, both because of the potential for substantial mortality to occur through exposure and for it to be selective through a clear genetic basis to population differences in resistance.

2.3. Ecological and non-reproductive genetic effects through predation

Increased predation associated with salmon aquaculture activities could result in both declines in abundance and selective mortality. Although direct estimates are lacking, some evidence exists to support the possibility of such a link, most likely through predators being attracted to aquaculture activities (Table 1C). Aquaculture sites have been shown to attract wild fish, invertebrates, marine mammals, and birds, likely due to the addition of food, and the farmed salmon themselves (see review in Callier et al. 2018), and the end result may be increased predation on wild individuals in the vicinity. Although it is possible that escapees could distract predators and reduce predation on wild populations through predator swamping, there is no evidence to date to support this. In fact, Kennedy & Greer (1988) reported heavy predation on hatchery smolts and wild Atlantic salmon and brown trout from the river Bush in Northern Ireland by the great cormorant *Phalacrocorax carbo*. This suggested a link between the release of captive bred smolts (a proxy for farm escapes), the attraction of increased numbers of these predatory birds to the river, and increased predation on the

river's wild Atlantic salmon and brown trout. Similarly, Hamoutene et al. (2018) conducted experimental releases and tracking of aquaculture Atlantic salmon near cage sites in southern Newfoundland, Canada. They found that most released fish were not detected beyond a few weeks of release, with temperature and movement data supporting predation as a cause. Increased predation of wild salmon smolts or adults near sea cages could therefore drive demographic decline or potentially act as a selective agent if predators cued on size, behaviour, or other traits. Moreover, rates of predation may be higher for individuals already experiencing infections, such as sea lice (see Section 2.2). Krkošek et al. (2011b) reported experimental evidence that predators selectively consuming infected prey which could simultaneously impose predation associated impacts and amplify disease or parasite associated selection and mortality.

2.4. Ecological and non-reproductive genetic effects through competitive interactions

Ecological and non-reproductive genetic effects have also been suggested via evidence for competitive interactions among farm and wild salmon. These competitive effects could be the result of ecological interactions among wild, farm escaped and hybrid offspring involving differences in behaviour among cross types such as in aggression, dominance, risk proneness, feeding/foraging activity. And as such, competition associated with these behavioral differences may influence survival and the selective environments experienced by wild fish. Given the clear overlap in habitat use-, and evidence for density dependence, these seem most likely to take place in freshwater during the juvenile stage (Table 1D). This has been illustrated by the work of Fleming et al. (2000), who released sexually mature farm and wild Atlantic salmon into the River Imsa in Norway. Despite the farm fish achieving less than one-third of the breeding success compared to wild fish, there was evidence of resource competition and competitive displacement, as the productivity of the wild fish was depressed by more than 30%. Fleming et al. (2000) concluded that invasions of farm fish have the potential for impacting wild population productivity both via changes to locally adaptive traits as well as reductions in genetic diversity. Skaala et al. (2012) documented similar effects in another natural system in Norway. These authors compared the performance of farm, wild, and hybrid Atlantic salmon and suggested that overlap in diets and competitions can impact wild productivity, which could reduce genetic variation in wild populations. Supporting this hypothesis, Robertsen et al. (2019) demonstrated that the presence of farmed–wild hybrids reduced the survival of wild half-sibs under semi-natural conditions. There is also clear evidence that escaped farmed salmon can compete for spawning habitats and may superimpose redds on top of those of wild Atlantic salmon (Webb et al. 1991, 1993a,b, Fleming et al. 1996). Such superimposition of redds could affect both spawning time and location of wild fish, as well as the growth and survival of wild offspring. Overall, it seems highly probable that increased competition can result in changes to the selective landscape experienced by wild individuals and in reductions in population size.

3. QUANTIFYING GENETIC EFFECTS OF NON-REPRODUCTIVE ECOLOGICAL INTERACTIONS

The studies reviewed above demonstrate strong potential for non-reproductive genetic interactions to occur in wild populations. However, quantifying these interactions between wild populations and domestic strains remains a major challenge, particularly when hybridization is occurring (i.e. direct genetic interactions). Dramatic increases in DNA sequencing capacity over the last decade present new opportunities for the use of genomic tools to quantify the impacts of net pen aquaculture on wild populations. Non-reproductive genetic interactions represent a special, more complex challenge, and the utility of genetic and genomic tools to resolve these genetic interactions will depend on the route and genomic scale of impact. That said, a large body of literature has been produced in recent years on the use of genetic/genomic tools to quantify both adaptive diversity and neutral diversity and effective population size or changes therein. As such, a clear opportunity exists to apply genetic and genomic methods to quantify these impacts.

3.1. Detecting changes in adaptive diversity

In the context of impacts due to changes in the selective landscape driven by ecological change, genomic change could be associated with a single gene, or many genes (i.e. polygenic). Genetic and genomic tools are increasingly being used to quantify the magnitude of natural selection in the wild (Vitti et al. 2013) and many approaches have been developed (Table 2A). One of the best approaches to quantify

Table 2. Summary of available genetic and genomic methods to evaluate non-reproductive genetic interactions

Method	Comparison	Statistics/tests	Reference
(A) Changes in adaptive diversity			
Time-series analysis	Changes in allele frequency	Empirical likelihood ratio test (ELR)	Feder et al. (2014)
	Changes in allele frequency	Frequency increment test (FIT)	Feder et al. (2014)
Temporal comparisons, pre- vs. post-impact	Changes in allele frequencies	Principal component analysis, outlier detection, genetic differentiation (F_{ST})	Bitter et al. (2019)
Temporal comparisons, pre- vs. post-impact	Changes in allele frequencies in response to size-selection gradients	% polymorphism, nucleotide diversity, & allele frequency shifts (controls vs. experimental samples)	Therkildsen et al. (2019)
Domestic ancestry estimation under different stocking intensities	Relationship between domestic ancestry and recombination rate at different genomic scales		Leitwein et al. (2019)
Outlier detection	Locus-specific comparison of posterior probabilities of models with and without selection	F_{ST} coefficient & and Bayes factor scores	Foll & Gaggiotti (2008)
Outlier detection	Tests of neutrality based on principal components analysis	Mahalanobis distance	Luu et al. (2017)
Impacted vs. non-impacted	Signatures of selection that covary with environmental stressor (e.g. pollution)	F_{ST}, population branch statistic, differences in nucleotide diversity along 20-kilobase sliding window	Oziolor et al. (2019)
Impacted vs. non-impacted	Signatures of selection associated with environmental stressor	F_{ST} outlier (FDIST2)	Dayan et al. (2019)
Genome-wide association studies	Polygenic associations with population decline involving genomic regions related to metabolism, developmental & physiological processes	Change in μ (signature of selective sweeps) between declining and non-declining population status of Atlantic salmon; Redundancy analysis (RDA) for detection of outliers, polygenic risk scores	Lehnert et al. (2019)
Soft selective sweeps	Identification of new alleles to intermediate frequency against a background of unusually long haplotypes of low nucleotide diversity	Integrated haplotype scores (iHS)	Voight et al. (2006)
Soft selective sweeps	Identification of selected alleles nearing or having achieved fixation in one population but that remains polymorphic in the wider group of populations.	Extended cross population haplotype homozygosity (XP-EHH)	Sabeti et al. (2007)
Soft selective sweeps	Detection of positive selection acting to increase haplotype homozygosity; combines distribution of fragment lengths between mutations and number of segregating sites between all pairs of chromosomes; ratio of haplotype homozygosity for derived & ancestral alleles.	Number of segregating sites by length (nSL); similar to iHS but (1) a genetic map is not required and (2) more robust to recombination and/or mutation rate variation	Ferrer-Admetlla et al. (2014)

Table continued on next page

Table 2 (continued)

Method	Comparison	Statistics/Tests	Reference
Machine learning	Correlates of habitat/environmental variables with observed genetic structure	Random Forest; PCA loadings; outlier detection	Sylvester et al. (2018a)
Machine learning	Detection of loci of small phenotypic effect on a key life-history variable (e.g. run timing) across multiple populations	Random forest; outlier detection; PCA	Brieuc et al. (2015)
(B) Changes in neutral diversity or effective population size			
Effective population size	Single-sample method based on linkage disequilibrium to estimate effective populations size	Contemporary N_e	Waples & Do (2010), Waples et al. (2016)
Effective population size	Single-sample method to estimate changes in contemporary N_e by comparing linkage disequilibrium estimates with recombination rates estimated from physical linkage or genomic position	Contemporary N_e estimates at various times in the past	Hollenbeck et al. (2016)
Effective population size	Application of Hollenbeck et al. (2016) for range-wide populations of Atlantic salmon and associations of genomic regions to decline status	Contemporary N_e estimates over time	Lehnert et al. (2019)

the presence of selection is either the comparison of representative pre- and post-impact genetic samples in the absence of hybridization or the examination of situations with the capacity to quantify and correct for signatures of recent or current hybridization (Leitwein et al. 2019). For time series analysis of changes in allele frequency associated with selection, differentiation measures such as the fixation index (F_{ST}) are commonly used, and several tests have been recently proposed using bi-allelic loci, including the empirical likelihood ratio test (ELRT) and the frequency increment test (FIT) (Feder et al. 2014). Recent temporal comparisons of natural selection in ecological, climate adaptation, and fishery-impact studies have revealed detectable increases in genomic differentiation over even short timeframes (e.g. 1 to 4 generations; Bitter et al. 2019, Leitwein et al. 2019, Therkildsen et al. 2019), indicating genomic tools show high power to detect changes in natural selection when recent pre-impact baselines are available. Where replicate temporal comparisons across sites can be made, this may allow uncovering parallel patterns and non-parallel signatures of adaptation. Knowledge of pre-impact genomic variation across replicates could quantify both the source and magnitude of non-reproductive genetic impacts; sites with similar starting genomic variation are more likely to show parallel responses, unless source or strength of selection differs.

In the absence of pre-impact samples, traditional tests for the presence of outliers (e.g. Foll & Gaggiotti 2008, Luu et al. 2017), trait associations, or selective sweeps (e.g. Nielsen 2005) may be applied using genome-wide polymorphism data, though the ability to attribute a given impact to these loci may be problematic. Similar to pre- and post-impact temporal comparisons, tests for genomic differentiation using metrics such as F_{ST} between sites with differing levels of exposure to stressors can be used to detect the magnitude and location of genomic change between these impacted and pristine sites (e.g. Dayan et al. 2019, Oziolor et al. 2019). Genome-wide association and genome environment association methods also show promise in measuring aquaculture impacts, but have traditionally been used to estimate correlations between genomic variants and trait or environmental variation (Rellstab et al. 2015, Santure & Garant 2018). A recent genomic study by Lehnert et al. (2019) instead used

decline status as the trait in genome-wide association and uncovered polygenic associations with population decline and variation in immune and developmental genes. This approach could be further refined in future studies by incorporating continuous measures of aquaculture exposure such as magnitude of escape, site proximity, or pathogen load.

Rapid evolutionary change is often associated with selection on standing genetic variation ('soft sweeps') rather than new mutations (Messer et al. 2016, Hermisson & Pennings 2017). Methods that utilize differences in frequency and diversity of haplotypes such as integrated haplotype score (iHS; Voight et al. 2006), extended cross population haplotype homozygosity (XP-EHH; Sabeti et al. 2007), and number of segregating sites by length (nSL; Ferrer-Admetlla et al. 2014) can identify signatures of soft selective sweeps. Identification of sweep signatures that are exclusive to aquaculture-impacted populations may provide an additional way of both validating genomic changes induced by non-reproductive genetic impacts and uncovering implicated target genes. Machine learning approaches have also shown promise in identifying subtle signatures of environment (Sylvester et al. 2018a), trait associations (Brieuc et al. 2015), and selective sweep signatures (Kern & Schrider 2018). These provide additional research areas for future studies into the genetic impacts of aquaculture exposure that may not be detected by traditional statistical approaches. Lastly, gene ontology (Rivals et al. 2007) and gene set (Daub et al. 2017) enrichment methods can be used to characterize functional impacts and parallel responses at biological levels above changes at individual genes (Jacobs et al. 2020) and can help clarify potential targets of selection from aquaculture interactions.

3.2. Detecting changes in neutral diversity or effective population size

Genomic approaches can also be applied in the context of resolving a loss of diversity due to demographic declines associated with non-reproductive genetic impacts and applied to quantify genome-wide trends in diversity over time or estimate trends in the effective population size (Table 2B; see Waples & Do 2010). Large genomic datasets offer new opportunities for enhanced estimates of effective population size (Waples et al. 2016) as well as retrospective estimates of changes in effective population size over time (e.g. Hollenbeck et al. 2016). For example, B. Watson (pers. comm.) evaluated the performance of estimates of effective population size (N_e) using large genomic datasets to assess and approximate population declines. This was used to establish a genomic baseline to detect non-reproductive genetic interactions in southern Newfoundland Atlantic salmon populations following the use of largely sterile Atlantic salmon in aquaculture. Their results suggest that large genomic datasets (≥1000 SNPs) were able to detect population declines significantly earlier, and with increased accuracy, than small genetic or genomic datasets (25 microsatellites or 100 SNPs). However, monitoring using effective size requires samples from multiple time points, which is not always possible. As an alternative, Hollenbeck et al. (2016) present a method that uses linkage information to bin loci by rates of recombination and reconstruct trends in N_e decades into the past. Lehnert et al. (2019) applied this method to Atlantic salmon across the North Atlantic and estimated that 60% of all populations have declined in recent decades. Finally, molecular approaches to mark-recapture abundance estimation (i.e. CKMR, Bravington et al. 2016) also offer the potential to quantify changes in population size over time and have been used in marine and freshwater fish species (Bravington et al. 2016, Waples et al. 2018, Ruzzante et al. 2019). Such approaches could be used to quantify population trends in effective size in the absence of assessment data and monitor for ecological and non-reproductive genetic interactions in future.

4. CONCLUSIONS

Ultimately, despite an abundance of relevant and informative research, the relative importance of hybridization and non-reproductive genetic interactions between domestic individuals and wild populations remains largely unresolved. Nonetheless, the literature suggests that ecological interactions arising from salmon aquaculture have the realistic potential to result in substantial genetic change in wild salmon populations, as well as other species. It is worth noting that, at present, there is a significant knowledge gap regarding the non-reproductive genetic impacts of increased predation or competition due to salmon aquaculture on wild populations. Fortunately, recent advances in genetic and genomic methods present a new scope for quantifying these impacts. However, careful experimental design and pre-impact comparisons will in most cases be needed to accurately attribute any genetic change to non-reproductive genetic interactions with salmon aquaculture activities.

Future research should explore the sensitivities and power of these approaches to detect changes in genetic diversity and character over time. Given that both reproductive and non-reproductive interactions co-occur within the native range of Atlantic salmon, there may be benefit to focus studies on instances where interbreeding is unlikely or impossible. This could involve the study of ecological and genetic impacts in other species such as Pacific salmon species or in Atlantic salmon in regions where sterility is employed as a containment or mitigation measure. Alternatively, genomic approaches could potentially be used to disentangle reproductive and non-reproductive interactions from indirect interactions based on the identification of hybrids, introgressed ancestry blocks, or signatures of selection.

Our review suggests that non-reproductive genetic interactions represent both a broad reaching and largely unresolved source of genetic impact on wild populations exposed to Atlantic salmon aquaculture activities. Thus, further study is urgently needed to support an integrated understanding of aquaculture–ecosystem interactions, their implications for ecosystem stability, and the identification of potential pathways of effect. This information will be essential to the development of potential mitigation and management strategies.

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Summary of Proceedings of the Symposium: What Works? A Workshop on Wild Atlantic Salmon Recovery Programs

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This document is a short summary of the symposium. A full summary of the presentations, discussions, and reccommendations can be found at http://www.asf.ca/proceedings-recovery-workshop.html.

Cover page photo by Tom Moffatt,/Atlantic Salmon Federation.

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Introduction

Wild Atlantic salmon populations in their natural range in Eastern North America have precipitously declined over the past three decades (ICES 2014). Although some of the more northern rivers have achieved conservation limits in recent years, many populations throughout the southern range are already extirpated or are on the verge of extirpation. Dozens of factors are hypothesized for the salmon's decline, some of which include chemicals, pollution, climate change, aquaculture, passage obstructions, prey availability, and predation (Cairns 2001).

Many Atlantic salmon recovery initiatives have been attempted over the past several decades with the goal to conserve, protect, and restore declining salmon populations. In many cases, programs focused on stocking to increase salmon numbers and overlooked key threats that might limit population recovery. Fifty years ago the quick answer would likely have been to produce smolts for stocking (U.S. Fish and Wildlife Service 1989; Marshall et al., 1994). Economically this may have been a reasonable approach, but the adult production and subsequent progeny may have been genetically inappropriate for long-term sustainability. Current thinking would suggest that the money should be spent on improving habitat (e.g., quality, connectivity, ecosystem health, etc.) with a smaller amount, if any, for supportive rearing programs.

In recent years, there has been a shift towards an ecosystem approach with new innovative ideas coming to the forefront (Saunders et al. 2006). Salmon are just one part of the ecosystem, other factors, including habitat, invasive species, and other diadromous fish must be considered in recovery.

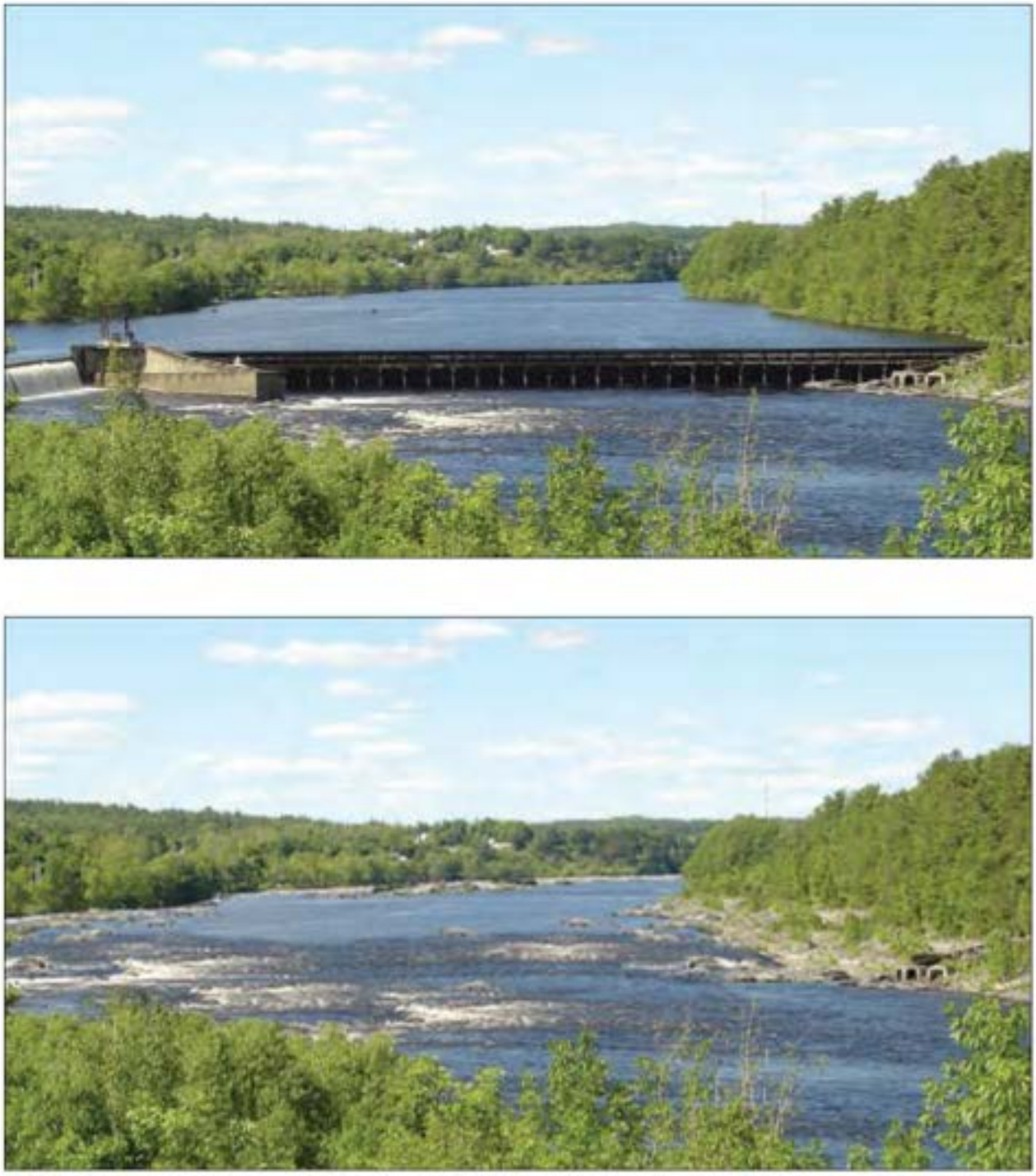
The workshop was intended as a forum for networking among river stakeholder groups, biologists, ecologists, scientists, policy makers and managers to foster collaborations and to pool all available data for wild Atlantic salmon recovery and rebuilding programs in eastern North America. The aim of the meeting was to review progress in the field, to present the latest research findings and to identify knowledge gaps, with the goal of integrating biological, socio-economic, and managerial perspectives.

Meeting Summary

The Atlantic Salmon Federation (ASF) hosted a workshop titled 'What works? A Workshop on Wild Atlantic Salmon Recovery Programs' in St. Andrews, New Brunswick, Canada from September 18-19, 2013. More than 100 people attended representing federal and provincial/state governments, First Nations, academia, river stakeholder groups, and non-government organizations (NGOs) from Canada, United States, United Kingdom, Netherlands, and France. Numerous others linked to the workshop remotely via live stream.

On the first day, the keynote address was given by Dr. Ian Fleming (Memorial University of Newfoundland, St. John's, Newfoundland, Canada) who spoke on the ecology and genetics of salmon recovery. This was followed by summaries of regional wild Atlantic salmon recovery programs in eastern North America that included population status, threats, role of hatcheries, and recovery actions. The next series of presentations focused on gene banking and life history stocking strategies. Day one concluded with presentations of case studies of various hatchery-assisted salmon stocking programs and an assessment of their effectiveness.

Throughout the keynote and session presentations on the first day, the repeated message was: stocking alone cannot produce recovery; it should not be the first and definitely not the only response to declining salmon populations in a watershed; and, when used, the goal must ultimately be to maximize wild or "wild-like" exposure in order to prevent loss of fitness. Fleming highlighted that salmon need to be adapted (population genetics) to their watersheds (ecology). He proposed that for hatchery intervention to be a success, hatchery products must be from river specific broodstock, survive, breed, and produce offspring that contribute to natural production. A stocking program that simply replaces or displaces wild production is not a success and will likely damage the wild population. As a temporary tool, management decisions to begin or end stocking hatchery products in a watershed need to be supported by data.



Artistic rendition of conditions in the lower Penobscot River prior to the removal of Veazie Dam (top) and post-removal of Veazie Dam (bottom). Expectations are that immediately below the dam site there will be minimal change, but water flow will change to follow the natural contours of the river.
Image credit: MMI Engineering.

There were 11 presentations that discussed the relative effectiveness of stocking different life stages of Atlantic salmon. Few had assessed the lifetime contribution to the population of stocking cohorts. None of the case histories told of successful hatchery based restoration of declining or extirpated populations. Each highlighted that recovery also requires addressing the threats to freshwater and marine survival to improve the chances that hatchery Atlantic salmon can contribute to future generations.

Habitat recovery actions were the focus of day two. The keynote speaker was Dr. Jamie Gibson (Fisheries and Oceans Canada, Dartmouth, Nova Scotia, Canada). He provided an overview of the role of population dynamics in recovery planning for Atlantic salmon. Population dynamics studies short-term and long-term changes in the size and age structure of populations, and the biological and environmental processes that influence those changes. His presentation was followed by sessions on habitat recovery initiatives, dams and fish passage, and water quality. The day concluded with a discussion panel based on three questions. Responses to these contributed to a workshop synthesis (conclusions).

On this day the repeated message was that habitat restoration projects need to re-establish natural stream processes and must focus on addressing the root cause of problems, not the symptoms. Most of the restoration projects described were directed at addressing the root cause of an identified problem (e.g., low pH, importance of marine derived nutrients, poor fish passage, sedimentation, human activity) and reported success (e.g. restored stream function). Small scale projects (e.g., digger logs, rock sills, deflectors) were less likely to be successful when the root causes were not identified.

Synthesizing the diverse information presented in the workshop to answer the question posed in the title ‘What works? A Workshop on Wild Atlantic Salmon Recovery Programs’ was not an easy task. One reason for this difficulty is that each person has a different idea of what the word “works” or “success” means in the context of population recovery. Recovering robust self-sustaining wild Atlantic salmon populations that could support commercial and recreational fisheries was a primary goal among attendees. Some envisioned a catch and release fishery; others a retention fishery. Regardless of this intention, where populations are currently listed as threatened or endangered, an initial recovery goal should be to recover and rebuild populations robust enough to be removed from these protections for the long-term.

Based on the data and experiences workshop participants shared, five guiding principles emerged that will assist in developing salmon recovery programs. The following guiding principles are described in more detail in the Workshop conclusions:

1.Team
2.Holistic Approach
3.Long-term commitment (funding and leadership)
4.Monitoring and evaluation
5.Outreach and communication

Conclusions

Developing a salmon restoration plan is a complicated undertaking. There are numerous factors that need to be considered from the state of the salmon resource in question, to the state of the riverine, estuarine, and marine environments as well as the societal and political factors. The complexities of these issues were clearly exemplified by the content of the presentations, posters and panel discussion associated with this workshop. There is not one clear universally agreed upon approach or menu that practitioners can apply to create a successful salmon restoration program. There are however, general guiding principles that we can recommend based on our experiences from this workshop.

Suggested Approach

In a completely natural state, Atlantic salmon survival and productivity will vary over time. Significant decreases in adult abundance due to natural variation can be interpreted as a call for concern and action. However, it is important to consider population abundance trends over some specified time-frame. Short-term population fluctuations are expected and therefore, should not carry the same weight or level of concern as long-term population declines. Maintaining long-term monitoring programs allows for the detection of these types of population trends and allows the increases and decreases to be put into historical context. It is difficult for local, provincial/state and federal agencies to maintain the funding needed for these types of programs as they often do not compete well against other short-term projects and investigations. However, maintaining these programs is essential to the responsible management of any salmon population. In the absence of long-term monitoring, contemporary field data can provide information on population status. In the absence of any contemporary data, expert opinion may be the best information available, including that provided by local and traditional knowledge. This hierarchy highlights the importance of long-term monitoring data and underscores that it is never too late to start a monitoring program.

Healthy and diverse freshwater, estuarine, and marine habitats are fundamental requirements to having healthy wild salmon populations. These provide the key elements needed for salmon survival and productivity and the basis for life history complexity within a population. Life history complexity (e.g., multiple river ages, multiple sea ages, 'early' and 'late' returns, repeat spawners, etc.) enables the development of increased population complexity. Diverse populations and ecosystems are more resilient, thereby providing greater buffering against environmental variation. When population diversity decreases it can lead to increased annual fluctuations in returning salmon and a higher probability of major population declines (Schindler et al. 2010). Long-term population declines and loss of life history and ecosystem diversity can often be caused by anthropogenic (i.e., human induced) impacts on aquatic communities (e.g., out of balance predator-prey relationships, declining co-evolved diadromous complex, excessive indirect or direct harvest etc.), habitat conditions (e.g., decrease water quality and quantity, decrease habitat quality and quantity etc.) and/or connectivity (limited access to the full suite of habitats types needed). Therefore, the first principles of any recovery program will need to be founded on the restoration and protection of habitat and ecosystem functions combined with sound management based on population monitoring.

For the reasons above, the process of developing a salmon restoration plan is complicated and there is no one template available that will fit all possible situations. The development of an effective restoration program for Atlantic salmon requires:

- An understanding of the problem;
- A clear statement of desired outcomes;
- An evaluation of available options; and
- A long-term commitment to the program.

The following flow chart is intended to provide guidance on the steps that should be taken when assessing the status of the salmon population and habitat in the watershed, both of which are essential components for the development of an effective restoration plan:

*Gibson (see Section 5 in full report, http://www.asf.ca/proceedings-recovery-workshop.html) provided clear examples of how population modeling can allow scientists and managers to investigate 1) how the dynamics of the populations have changed, resulting in the population decline and 2) how populations would be expected to respond to specific recovery actions based on those dynamics. Understanding the impacts of threats to the population through these types of modeling effort are absolutely essential to effective and efficient restoration planning. Following the above process will aid managers in determining what root-cause problems are affecting the productivity of the salmon population(s) they are focused on so that suitable plans can be developed to address them.

Stocking

For many years, stocking has been used as the default method of countering low fish numbers. However, stocking has often resulted in unforeseen consequences (e.g., deleterious genetic changes resulting in loss of wild traits) and as such, must be very carefully considered before incorporating into a restoration or recovery program. Otherwise, the "stock first" approach is knee-jerk and could eventually inflict more harm than it does good for the population under recovery. Hatcheries were originally thought of as a "techno" fix to the problem of declining salmon populations. Instead of analyzing and fixing the habitat problems and/or reducing the excess harvest of adult spawners, hatcheries were designed to simply increase the number of salmon available. This practice often simply disguised the problems limiting production. The flow chart above will focus the manager's attention on the task of identifying the limiting factors for the population. Unless the factors limiting the population are identified and mitigated, stocking will not achieve population recovery.

Through continued research and innovation of hatchery and rearing practices, our understanding of how to effectively use and manage hatcheries is continually growing, but remains far from complete. There are significant ecological and genetic risks associated with the use of hatcheries. Salmon stocks were once viewed as interchangeable (i.e. transferrable from one region or watershed to another), which is in contrast to the contemporary knowledge of unique populations within and among rivers.

Despite these concerns, the use of hatcheries to rear Atlantic salmon for stocking may be justified in some cases. A clear example for hatchery intervention is when populations are in danger of extirpation. In other situations stocking should only be considered after all available fishery management measures have been exhausted and a full understanding of the threats has been developed (see figure above) and actions have been undertaken to improve habitat quality and quantity, and fish passage. Simply put, stocking fish into poor habitat and/or areas with poor fish passage will likely yield few, if any, benefits toward recovery.

If stocking is to be considered as part of the overall recovery plan, it is important to have an understanding of the goals and timelines for hatchery intervention. There are a number of guiding principles that should be considered for hatchery intervention:

- First, consult with population dynamics and genetics experts to fully understand the pros and cons of the proposed effort.
- If the objective of the program is recovery of wild populations, then human intervention should be minimized so as not to interfere with natural smolt recruitment processes.
- Criteria for initiating and ending a stocking program should be predetermined.

SPAWNING AND REARING

- Use local wild broodstock if available.
- Use a large number of randomly selected breeders (e.g., mix sizes of fish), unless demographic or genetic criteria indicate otherwise.
- Obtain a representative genetic composition to balance the demographic gains with genetic diversity. Minimize time spent in the hatchery.
- Maximize wild or “wild-like” exposure.
- Alter artificial rearing environments to promote fish traits that may be more favorable in nature.
- Wild exposure of hatchery products can improve short (within generation) and long term (transgenerational) success of artificially reared fish.

RELEASES

- Identify and fix limiting factors that may impede survival at each life stage and plan releases accordingly.
- Carefully consider the most appropriate choice of life stage to be stocked, based on the tenets of minimizing hatchery involvement and maximizing wild exposure.
- Long term monitoring is essential to understanding long-term contribution of the stocked fish and therefore to measuring success (egg to at least F1 generation).

AND REMEMBER THAT:

- Stocking should be considered a temporary tool.
- Stocking should not inhibit other restoration/recovery measures.
- Stocking, by itself, will not be sufficient to recover/restore populations.

Recommendations

The information presented at this workshop demonstrates the significant progress that has been made in our knowledge of wild Atlantic salmon recovery and restoration programs. In this workshop there were a series of presentations that described advantages and disadvantages of various hatchery techniques, stocking strategies, habitat restoration and fish passage improvement methods. The workshop presentations did not span the full range of human intervention but highlighted various approaches along the spectrum. Some techniques showed promise, but in all cases hatchery intervention alone did not result in recovery.

For many years fisheries professionals have focused on monitoring for the primary purpose of assessing stock abundance. Stock restoration and enhancement techniques were often undertaken without a firm understanding of the full suite of threats in the watershed; the effect of these on the population; and the risks, limitations, and benefits associated with particular recovery actions. The lessons highlighted and demonstrated within this workshop show the benefit of, and our progress towards, moving away from this paradigm.

The existing approach to resource management often has not achieved long term conservation goals. Decisions have been made based on short term government priorities and the needs of dominant stakeholders, and are not always fully science-based. This often leads to short term band aid approaches (e.g. stocking) rather than addressing long term management of habitat and harvest. These approaches need to change. More stakeholders (NGOS, recreational anglers, scientists, First Nations) need to become involved to create an active and committed decision making body to develop locally tailored solutions.

The lessons highlighted within this workshop are not unique to salmon recovery initiatives. They are reflective of the general evolution towards an ecosystem approach to natural resource management and restoration. There are many other recent examples of ecosystem and holistic based natural resource management, which can be helpful guides when developing an Atlantic salmon management plan. For example, Palmer et al. (2005) proposed five criteria that could be used to measure the success of river restoration projects. These criteria help bring an ecological perspective to processes of river restoration. Given that salmon restoration and river restoration activities often overlap (Fleming, refer to full report), the criteria proposed by Palmer et al. (2005) may provide a solid foundation for both evaluating the potential effects of proposed salmon restoration actions, as well as the outcomes of salmon restoration efforts post-implementation.

The five criteria proposed by Palmer et al. (2005) are summarized below:

1. There should be a specific guiding image of the restoration effort under consideration that envisions a more dynamic and healthy state than currently exists;
2. The ecological condition of the system/population must be measurably improved;
3. The population should be more self-sustaining and resilient to external perturbations so minimal follow-up is needed;
4. No lasting harm should be inflicted; and
5. Both pre- and post-assessment activities must be completed and data must be made publicly available.

This workshop focused on the science and management of Atlantic salmon, with particular emphasis on the biology and ecology of the species and new techniques in restoration. However, the successful restoration and management of the species will involve a full suite of additional considerations such as regional economics, the available resources (e.g. fiscal, standing stock, infrastructure, etc.), and political and societal views of the effort. The development of an effective management and or restoration plan for the species will require that all of these additional factors be taken into account.

It is impossible for us to suggest a recovery plan that would meet the needs of your watershed and salmon population. The particulars of what you are dealing with within your watershed (e.g., population status, habitat status, politics and local engagement) will determine the best course of actions. We can, however, suggest a number of building blocks or principles that should form the foundation of any recovery plan. Below we present five guiding principles.

1. Team

a. The foundation of a recovery plan requires a solid and committed team to create a local decision making body.

b. A 'champion' (individual or organization) needs to be identified as project leader.

   i. Teams need a good leader, someone who has passion for the watershed, restoration tasks, and can leverage the strengths of each member to ensure the work identified as needed by the team is accomplished. Finding effective leaders is no simple task, but is essential to success.

c. The team should consist of a diverse group of stakeholders (e.g. NGOs, First Nations, recreational anglers, scientists, and watershed users), government officials (i.e. science and management) and policy makers (i.e. elected officials).

d. Partnering allows for the pooling of resources, increases funding options and allows for the addressing of critical questions at a broader level.

e. Team members must share knowledge, discuss options for best recovery strategies, and work together to plan and prioritize projects using science based decision processes that include and take into consideration local and traditional knowledge wherever possible.

f. The team must meet regularly to review progress (e.g., stock status re ports, research projects, etc.) and determine best management options

2. Holistic Approach

It is now generally recognized in conservation circles that any given population cannot be recovered in isolation of other co-existent native fish populations and ecosystem circumstances, nor is there much chance at recovery if the strategy is to address symptoms as opposed to root cause issues. As such, we suggest that any recovery strategy must take a holistic approach, taking into consideration the following:

a. Need to take a multi-species and ecosystem-wide approach if you want to achieve the best chance of salmon recovery (e.g., status of population in nearby rivers/watersheds, status of other native fish communities).
b. Must identify and understand the root cause(s) of limiting factors and how they relate to the entire ecosystem.
c. Coupling salmon restoration interests with those of the diadromous species complex will ensure that:
    i. The salmon's long-term interests are represented;
    ii. Actions taken will provide greater benefit to the entire ecosys tem that supports wild Atlantic salmon;
    iii. There is a broader ecosystem recovery potential; and
    iv. An expanded potential resource pool is available to support restoration efforts.
d. Practical, management plans should be developed for each watershed. A practical management plan accurately characterizes the status of the salmon resource as best as can be accomplished with combined scientific, local and traditional knowledge. It will also characterize the effects of individual threats allowing managers to identify and prioritize restoration actions on a watershed by watershed basis.
    i. Specific issues/threats are often not limited to a single tributary, but rather are occurring within the larger watershed. For example, conducting targeted stream bank restoration pro grams to address localized erosion issues often only serve as applying "band-aids" on issues that are symptomatic of larger scale issues that should be addressed.
    ii. This should not be considered an indictment of in-stream work. It can often provide important short-term benefits. However, the larger watershed level issues (i.e. the root causes) must be properly identified and addressed to support a long term solution so as to avoid or prevent similar problematic symptoms in the future.
e. Prioritizing actions should occur independently of fiscal concerns, and perhaps more importantly political concerns.

f. A multilevel approach is needed: (local, regional, national, international).
    i. Local groups should focus efforts in freshwater and estuarine areas, i.e. areas within their sphere of influence.
    ii. Larger efforts (e.g., marine mortality) must be taken on by larger entities, with the support of local groups.

g. The causes of marine mortality and an understanding of post-smolt to adult migration behavior and mortality (where, when, and how), including indirect bycatch and directed harvest, must be identified. Increase support to study marine mortality using the state of the art technologies.

h. Productivity limitations caused by low marine survival should not be considered a reason to prevent freshwater actions. One of the fundamental goals of any recovery effort should be to improve or maximize fresh water production of highly fit juvenile salmon to help offset the effects of high marine mortality.

3. Long-term commitment (funding and leadership)

a. Any recovery effort requires a long term commitment by the team involved.

b. Clear goals and timelines (e.g., start and end dates) must be defined for each phase of the project.

c. Performance measures must be established for each phase of the project.

d. Funding sources must be confirmed and reviewed periodically.

4. Monitoring and evaluation

a. Monitoring and evaluation must be fundamental components of any recovery program.

b. There must be a clear understanding of the project purpose, experimental design, and performance measures when designing a monitoring program so that the outcomes of the recovery effortcan be understood and adjustments can be made as necessary.

c. Spatially and temporally representative monitoring of all restoration efforts is needed to assess effectiveness.

d. Thorough monitoring and evaluation of a recovery program can take multiple generations, extending well beyond the time frame of the recovery actions (it takes 4 to 8 years to complete a single salmon generation from egg to returning adult).

5. Outreach and communication

a. Recovery and management plans that are based on science and local/traditional knowledge must be communicated to policy makers and politicians.

b. The science and management information needs to be transferred to policy makers and politicians.

c. A collective vision (from the team) would help inform and influence decision makers (i.e. elected officials) and others (e.g., industry, philanthropist foundations who can influence policy and funding actions).

d. Documenting and sharing lessons learned from failed restoration programs is just as important as for successful programs to prevent future failures.

e. Ultimately, political will is needed to accomplish on the ground recovery actions, and this of course depends entirely on the presence of a strong team with strong leadership.

One final thought

There are no guarantees that a holistic recovery program that addresses multiple threats within a watershed in support of either a wild population, or a live gene banking program, will be successful in recovering salmon. However, by ensuring that freshwater habitat is as productive as possible, it puts the watershed and its salmon population in a better position so that the chances of recovery are improved.

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Farmed salmonids drive the abundance, ecology and evolution of parasitic salmon lice in Norway

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ABSTRACT: Sea cage fish farming is typically open to the environment, with disease transmission possible between farmed and wild hosts. In salmonid aquaculture, salmon louse *Lepeophtheirus salmonis* infestations cause production losses, reduce welfare for farmed fish and increase infestation rates for wild fish populations. The high density of hosts in farms likely also shifts the coevolutionary arms race between host and parasite, with ecological and evolutionary consequences for the salmon louse. Using farm-reported salmon and louse abundances and publicly reported estimates of wild salmonid host abundances and the salmon lice they carry, we estimated (1) the relative abundance of farmed and wild salmonid hosts and (2) the relative importance of each for the abundance of salmon lice for the coastal zone of Norway from 1998 to 2017. Farmed hosts increased in importance over time with the expansion of the industry. From 2013 to 2017, farmed salmonids outnumbered wild salmonids by 267–281:1. By 2017, farmed salmonids accounted for 99.6% of available hosts and produced 99.1% of adult female salmon lice and 97.6% of mated (ovigerous) adult female salmon lice in Norwegian coastal waters. The persistent dominance of farmed hosts has clear implications: (1) management decisions that aim to limit lice abundance can be guided by lice data from farms alone, as lice on wild salmonids make a trivial contribution to the national lice population; and (2) strategies to prevent or treat lice infestations are vulnerable to the evolution of resistance, as the pool of wild hosts is inconsequential and will not act as a refuge large enough to stem the evolution of resistance. As the Norwegian salmon industry expands and salmon lice infestations continue, farmed salmon will drive the ecology and evolution of salmon lice.

KEY WORDS: Aquaculture · Host–parasite coevolution · Host availability · *Lepeophtheirus salmonis* · Resistance · *Salmo salar* · Sea lice

1. INTRODUCTION

Industrial aquaculture is a relatively modern phenomenon (Duarte et al. 2007), with most farmed species still at the lowest level of domestication (Teletchea & Fontaine 2014) and held in systems that are open to the environment, leaving stock vulnerable to pathogens. Once present, the high density of hosts within farms facilitates rapid pathogen propagation, and as a result, severe outbreaks have occurred for almost all fish species cultured in open systems (Kent 2000, Bondad-Reantaso et al. 2005).

Parasites are a key concern for fish farming, leading to production losses and poor welfare for billions

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of animals either directly (Barber 2007) or indirectly via the side effects of control measures (Overton et al. 2019). Moreover, because industrial farming increases the abundance of farmed hosts, infestations on farmed fish can have serious consequences for wild fish populations; the large number of hosts within farms typically amplifies the number of infective stage parasites that flow to the wider environment, spilling over to increase infestation in wild fishes (Krkošek et al. 2013, Serra-Llinares et al. 2016, Bouwmeester et al. 2021). Artificial conditions within farms also establish new settings for the coevolutionary arms race between host and parasite, with ecological and evolutionary consequences for parasites as they adapt to farmed fish and farming practices (Mennerat et al. 2010, Kennedy et al. 2016, Nowak 2007, Coates et al. 2021).

Salmonids (principally Atlantic salmon *Salmo salar* but also rainbow trout *Oncorhynchus mykiss*) are the most produced fish in the marine environment, with a global production of ~3 million t yr^{-1} (FAO 2020). The largest producer is Norway, where nearly 700 farming locations in coastal waters hold >400 million Atlantic salmon and rainbow trout in open sea cages (Norwegian Directorate of Fisheries 2020a). Rapid expansion since the birth of the industry in the 1960s has fundamentally shifted the transmission dynamics for ectoparasitic salmon lice *Lepeophtheirus salmonis* (Caligidae) in the Norwegian coastal zone, with important implications for the ecology and evolution of wild salmonids as well as salmon lice (Torrissen et al. 2013). Rapid evolution of louse life history traits has already occurred in Norway (Mennerat et al. 2017), along with resistance to multiple common delousing chemicals (Besnier et al. 2014, Aaen et al. 2015). Strategies that seek to prevent infestations from occurring may also be vulnerable to the evolution of resistance if farmed salmon make up a sufficient proportion of available hosts for salmon lice (Barrett et al. 2020, Coates et al. 2021).

High lice loads on wild salmonids during the 1990s in Norway were partly attributed to salmonid farming and prompted the introduction of maximum lice thresholds in farms (updated legislation: Norwegian Ministry of Trade and Fisheries 2012), together with annual monitoring programs designed to track lice loads on wild Atlantic salmon, Arctic charr *Salvelinus alpinus* and wild brown sea trout *Salmo trutta* (Bjørn et al. 2001, 2002, 2003, 2005, 2007, 2008, 2009, 2010). Simultaneously, a Norwegian salmon lice dispersal model was developed to complement these efforts by predicting infestation pressure based on lice abundance in farms and environmental parameters that influence the dispersal of lice larvae (Asplin et al. 2004, 2014, Johnsen et al. 2014, Sandvik et al. 2016). Predictions from the lice dispersal model are used to calculate likely infestation pressures on outmigrating salmon in spring, and this contributes to the evidence an expert panel uses to set limits on farmed biomass for each farming region (the 'traffic light' system) with the goal of minimising infestation pressure on wild salmonids (Norwegian Ministry of Trade and Fisheries 2017, Myksvoll et al. 2018, Johnsen et al. 2021). Lice dispersal model predictions have generally mapped closely to observed infestation pressure in sentinel cages (Sandvik et al. 2016, 2020) and on wild salmon (Myksvoll et al. 2018), and they do this without accounting for lice larvae that arise from wild hosts (e.g. Johnsen et al. 2014, Skarðhamar et al. 2018). Soon after the legislation was passed to first set lice limits on farms, Heuch & Mo (2001) modelled salmon lice egg production under past and future scenarios and suggested that as early as 2001, farms were responsible for most louse eggs produced.

Since Heuch & Mo's (2001) initial salmon lice production estimate, farmed salmonid numbers have increased 2.4 times in Norwegian coastal waters, and ~20 yr of lice density data have been collected from farmed and wild salmonids. Here, we use publicly available data on wild and farmed host numbers and reported lice abundances on farmed and wild hosts to estimate the relative importance of farmed and wild hosts as reservoirs for salmon lice over the last 2 decades.

2. METHODS

For each year from 1998 to 2017, we estimated the proportion of total host and louse populations contributed by hosts and lice within farms (P_H and P_L, respectively). These metrics for the relative importance of farmed salmon hosts account for the seasonal dynamics of fish movement by only counting fish (and lice) that are in the coastal waters during the given year. Lice transmission is considered negligible in offshore locations, and lice cannot survive in freshwater rivers. To make these estimates, we obtained data allowing us to calculate 13 variables describing the mean abundance per fish for farmed and wild salmonids, as well as their seasonal usage of coastal waters. H denotes host numbers (Atlantic salmon *Salmo salar* and rainbow trout *Oncorhynchus mykiss*), L denotes mean number of adult female lice per host, and T denotes the proportion of time each host spends in coastal waters. Arctic charr *Salvelinus*

alpinus were considered of negligible importance following Heuch & Mo (2001), as while they are vulnerable to salmon lice infestation, seagoing individuals are rare in all but the northernmost part of the study area. In addition, they stay in the sea at low temperatures and for short durations, (<6 wk) so that any lice they catch seldom develop into adult females (Klemetsen et al. 2003).

The 13 variables we used were as follows:

(1) H_F: Average number of farmed salmon and rainbow trout hosts in the sea throughout each year. Monthly number of fish data for 2005 to 2017 were available from the Norwegian Directorate of Fisheries, but monthly data prior to 2005 were not. Therefore, we used the number of farmed salmon and rainbow trout sold as a proxy for the number farmed each year prior to 2015 (Norwegian Directorate of Fisheries 2020a). However, not all fish that are farmed are sold, so a correction factor was necessary. We estimated the correction factor by dividing the number of salmon or trout farmed by the number sold, for each year in 2005 to 2017 (a period for which both data sources were available). On average, there were 1.22 fish farmed for every fish sold, so the annual estimates for 1998 to 2004 were multiplied by 1.22.

(2) H_{ES}: Number of salmon that escaped from farms, based on data from the Norwegian Directorate of Fisheries (2020b). Research indicates that the total number of farmed salmon escapees is between 2 and 4 times higher than the numbers reported to authorities (Skilbrei et al. 2015). Therefore, we multiplied the reported number of escapees by a factor of 3.

(3) H_{ET}: Number of rainbow trout that escaped from farms, based on data from the Norwegian Directorate of Fisheries (2020b). To adjust for the underreporting of the number of farmed trout escapees (as above), we also multiplied the reported number of escapees by a factor of 3.

(4) H_{WS}: Number of wild salmon returns in a given year. Data were sourced from Thorstad & Forseth (2017). It is assumed that non-returning salmon are offshore of the coastal zone and not important for lice abundance.

(5) H_{WT}: Number of wild brown sea trout in Norway. No comprehensive assessment of wild brown sea trout abundance exists for Norway. In their earlier model, Heuch & Mo (2001) used an estimate of 1 million, which we have implemented as a constant across years.

(6) L_F: Mean adult female lice per farmed salmon and trout. Data for 2005 to 2017 are sourced from legislated reporting from the Norwegian Food Safety Authority. Data from earlier years are sourced from a publicly available database at Lusedata (http://lusedata.no/statistikk/excel). Data were not available for 2000 to 2001, so these years were interpolated by taking the mean of 1999 and 2002.

(7) L_E: Adult female lice per fish on escaped salmon and trout. As no data are routinely collected for this in Norway, we used the same values as for L_{WT}.

(8) L_{WS}: Mean adult female lice per wild salmon. As no data were routinely collected for this in Norway from 2000 onwards, we used the same values as for L_{WT}. For 1998 to 1999, we estimated densities of ovigerous adult female lice per fish according to Norwegian Institute for Nature Research (NINA) reports by Grimnes et al. (1999, 2000). These values were also used to estimate the total number of adult female lice per fish by assuming that ovigerous female lice represented 95% of all adult female lice (Murray 2002).

(9) L_{WT}: Mean adult female lice per wild sea trout. Data for 2010 to 2017 were sourced from the National Aquaculture Legislation Overview (national monitoring program for salmon lice). Estimates for 2000 to 2004 and 2006 to 2009 were obtained from annual NINA reports (Bjørn et al. 2001, 2002, 2003, 2004, 2005, 2007, 2008, 2009, 2010). For each year, the number of adult female lice per fish and, if specified, the number of adult female lice with eggstrings per fish were extracted from these reports. Where female and male adult lice were not reported separately, we divided values by 2 to give an estimated mean number of adult female lice per fish. Where there were multiple samples from a site, we took the weighted mean. Due to a shift in funding from the Norwegian Directorate for Nature Management (now Norwegian Environment Agency) to the Norwegian Food Safety Authority, no monitoring data were available from 2005. Therefore, we used the mean of 2004 and 2006 for 2005. For 1998 to 1999, lice data for wild salmon were used (see L_{WS}).

(10) T_F: Proportion of the year that farmed salmon and trout spend in coastal waters. As fish are held in the ocean for the full grow-out period following smoltification, they spend all 12 mo of the year in the ocean. Thus, T_F in all years.

(11) T_E: Proportion of the year that escaped salmon and trout spent in coastal waters. As there has been no published information regarding the duration farmed salmon and trout escapees spend in coastal waters, we conservatively estimated that the duration was 6 mo per year (T_F = 0.5), which assumed that escape events occur evenly throughout the year.

(12) T_{WS}: Proportion of the year that returning wild salmon spend in coastal waters. Estimated at 16 d per year (T_{WS} = 0.044), as per Karlsen et al. (2017).

(13) T_{WT}: Proportion of the year that wild sea trout spend in coastal waters. Trout smolts typically leave rivers in spring, and post-smolts may remain at sea during summer and return to freshwater over winter (Thorstad et al. 2016). Adults spend summers at sea and winters in freshwater, but some can remain at sea until they later return to freshwater for spawning. Therefore, we assumed that, on average, wild sea trout spend 6 mo of the year in coastal waters (T_{WT} = 0.5).

To calculate the proportion of the total host population represented by farmed fish in each year, we divided the number of salmonids in farms by the estimated total number of salmonids in the environment (farmed, wild and escapee salmon and trout) according to the following equation:

$$P_L = \frac{H_F L_F T_F}{H_F L_F T_F + (H_{ES} + H_{ET}) + L_E T_E + H_{WS} L_{WS} + H_{WT} L_{WT} T_{WT}} \quad (1)$$

To calculate the proportion of the reproductive lice population that is on farmed hosts, we factored in data on mean lice abundance per fish, and we weighted these numbers by the proportion of time infected hosts spend in coastal waters in any given year. Our proportion is, thus, an estimate of the likely proportional contribution that farmed fish make to future infestation pressure:

$$P_H = \frac{H_F}{H_F + H_{ES} + H_{ET} + H_{WS} + H_{WT}} \quad (2)$$

The calculation of P_L assumes that adult female lice on farmed and wild salmonids produce the same number of larvae and that larvae produced by adult female lice on farmed and wild salmonids have the same probability of contributing to future infestation pressure. This assumption will result in a slight underestimation of the importance of farmed hosts if a high density of hosts and conspecifics at farms increases mate-finding success and facilitates higher reproductive output (e.g. Mennerat et al. 2017) or will overestimate their importance if regular lice control by farmers can maintain low infestation densities and reduce mate availability. To correct for differences in fertility based on mate availability (essentially an Allee effect, Krkošek et al. 2012), we used predictions from mate limitation models for farmed salmon (Stormoen et al. 2013) and wild sea trout (Murray 2002) to estimate the proportion of mated (i.e. ovigerous) adult female salmon lice based on the mean infestation density for each year. The proportional contribution of farms to lice reproduction was also calculated taking this effect into account, yielding P_{LO} (the O for ovigerous adult female salmon lice). This alternative measure was calculated as for P_L but with L terms each multiplied by the predicted proportion of ovigerous females given the annual mean lice density of the host population. We are not aware of equivalent models for wild Atlantic salmon or escaped farmed salmon, so we used the sea trout model (Murray 2002) for all salmonids in the wild.

Some of our variables are uncertain, particularly those related to abundance and residency of wild salmonids (including farm escapees). To assess the sensitivity of the model to changes in these parameters, we recalculated P_L and P_{LO} with each of the following parameters increased by high but conceivable amounts to increase the importance of wild salmonids for salmon lice populations:

(1) T_E: Escaped salmon may be more likely to remain in coastal waters than wild salmon. We tested the effect of increasing T_E from 0.5 to 0.75.

(2) H_{ES} and H_{ET}: The literature suggests that actual numbers of escapees are 2 to 4 times higher than reported. We tested the effect of multiplying reported escapes by 4 instead of 3.

(3) H_{WS}: Some proportion of non-returning wild salmon is likely present in the coastal zone. We tested the effect of doubling estimates for H_{WS} to account for such individuals.

(4) T_{WS}: Based on the available literature, we assumed that wild salmon spend the majority of their time at sea. We tested the effect of increasing T_{WS} from 0.044 to 0.5.

(5) H_{WT}: Sea trout abundance is poorly understood and likely fluctuates slightly year to year. We tested the effect of increasing this estimate by 50% to 1.5 million.

(6) T_{WT}: Sea trout use both coastal and offshore environments, but the proportion of time spent in each is uncertain. We tested the effect of increasing T_{WT} to 0.75.

We first adjusted each of these parameters sequentially to assess the sensitivity of the model to each one and then re-ran the model with all adjustments simultaneously to show the outcome of a severe underestimate of the contribution of lice on wild salmonids.

3. RESULTS

The number of farmed salmonids increased most years from 1998 to 2017 (Fig. 1A). The number of salmon and trout in the wild either declined or

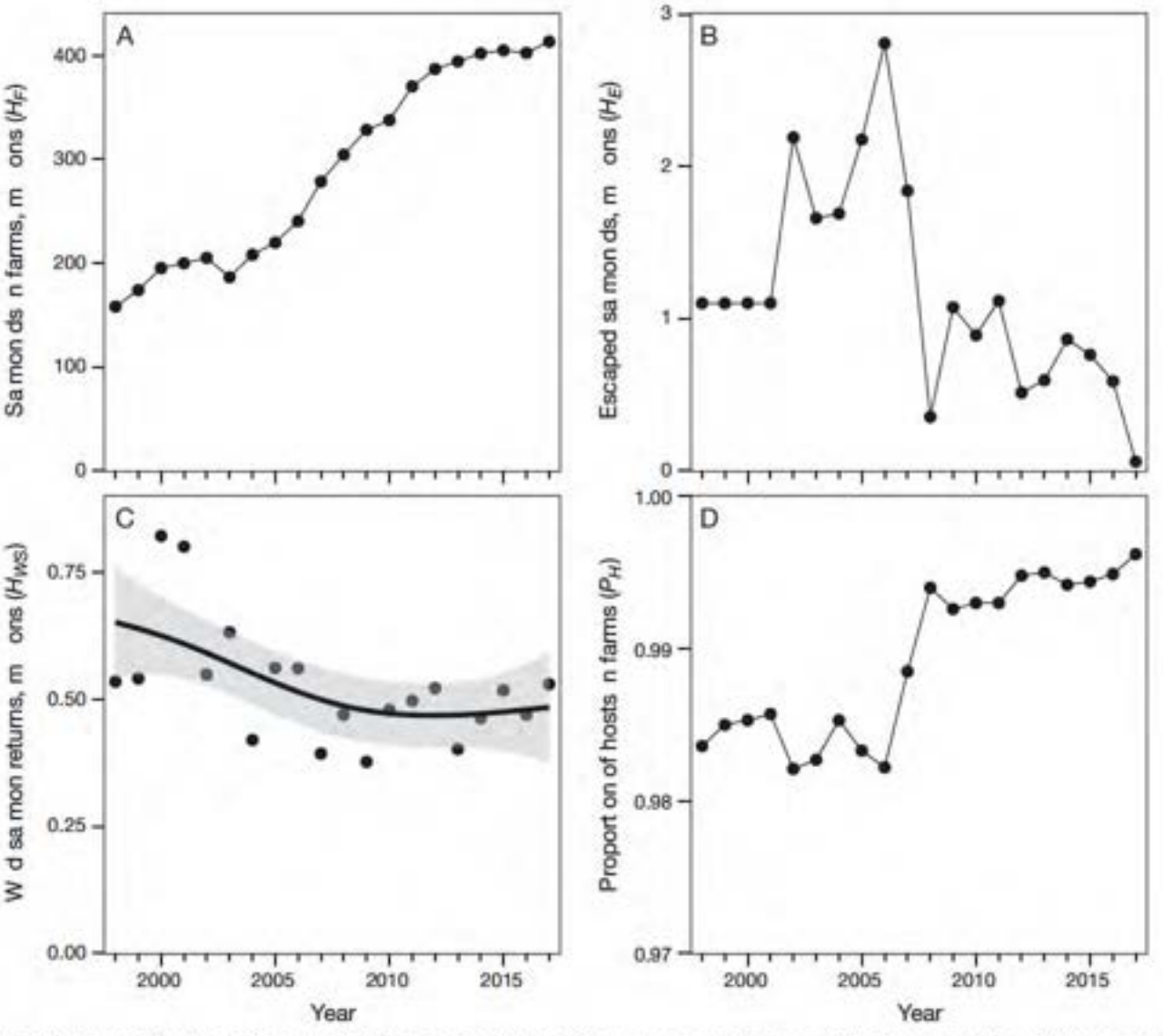

Fig. 1. Temporal trends in host availability for salmon lice in Norwegian coastal waters: (A) salmonids in farms, (B) escaped salmonids (reports multiplied by a factor of 3 to account for systemic underreporting), (C) wild salmon returns plotted with a generalised additive model fit and 95% CIs and (D) relative availability of salmonid hosts in farms vs. in the wild. Wild salmonid population includes farm escapees

remained stable over the same period, with the number of farm escapees peaking in 2006 before declining sharply (Fig. 1B) and wild salmon returns peaking in 2000 to 2001 before declining slightly and apparently stabilising around 500000 yr^{-1} (Fig. 1C). Together, these trends have caused the relative host availability of farmed vs. wild salmonids to increase throughout 1998 to 2017 (Fig. 1D), and by 2017, farmed salmonids accounted for the vast majority (P_H = 0.996) of available hosts for salmon lice in the Norwegian coastal zone. From 2013 to 2017, farmed salmonids outnumbered wild salmonids (sea trout and returning salmon) by 267–281:1.

Mean lice infestation densities on both farmed and wild fish fluctuate considerably year to year but have generally declined over time (Fig. 2A–C). Despite this variation, the proportion of lice emanating from farms has been consistently high (2010–2017: P_L = 0.97–0.99; Fig. 3). In other words, hosts in farms have accounted for >97% of all adult female salmon lice in the Norwegian coastal zone for each year since 2010, while in 2017, we estimate that 99.1% of adult female salmon lice were in farms (Fig. 3). The number of lice per host tends to be lower on farmed salmonids, and limiting the model to ovigerous adult female lice slightly reduces the relative importance

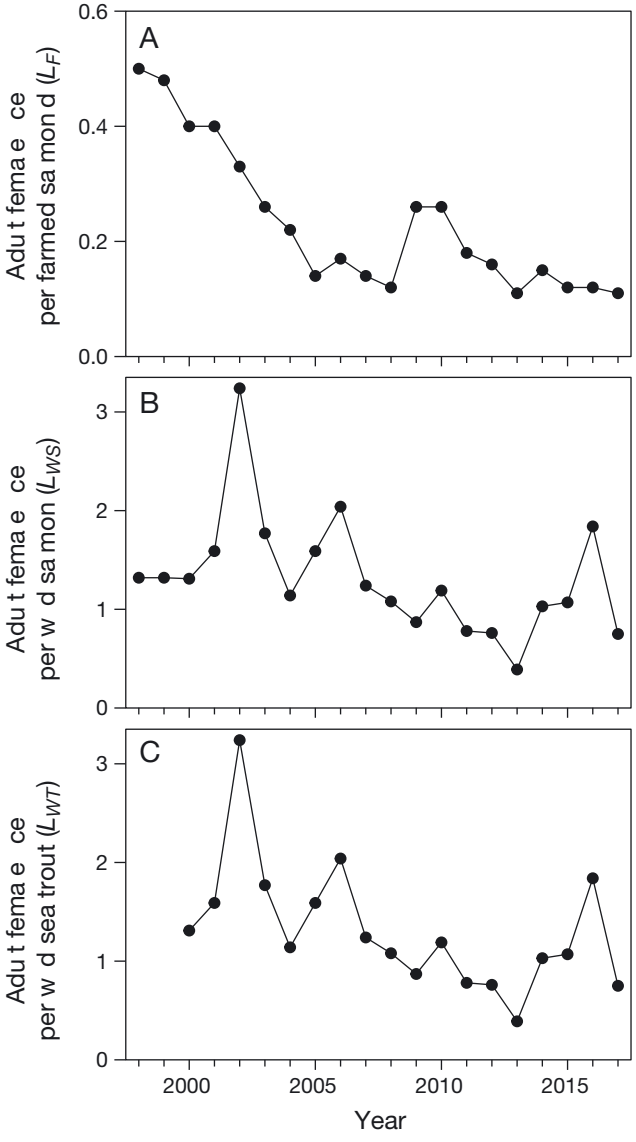

Fig. 2. Temporal trends in adult female salmon lice infesta tion density on (A) farmed salmon, (B) wild salmon and (C) wild sea trout

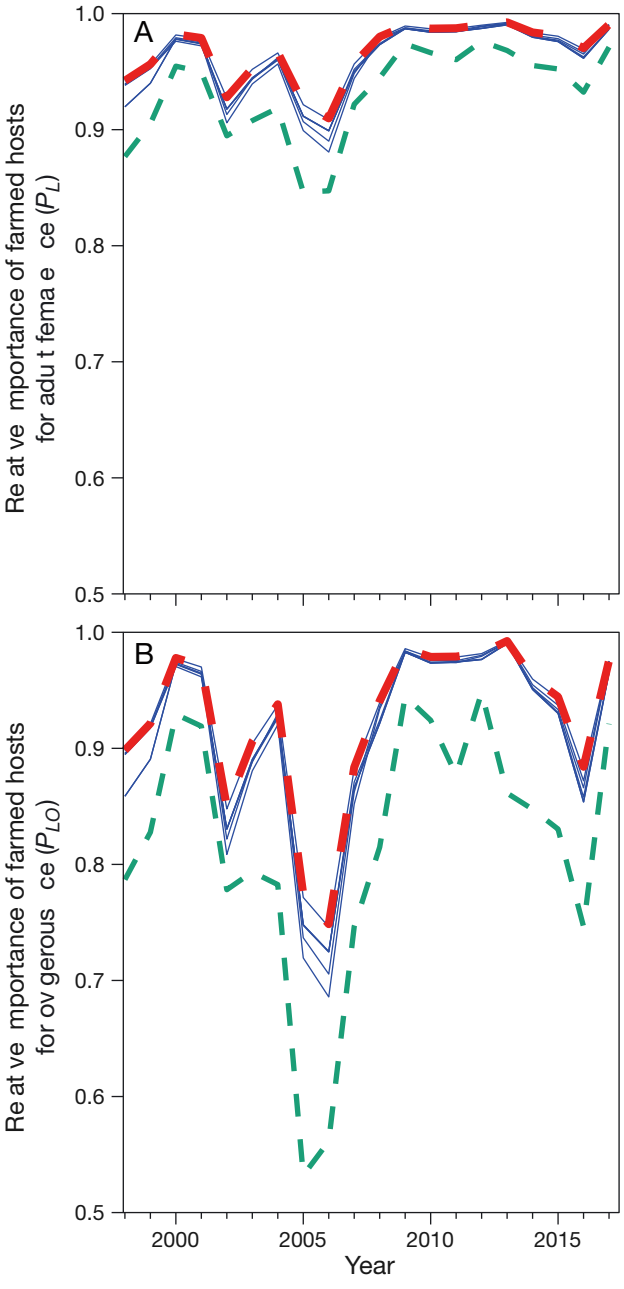

Fig. 3. Temporal trends in relative importance of farmed and wild salmonid hosts for adult female salmon lice: (A) all adult females and (B) mated (ovigerous) adult females only. Lines show proportional contributions based on (1) the best esti mate of the number of hosts in farms per host in the wild (thick red dashed line), (2) adjustments for each of 6 uncer tain parameters relating to wild host availability (grey solid lines) and (3) the worst case underestimate of wild host availability (green dashed line)

of farmed hosts in recent years, but the effect is qualitatively unchanged (in 2017, P_{LO} = 0.98; Fig. 3).

Of the 6 parameters that were informed by uncertain data, the model was most sensitive to adjustments to T_{WS}, H_{WT} and T_{WT} (Table 1). These are parameters that describe the availability of wild salmon and sea trout hosts in the Norwegian coastal zone. However, none of these parameters, when adjusted in isolation, caused P_L to fall below 0.96 in 2017 (Table 1; Fig. 3). Adjusting all the parameters simultaneously resulted in a lower P_L, but farmed salmon were still the dominant source of new lice (P_L = 0.92: Table 1; Fig. 3). Findings were qualitatively similar for P_{LO} (Table 1; Fig. 3). Together, this indicates that the model is highly robust to uncertainty around these parameters or fluctuations in abundance of wild salmon and sea trout.

Table 1. Outcomes of a sensitivity analysis considering 6 uncertain parameters that relate to the availability of wild salmonid hosts in 2017. Using our best estimates for each of these parameters indicates that 99.1 % of adult female salmon lice (P_L = 0.991) and 97.6 % of ovigerous females (P_{LO} = 0.976) were within salmon farms in 2017. However, P_L and P_{LO} are both reduced under each of the following scenarios: (1) Escaped salmon are more likely to remain in coastal waters than wild salmon; (2) Rates of escape by farmed salmonids are higher than our best estimate; (3) More non returning wild salmon are present in the coastal zone than our best estimate; (4) Wild salmon spend more time in coastal waters than our best estimate; (5) Sea trout abundance is higher than our best estimate; (6) Sea trout spend more time in the coastal zone than our best estimate; All: Scenarios 1 6 applied simultaneously

Scenario	Affected parameter	Best estimate	Alternative estimate	P_L based on alternative estimate	P_{LO} based on alternative estimate
1	T_E	0.5	0.75	0.990	0.975
2	H_E	45000	60000	0.990	0.975
3	H_{WS}	530000	1060000	0.990	0.975
4	T_{WS}	0.044	0.5	0.987	0.965
5	H_{WT}	1000000	1500000	0.986	0.965
6	T_{WT}	0.5	0.75	0.986	0.965
All	All	All	All	0.971	0.921

4. DISCUSSION

4.1. Overwhelming importance of farmed hosts for salmon lice

We estimate that the vast majority (99.1 %) of adult female salmon lice in Norwegian coastal waters occur on farmed salmonids. Farmed hosts are clearly the reproductive engine for the lice population. While the model includes several data-poor parameters related to the abundance and distribution of wild salmonids, the abundance of farmed hosts is now so large that the model is highly robust to changes in the estimated population size of wild hosts. According to our most conservative estimate, which greatly increased the number of wild hosts in the coastal zone, farmed salmon still hosted 97.1 % of adult female lice and 92.1 % of ovigerous adult female lice in 2017. For at least the past 2 decades, farmed hosts have driven lice abundance with negligible contributions from wild hosts.

4.2. Model assumptions and uncertainty

For the variables informed by uncertain data, and other model variables, we used conservative values which would have overestimated the contribution of lice on wild salmonids to overall lice abundance. Evidence suggests that some of these variables likely have lower values which would diminish the contribution of lice on wild salmonids and thus increase the estimate of P_L. As the model was sensitive to T_{WS}, it is worth exploring the estimate used, in addition to H_{WS} (number of wild salmon returns in a given year), which was not sensitivity tested as yearly estimates were available but behaves in a similar way in the model as H_{WT}. The Norway scale values used for H_{WS} and H_{WT} assumed that lice infesting returning salmon and sea trout contribute equally to generating salmon lice, regardless of where these fish occur geographically. We estimate that only ~55 % of the total number of salmon that return to rivers in Norway each year do so to areas where intensive salmon farming occurs (intensive salmon farming areas: western Norway = 40 000 returnees, middle Norway = 18 0000 and half of northern Norway = 64 500), with 45 % returning to areas with little or no salmon farming (southern Norway = 124 000, half of northern Norway = 64 500, Tana River = 39 000; Thorstad et al. 2020). Sea trout population numbers are far less certain but are believed to be in long-term decline with only 20 % of 430 populations classified as being in good condition, 31 % in moderate condition and 48 % in poor condition, largely due to the negative impacts of salmon lice infestations (Thorstad et al. 2019). Our national estimate would also include many individuals in coastal areas where no salmon farming occurs in southern and northern Norway. Wild salmon and sea trout populations in areas where salmon farming is absent or limited are less likely to become infected with lice than intensive farming regions, and thus the contribution of lice that infest them to the overall lice population will be relatively small. This leads to a broader point that the model operates at nation scale, using national level averages. Refining the model to address regional variability across many of the variables would likely reveal areas where estimates of P_L are higher and lower than the nationwide average of 0.97 to 0.99 from 2010 to 2017.

4.3. Implications for salmon lice ecology and evolution

Evidence exists that the farm environment has already driven lice evolution (e.g. Mennerat et al. 2012, Ugelvik et al. 2017), and our results suggest this process will continue with little mitigation provided by gene flow from lice on wild hosts. Salmon farming in Norway is now over 40 yr old. As 8 to 10 lice generations are possible per year, depending on temperatures (Samsing et al. 2016, Hamre et al. 2019), there have been up to 300 generations of salmon lice since farming began. Lice of all life stages face novel conditions and selection pressures at farms, including a high density and abundance of both hosts and conspecifics, along with periodic human intervention in the form of lice management (preventative or post-infestation delousing) and harvesting of their salmonid hosts. Coates et al. (2021) assessed the potential for salmon lice to adapt to the main prevention and control methods (chemotherapeutants, mechanical and thermal treatments, cleaner fish, freshwater treatments, depth-based preventions [e.g. skirts and snorkels] and selective breeding). Lice have evolved resistance to at least 4 of 5 chemical therapeutants (Aaen et al. 2015, Myhre Jensen et al. 2020), and while evidence is incomplete for the other louse control methods, Coates et al. (2021) concluded that the evolution of resistance to non-chemical methods is a strong possibility given the variation that exists in and between louse populations (Jacobs et al. 2018) on which non-chemical selection pressures could act and that this variation may have a genetic basis.

In other parts of the world, wild hosts constitute a much higher proportion of the total host population. In these areas, we can expect adaptation by lice to farmed conditions to be slowed through a constant flow of farm-maladapted genes from the wild population (Kreitzman et al. 2018). This gene flow is an evosystem service provided by a robust wild population of hosts. Our data show that the situation in Norway is vastly different: here, the size of the industry means the farmed population numerically dominates the wild population. Maladaptive gene flow in this case will be outwards, towards the louse population held on wild salmonids.

In this system, wild salmonids may still be influential, not as a reservoir but as vectors that boost lice population connectivity between farming regions. Indeed, where farms are oceanographically distant from upstream farms (i.e. beyond the planktonic dispersal distance of a single cohort of larval lice), highly mobile wild hosts may act as a vector that facilitates the spread of lice and genes that confer resistance to control measures throughout a farming network. With infectivity of salmon lice copepodids almost negligible after 14 d at 10°C (Skern-Mauritzen et al. 2020), there are likely many sites that rarely receive infestation pressure directly from upstream farms. So-called firebreaks, or areas of no farming that disrupt dispersal pathways, targeted to decrease connectivity in planktonic lice dispersal pathways are projected to provide benefits by slowing the dispersal of genes that confer resistance to specific treatments and reducing the pool of available infective stages to create first infestations after stocking (Samsing et al. 2019). High connectivity of salmon lice populations facilitated by wild hosts could weaken the effectiveness of firebreaks and the advantages they confer. Given this situation, it is interesting to consider the selection pressures that act on lice attached to farmed hosts and how this may affect lice fitness on wild hosts.

4.3.1. Life history traits

Evolutionary theory predicts that host–parasite systems with high parasite transmission rates will select for high virulence; where there is a high availability of new hosts, parasite fitness is maximised by early maturation and high fecundity even if it damages the host. Fish farming creates such conditions (Nowak 2007, Mennerat et al. 2010). In contrast, salmon lice that infest wild hosts can have a relatively long lifespan before the host dies or returns to freshwater. Furthermore, lice on wild hosts may sometimes have little choice but to await the arrival of a potential mate to the same host, as it is inherently risky to attempt to switch hosts when hosts are infrequent. Conversely, farming conditions favour a rapid life cycle, driven by (1) an abundance of mates; (2) high host availability, which could facilitate host switching as adult lice and increase the likelihood of offspring finding a host; and (3) the need to reproduce before the farmer delouses or harvests. Individuals that invest heavily in reproduction early in life (even at the expense of somatic growth) are more likely to produce offspring before delousing or harvesting occurs.

Common garden experiments demonstrate that salmon farming has altered the virulence and life history of salmon lice. Lice strains collected from farmed salmon in areas with intensive aquaculture caused more severe skin damage, achieved higher infestation densities and produced more eggs in their first batch (and fewer in later batches) than strains collected

from wild hosts in unfarmed locations (Mennerat et al. 2017, Ugelvik et al. 2017). These differences were observed after lice strains were reared in the laboratory for at least 3 generations, pointing to underlying genetic variation in virulence and reproduction.

4.3.2. Host availability and host-finding traits

Louse larvae that do not find and attach to a host do not reproduce; therefore, there should be strong selection pressure on traits that affect encounter or attachment success. To the extent that such traits are heritable, rapid evolution likely drives responses to changes in the availability and distribution of hosts from the expansion of farming. This has clear implications for the evolutionary ecology of lice–salmon interactions. Farming increases the abundance of potential hosts, which could conceivably dampen or alter selection pressure on host-finding traits. However, farmers may also intervene with barriers to infestation, including technologies to prevent encounters between lice and hosts in the surface layers where lice are most common, such as snorkel cages (Geitung et al. 2019) and skirts (Grøntvedt et al. 2018, Stien et al. 2018), and behavioural manipulation of swimming depth using deep lights and feeding (Frenzl et al. 2014). Swimming depth of the infectious copepodid larval stage varies among families (Coates et al. 2020) and may have a genetic basis. If the vertical distribution of lice is influenced by heritable traits, then the increasing mean depth of available hosts could drive the evolution of lice larvae with deeper distributions. Intriguingly, this could be a benefit for wild salmon if the widespread adoption of deeper farming leads to a gradual decoupling of the preferred shallow swimming depths of outmigrating wild salmon smolts (Plantalech Manel-la et al. 2009) and sea trout in coastal waters (Rikardsen et al. 2007) and salmon lice, reducing the infestation pressure for wild salmonids.

4.3.3. No wild refuge to slow the development of treatment resistance

While effective delousing reduces life expectancy for lice and thus selects for faster life history and increased virulence, treatments that allow some survivorship will also drive the evolution of treatment resistance. The rapid evolution of treatment resistance is a recurring story in human health and intensive agriculture (e.g. antibiotics: Raymond 2019). Salmon aquaculture in Norway is similarly vulnerable because the number of farmed salmon is far greater than the number of wild salmon, such that the main source for re-infestation comes from hydrodynamically connected farms. Frequent parasite treatments apply constant selection pressure on traits for resistance, and the vast majority of the lice population is exposed to the selection pressure from these treatments (Overton et al. 2019, Coates et al. 2021).

Genes that encode resistance to chemotherapeutants are already common in the salmon lice population in the Atlantic. Resistance to the treatments emamectin benzoate and azamethiphos each emerged at single point sources, before rapidly spreading across the North Atlantic (Besnier et al. 2014, Kaur et al. 2016, Fjørtoft et al. 2020). This situation contrasts sharply with the common use of emamectin benzoate on the Pacific coast of North America. Emamectin benzoate has remained highly effective, at least until very recently (Messmer et al. 2018), presumably because there was enough gene flow from lice on abundant wild hosts that are not exposed to the treatment (Kreitzman et al. 2017). As a simple comparison of farmed and wild salmonid numbers on the Canadian–US west coast, Kreitzman et al. (2017) compared wild salmonid capture and aquaculture production to show wild salmonids were 5 times more abundant. Using an agent-based model to predict how important wild salmon population size is as a wild refugium to the evolution of resistance of salmon lice to chemotherapeutants, McEwan et al. (2015) revealed that while equal numbers of farmed and wild salmon tempered the evolution of resistance, ratios of 10:1 farmed to wild salmon resulted in high levels of evolved resistance. Norway is far beyond this level (267–281:1 farmed to wild from 2013 to 2017), and other major farming regions in the Atlantic (e.g. Scotland and the east coast of North America) likely also exceed the 10:1 farmed to wild salmon threshold for high levels of evolved resistance.

Treatment resistance can be costly for the farming industry, as stock must be harvested early or culled when they cannot be treated, and resistance is not limited to pharmaceuticals: there are now reports of farmers needing to use higher concentrations in hydrogen peroxide baths, higher temperatures or longer durations in thermal delousing systems, and longer durations in freshwater baths. Each of these traits is thought to have a heritable basis (Helgesen et al. 2015, Ljungfeldt et al. 2017, Treasurer et al. 2000).

4.4. Implications for salmon lice dispersal modelling

The Norwegian salmon lice dispersal model is a spatially explicit biophysical model combining a hydrodynamic model and particle-tracking module (Asplin et al. 2014, Johnsen et al. 2014, Myksvoll et al. 2018). Larval supply (rate of hatching) is calculated from weekly reports of the number of farmed fish, adult female lice per fish and water temperature at each site (Johnsen et al. 2020). The likely dispersal of released larval particles is modelled primarily using the horizontal current component coupled with aging and mortality of larvae. Managers assess the model outputs and sample wild fish for ground truthing of model predictions. Modelled and observed infestation data are then reported to the Norwegian Food Safety Authority. At the end of each season, the model is re-run with updated data and the outputs used to inform the annual risk assessment of salmon lice infestation pressure on wild salmonids, which in turn assists the Norwegian Ministry of Trade and Fisheries in setting farmed biomass limits for the following year across each of Norway's 13 production zones (the traffic light system: Norwegian Ministry of Trade and Fisheries 2017). The predictive methodology has been criticised for its assumption that farmed salmon are the overriding driver of larval supply and that lice derived from wild salmonids are not considered. The findings from our model indicate that accounting for releases of larvae from wild hosts, even if possible, would not provide meaningful improvement to predictions of infestation pressure.

4.5. Conclusions

Farmed salmonids are of overwhelming importance for the ecology and evolution of salmon lice in Norway. In 2017, salmonids in farms accounted for 99.6% of available hosts and 99.3% of adult female salmon lice in Norwegian coastal waters. As such, we suggest that modelled estimates of infestation pressure can safely be informed by data on lice populations in farms alone. Moreover, the wild salmonid population is unlikely to function as a meaningful refuge from selection pressures in the sea cage environment and will not slow the evolution of resistance to lice management strategies applied within farms. Rather, wild salmon are likely to be parasitised by lice that are increasingly well adapted to farm conditions. Whether this will result in a lower or higher impact on wild salmon remains unclear. Through dispersal, however, wild fish may still connect the broader lice population and ensure gene flow. The extent to which this will be true will depend on how effectively farm-adapted lice can infest and be dispersed by wild fish, an outcome difficult to predict as the evolutionary trajectory of salmon lice becomes increasingly attuned to their farmed hosts.

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Maritimes Region

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RECOVERY POTENTIAL ASSESSMENT FOR SOUTHERN UPLAND ATLANTIC SALMON

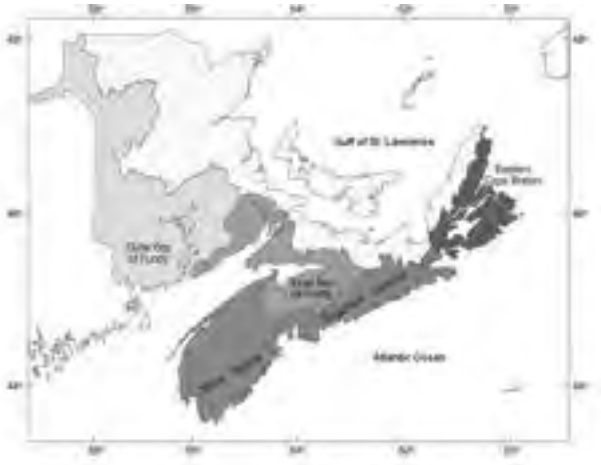

Figure 1. Map showing the location of the Southern Upland relative to the three other designatable units for Atlantic salmon in the Maritimes Region.

Context

The Nova Scotia Southern Upland (SU) population of Atlantic salmon was evaluated as Endangered by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) in November 2010. This population assemblage (designatable unit) occupies rivers on the mainland of Nova Scotia, including all rivers south of the Canso Causeway on both the Eastern Shore and South Shore of Nova Scotia draining into the Atlantic Ocean (Figure 1), as well as the Bay of Fundy rivers southwest of Cape Split. The unique phylogenetic history of SU Atlantic salmon, the minimal historical gene flow between the SU and surrounding regions, the low rates of straying from other regions, and the evidence for local adaption to environmental conditions in the SU region support the view that SU salmon differ from salmon in other areas.

A Recovery Potential Assessment (RPA) process has been developed by Fisheries and Oceans Canada (DFO) Science to provide the information and scientific advice required to meet the various requirements of the Species at Risk Act (SARA). The scientific information provided in the RPA serves as advice to the Minister regarding the listing of the species under SARA and is used when analyzing the socio-economic impacts of listing, as well as during subsequent consultations, where applicable. It is also used to evaluate activities that could contravene the SARA should the species be listed, as well as in the development of a recovery strategy. This assessment considers the scientific data available to assess the recovery potential of SU Atlantic Salmon.

This Science Advisory Report is from the May 22-25, 2012, Recovery Potential Assessment for Southern Upland Atlantic salmon. Additional publications from this meeting will be posted on the *Fisheries and Oceans Canada (DFO) Science Advisory Schedule* as they become available.

SUMMARY

- Available indices show that abundance of Atlantic salmon is very low in the Southern Upland designatable unit and has declined from levels observed in the 1980s and 1990s.
- Annual adult abundance in four rivers declined 88% to 99% from observed abundance in the 1980s, a similar trend is observed in the recreational catch.
- Region-wide comparisons of juvenile density data from more than 50 rivers indicate significant ongoing declines between 2000 and 2008/2009 and provide evidence for river-specific extirpations.
- Population modeling for two of the larger populations remaining in the Southern Upland designatable unit (LaHave and St. Mary's) indicates a high probability of extirpation (87% and 73% within 50 years for these two populations respectively) in the absence of human intervention or a change in survival rates for some other reason.
- Population viability analyses indicate that the loss of past resiliency to environmental variability and extreme environmental events is contributing to the high risk of extinction.
- Juvenile Atlantic salmon were found in 22 of 54 river systems surveyed in 2008/2009. Given the reductions in freshwater habitat that have already occurred and the current low population size with ongoing declines, all 22 rivers include important habitat for Southern Upland Atlantic salmon. Restoration of these populations is expected to achieve the distribution component of the recovery target. If additional rivers are found to contain salmon, the consideration of these rivers as important habitat would have to be re-evaluated.
- The estuaries associated with these 22 rivers are considered to be important habitat for Atlantic salmon as successful migration through this area is required to complete their life cycle.
- While there is likely to be important marine habitat for Southern Upland Atlantic salmon, given broad temporal and spatial variation, it is difficult to link important life-history functions with specific marine features and their attributes.
- Proposed recovery targets for Atlantic salmon populations in the Southern Upland designatable unit have both abundance and distribution components. Abundance targets for Southern Upland Atlantic salmon are proposed as the river-specific conservation egg requirements. The distribution target should encompass the range of genetic and phenotypic variability among populations, and environmental variability among rivers, and would include rivers distributed throughout the designatable unit to allow for gene flow between the rivers/populations. There is the expectation that including a wider variety of populations in the distribution target will enhance persistence as well as facilitate recovery in the longer term.
- Interim recovery targets for Southern Upland Atlantic salmon can be used to evaluate progress towards recovery. First, halt the decline in abundance and distribution in rivers with documented Atlantic salmon populations. Next, reduce the extinction risk in rivers with documented Atlantic salmon populations by increasing the abundance in these rivers. Then, as necessary, expand the presence and abundance of Atlantic salmon into other rivers currently without salmon to fill in gaps in distribution within the Southern Upland designatable unit and facilitate metapopulation dynamics.
- Recovery targets will need to be revisited as information about the dynamics of the recovering population becomes available. Progress towards recovery targets can be evaluated using survival and extinction risks metrics.
- Two dwelling places were evaluated for their potential consideration as a residence for Atlantic salmon. Of these, redds most closely match the definition of a residence because they are constructed, whereas home stones are not.
- Threats to persistence and recovery in freshwater environments identified with a high level of overall concern include (importance not implied by order): acidification, altered hydrology,

invasive fish species, habitat fragmentation due to dams and culverts, and illegal fishing and poaching.

- Threats in estuarine and marine environments identified with a high level of overall concern are (importance not implied by order): salmonid aquaculture and marine ecosystem changes.
- From analyses of land use in the Southern Upland region, previous and on-going human activities are extensive in the majority of drainage basins and have likely altered hydrological processes in Southern Upland watersheds. Watershed-scale factors have the potential to override factors controlling salmon abundance at smaller spatial scales (i.e., within the stream reach).
- River acidification has significantly contributed to reduced abundance or extirpation of populations from many rivers in the region during the last century. Although most systems are not acidifying further, few are recovering and most are expected to remain affected by acidification for more than 60 years.
- Acidification and barriers to fish passage are thought to have reduced the amount of freshwater habitat by approximately 40%, an estimate that may be conservative. However, given the low abundance of salmon at present, habitat quantity is not thought to be currently limiting for populations in rivers where barriers and acidification are not issues. Whether freshwater habitat becomes limiting in the future depends on the dynamics of recovered populations.
- Population modeling for the LaHave River (above Morgan Falls) and the St. Mary's River (West Branch) salmon populations indicated that smolt-to-adult return rates, a proxy for at-sea survival, have decreased by a factor of roughly three between the 1980s and 2000s. Return rates for Southern Upland salmon are currently about ten times higher than they are for inner Bay of Fundy salmon populations.
- In contrast with inner Bay of Fundy salmon populations, for which at-sea survival is so low that recovery actions in fresh water are expected to have little effect on overall viability, recovery actions focused on improving freshwater productivity are expected to reduce extinction risk for Southern Upland salmon.
- Remediation actions to address land use issues will not produce immediate population increases for Southern Upland salmon. However, large-scale changes are the most likely to bring about substantial population increase in Southern Upland salmon because they should have a greater impact on total abundance in the watershed rather than on localized density, and they would address issues at the watershed scale. Coordination of activities at small scales may produce more immediate effects but of shorter duration than addressing landscape-scale threats.
- Population viability analyses indicate that relatively small increases in either freshwater productivity or at-sea survival are expected to decrease extinction probabilities. For example, for the LaHave River (above Morgan Falls) population, increasing freshwater productivity by 20% decreases probability of extinction within 50 years from 87% to 21%, while a freshwater productivity increase of 50% decreases the probability of extinction within 50 years to near zero. Larger changes in at-sea survival are required to restore populations to levels above their conservation requirements.
- Sensitivity analysis examining the effect of starting population size on population viability highlights the risks associated with delaying recovery actions; recovery is expected to become more difficult if abundance continues to decline, as is predicted for these populations.
- Atlantic salmon is one of the most-studied fish species in the world. Readers are referred to the supporting research documents, which form part of the advisory package for this designatable unit, for more information than is contained in this summary document.

BACKGROUND

Rationale for Assessment

When the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) has assessed aquatic species as Threatened or Endangered, Fisheries and Oceans Canada (DFO), as the responsible jurisdiction under the *Species at Risk Act* (SARA), is required to undertake a number of actions. Many of these actions require scientific information on the current status of the species, population or designable unit (DU), threats to its survival and recovery, habitat needs, and the feasibility of its recovery. Formulation of this scientific advice has typically been developed through a Recovery Potential Assessment (RPA) that is conducted as soon as possible after the COSEWIC assessment. This timing allows for the consideration of peer-reviewed scientific analyses into SARA processes including listing decisions and recovery planning.

Southern Upland (SU) Atlantic salmon (*Salmo salar*) was assessed as Endangered by COSEWIC in November 2010 (COSEWIC 2011). DFO Science was asked to undertake an RPA for the Nova Scotia Southern Upland DU based on DFO's protocol for conducting RPAs (DFO 2007). Information on 22 Terms of Reference was reviewed at this meeting.

Southern Upland DU

The Southern Upland DU of Atlantic salmon consists of the salmon populations that occupy rivers in a region of Nova Scotia extending from the northeastern mainland near Canso, into the Bay of Fundy at Cape Split (COSEWIC 2011). This region includes rivers on both the Eastern Shore and South Shore of Nova Scotia draining into the Atlantic Ocean (Figure 1), as well as Bay of Fundy rivers south of Cape Split. Historically, it has been divided into three Salmon Fishing Areas (SFAs): SFA 20 (Eastern Shore), SFA 21 (Southwest Nova Scotia), and part of SFA 22 (Bay of Fundy Rivers inland of the Annapolis River).

Based on genetic evidence, regional geography and differences in life history characteristics SU Atlantic salmon is considered to be biologically unique (Gibson et al. 2011) and its extirpation would constitute an irreplaceable loss of Atlantic salmon biodiversity. Additional information on the genetic analysis of SU Atlantic salmon is provided in O'Reilly et al. (2012).

The exact number of rivers inhabited by SU Atlantic salmon is not known, but salmon likely used most accessible habitat in this area at least intermittently in the past. There are 585 watersheds (streams of various sizes draining directly into the ocean) in the region; 72 are considered to have historically contained Atlantic salmon populations (Figure 2).

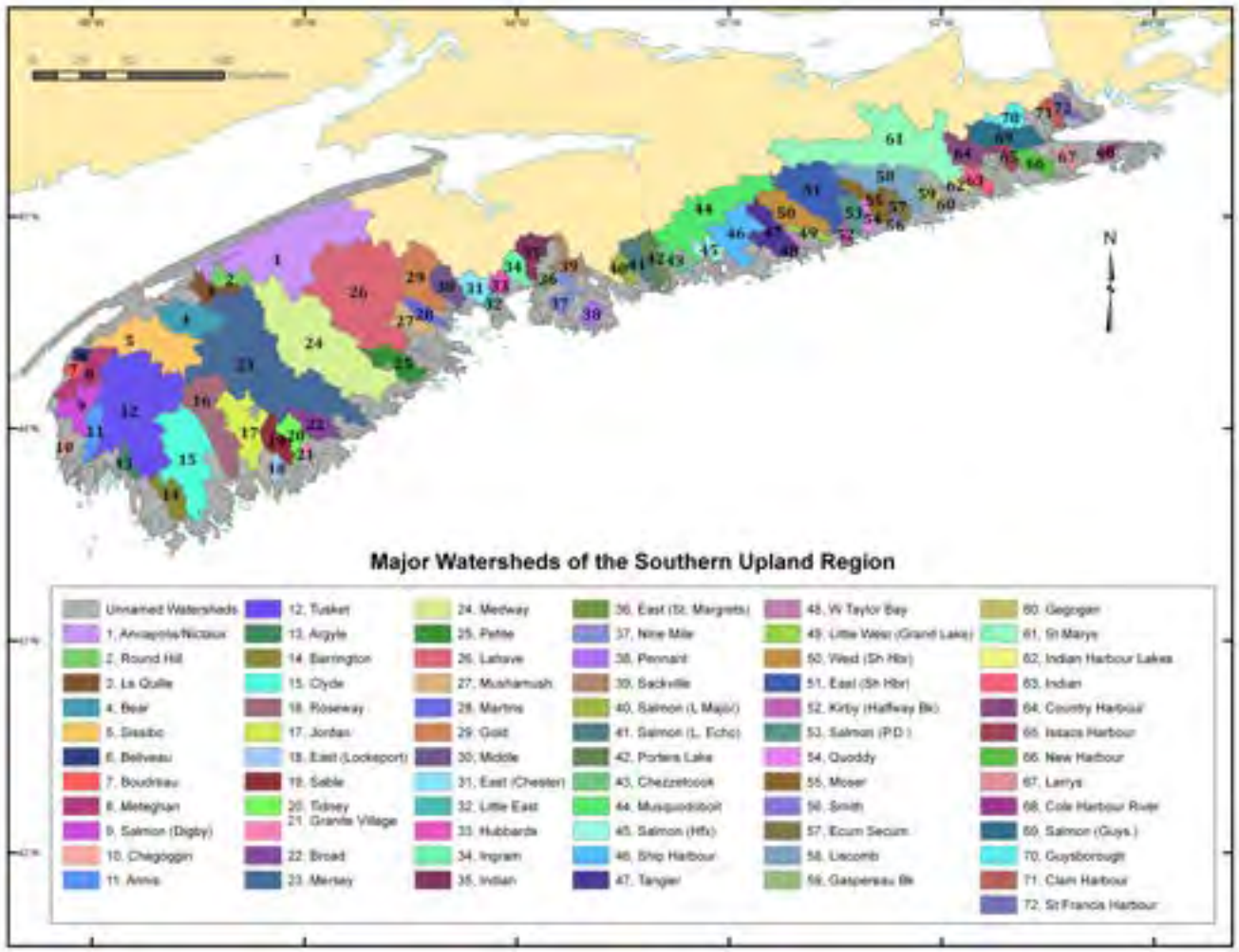

Figure 2. Map of the watersheds contained in the Southern Upland region, labelled by number and colour, where the boundaries were determined from the Secondary Watersheds layer for ArcGIS developed by the Nova Scotia Department of the Environment. Watersheds that are not labelled by number, but are still contained within the Southern Upland region are shown in grey.

Information on the life cycle of SU Atlantic salmon is contained in Gibson and Bowlby (2013). Within the SU populations, salmon mature after either one or two winters at sea (called “one sea-winter salmon” or 1SW, “two sea-winter salmon” or 2SW, respectively), although historically a small proportion also matured after three winters at sea (called “three sea-winter salmon” or 3SW). The proportion of salmon maturing after a given number of winters at sea is highly variable among populations and 3SW salmon are now very rare or absent from most populations in the Southern Upland.

Atlantic salmon is one of the most-studied fish species in the world. Readers are referred to the supporting research documents, which form part of the advisory package for this DU, for more information than is contained in this summary document.

ASSESSMENT

Status and Trends

Data available for evaluating the abundance and trends of SU Atlantic salmon include assessments of adult salmon returning to the St. Mary's River (West Branch), LaHave River (above Morgan Falls), and East River (Sheet Harbour) populations, estimates of smolts abundance for these populations, and estimates of the abundance of juvenile salmon (fry and parr) in many rivers. In the past, abundance has been assessed for the Liscomb River population as well. A detailed discussion of the abundance and trends of SU Atlantic salmon is contained in Bowlby et al. (2013a).

Adult Abundance

Available indices show that abundance of Atlantic salmon is very low in the SU DU and has declined from levels observed in the 1980s and 1990s. Annual adult abundance in four rivers declined by 88% to 99% from observed abundance in the 1980s (Figure 3); a similar trend is observed in the recreational catch time series.

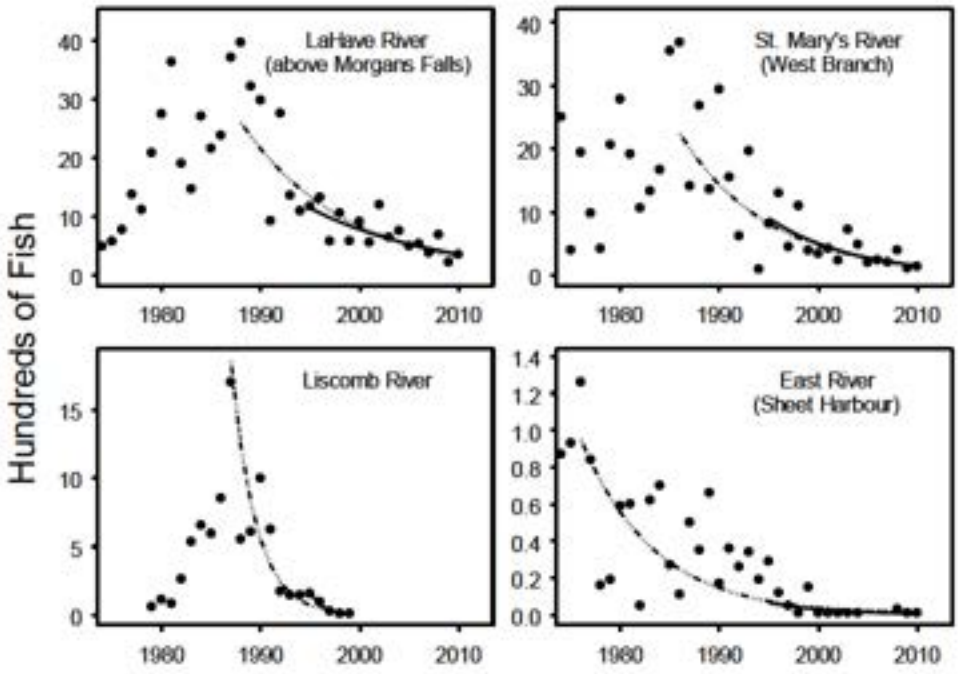

Figure 3. Atlantic salmon adult abundance time series based on adult count data (points) for four rivers in the Southern Upland from 1974 to 2010. The lines show the trends estimated by log-linear regression over the previous 3 generations (solid lines) and from the year of maximum abundance (dashed lines).

Juvenile Abundance and Distribution

Region-wide comparisons of juvenile density data (obtained by electrofishing) from more than 50 rivers indicate significant ongoing declines between 2000 and 2008/2009 and provide evidence for river-specific extirpations. In 2008/2009, juvenile Atlantic salmon were found in 22 of 54 surveyed rivers within the DU, but were not found in 4 rivers where they had been found in 2000 (Figure 4). Despite fishing effort in the two surveys being similar, only one quarter as many salmon juveniles were captured in the 2008/09 survey as in 2000 (1,019 versus 3,733).

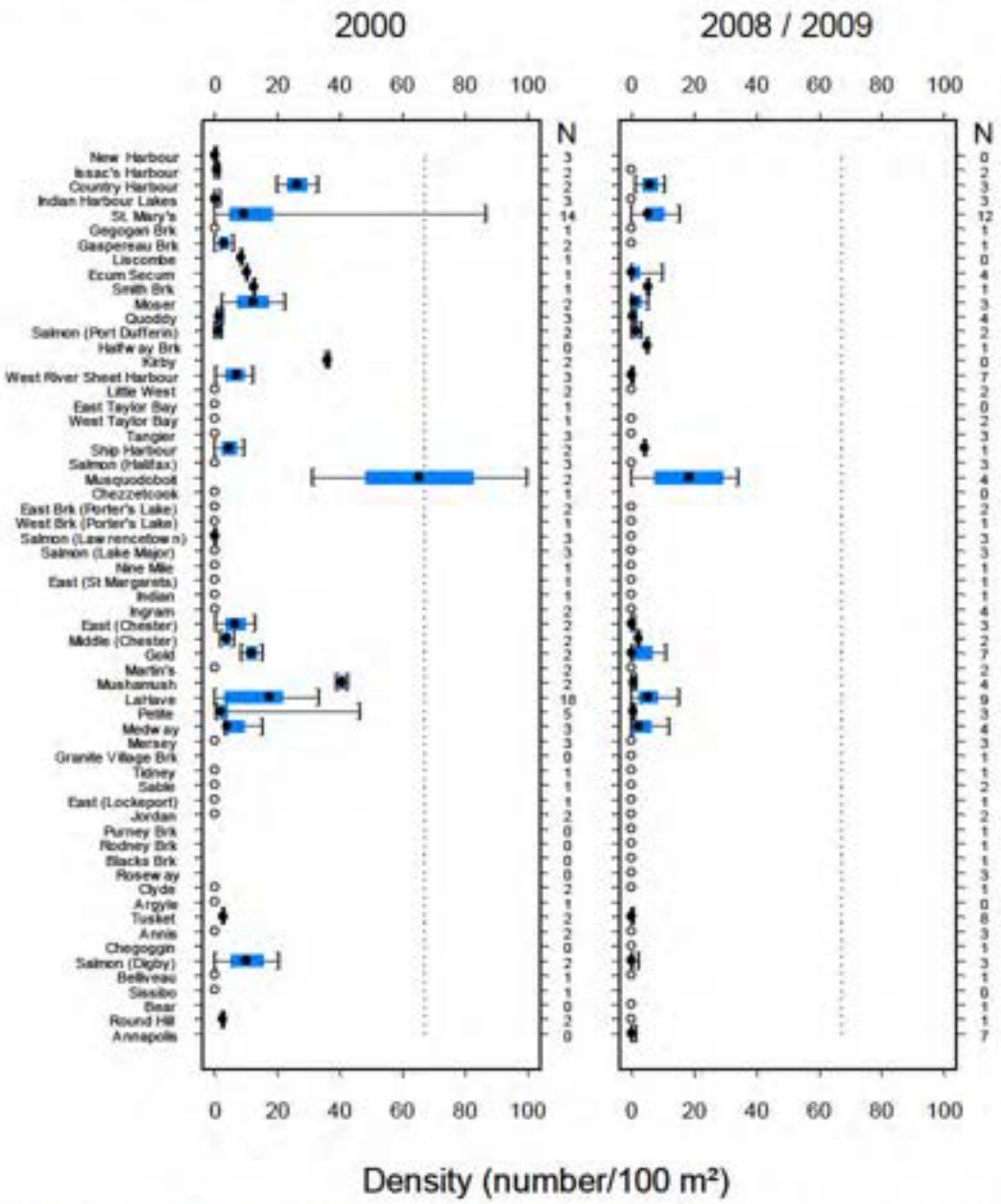

Figure 4. Boxplots of Atlantic salmon juvenile densities (age 0, age 1, and age 2+ combined) in rivers sampled by electrofishing during the survey in 2000 (left panel) and in 2008/2009 (right panel). The number of sites sampled in each river is given on the right-hand axis in both panels, and sites in which no salmon were captured are represented by open circles. The vertical dotted line shows Elson's norm for total juvenile abundance in both panels. Box plots are interpreted as follows: the black symbols are the medians, the rectangle shows the interquartile range and the whiskers the minimum and maximum values. Reprinted from Gibson et al. (2011).

Where present in 2008/2009, the observed densities of juvenile salmon ranged from 0.3 to 33.8 fish per 100 m^2 (Figure 4). Observed densities of fry (age 0) ranged from 0.3 to 28.0 fish per 100 m^2 and of parr (age 1 and age 2+) ranged from 0.2 to 16.1 fish per 100 m^2, with the highest values being recorded on the Musquodoboit River. In general, the mean density of either age class was lower than Elson's norm (30 age 0 fish per 100 m^2 and 24 age 1 and older fish per 100 m^2), values that have been used as a reference for juvenile production in fresh water.

Range and Distribution

The evaluation of range and present distribution of SU Atlantic salmon in fresh water is based on juvenile salmon surveys (Figure 4), although salmon may be present in some rivers not included in the survey. The full extent of the marine range of SU Atlantic salmon is not known, but tagging studies indicate that SU Atlantic salmon can be found along the entire coast of Nova Scotia, from the inner Bay of Fundy to the tip of Cape Breton, throughout most, if not all, of the year. Additionally, they may be found along the coast of northern New Brunswick, Newfoundland, northern Quebec, and the tip of Labrador, migrating northward until a proportion reach the Labrador Sea, Irminger Sea, or along the coast of West Greenland. For the high-seas fisheries in Labrador and West Greenland, few of the tag recaptures were assigned a latitude and longitude when recovered; therefore, it is not possible to determine how far off-shore Atlantic salmon may frequent in these areas. Assuming that these data represent general distribution patterns in the marine environment, there appears to be limited use of the Gulf of St. Lawrence (including the coastal areas around the Magdalen Islands, northern New Brunswick, or Quebec near Anticosti Island) by SU Atlantic salmon. Further details of the analysis of the tagging data are provided in Bowlby et al. (2013b).

Population Dynamics

A life history-based population dynamics model was used to evaluate population viability. The population dynamics model consists of two parts: a freshwater production model that provides estimates of the expected smolt production as a function of egg deposition, and an egg-per-smolt (EPS) model that provides estimates of the rate at which smolts produce eggs throughout their lives. These components are combined via an equilibrium analysis that provides estimates of the abundance at which the population would stabilize if the input parameters remained unchanged. This combined model is then used to evaluate how equilibrium population size has changed through time, as well as how the population would be expected to change in response to changes in carrying capacity, survival, or life stage transition probabilities. Parameter estimates from the model are used in the population viability analysis (PVA) for the recovery scenarios. Analyses are presented for the two larger rivers for which there are sufficient monitoring data: the LaHave River (above Morgan Falls), and the St. Mary's River (West Branch).

Life-History Parameter Estimates

Life-history parameter estimates were derived using a statistical, life history-based population dynamics model. Methods and results of this analysis are described in detail in Gibson and Bowlby (2013). Some key parameters are described below, including indications of where these have changed over time.

Freshwater Productivity

Analyses for LaHave River (above Morgan Falls) indicate that for the 1974 to 1985 time period, the maximum number of smolts produced per egg was 0.017 and that this value decreased to 0.013 in the 1985 to 2010 time period. Similarly, the carrying capacity for smolt production decreased from 147,700 to 119,690 (5.7 to 4.6 smolts per 100 m^2) between the two periods. For the St. Mary's River (West Branch), the carrying capacity of age-1 parr was estimated to be 11.76 parr per 100 m^2 and is considered to be low relative to other populations. The estimated number of smolts produced per egg is 0.034 and the carrying capacity for smolt is estimated to be 104,120 smolts (4.7 smolts per 100 m^2) (average values for the time period 1974 to 2010).

Details about these analyses, as well as age- and stage-specific survival rates for these populations, are provided in Gibson and Bowlby (2013).

Survival of Emigrating Smolts and Kelts in Rivers and Estuaries

The survival of emigrating SU smolts and kelts in rivers and estuaries is reasonably well studied, and provides an indication of how much survival could be changed by recovery actions that were focused on this life history event.

The survival of emigrating smolts in the LaHave, St. Mary's and Gold rivers was studied during 2010, and in West River (Sheet Harbour), during 2008, 2009 and 2010. Observed survival from release to the head of tide (the freshwater zone) ranged from 71.9% to 100%, and survival to the open ocean ranged from 39.4% to 73.5% (Table 1).

There are two studies of kelt survival in SU estuaries. In the St. Mary's River, 24 acoustically tagged kelts were detected leaving the river in the spring and all these fish survived to leave the estuary. In a study of the survival and behaviour of migrating kelts in freshwater, estuarine, and coastal habitat using LaHave River salmon, 27 of 30 acoustically tagged fish were detected leaving coastal habitat, indicating that survival was at least 90% while migrating through those environments. Further details on these studies are provided in Gibson and Bowlby (2013).

Table 1. Cumulative survival (%) and standardized survival (% per km of habitat zone length) of smolts upon exit from four habitat-zones (FW – freshwater; IE – inner estuary; OE – outer estuary; Bay / Overall). Smolts detected dead less than 1 km from release were excluded from estimates of observed survival. Reprinted from Halfyard et al. (2012).

	Observed Cumulative Survival Upon Exit			
River-Year	**FW**	**IE**	**OE**	**BAY / Overall**
LaHave	76.5% 98.9% ·km^{-1}	76.5% 100.0% ·km^{-1}	73.5% 99.7% ·km^{-1}	73.5% 100.00% ·km^{-1}
Gold	100.0% 100.0% ·km^{-1}	88.2% 92.4% ·km^{-1}	79.4% 97.8% ·km^{-1}	61.8% 97.6% ·km^{-1}
St. Mary's	79.4% 99.3% ·km^{-1}	76.5% 98.7% ·km^{-1}	73.5% 98.7% ·km^{-1}	67.6% 98.3% ·km^{-1}
West 2008	78.9% 97.0% ·km^{-1}	52.6% 83.8% ·km^{-1}	47.4% 96.5% ·km^{-1}	47.4% 100.0% ·km^{-1}
West 2009	96.0% 99.5% ·km^{-1}	76.0% 90.5% ·km^{-1}	72.0% 98.3% ·km^{-1}	68.0% 98.8% ·km^{-1}
West 2010	71.9% 95.5% ·km^{-1}	54.5% 91.0% ·km^{-1}	51.5% 98.0% ·km^{-1}	39.4% 95.0% ·km^{-1}

At-Sea Survival of Smolts and Kelts

One of the main threats to SU Atlantic salmon is thought to be the change in smolt-to-adult return rates, although estimates of the return rates for wild smolts are not available prior to the mid-1990s because smolt abundance was not being monitored before then. To resolve this

issue, a model was set up to estimate past return rates using time series of estimated egg depositions, age-specific abundances of fry and parr, and the more recent age-specific smolt abundance time series.

The observed and estimated return rates of 1SW and 2SW salmon to the river mouth for the LaHave River (above Morgan Falls) population increased in the mid-1980s coincident with the closure of the commercial fisheries on Nova Scotia's coast (Figure 5). Return rates generally declined from 1985 to 1995 and have fluctuated without a clear trend since. In the 1980s, return rates varied between 2.87% and 17.60% for 1SW salmon and between 0.31% and 1.21% for 2SW salmon for the LaHave River (above Morgan Falls) population (Table 2); whereas, in the 2000s, return rates varied between 2.25% and 4.14% for 1SW salmon and between 0.31% and 1.21% for 2SW salmon. Similarly, for the St. Mary's River (West Branch) population, return rates in the 1980s varied between 1.17% and 5.52% for 1SW salmon and between 0.54% and 2.11% for 2SW salmon. In the 2000s, return rates varied between 0.18% and 2.11% and between 0.00% and 0.30% for 1SW and 2SW salmon respectively (Table 2). Return rates for Southern Upland salmon are currently about ten times higher than they are for inner Bay of Fundy salmon populations.

Population modeling for the LaHave River (above Morgan Falls) and the St. Mary's River (West Branch) salmon populations indicated that smolt-to-adult return rates, a proxy for at-sea survival, have decreased by a factor of roughly three between the 1980s and 2000s.

Table 2. A summary of the average return rates (percent) of one sea-winter and two sea-winter wild Atlantic salmon for the 1980 to 1989 and 2000 to 2009 time periods for the populations in the LaHave River (above Morgan Falls) and in the West Branch of the St. Mary's River.

	LaHave River (above Morgan Falls)		St. Mary's River (West Branch)	
	1980-1989	2000-2009	1980-1989	2000-2009
Return rates to river mouth (%)				
1SW mean	7.28	2.25	3.33	1.18
1SW minimum	2.87	1.19	1.17	0.54
1SW maximum	17.60	4.14	5.52	2.11
2SW mean	0.74	0.33	0.74	0.09
2SW minimum	0.31	0.10	0.18	0.00
2SW maximum	1.21	0.52	1.54	0.30

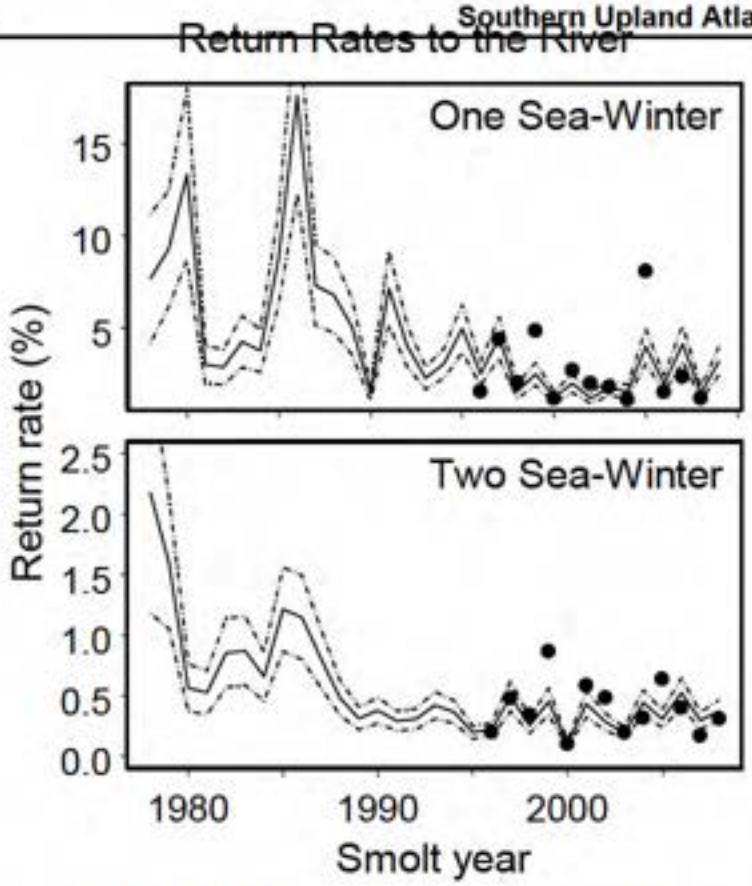

Figure 5. Observed (points) and estimated (lines) return rates for one sea-winter and two sea-winter wild Atlantic salmon for the LaHave River (above Morgan Falls) population, as estimated with the life history model. The broken lines show 95% confidence intervals based on normal approximations. Return rates are to the mouth of the river.

In addition to the changes in survival of smolts, the survival of adult salmon has also decreased since the 1980s. Details of research based on LaHave River salmon are summarized in Gibson and Bowlby (2013). The resulting estimates of mortality in the first year between spawning events increased throughout the time series, whereas mortality in the second year between spawning events increased but tended to oscillate (Figure 6). Decadal comparisons of parameter estimates indicate that mortality in the first year has continued to trend upward, indicating increasing mortality in freshwater or marine near-shore regions (near-field), whereas average second-year mortality values increased from the 1980s to the 1990s, consistent with a regime shift in the oceanic (far-field) environments. The probability of consecutive spawning varied during the time without any obvious trend in period. Fluctuations in the second-year mortality parameter matched fluctuations in the winter North Atlantic Oscillation Index (Figure 6), although this relationship was less apparent after 2000, possibly indicating a change in the regulatory mechanism in the later time period.

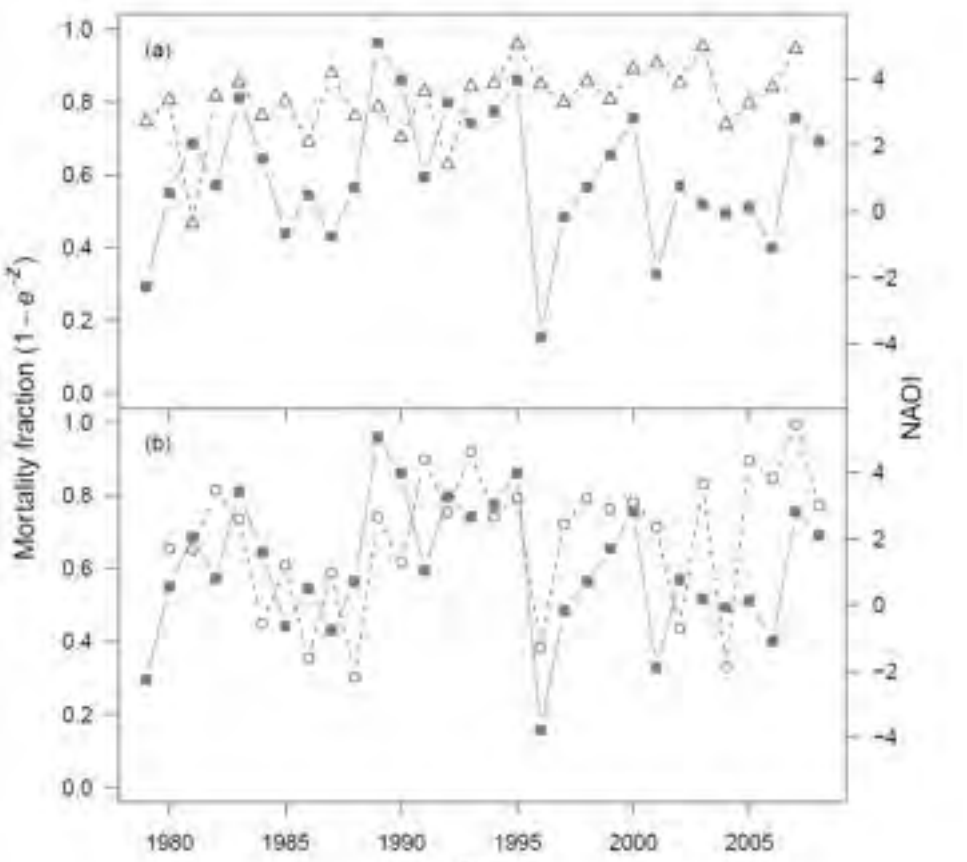

Figure 6. Annual mortality rate of LaHave River salmon as the proportion of potential mature Atlantic salmon that die in a given first year plotted alongside the winter North Atlantic Oscillation Index (NAOI) (■), an environmental variable thought to influence the marine ecology of Atlantic salmon. The NAOI is compared to mortality in the first year (Δ), which occurs mainly in freshwater (a) and mortality in the second year (○), which occurs mainly in the marine environment (b). A horizontal dashed line is provided for reference and represents an NOAI of 0 or an annually mortality rate of 50%. Reprinted from Hubley and Gibson (2011).

Population Dynamics: Past and Present

Due to the decreases in survival described above, the number of eggs expected to be produced by a smolt through its life (EPS) has also decreased. For the LaHave population, EPS values ranged between 87 and 489 eggs/smolt in the 1980s and between 29 and 111 eggs/smolt in the 2000s, a statistically significant decrease. Similar changes were estimated for the St. Mary's population, although the EPS values were generally lower.

The estimates of freshwater productivity (the rate at which eggs produce smolts) and the EPS estimates (the rate at which smolts produce eggs) were combined via an equilibrium analysis to provide estimates of the abundance at which the population will stabilize if the input parameters remain unchanged. This combined model is then used to evaluate how equilibrium population size has changed through time, as well as how the population would be expected to change in response to changes in carrying capacity, survival, or life stage transition probabilities.

The equilibrium population size for the LaHave River population varied substantially in the 1980s because of changes in the return rates and the repeat spawning component (Figure 7). However, even at the minimum values observed during that time period, the equilibrium population was greater than one. During the 2000s, the mean equilibrium for the LaHave

population was zero (Table 3), indicating that the population will extirpate in the absence of human intervention or another factor that causes a change in the life history parameter values. The equilibrium population size for the St. Mary's population is slightly greater than zero (Table 4), but is low enough that the population is expected to be at high risk of extirpation due to the effects of random environmental variability.

Maximum lifetime reproductive rates for the LaHave and St. Mary's populations (Table 4) have decreased from averages of 3.59 and 4.44 in the 1980s, respectively, to averages of 0.84 and 1.02 during the 2000s. These values mean that during the 2000s, at low abundance and in the absence of density dependence (which further lowers reproductive rates), a salmon in the LaHave River produces on average a total of 0.84 replacement salmon throughout its life. Because this value is less than one (which would indicate that each spawner could replace themselves), the population is not considered viable. In the St. Mary's River, a salmon produces on average a maximum of 1.02 replacement salmon throughout its life, indicating that the population has almost no capacity to rebuild if environmental events such as floods or droughts lower survival at some point in time. Note that the minimum rate indicates that there are years of low survival, which is why this population is at risk from environmental stochasticity.

Additional information about the population dynamics of SU salmon is provided in Gibson and Bowlby (2013).

Table 3. A summary of the equilibrium population sizes and maximum lifetime reproductive rates for wild Atlantic salmon for the 1980 to 1989 and 2000 to 2009 time periods for the populations in the LaHave River (above Morgan Falls) and in the West Branch of the St. Mary's River. The values are the maximum likelihood estimates from the life history models. Two sets of values are provided: those derived using return rates to the river mouth, and those derived based on survival to the time of the assessments during the fall. The difference in the values is an indicator of the effect of the recreational fishery on the population dynamics in each time period.

	LaHave River (above Morgan Falls)		St. Mary's River (West Branch)	
	1980-1989	2000-2009	1980-1989	2000-2009
Values using return rates to river mouth				
Equilibrium egg deposition				
mean	23,188,000	0	10,651,000	71,262
minimum	3,898,900	0	1,179,800	0
maximum	63,289,000	4,378,700	21,864,000	3,428,700
Equilibrium smolt abundance				
mean	106,590	0	80,646	2,339
minimum	44,841	0	28,703	0
maximum	129,410	39,342	91,189	54,680
Max. lifetime reproductive rate				
mean	3.59	0.84	4.44	1.02
minimum	1.44	0.39	1.38	0.39
maximum	8.08	1.49	8.05	2.11

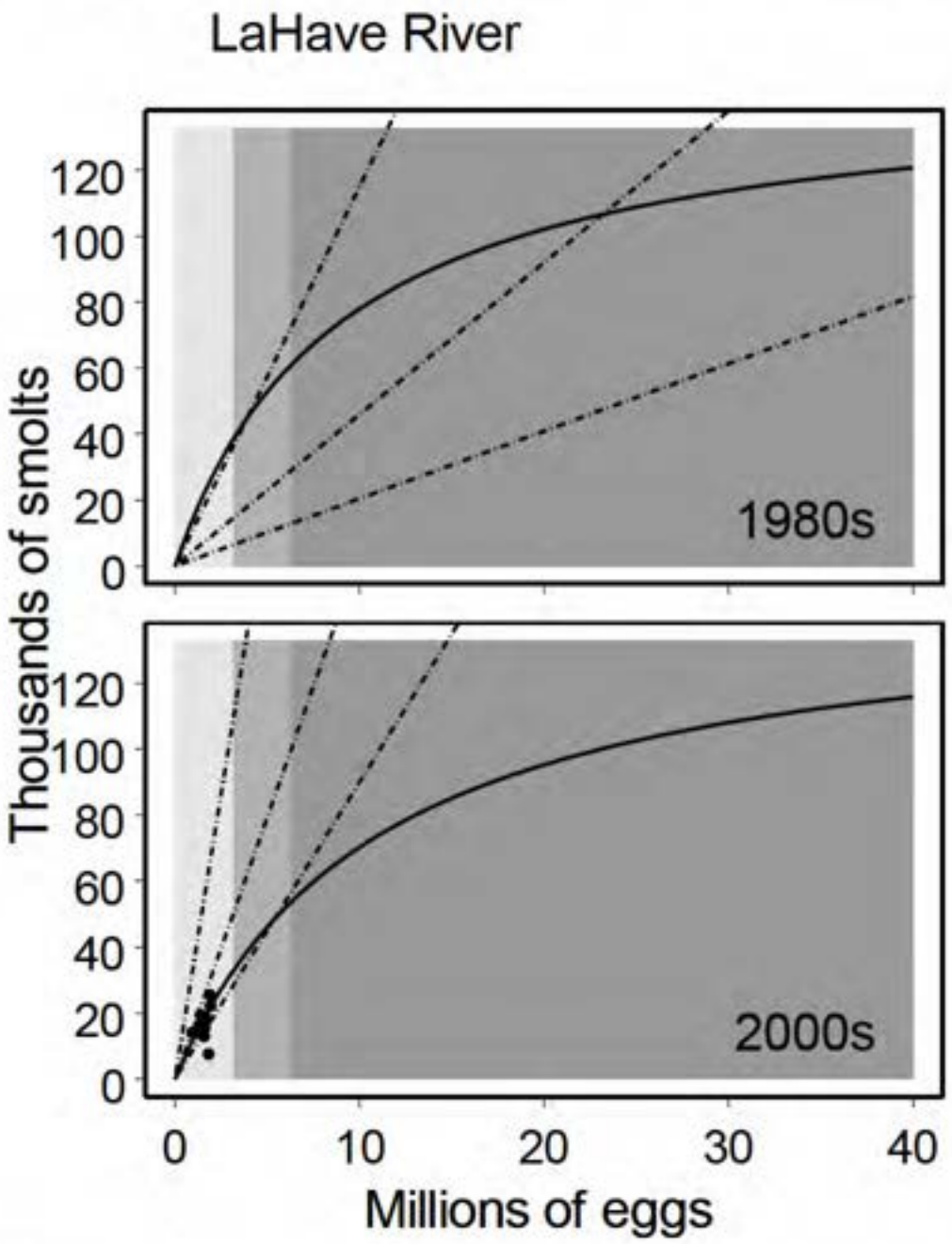

Figure 7. Equilibrium analysis of the dynamics of the Atlantic salmon population in the LaHave River, above Morgan Falls. The points are the observed egg depositions and smolt production for the 2000 to 2008 (lower panel) egg deposition years. The curved, solid line represents freshwater production. The straight, dashed lines represent marine production as calculated at the minimum observed return rates, the mean observed return rates, and the maximum observed return rates for 1SW and 2SW adults during the two time periods. Dark shading indicates egg depositions above the conservation egg requirement, medium shading is between 50% and 100% the egg requirement, and the light shading is below 50% of the requirement.

Population Viability under Present Conditions

Population viability analyses were carried out for both the LaHave River (above Morgan Falls) and the St. Mary's River (West Branch) salmon populations, using both the 1980s ("past") and 2000s ("present") dynamics. Populations are modeled as closed populations, meaning that they are not affected by either immigration or emigration. For each scenario analyzed with the PVA, 2000 population trajectories were simulated and the extinction and recovery probabilities were calculated as the proportion of populations that go extinct by a specified time. For both the past and present scenarios, the population was projected forward from a starting abundance equal to the estimated adult population size in 2010. The numbers of eggs, parr, smolt and adults, as well as their age, sex and previous spawning structure, at the start of each simulation were calculated from the adult abundance using the life-history parameter values specific to the simulation. Populations were assumed to be extinct if the simulated abundance of females dropped below 15 females for two consecutive years. When evaluating recovery probabilities, the conservation requirement was used as the recovery target.

Abundances for each life stage were projected forward for 100 years even though there is considerable uncertainty about what the dynamics of these populations will be at that time. The reason for using these projections is to evaluate longer term viability for each scenario (i.e. does it go to zero or not) and not to estimate abundance at some future time. These projections are used to determine whether the populations are viable for each combination of life history parameters, random variability and extreme events included in the scenario. In the results that follow, emphasis is placed on the LaHave River (above Morgan Falls) population.

Population modeling for two of the larger populations remaining in the Southern Upland DU (LaHave and St Mary's) indicates a high probability of extirpation (87% and 73% within 50 years for these two populations respectively) in the absence of human intervention or a change in survival rates for some other reason.

Abundance trajectories for the LaHave River (above Morgan Falls) salmon population (Figure 8) indicate that, given the present (2000s) population dynamics, this population will extirpate and has zero probability of reaching its recovery target (Figure 9; Table 4). The probability of extinction increases rapidly after about 15 years, with 31% of the simulated populations being extinct within 30 years and >95% going extinct within 60 years (Table 4). None of the 2000 simulated population trajectories met the recovery target within 100 years. This result is consistent with the maximum lifetime reproductive rate estimate of less than one (indicating that the population should continually decline under current dynamics) and the equilibrium population size of zero.

The results for the St. Mary's River (West Branch) salmon population (details in Gibson and Bowlby 2013) are similar. Even though the St. Mary's River (West Branch) salmon population has a maximum lifetime reproductive rate estimate of just over one, this population is also expected to extirpate due to the effects of natural variability in survival. Extinction probabilities also increased rapidly, with 30% of the simulated populations extirpating within 30 years, and 86% of the simulated populations becoming extirpated within 60 years. None of the 2000 simulated populations met the recovery target at any point within 100 years indicating a recovery probability of near zero based on the present dynamics.

Table 4. Probabilities of extinction and of recovery within 1 to 10 decades for the LaHave River (above Morgan Falls) Atlantic salmon population. Two scenarios are shown, one based on the 1980s dynamics (past dynamics) and one based on the 2000s dynamics (present dynamics). The same random numbers are used for each scenario to ensure they are comparable. Probabilities are calculated as the proportion of 2000 Monte Carlo simulations of population trajectories that either became extinct or met the recovery target.

	Probability of Extinction		Probability of Recovery	
Dynamics:	Present	Past	Present	Past
Year				
10	0.00	0.00	0.00	0.34
20	0.05	0.00	0.00	0.97
30	0.31	0.00	0.00	1.00
40	0.66	0.00	0.00	1.00
50	0.87	0.00	0.00	1.00
60	0.96	0.00	0.00	1.00
70	0.99	0.00	0.00	1.00
80	1.00	0.00	0.00	1.00
90	1.00	0.00	0.00	1.00
100	1.00	0.00	0.00	1.00

Population Viability under Past Conditions

In contrast, abundance trajectories using the past (1980s) dynamics (Figure 8) indicate rapid population growth. None of the simulated population trajectories extirpate within 100 years (Figure 9; Table 4) and all simulations reach the recovery target within 30 years.

As was the case with the LaHave River (above Morgan Falls) population, abundance trajectories using the past (1980s) dynamics for St. Mary's River (West Branch) indicate rapid population growth. None of the simulated population trajectories extirpate within 100 years and 97% of the simulated populations reach the recovery target within 30 years. Not all populations remain above the recovery target all of the time because of the low carrying capacity for age-1 parr estimated for this population.

Effects of Extreme Environmental Events

The population viability analyses indicate that the loss of past resiliency to environmental variability and extreme environmental events is contributing to the high risk of extinction. Extreme environmental events that markedly reduce the abundance of juvenile Atlantic salmon do occasionally occur. One such event potentially occurred in the fall of 2010 with very high water levels occurring shortly after the spawning season. Extremely high water events can lead to disturbance or destruction of redds or overwintering habitat for juveniles resulting in higher mortality. The effects of environmental variability and extreme events were investigated using the St. Mary's River (West Branch) population model. The St. Mary's example was chosen rather than the LaHave because it has an equilibrium population size greater than zero, and, therefore, would not become extinct in the absence of environmental variability. However, when random variability is added to the projections (using the same life history parameter values as in the base model), the median time to extinction becomes just under 70 years with 10% of the populations becoming extinct within 40 years. When extreme events are added, 10% of the populations are extinct in 22 years, and half of the populations are extinct within 40 years. Changing the frequency and magnitude of the extreme events changes the extinction probabilities as expected. However, when the same random variability and extreme event scenarios are modeled using the 1980s dynamics, none of the 10,000 simulated population trajectories become extinct and most met the recovery target. This highlights the resiliency that

these salmon populations had in the past to environmental variability. Restoring this resiliency, resulting from distributing reproductive effort over multiple years coupled with higher survival, will be an important component of recovering SU Atlantic salmon.

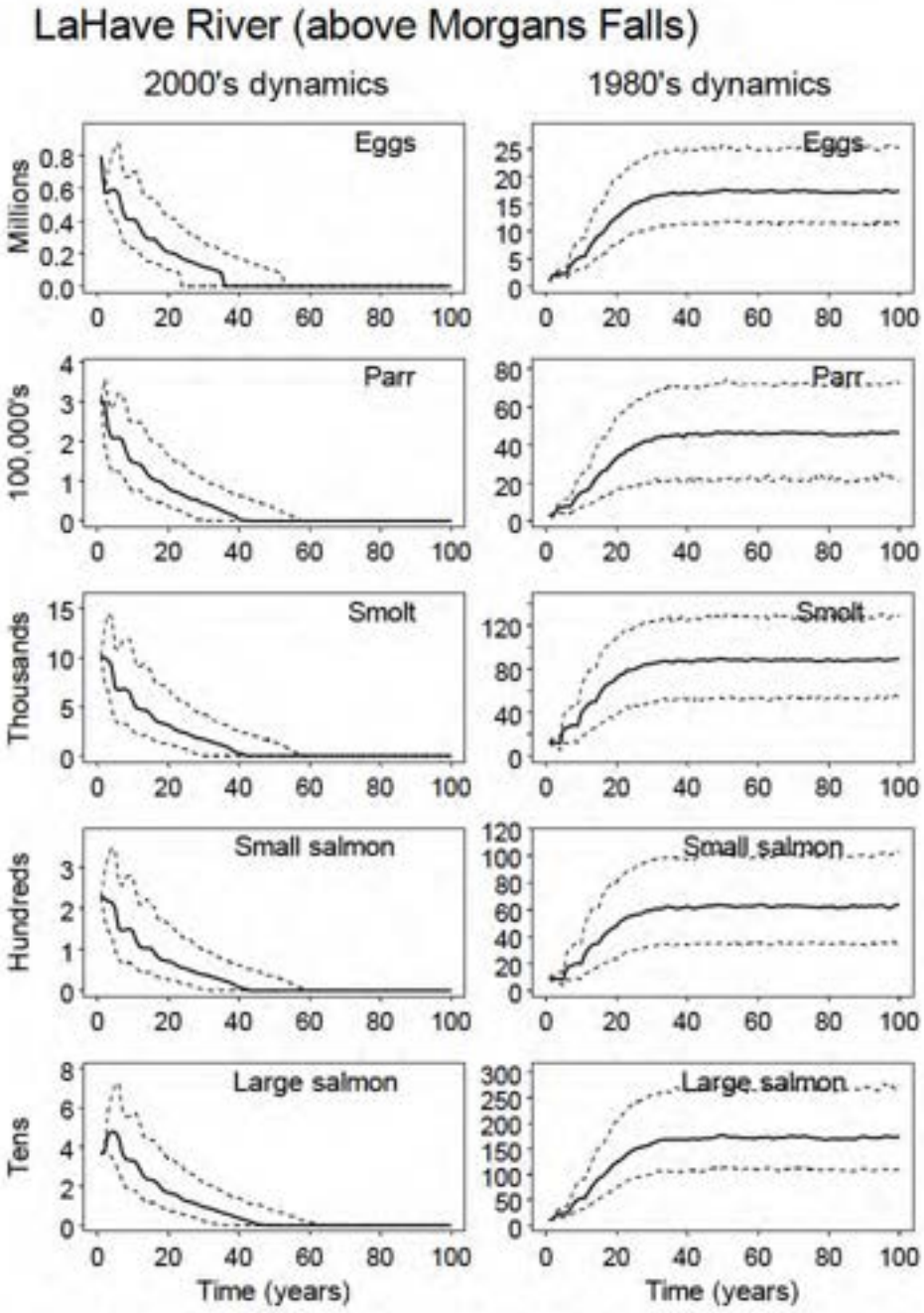

Figure 8. Simulated median abundance (solid line) with the 10th and 90th percentiles (dashed lines) for each of five life history stages from Monte Carlo simulations of the LaHave River (above Morgan Falls) Atlantic salmon population viability model. Two scenarios are shown, one based on the 1980s dynamics (right panels) and one based on the 2000s dynamics (left panels). The graphs summarize 2000 simulations for each scenario.

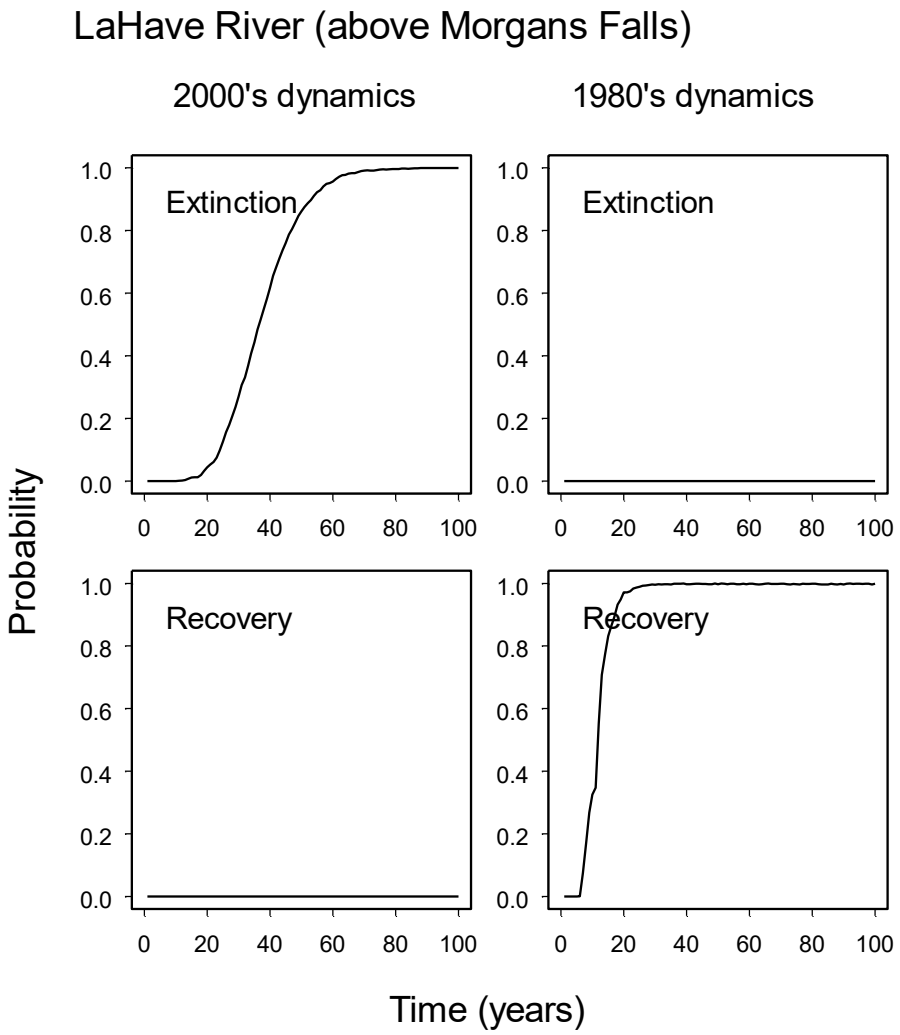

Figure 9. The probability of extinction and the probability of recovery as a function of time for the LaHave River (above Morgan Falls) Atlantic salmon population. Two scenarios are shown, one based on the 1980s dynamics (right panels) and one based on the 2000s dynamics (left panels). Probabilities are calculated as the proportion of 2000 Monte Carlo simulations of population trajectories that either went extinct or met the recovery target.

Habitat Considerations

Functional Descriptions of Habitat Properties

Detailed descriptions of aquatic habitat that SU Atlantic salmon need for successful completion of all life-history stages can be found in Bowlby et al. (2013b).

Freshwater Environment

Adult Atlantic salmon return to rivers in the SU as early as April and as late as November, but the largest proportion of the population enters the rivers in May to August, and fish can spend up to 6 to 7 months in fresh water prior to spawning. The upstream migration appears to generally consist of a migration phase with steady progress upriver interspersed with stationary resting periods, and a long residence period called the holding phase. Habitat properties required for successful migration into rivers include: appropriate river discharge (e.g. it has been

suggested that upstream migration will initiate at a river discharge rate of >0.09 m^3/s per meter of river width), pools of sufficient depth and proximity in which to hold (spending weeks to months in a single pool), and unimpeded access throughout the length of the river.

Atlantic salmon in the SU spawn in October and November, with eggs incubating in redds through the winter and hatching in April. Successful incubation and hatching depends on: river discharge, water depth (e.g. generally between 0.15 to 0.76 m for redd construction) and velocity (e.g. 0.3-0.5 m/s preferred at spawning sites), substrate composition (e.g. coarse gravel and cobble with a median grain size between 15 and 30 mm forms the majority of the substrate of redds, with fine sediments found at low concentrations), water temperature (e.g. stable cold temperatures for egg development), and water quality (e.g. uncontaminated water with a pH >5.0 for development of embryos and alevins).

Juvenile SU Atlantic salmon remain in fresh water for one to four years after emergence, with most migrating to the sea two years after emergence. Habitat properties that are important for the successful rearing of juveniles (fry and parr) include: water depth (e.g. age 0 fry tend to occupy water 15-25 cm deep) and velocity (e.g. fry tend to be found in riffles with surface velocities >40 cm/s, parr are found in a wider range of velocities with an optimum between 20-40 cm/s; juvenile Atlantic salmon are rarely found at water velocities <5 cm/s or >100 cm/s, and, in the winter juveniles seek out lower velocity water, presumably to minimize energy expenditure); substrate composition (e.g. preferred substrate for age 0 salmon is in the range 16-256 mm diameter (gravel to cobble) and 64-512 mm diameter (cobble to boulder) for age 1 and older parr); the presence of cover; water temperature (typically between 15ºC and 25ºC); and water quality (uncontaminated water of pH > 5.4).

Salmon smolts do not have the same freshwater habitat requirements as parr, but rather require the environmental conditions necessary to trigger the changes associated with smoltification as well as to successfully emigrate to salt water. Environmental characteristics influencing the process of smoltification are: photoperiod, water temperature, and river discharge. The main characteristics influencing successful emigration from the river are: unimpeded access throughout the length of the river, and sufficient water discharge.

Relatively little is known about freshwater habitat use by post-spawning adult salmon (kelts) in the SU. Kelts have been shown to over-winter in deep water habitats and descend the river in the spring, although some kelts may exit the river relatively soon after spawning. Whether some SU kelts over-winter in estuaries is unknown. The proportion of the population that remains in the river during winter likely depends on the availability of pools, lakes, and stillwaters in the watershed. In a 2010 and 2011 acoustic tagging study in the St. Mary's River, all 24 of the tagged salmon left the river in spring after spawning; no kelts emigrated immediately after spawning or during the winter. The earliest observed salmon leaving the river was on March 16th, but most salmon exited the river between April 22nd and May 11th. This suggests that the proportion of adults remaining in SU rivers after spawning to overwinter in fresh water is high, particularly in rivers with suitable overwintering habitat.

Estuarine Environment

Once smolts leave fresh water, they swim actively, moving continuously through the estuary without a long period of acclimation to salt water. Migration patterns are not necessarily directly toward the open ocean, and residency times in the estuary are varied. This cyclical movement pattern has been exhibited by SU smolts. Residency patterns only suggest where and when smolts occupy estuaries, not the physical habitat characteristics that may be required. Given that smolts are thought to swim near the surface within the fastest flowing section of the water column, and use an ebb tide pattern of migration, habitat choice is unlikely to be based on

physical habitat characteristics (e.g. substrate type). It is more likely that the oceanographic conditions in estuaries and coastal areas influence movement and habitat choice in estuaries.

Adult Atlantic salmon return to rivers in the SU throughout the spring, summer, and fall months. Similar to smolt use of estuaries, a variety of estuarine residency times for adults have been observed, from moving through estuaries in a matter of days to spending 3.5 months holding in an estuary before moving into the river. Estuaries appear to be mainly staging areas, and movements within them are frequently slow (<0.2 body lengths per second), following the sinusoidal pattern of the tidal currents. While holding in the estuary, adults seem to favour deep water of intermediate salinities ranging from 5 to 20 parts per thousand.

The limited information on residency times or habitat use by kelts in estuaries suggests that estuaries are used predominantly as staging areas and migratory corridors in the spring. In spring, kelts pass relatively quickly through estuaries on their way to open ocean. The one study on acoustically tagged kelts in the LaHave River found that kelts tagged in fresh water in April exited the estuary within five weeks of release. There was no typical migration pattern; one kelt exhibited non-stop migration seaward and others interspersed periods of continuous movement, residence, and backtracking.

Marine Environment

Habitat use in the marine environment for immature Atlantic salmon (individuals that have undergone smoltification, migrated to the ocean, but have not yet returned to fresh water for the first time to spawn, also known as post-smolts) has been mainly hypothesized based on physiological requirements and/or tolerances of Atlantic salmon in the marine environment. At sea, salmon tend to be found in relatively cool (4^{o}C to 10^{o}C) water, avoiding cold water (<2^{o}C), and modifying their migratory route in space and time in response to ocean temperature conditions. For example, in years where coastal water temperatures are warmer, salmon arrive at home rivers earlier. Tagging studies suggest that immature salmon are pelagic, spending the majority of their time in the top few meters of the water column, following the dominant surface currents and remaining in the warmest thermocline. Although movement patterns and distribution have been correlated with water temperature and other abiotic factors, the availability of prey and potential for growth are assumed to determine actual distribution at sea. As such, marine distribution patterns would be expected to vary in space and time as well as among years, based primarily on the distribution of suitable prey items.

Recent studies in the Northeast Atlantic demonstrate that immature salmon begin to feed extensively on marine fish larvae and to a lesser extent on high-energy crustaceans, experiencing a rapid increase in growth in the near-shore environment. Atlantic salmon are opportunistic feeders, leading to geographical differences in the type and amount of prey consumed. There is some indication that Atlantic salmon in the Northwest Atlantic have a larger proportion of insects and crustaceans in their diet than those in the Northeast Atlantic, but gadoids, herring and sand lance are also important prey items.

Growth patterns of scale circuli from two populations in the SU region combined with tag returns from commercial fishing suggest that these populations experience similar oceanographic conditions and use similar temporal and spatial routes during marine migration. A coastal or near-shore migration route along the North American continent is generally accepted (as described in the Spatial Extent of Habitat section). The location of primary feeding and staging grounds for immature salmon destined to return after one winter at sea to rivers in the SU is less well known. It may include all near-shore areas along the North American coast with suitable surface temperatures, extending northward to the Labrador Sea, but is more likely to correspond to areas of high prey density within that broad range.

After spawning, the majority of adults exit rivers in the spring of the following year for a period of reconditioning before spawning again. The length of time adults spend in the ocean between spawning events likely determines marine habitat use and distribution patterns. Consecutive spawners return in the same year as their kelt migration and have a relatively short ocean residence period (< 6 months), while alternate spawners return the following year and can spend up to a year and a half in the marine environment. Tagging studies demonstrate that alternate spawners travel as far north as West Greenland, likely following a similar migration route as immature salmon along the coastal or near-shore habitats of North America. The marine habitat use of consecutive spawning adults is less well known, but it is very unlikely that individuals would be able to reach the Labrador Sea or West Greenland in the time between spawning events. One acoustically tagged kelt from the LaHave River reconditioned over a period of 79 days before re-ascending the river, but spent this time outside the estuary. As with immature salmon, marine distribution and habitat use of adults is thought to be determined primarily by the distribution and abundance of suitable prey. Fish are the majority of the diet of adult salmon, and the species consumed include capelin, sand eels, herring, lanternfishes and barracudina. Amphipods, euphausids (krill) and other invertebrates are also consumed, and there is some indication that the proportion of invertebrates consumed increases in more southerly feeding areas.

Spatial Extent of Habitat

Freshwater Environment

Wild Atlantic salmon exhibit nearly precise homing to natal rivers, which results in significant population structuring at the river scale. There is no information which suggests that salmon do not use all available rivers in the SU at least intermittently, and assessment data demonstrates that there is no apparent minimum watershed size for occupancy. As described in the Background section, the number of watersheds that are known to have contained salmon populations is 72 (Figure 2). However, 513 additional watersheds in the SU have been identified by the Nova Scotia Department of the Environment (NS DoE), of which 256 are larger than Smith Brook (the smallest watershed known to have contained salmon). These other watersheds have a total drainage area of 6,586 km^2 (excluding coastal islands), and each has the potential to support Atlantic salmon.

Combining information from all watersheds known to have contained salmon (Figure 2), there is an estimated 20,981 km^2 of drainage area, which contains 783,142 habitat units (100 m^2) of rearing area for Atlantic salmon. The 10 largest systems contain slightly more than half of this productive area (436,572 habitat units), and only 4 watersheds have an estimated rearing area that is less than 1,000 habitat units.

Estuarine Environment

The use of particular habitat types within estuaries by smolts, adults and kelts is relatively unknown for SU Atlantic salmon, but estuarine habitat availability is not thought to be limiting.

Marine Environment

Marine distribution patterns for SU Atlantic salmon were assessed based on recovery locations of tagged smolts and adults reported by commercial and recreational fisheries.

In total, there were 5,158 recaptures of individuals tagged in the SU region (1,899 from SFA 20 and 3,259 from SFA 21). Recapture rates from groups of tagged fish were extremely low, generally less than 5% (mean = 3.9%, median = 0.8%, range: 0.02% - 73%). All of the higher

recapture rates were associated with releases upstream of continuously monitored facilities, like Morgan Falls fishway on the LaHave River. There were very few release events of exclusively wild-origin fish (either adult or smolt) or of adults (either hatchery or wild. Therefore, the data presented are based entirely on recaptures of hatchery-origin or mixed-origin (wild plus hatchery in the same release group) smolts. Due to the relative scarcity of recapture information, marine distribution patterns of SU Atlantic salmon are presented as a group, although there are likely differences among populations in marine habitat use. Three time periods were evaluated: distribution in the year of release, distribution in the year following release, and distribution two years following release.

First Year Following Release (Figure 10): The majority of tagged smolts were released in fresh water in April and May. By late May and throughout June, smolts had begun leaving fresh water and moving along the coast of Nova Scotia, both in a southern and northern direction (Figure 10). By July, individuals had spread out along the entire coast of Nova Scotia, from the inner Bay of Fundy to the tip of Cape Breton, while a smaller proportion had moved substantially farther northward, to Eastern Newfoundland, Northern Quebec and the tip of Labrador (Figure 10). A similar pattern exists during August. From September until the following March, there were very few tag recaptures; these indicated that a proportion of SU salmon remained along the coast of NS during the winter months. Interestingly, there were no recaptures of immature SU Atlantic salmon off the coasts of Newfoundland, Quebec, and Labrador after September. This may suggest that immature Atlantic salmon from the SU do not over-winter this far north in their first winter at sea, or that they arrive after the close of the various fishing seasons (i.e. after November). Additionally, immature salmon were not captured in the West Greenland fishery in the first year following release (based on a total of 430 recapture events), which may indicate that they do not travel this far north in their first year or are too small to be captured by the fishing gear.

Second Year Following Release (Figure 11): In the second year, there would be salmon that return to natal rivers to spawn after 1SW as well as salmon that remain at sea for the second year (and will return as 2SW or older). The earliest recaptures in the spring were still off the coast of Nova Scotia (Figure 11), suggesting that a proportion of the individuals remained relatively localized for their entire first year at sea. Beginning in May, the largest number of recaptures was along the northern coast of NL and spread to more southerly locations in June, concentrated off the coast of Nova Scotia (Figure 10). Recaptures in the high-seas fishery off West Greenland took place from July to November (Figure 10), and the relative scarcity of recaptures in July, October and November may reflect reduced fishing effort rather than movement into or out of this area. The catch from the West Greenland fishery is thought to consist almost entirely of individuals destined to return to natal rivers as 2SW spawners, so these tag returns represent the 2SW component of populations. It is possible that the recaptures off the northern coast of Newfoundland and Labrador during the spring, summer and fall months (Figure 11) also consist of a proportion of 2SW individuals, as well as those returning to their natal rivers to spawn. It is likely that most of the recaptures of salmon off the coast of Nova Scotia in the summer months represent 1SW individuals (Figure 11). It is similarly likely that the distribution of 1SW and 2SW fish partially overlap during the summer months.

Third Year Following Release (Figure 12): In the third year, there would be salmon returning to the marine environment after spawning as 1SW salmon and salmon returning to natal rivers to spawn as 2SW adults. Based on results of kelt tagging in the LaHave River, it is likely that some portion of the marine recaptures off the coast of Nova Scotia in April and early May (Figure 12) are salmon that over-wintered in fresh water and returned to recondition in the marine environment. The other portion of the recaptures was likely first-time spawners. There were recaptures off the coast of Newfoundland from May to November (Figure 12), potentially representing two groups: salmon moving from West Greenland and the Labrador Sea on their

way to natal rivers (2SW spawners) and salmon moving northward to recondition after previously spawning.

Assuming that these data represent general distribution patterns in the marine environment, there seems to be very limited use of the Gulf of St. Lawrence (including the coastal areas around the Magdalen Islands, northern New Brunswick, or Quebec near Anticosti Island) by SU Atlantic salmon. However, they do move along both coasts of Newfoundland, and they have been recaptured at locations south of where they were released. Contrary to predictions of progressive northward movement for immature individuals to over-wintering areas in the Labrador Sea or West Greenland, these tagging data suggest that SU Atlantic salmon are widely distributed in coastal marine habitats throughout their first year, particularly during the summer months.

Although it is not possible to explicitly describe the movement patterns of the various life stages of SU Atlantic salmon from these data, the inferences above highlight a crucial point when designating critical habitat in the marine environment. Although different life stages may transiently occupy similar habitats, their overall direction of movement could be in opposite directions, potentially leading to a relatively ubiquitous distribution from Nova Scotia to the Labrador Sea and West Greenland throughout most of the year. Given the variability in run-timing, both within and among populations, similar variability is likely to exist in movement of SU Atlantic salmon along the near-shore environments of the Northeast Atlantic, meaning that marine distribution (and therefore habitat use) cannot be clearly delineated on a seasonal basis.

Freshwater Spatial Constraints: Influence of Barriers and Water Chemistry on Habitat Accessibility

Assessing the impact of physical barriers on the amount of habitat in a watershed is difficult because structures can be entirely or seasonally impassable for various life stages depending on stream flow. An ArcGIS layer detailing available information on barriers in SU watersheds was compiled jointly by the NS DoE and the DFO Habitat Management (HM). This layer contains the characteristics of known barriers, including fish passage capabilities (e.g. classified as passable to fish or not). These data represent the best regional information, but data were collected over multiple years. The most recent updates to specific records span the years from 2007 to 2010 (a total of 37 out of 586 records do not list a date). Any recent changes would not have been captured in the database.

By intersecting the stream network from the National Hydrographic Service with the barrier locations, it was possible to calculate the percentage of the flow network (stream length) affected by barriers in each of the SU watersheds. There is an essentially linear relationship between the length of the flow network and the drainage area in watersheds in the SU (data not shown), so these percentages were multiplied by the amount of rearing area in a watershed to approximate the impact of barriers on habitat availability. The accessible rearing area was estimated at 57.0 million m² (73.2% of total rearing area) and the inaccessible area was estimated to be 21.0 million m² (26.8% of total rearing area).

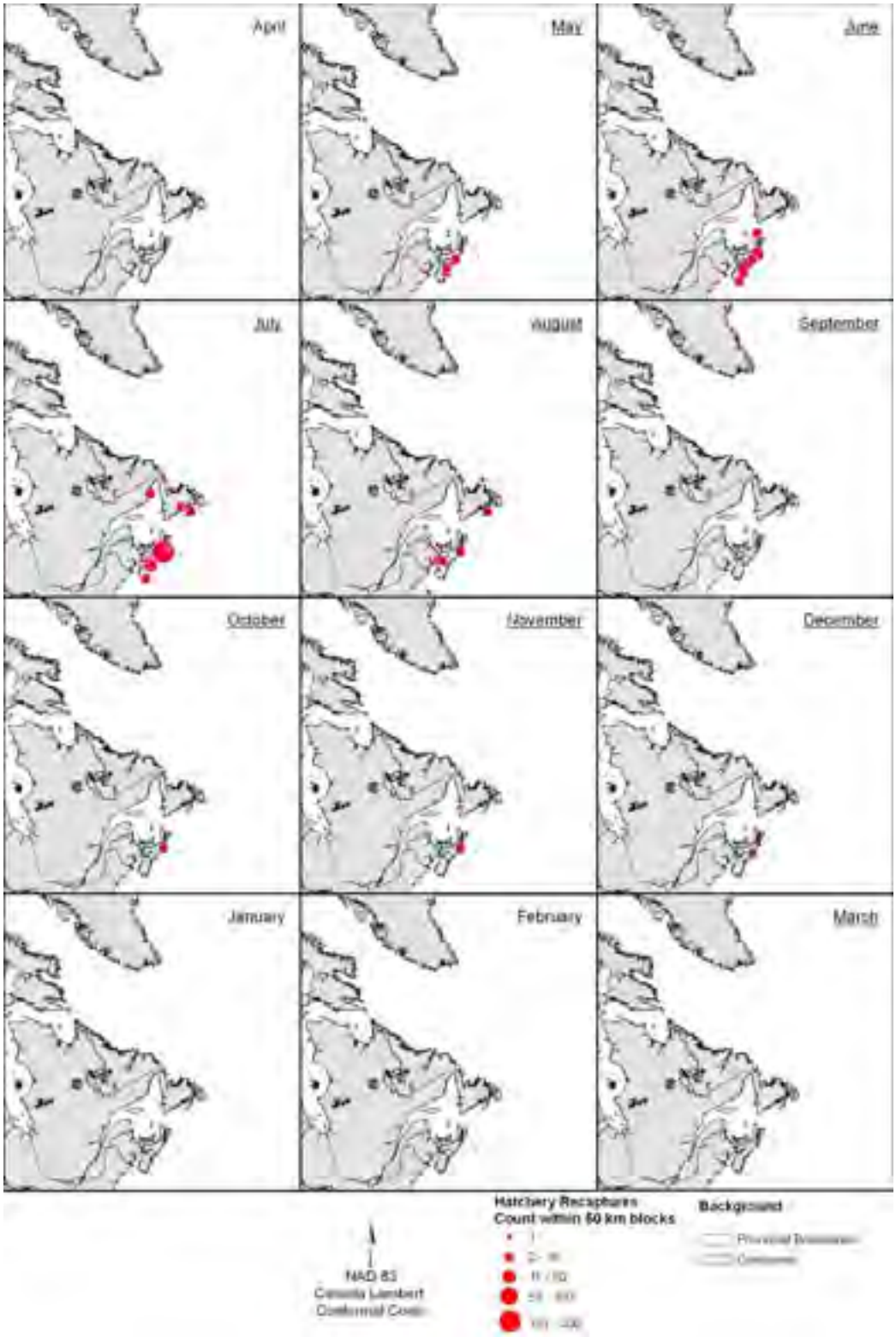

Figure 10. Recapture locations in the marine environment of individually tagged, hatchery-origin smolts in the first year following release, where the size of the point on the map is proportional to the number of recaptures within a 50 km^2 grid.

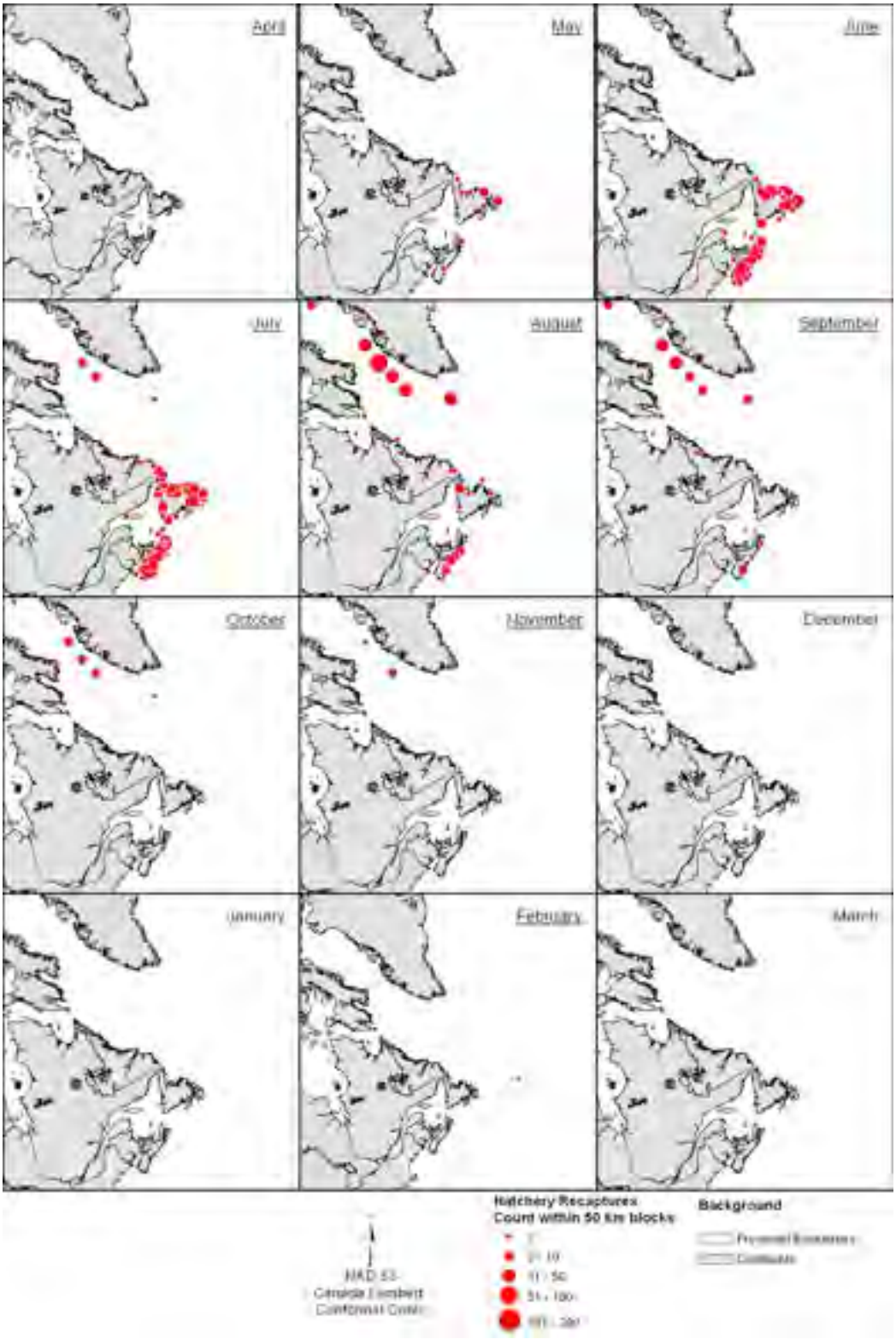

Figure 11. Recapture locations in the marine environment of individually tagged, hatchery-origin smolts in the second year following release, where the size of the point on the map is proportional to the number of recaptures within a 50 km^2 grid.

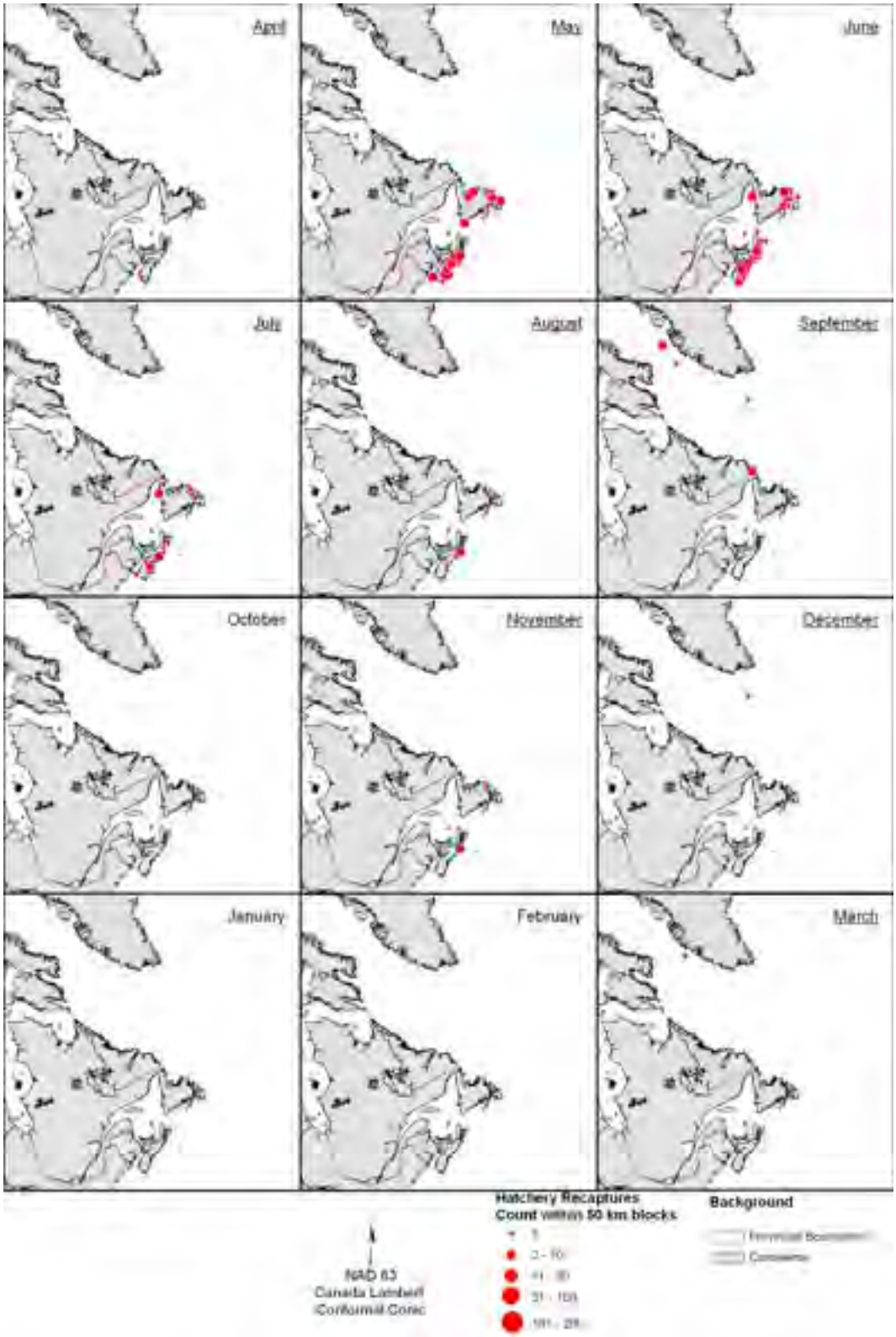

Figure 12. Recapture locations in the marine environment of individually tagged, hatchery-origin smolts in the third year following release, where the size of the point on the map is proportional to the number of recaptures within a 50 km^2 grid.

Acidification (low pH) is a major factor limiting the production of Atlantic salmon in many SU rivers. It can partially or completely eliminate suitable habitat within a watershed. Highly acidified water is not a barrier *per se* because adults can still enter the river and spawn;

however, the habitat is unsuitable because their progeny die. Thirteen rivers are considered to be unsuitable for spawning and juvenile rearing based on their acidity level (mean annual pH < 4.7), conclusion supported by the juvenile density estimates from the electrofishing surveys (0/100m^2). These 13 rivers contain a total of 100,198 habitat units (100 m^2) [or 10 million m^2] that is considered unsuitable for Atlantic salmon production.

None of the 5 watersheds that are identified as impassable due to barriers at head-of-tide are among the 13 watersheds that unsuitable for Atlantic salmon due to acidification. Thus, 18 watersheds have very little or no rearing area available for Atlantic salmon. Of the remaining 54 rivers, 25 contain total barriers that block from 0.1% to 94.5% of the watershed. There are 29 rivers that do not contain a known total barrier, and these tend to be either smaller systems or watersheds along the Eastern Shore of Nova Scotia. Of the 783,142 habitat units (100 m^2) available in rivers in the SU region, only 476,746 (61%) remain accessible to Atlantic salmon populations (Figure 13).

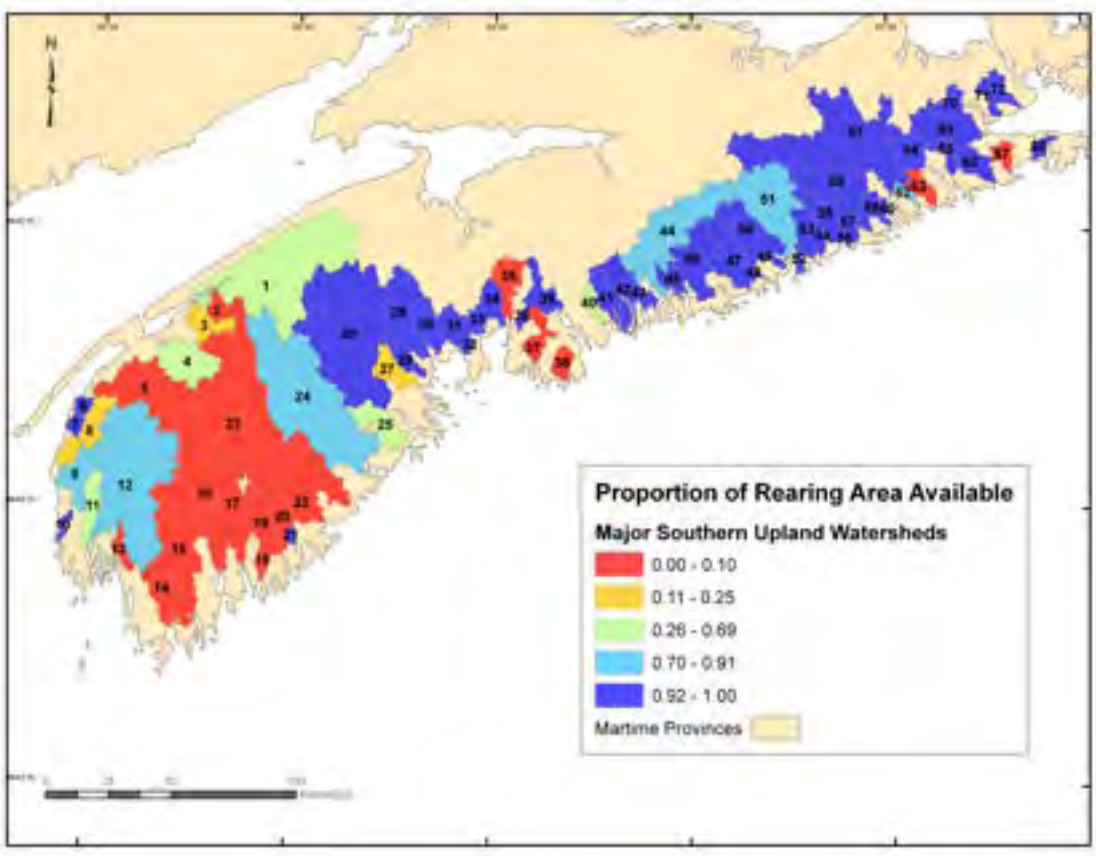

Figure 13. Proportion of rearing area available to Atlantic salmon for watersheds in the Southern Upland based on accessible habitat area (i.e. area below impassable dams) as well as pH category (where mean annual pH < 4.7 is considered unusable). Watershed numbers correspond to the legend in Figure 2.

Thus, together, acidification and barriers to fish passage are thought to have reduced the amount of freshwater habitat by approximately 40%. Thirteen individual watersheds are thought to contain essentially no useable habitat (based on acidification) and a range of 0.1% to 95% of habitat (based on stream length) is lost in other watersheds. These estimated reductions in habitat quantity are likely conservative. However, given the low abundance of salmon at present, habitat quantity is not thought to be currently limiting in rivers unaffected by barriers and acidification. .

Supply of Suitable Habitat

Current juvenile densities estimated for rivers in the SU are very low (Figure 4), particularly when compared to historical estimates of juvenile salmon production that have been used as a reference levels in the past (29 age 0 fish/100 m^2 and 38 age 1 and older fish/100 m^2: known as Elson's norm) . In other regions, where Atlantic salmon populations are thought to be meeting or close to conservation requirements, juvenile density estimates for all age classes regularly exceed Elson's norm. Although rivers in the SU may have lower productive potential than those in other areas because of their underlying geology, the amount of rearing habitat for juveniles in a given watershed (i.e. habitat of suitable gradient) is unlikely to be limiting population size for unobstructed systems and non-acid impacted systems at present. Low juvenile abundance is more likely the result of low adult abundance (in part due to low at-sea survival) and effects of human activity in these watersheds. As described above, physical barriers and water quality have likely reduced the quantity of freshwater habitat available to spawning adults by at least 40%, which would be expected to reduce adult abundance by the same amount if other life history parameters remained unchanged. In these rivers, supply of suitable habitat likely would not meet the demand.

The production of juvenile Atlantic salmon in freshwater habitats is governed by density dependent growth, survival, and habitat use. However, potential for growth is inversely related to density and, as populations become larger (with no change in the quality and quantity of available habitat), the potential rate of population growth declines. At high abundance, populations exhibit relatively constant juvenile production over a very large range of egg deposition values. In the context of habitat limitation for SU Atlantic salmon at very high abundance, this demonstrates that the productive capacity of freshwater habitats (i.e. habitat quality and quantity) will ultimately limit population size.

Regardless of the present value for carrying capacity in a specific river, the marine survival rates experienced by populations would affect whether freshwater habitat is limiting population growth at a given level of abundance. The equilibrium analysis presented earlier shows that the mean marine survival rates observed on the St. Mary's and LaHave rivers were sufficient to enable population growth in excess of the conservation requirement during the 1980s. However, under current dynamics, these populations would not be predicted to reach the conservation requirement even at the maximum observed marine survival rates during the 2000s. Ultimately, whether freshwater habitat becomes limiting in the future depends on the dynamics of recovered populations. If survival in the marine environment were to meet or exceed levels of the 1980s, freshwater habitat is not expected to become limiting until the population had reached abundance levels in excess of the conservation requirement. Conversely, if marine survival remains at current levels or undergoes a modest increase, it is predicted that increases in freshwater productivity would be necessary to reduce extinction risk or promote population increase for SU Atlantic salmon populations. The question of whether available habitat will become limiting as populations increase depends on the productive capacity of freshwater habitats as well as the mortality rates experienced by Atlantic salmon in the marine environment.

Trade-offs Associated with Habitat Allocation Options

Allocation of freshwater habitat (i.e. for consideration as critical habitat for SU salmon) can occur on at least two scales: at the watershed scale and within a watershed. At a watershed scale, freshwater habitat should be allocated to minimize extinction risk for SU Atlantic salmon populations by ensuring that the remaining genetic diversity of SU Atlantic salmon is protected, and by facilitating the re-establishment of wild self-sustaining populations in other rivers.

Specifically, watersheds that are currently known to contain Atlantic salmon and those that have a high probability of containing useable freshwater habitat are considered priorities.

Juvenile Atlantic salmon were found in 22 of the 72 (54 surveyed) river systems in 2008/2009, with knowledge of others. Given the reductions in habitat that have already occurred and the current low population size with ongoing declines, all 22 rivers include important habitat for SU Atlantic salmon. Restoration of these populations is expected to achieve the distribution component of the recovery target described below. If additional rivers are found to contain salmon, the consideration of these rivers as important habitat would have to be evaluated.

Barriers and pH are two factors that have a large effect on freshwater habitat availability and quality, respectively, and depending on the extent of each, can be difficult or costly to remediate. Therefore, rivers or parts of rivers that remain accessible to Atlantic salmon (due to the absence of total barriers) or rivers that remain mildly or un-impacted by acidification (mean annual pH that is greater than 5.0; category 3 and 4 rivers) should also be considered very important in terms of habitat allocation for SU Atlantic salmon (Figure 14). Even if the specific river does not contain Atlantic salmon at present, these areas likely contain useable freshwater habitat that could support populations in the future. Including some rivers with varying levels of pH should also help to protect the remaining genetic diversity among populations in the SU, given that there are wild populations remaining with greater tolerance to low pH (e.g. salmon in the Tusket River have a higher tolerance of low pH than other populations in Nova Scotia).

At smaller spatial scales, habitat allocation decisions can be made to ensure that habitat availability for a single life stage does not become limiting. Atlantic salmon have a complex life cycle with different habitat requirements for each life stage. Habitat for all life stages, as well as habitat connectivity, needs to be considered when identifying priority habitats for allocation, to avoid having one habitat type limiting population growth.

In addition, the estuaries associated with these rivers are considered to be important habitat for Atlantic salmon, with successful migration through this area essential to the completion of their life history.

While there is likely important marine habitat for SU Atlantic salmon, given broad temporal and spatial variation, it is difficult to link important life-history functions with specific marine features and their attributes. Further research into marine distribution patterns is unlikely to reveal distinct areas that should be considered for marine habitat allocation. Habitat allocation decisions could potentially be made at a broad scale, and the evaluation of activities likely to impact this habitat could be based on the extent to which they reduce the capacity of the larger area to provide salmon habitat.

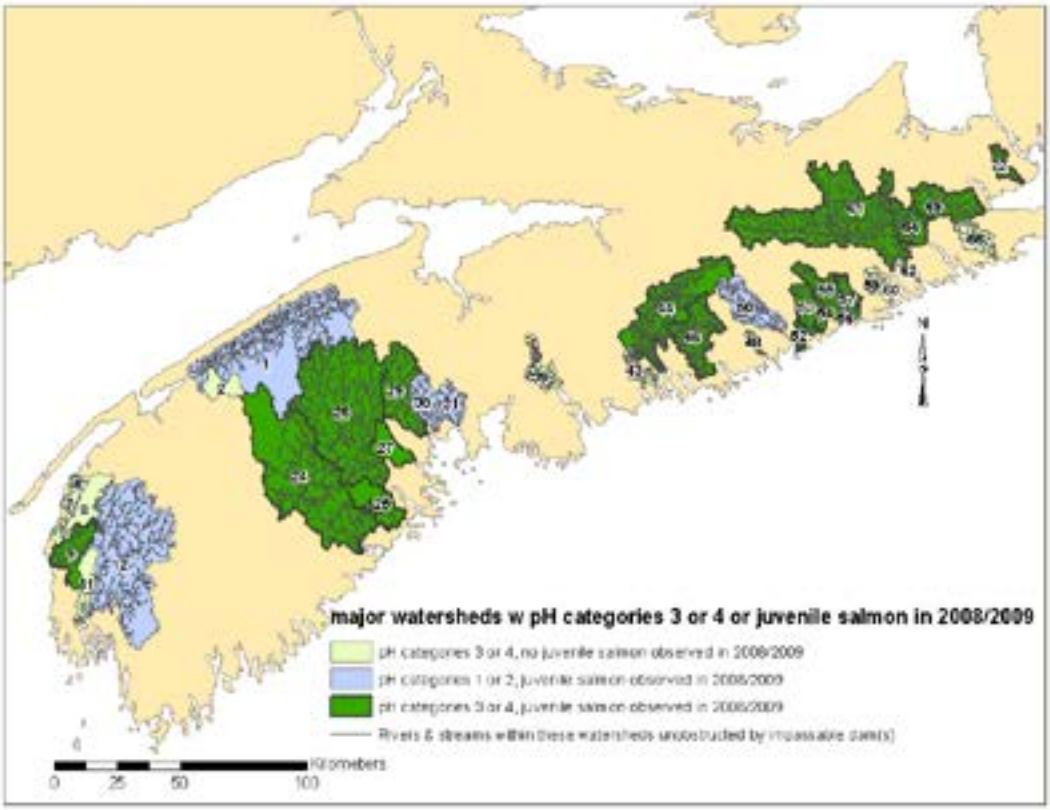

Figure 14. Location of freshwater habitats that exhibit one (or more) of three characteristics: have a pH greater than 5.0 (rivers in pH categories 3 or 4; see also Figure 16), have a high proportion of the watershed not impacted by barriers to fish passage, and/or contained Atlantic salmon in the most recent (2008/09) electrofishing survey. Watershed numbers correspond to the legend in Figure 2.

Recovery Targets

Long-term goals for the recovery of Atlantic salmon in the SU region include increasing the size and total number of populations, as well as their distribution. However, determining how many populations are needed to attain this long-term goal or how large they must be to ensure recovery of SU Atlantic salmon is not possible from a quantitative perspective because the dynamics of recovered populations of SU Atlantic salmon are not known. Previous research on abundance targets as well as theoretical research on how species distribution relates to persistence or recovery can be used as a basis for decision-making.

Proposed recovery targets for Atlantic salmon populations in the Southern Upland DU have both abundance and distribution components.

Abundance targets for Southern Upland Atlantic salmon are proposed as the river-specific conservation egg requirements, which are based on the estimated amount of juvenile rearing area and an egg deposition rate of 2.4 eggs/m^2. Attaining the conservation requirement is consistent with attaining long-term population persistence, maintaining the ecological function of the watersheds in which salmon formerly resided, and increasing the potential for human benefits if populations were recovered in as many rivers as possible. Overall population size is positively related to population persistence for a range of fish species, which suggests that increasing population size for salmon in the SU region is important for recovery. However, population size alone is not an indicator of population viability, and precisely how large populations need to be depends on their dynamics during population rebuilding.

The distribution target should encompass the range of genetic and phenotypic variability among populations, environmental variability among rivers, and include rivers distributed throughout the DU to allow for gene flow between the rivers/populations. There is the expectation that including a wider variety of populations in the distribution target will enhance persistence as well as facilitate recovery in the longer term. The following criteria can be used to help prioritize among river systems when setting distribution targets: current population size, complexity (in population life history, local adaptation and genetic distinctiveness), connectivity with surrounding populations (metapopulation structure), and the number and location of source populations.

There is population and genetic structuring within the SU region, which means all populations of Atlantic salmon cannot be considered equivalent. Furthermore, each population has the potential to contribute genetically and/or demographically to the long term persistence of SU Atlantic salmon (and possibly the species itself) so it is intrinsically important. Preserving the maximum amount of genetic variation will maximize the evolutionary potential of SU Atlantic salmon, ensuring that the DU as a whole will have the ability to respond or adapt to environmental change and a chance of re-colonizing rivers that have been extirpated. Preserving both populations with high genetic variation and populations with high genetic divergence will be important for recovery. If populations were prioritized for recovery based on within-river genetic variation, the Medway, St. Mary's (East Branch) and Salmon River (Guysborough) would all be important populations (see O'Reilly et al 2012). If populations were prioritized based on genetic divergence, the Moser and Musquodoboit rivers would become important (see O'Reilly et al. 2012).

Local adaptation among populations is thought to result primarily from environmental heterogeneity (i.e. habitat variation), and to be maintained by the homing behavior of Atlantic salmon. A cluster analysis identified 3 main groupings of rivers and 6 subgroupings (Figure 15) that could be representative of environmental heterogeneity within the region (see Bowlby et al. 2013b for details). At a minimum, all three groups should be represented in the distribution target for SU Atlantic salmon but choosing populations representative of the six smaller groupings would further increase the diversity in the target populations. It is generally accepted that larger rivers (populations) are better source populations for emigration and colonization than are smaller rivers. Further, having as many populations included in the distribution target as is practically feasible is expected to increase the long-term persistence of the DU. Having more than one population from each group is expected to help protect against catastrophic loss.

Interim recovery targets for SU Atlantic salmon can be used to evaluate progress towards recovery. Progress towards recovery targets, particularly with respect to halting the decline, can be evaluated using survival and extinction risks metrics. Proposed interim targets are:

- First, halt the decline in abundance and distribution in rivers with documented Atlantic salmon populations.
- Next, reduce the extinction risk in the rivers with documented Atlantic salmon populations by alleviating threats in these rivers.
- Then, as necessary, expand the presence and abundance of Atlantic salmon into other rivers currently without salmon to fill in gaps in distribution within the SU DU and facilitate metapopulation dynamics.

Recovery targets will need to be revisited as information about the dynamics of the recovering population becomes available.

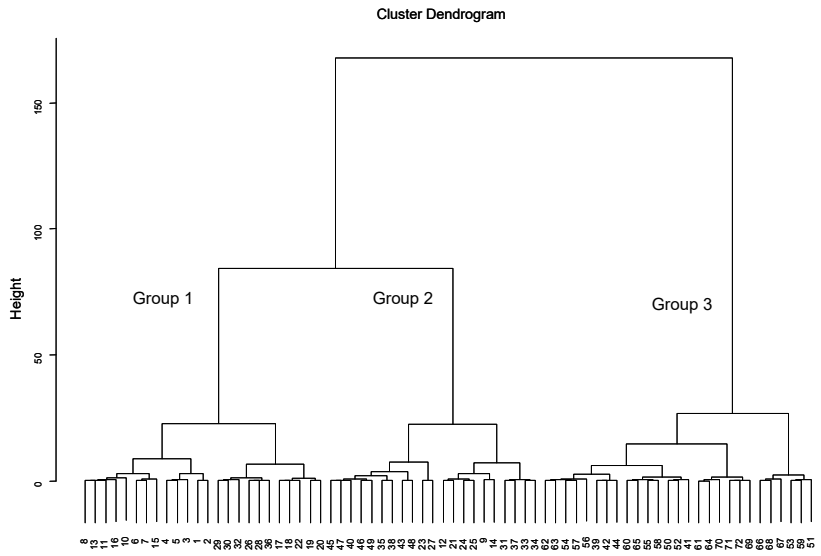

Figure 15. Dendrogram representing the degree of dissimilarity among watersheds (refer to Figure 2 for the names corresponding to each river number) as identified by the hierarchical cluster analysis. More similar watersheds are more closely joined.

Residence Requirements

Under *SARA*, a residence is defined as a dwelling-place that is occupied or habitually occupied by one or more individuals during all or part of their life cycles, including breeding, rearing, staging, wintering, feeding or hibernating (*SARA* section 2.1). DFO's Draft Operational Guidelines for the Identification of Residence and Preparation of a Residence Statement for an Aquatic Species at Risk (DFO, unpublished report) uses the following four conditions to determine when the concept of a residence applies to an aquatic species: (1) there is a discrete dwelling-place that has structural form and function similar to a den or nest, (2) an individual of the species has made an investment in the creation, modification or protection of the dwelling-place, (3) the dwelling-place has the functional capacity to support the successful performance of an essential life-cycle process such as spawning, breeding, nursing and rearing, and (4) the dwelling place is occupied by one or more individuals at one or more parts of its life cycle.

Two dwelling places (used by three life stages) were evaluated for their potential consideration as a residence for Atlantic salmon. These were redds (used by eggs and alevins) and home stones (used by juvenile salmon in fresh water). Each of these is habitually occupied during part of the salmon's life cycle, individuals invest energy in its creation or defense, and it provides specific functions to enable the successful completion of the Atlantic salmon's life-cycle. Of these, redds most closely match the definition of a residence because they are constructed, whereas home stones are not.

Eggs and alevins reside in redds from late October/early November until spring (mid-May or early June) when fry emerge and begin feeding. Redds are essential to protect eggs and alevins from disturbance (e.g. ice scour, bedload transport, physical impact by debris), currents, changing water levels and predators. Redds provide hydraulic eddies that capture expressed eggs and, after being covered with gravel by the adult salmon, provide interstitial space for water flow and oxygen for the incubation of the eggs and development of alevins prior to emergence. As such, they minimize movement of the eggs, prevent eggs from being displaced into unfavorable habitats, and can provide protection from some predators. Redds are typically 2.3 and 5.7 m^2 in size, and consist of a raised mound of gravel or dome under which most of the eggs are located and an upstream depression or 'pot'. Burial depths are about 10 to 15 cm^2.

Redds are typically constructed in water depths of 17 to 76 cm and velocities between 26 to 90 cm/s^2.

Juvenile Atlantic salmon are territorial, remaining relatively stationary near a home stone that they actively defend from other juveniles. Occupancy (prior residency) is a key determinant for successful defense. Home stones provide eddies that shelter parr from instream currents and cover for predator avoidance, as well as influence the availability of invertebrate drift for feeding (depending on the location of the stone relative to water flow). Therefore, the choice of a territory or home stone directly impacts the potential for individual growth and successful rearing in the freshwater environment. The ability to obtain and defend a territory has been linked to growth, age-of-smoltification, and hence age-at-maturity, a key life history parameter. Although juvenile salmon may change home stones intermittently, movement is thought to be limited. For example, one study found that 61.8% of young-of-the-year salmon moved less than 1 meter during July and August. Typical home stones range from <10 cm to > 40 cm in diameter, and there is some indication that the size of stone selected increases from summer to autumn, i.e. preferred sizes increase as juveniles grow. Home stones are occupied soon after emergence from the gravel in the spring and used until juveniles return to the substrate in late autumn.

Threats

Threats are defined as any activities or processes that have, are, or may cause harm, death or behavioural changes to populations, and/or impairment of habitat to the extent that population-level effects occur. This definition includes natural and anthropogenic sources for threats. Current SU salmon populations have little ability to increase in size, so it is expected that threats that act intermittently would have longer-lasting effects on populations than when productivity was higher. Additionally, human activities that reduce Atlantic salmon populations often represent an assemblage of threats to fish and fish habitat. Thus, it is difficult to discuss a specific threat in isolation given the cumulative and correlated nature of the majority of threats.

Detailed information on each major potential threat to SU Atlantic salmon individuals and their habitat is contained in Bowlby et al. (2013b), with a summary provided here in Appendix A. The overall level of concern ascribed to a specific threat takes into account the severity of impacts on populations, how often they occur, as well as how widespread the threat is in the SU DU.

In general, there is a lot of information on how threats affect Atlantic salmon in terms of changes to growth, survival or behaviour of a given life stage (predominantly juveniles). However, comparatively little research links threats in SU watersheds with changes in adult abundance of specific Atlantic salmon populations. From analyses of land use in the SU region (Bowlby et al. 2013b), previous and on-going human activities are extensive in the majority of drainage basins and have likely altered hydrological processes in SU watersheds. Landscape factors controlling hydrology operate at hierarchically nested spatial scales (regional, catchment, reach, instream habitat), which means they often override factors controlling salmon abundance at small spatial scales.

Threats with a high level of concern are discussed below. Threats to persistence and recovery in freshwater environments identified with a high level of overall concern include (importance not implied by order): acidification, altered hydrology, invasive fish species, habitat fragmentation due to dams and culverts, and illegal fishing and poaching. Threats in estuarine and marine environments identified with a high level of overall concern are (importance not implied by order): salmonid aquaculture and marine ecosystem changes.

Acidification

Watersheds in the SU region have been heavily impacted by acidification, which has predominantly originated from atmospheric deposition (i.e. acid rain) due to industrial sources in North America. The underlying geology of the SU is such that rivers have little buffering capacity and have mildly to substantially decreased in pH. River acidification has significantly contributed to reduced abundance or extirpation of populations from many rivers in the region during the last century. In addition to ongoing effects of acidification, contemporary declines in non-acidified rivers indicate that other factors are also influencing populations. Although most systems are not acidifying further, few are recovering and most are expected to remain affected by acidification for more than 60 years. Rivers in the southwestern portion of the SU tend to be more highly acidified than those in the northeastern portion.

Low pH reduces the survival of juvenile Atlantic salmon through direct mortality or increased susceptibility to predation or disease, as well as reduced ability to compete for food or space and interference with the smoltification process. Fry (age 0) are thought to be the most severely affected life stage, with cumulative mortality curves predicting 50% mortality at a pH of 5.3. Mean annual pH values of <4.7 are considered insufficient for the continued maintenance of Atlantic salmon populations. Korman et al. (1994) developed toxicity functions by life stage based on studies available in the literature and used these to estimate egg-to-smolt mortality rates associated with pH for specific periods. Mortality estimates by life stage from these functions for surface pH values of 4.5 to 5.5 are provided in Table 5. These rates are in addition to natural mortality and mortality from other causes.

Table 5. Mortality rates (%) and toxic accumulation (TD - proportion dying weekly) of juvenile Atlantic salmon as a function of surface pH as derived from the toxicity functions in Korman et al. (1994). Values outside the interval 0-100% were assigned the limit value. Rates and pH values are specific to the time period. Mortality rates are in addition to natural mortality and mortality from other causes. Adapted from Korman et al. (1994).

Life Stage	Time Period	Rate	Average Surface pH				
			4.50	4.75	5.0	5.25	5.50
Egg	Nov. – Apr.	Mortality	57.1%	37.3%	17.6%	0%	0%
Alevin	May	Mortality	36.3%	16.6%	7.6%	3.5%	1.6%
Fry	June	Mortality	100%	100%	56.7%	31.7%	17.7%
Parr	All year	TD	0.19	0.017	0.0016	0.0001	0.0000
Wild smolt	May	TD	0.19	0.017	0.0016	0.0001	0.0000
Hatchery Smolt	May 15-25	TD	0.19	0.017	0.0016	0.0001	0.0000

Sixty rivers in the SU have been classified based on mean annual pH (Figure 16). Salmon populations in extremely acidified systems (pH <4.7) are thought to be extirpated (13 rivers), reduced by 90% in moderately impacted systems (pH = 4.7-5.0; 20 rivers), reduced by about 10% in slightly impacted systems (pH = 5.1-5.4; 14 rivers), and apparently unaffected when pH >5.4 (13 rivers) based on research in the 1980s. However, juvenile densities calculated in the 2008/09 electrofishing survey suggest that reductions in productivity could be even higher (95% and 58% respectively for moderately and slightly impacted systems). This means 316,726 to 334,322 habitat units (out of a total of 351,918) from moderately impacted rivers, and 19,431 to 112,701 habitat units (out of a total of 194,312) from mildly impacted rivers would be unsuitable for juvenile production.

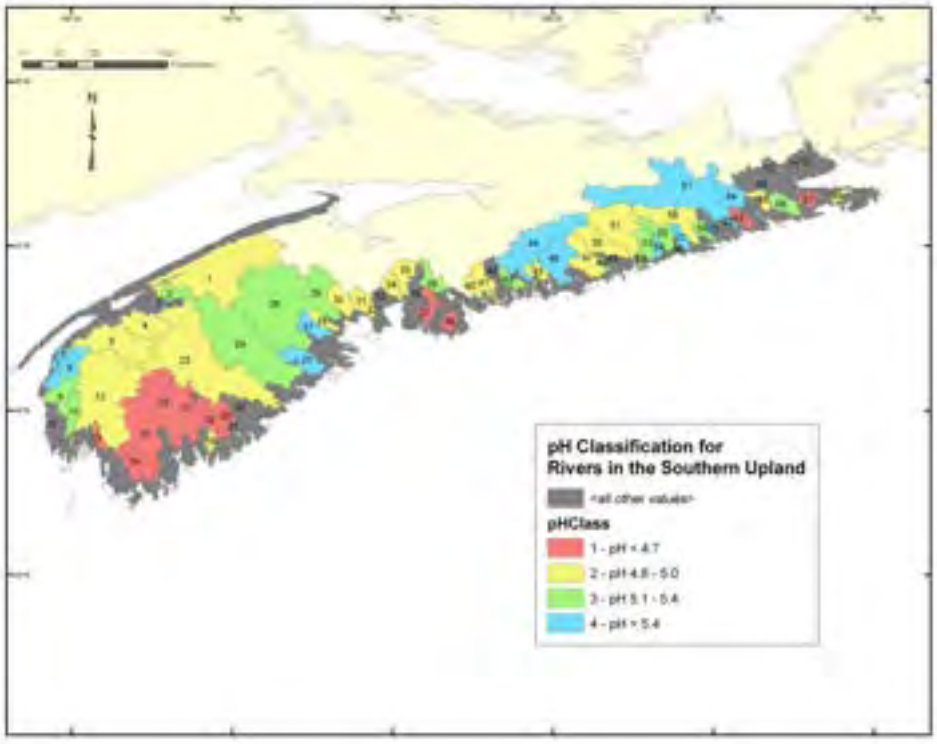

Figure 16. Classification of mean annual pH for rivers in the Southern Upland region; data are from Amiro (2006). Watershed numbers correspond to the legend in Figure 2.

Altered Hydrology

The hydrological regime of a riverine system may be altered by a large variety of human activities. These include direct withdrawal of water for industrial, agricultural or municipal purposes, intensive land use affecting overland and groundwater flow, water diversions for power generation, and an operating schedule of water release at power generating stations not consistent with the natural flow regime. These activities can have significant effects on salmon spawning and rearing habitat, especially when stream base flows are substantially reduced.

River discharge in systems of the SU DU is highly variable among years. However, natural variability may be exacerbated by intensive land use (e.g. forestry, agriculture, urbanization), which can accelerate the rate of runoff from land and entrance into stream channels. This can make a river more prone to flooding and increase the frequency and duration of both large freshets and droughts. Extreme low flows can increase the incidence of temperature extremes, reduce seasonal habitat availability in a watershed and influence food supply. The survival of eggs, alevins and juveniles has been directly linked to stream discharge, with better survival in years with higher flows during the summer and winter months. Extremely high flows can cause large scale erosion and significant changes in channel and bed morphology. All of these processes influence the quality and quantity of habitat available in fresh water. Under extremely high flows, juvenile salmon tend to seek refuge in the substrate, but can experience increased mortality from physical displacement, turbulence, abrasion, and transportation of the substrate.

Altered hydrological regimes directly affect water temperature thereby affecting the behaviour, growth, and survival of all freshwater life stages of Atlantic salmon, and can limit the amount of useable habitat in a watershed. Extreme high temperatures can lead to direct mortality of juveniles if they cannot move to cold water refugia, or can reduce survival indirectly through

impacts on growth, predator avoidance responses, or individual susceptibility to disease and parasites. Extreme low temperatures during winter can result in direct mortality by freezing redds or physical disturbances from ice scour, in addition to reducing developmental rates of eggs and alevins. In addition to extreme hydrological events, loss of riparian cover, excessive groundwater extraction as well as water management at reservoirs and hydroelectric generating stations can contribute to extreme temperature events.

Additionally, returning adult spawners have been found to initiate spawning migrations as water levels rise, as well as to require sufficient water for distribution throughout the river system and to hold in pools. Spring high water is potentially a trigger for smolt migration, and survival of smolts has been shown to be higher under years of high discharge than low in some systems.

Invasive Species (Fish)

Chain pickerel and smallmouth bass have substantially increased in abundance and distribution since first being introduced into the SU region. Chain pickerel are currently found in 69 documented locations in the SU, while smallmouth bass are more widely distributed in 174 documented locations (see Bowlby et al. 2013b). Both are recognized as being significant piscivores. Chain pickerel are thought to influence Atlantic salmon populations directly through predation rather than through competition. Preliminary studies in the SU region suggest that pickerel presence in a lake substantially reduces the abundance and species richness of the native fish community. Introduced smallmouth bass influence fish communities through competition as well as predation, and their presence has been linked to community shifts and extirpations of native fishes. Atlantic salmon juveniles have been found to shift habitat use in areas where smallmouth bass are also found, although these results were dependent on water temperature and discharge conditions.

Habitat Fragmentation Due to Dams, Culverts and Other Permanent Structures

Permanent structures are often placed in or along rivers for three main purposes: water impoundment (reservoirs for hydro, municipal drinking water, or other industrial uses), bank stabilization (to prevent movement of the stream channel), or water diversion (for industrial and recreational uses or flood prevention). There are 233 dams or barrier structures identified by the NS DoE and DFO HM in watersheds in the SU region (Figure 17), 44 of which are thought to be passable to fish populations.

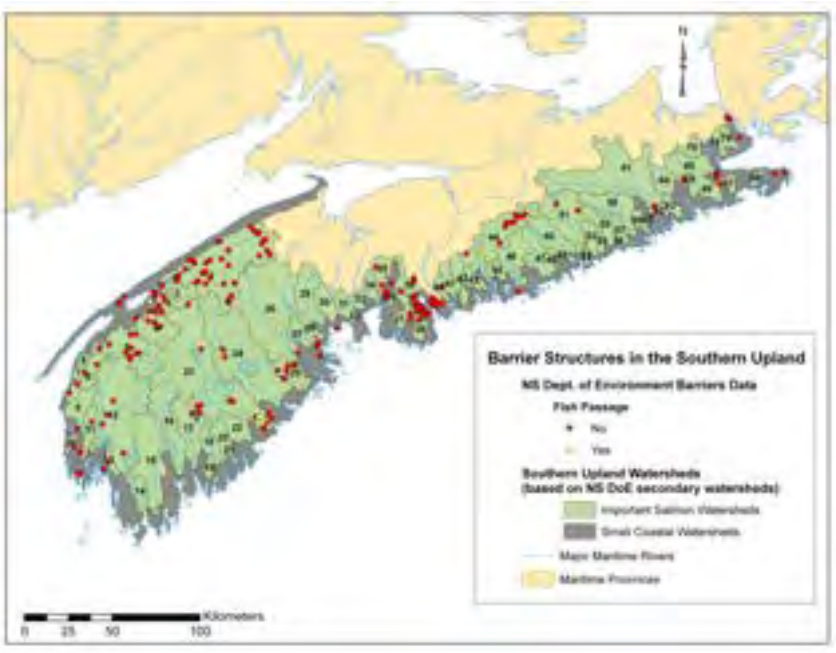

Figure 17. All barrier structures in the Southern Upland region listed on the barriers layer from the Nova Scotia Department of the Environment and DFO Habitat Management (Maritimes). Those without fish passage are shown in red, while those with at least partial passage are shown in blue. Watershed numbers correspond to the legend in Figure 2.

Due to poor design, improper installation or inadequate maintenance, culverts contribute to habitat fragmentation in watersheds by becoming seasonal or complete barriers to fish movement. Recent surveys of culverts in Nova Scotia suggest that barriers to fish passage are prevalent, with 37% assessed as full barriers and 18% assessed as partial barriers in the Annapolis watershed, and 61% assessed as full barriers from a random sample of 50 culverts in Colchester, Cumberland, Halifax and Hants Counties. Out of 62 culverts assessed on the St. Mary's River, 40 did not meet criteria for water depth, 35 exceeded velocity criteria, and 24 had an outfall drop potentially preventing passage. Similar results have been obtained for watersheds containing Atlantic salmon in Newfoundland and the continental U.S. as well watersheds containing Pacific salmon and other trout species in Alaska and British Columbia. Activities such as timber harvesting, urbanization, infrastructure (like new highways) or other land development tend to increase the number of culvert installations in a watershed. Using road crossings as a proxy for culverts (Figure 18), SU watersheds in more populated areas as well as those impacted the most heavily by forestry or agriculture had the highest road densities and thus the greatest potential for impact from culverts.

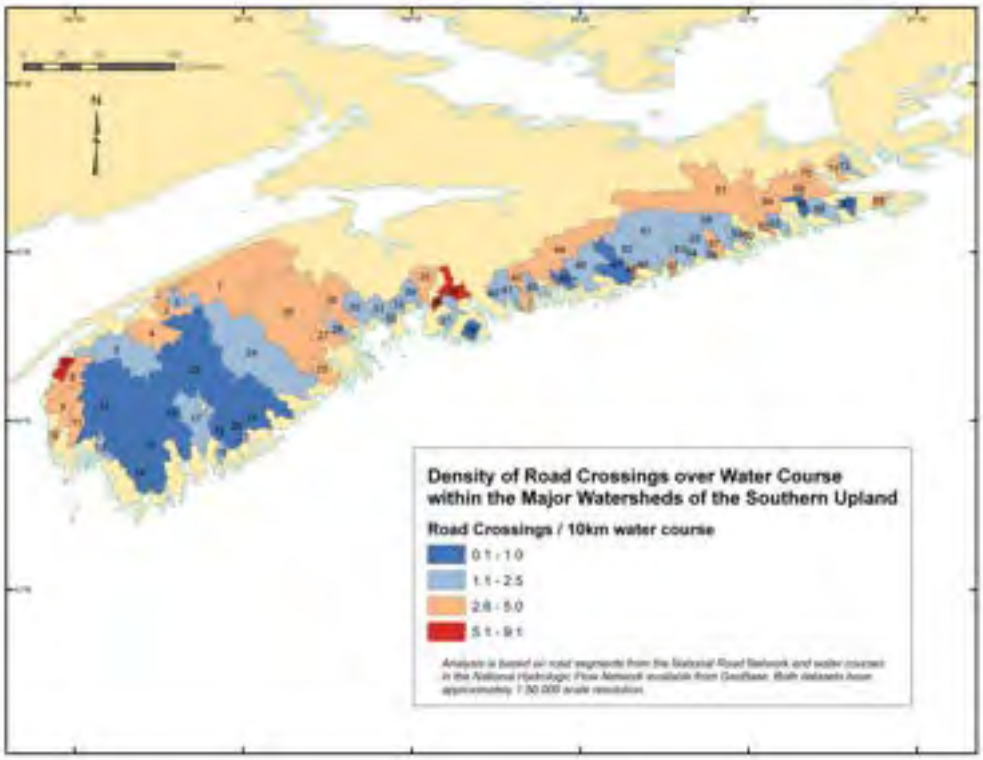

Figure 18. The density of road crossings within watersheds of the Southern Upland region. Watershed numbers correspond to the legend in Figure 2.

Illegal Fishing and Poaching

There have been many anecdotal reports of illegal fishing (e.g. targeting salmon while fishing with a general license) and harvests (i.e. poaching) of Atlantic salmon in the SU region, either using recreational fishing gear, gillnets, or other capture methods. The magnitude of this threat to specific populations is not possible to quantify; however, poaching would be expected to have the greatest impact when population sizes are small (as they are at present) because a larger proportion of the population would be affected. Additionally, the population dynamics modeling presented here indicates that populations have very little capacity to recover from any illegal removals (i.e. are not able to quickly increase in size).

Population Level Effects of Recreational Fishing

While recreational fishing is currently identified as a low threat (Appendix A) to SU Atlantic salmon, the population level effects of recreational fishing are described here.

Recreational fishing seasons, regulations and practices in the SU have changed through time from fisheries that were primarily retention fisheries for both large and small salmon, to virtually all hook-and-release fisheries, to closures throughout the SU Region in 2010.

Hook and release recreational fisheries provide an intermediate management strategy between a full retention fishery and fishery closure for populations that are below target levels. The effects are conditional on the life history and dynamics, such as freshwater productivity, survival at-sea and repeat spawning frequency. Catch and release fisheries would be expected to result in populations sizes that are higher than those in a full retention fishery, but lower than those expected to result from fishery closure. A similar relationship is expected for the lifetime reproductive rates. As such, they have the potential to slow recovery rates relative to fishery

closures, although population growth is expected to be more rapid with a catch and release fishery than a full retention fishery.

Highly variable rates of fish mortality associated with a fish being hooked and subsequently released have been reported in the literature. Water temperature is cited as an important factor; angling at low temperatures (i.e. below 17-18^{o}C) generally results in lower mortalities than catch-and-release angling that occurs at higher water temperatures. In addition to temperature, fish mortality associated with catch-and-release angling is also believed to be affected by an angler's level of experience; fish mortality is believed to be lower for more experienced anglers than for less experienced anglers. Although there are several studies that show low direct mortality associated with catch-and release recreational fisheries if conducted at low water temperatures (i.e. below 17-18 C), there is little information available about other effects of catch and release salmon fishing (e.g. potential effects on migration, reproduction, habitat impacts, transfer of pathogens).

The LaHave River (above Morgan Falls) salmon population is the only SU population with sufficient data to evaluate the effects of recreational fisheries on population dynamics. In the 1980s when retention fisheries were in effect, the recreational fisheries reduced survival to spawning escapement by up to 31% for 1SW salmon, with lesser effects on 2SW in part due to the timing of the increase in recreational fishing effort and the shift to hook-and-release fisheries for large salmon. This led to a reduction in the annual equilibrium population size of up to 48% and reductions in maximum lifetime reproductive rates of up to 23%. With the switch to hook-and release fisheries, the impact of the fishery on the dynamics of the population is much less (nearly negligible), although this conclusion is conditional on the assumed 4% hook-and-release mortality rate and on the assumptions that both the non-lethal effects of hook-at-release and habitat impacts are minor. These effects would be greater if the fishing season extends into periods with warmer water temperatures. Additionally, these values should be interpreted in the context of the past impacts of the fisheries on these populations. In the future, any impacts to populations from the recreational fishery would depend on fishing intensity and management regulations with respect to timing of the fishery, as well as the associated mortality rate.

Aquaculture

Commercial aquaculture of Atlantic salmon in the marine environment of Nova Scotia typically occurs in net pens anchored in coastal estuaries or sheltered near-shore sites. Effects on wild Atlantic salmon populations from aquaculture would occur either by interaction in the immediate vicinity of the net-pens or by interactions between escaped aquaculture salmon and wild salmon. Aquaculture escapes, migration of wild salmon to or past aquaculture sites, and a combination of escapes and migration can potentially result in predator attraction, disease and pathogen exchanges, competition and genetic effects.

Rivers in close proximity to existing aquaculture lease sites include many of those that contain the larger remaining populations of Atlantic salmon in the SU region. Individuals from populations such as the Annapolis/Nictaux have the potential to pass or interact with all salmonid aquaculture sites in the SU region as they move through coastal areas, while this would be less likely for more northern populations (e.g. those near Canso).

Interbreeding between wild populations and aquaculture escapes causes reduced fitness in the hybrids as they are less adapted to local conditions and, thus, exhibit lower survival rates and less resilience to environmental change. The larger the genetic difference between wild and farmed populations, the greater these effects will be. The use of broodstock from other areas leads to greater genetic differences. Such changes can be permanent when genes from farmed fish become fixed in the wild genome (introgression). Despite poor reproductive success, the

large number of escaped salmon in some areas of Canada has resulted in reports of significant numbers reproducing. For example, 20% of redds in the Magaguadavic River, New Brunswick were thought to belong to females of aquaculture origin in the 1992/1993 spawning period. Research in Europe has demonstrated that the number of farmed salmon entering rivers is proportional to the number of farms, and that escapes will enter multiple rivers in the vicinity of aquaculture sites. Aquaculture escapes in North American rivers have been reported in 54 of 62 (87%) rivers investigated within a 300 km radius of the aquaculture industry since 1984. Aquaculture escapes made up an average of 9.2% (range: 0% to 100%) of the adult population in these rivers. The prevalence of escapes suggests that farmed salmon pose a significant risk to the persistence of wild populations, and a recent meta-analysis has demonstrated that reduced survival and abundance of several salmonid species (including Atlantic salmon) are correlated with increases in aquaculture.

More direct sources of mortality to wild Atlantic salmon populations from aquaculture sites have been hypothesized to come from competition for resources, predator attraction to net-pens, as well as disease transfer from captive to wild fish. However, the available evidence suggests that growth and survival of immature Atlantic salmon in the marine environment are not limited by food, and predator attraction to net-pens has not been directly linked to increased mortality in wild populations. Similarly, there are no proven cases in Canada where disease or sea-lice outbreaks in wild populations can be directly linked to aquaculture sites, although research in epidemiology demonstrates that exposure and the frequency of exposure are important contributing factors to the spread of disease.

Aquaculture impacts would be expected to decline with distance from a specific site as well as with the recipient population size. For a given number of farmed salmon entering a river, the population-level impacts of interbreeding are expected to decrease with increases in size of the wild population, suggesting that one potentially important mitigation measure for this threat is to increase abundance of wild salmon by addressing other threats.

Marine Ecosystem Changes

The abundance and distribution of prey species and predators is thought to be an important factor affecting marine growth and survival of Atlantic salmon populations. Recent evidence of a whole ecosystem regime shift in the Eastern Scotian Shelf (ESS) demonstrates that significant change to the ecological communities experienced by wild Atlantic salmon populations at sea is likely, particularly if individuals use areas farther from the coast. The ESS ecosystem has shifted from dominance by large-bodied demersal fish, to small pelagic and demersal fish, and macroinvertebrates; a change that is also thought to be occurring in surrounding regions (i.e. Western Scotian Shelf (WSS)), albeit at a slower pace. One aspect of this shift is that strong trophic interactions between the remaining top predators, as well as fundamentally altered energy flow and nutrient cycling, appear to be maintaining the new ecological state. It has been hypothesized that changes in the abundance and distribution of small pelagic fishes affects food availability and thus marine survival of Atlantic salmon, and that increased grey seal (*Halichoerus grypus*) populations (as seen on the ESS) may lead to significantly higher predation pressure. However, empirical evidence of either impact has not been found for SU Atlantic salmon.

Large-scale changes to atmospheric and oceanographic conditions have been observed throughout the marine range of Atlantic salmon. For example, the WSS experienced a cold period during the 1960s, was warmer than average until 1998, and then significantly cooled after cold water intrusion from the Labrador Sea. The ESS cooled from about 1983 to the early 1990s and bottom temperatures have remained colder than average since. Sea-ice cover in the Gulf of St. Lawrence and off Newfoundland and Labrador in winter 2009/2010 was the lowest on

record for both regions since the beginning of monitoring in 1968/1969. This lack of ice was in part due to warmer temperatures, but also to early season storms breaking up and suppressing new ice growth. The NAO has been shifting from mostly negative to mostly positive values from the 1970s to the early 2000s. Winter NAO is strongly negatively correlated with sea-surface temperature and thus could influence Atlantic salmon overwintering behaviour and mortality rates at sea. Most research that has found a correlation between Atlantic salmon catches, sea-age at maturity, or smolt-to-adult survival and recruitment with winter NAO values has been from European populations, although there are weakly correlated examples in North America. However, as discussed previously, partitioning mortality of adult salmon between spawning events into that experienced predominantly in freshwater, estuarine and near-shore environments (first year) and that experienced in more distant marine environments (second year) demonstrated a strong correlation between NAO and survival in the second year for alternate-spawning Atlantic salmon from the LaHave River.

Highest marine mortality rates are hypothesized to occur soon after immature salmon reach the open ocean while they are still in the near-shore environment. One hypothesis is that faster growth and lower mortality of immature Atlantic salmon is associated with entry into the ocean at a time when larval fish prey are abundant and at a consumable size. Thus, the environmental factors controlling primary marine production (which would determine prey availability and size) may have a large impact on early marine survival and growth.

Mitigation and Alternatives

Restoring marine or freshwater habitat quality requires the ability to quantify the impact of a given threat on a given population, something that is much more likely in fresh water than in the marine environment. Threats in fresh water are also more localized and can be addressed with remediation actions in the short term. It is likely that increasing habitat quality and quantity in fresh water will prevent further extirpations and promote self-sustaining populations at low size. Some threats (like acidification) have well-known remediation actions (liming) that can lead to population growth. In other cases, recovery actions addressing multiple threats simultaneously might be required to increase abundance. It has been suggested that watershed restoration for salmon species should focus first on reconnecting isolated fish habitats (i.e. remediating barriers) before moving on to restoring hydrologic, geologic and riparian processes at a watershed scale, and lastly to focusing on in-stream habitat enhancement. When choosing rivers for restoration, an attempt should be made to capture the range of variation among systems in the SU and to prioritize the larger remaining populations for recovery.

Remediation actions to address land use issues will not produce immediate population increases for SU Atlantic salmon. For example, it would take many years before riparian vegetation would grow to a size that would significantly reduce sediment inputs, which would be expected to increase habitat quality and reduce juvenile mortality in the river. Such large-scale changes are the most likely to bring about substantial population increase in Atlantic salmon because they should have a greater impact on total abundance in the watershed rather than on localized density, and they would address issues at the watershed scale.

Remediation of landscape-level threats to watersheds (e.g. forestry, agriculture, urbanization, roads) requires working at a much larger scale than the stream reach, and typically includes actions that are distant from the actual streambed (e.g. replanting riparian vegetation, revisiting regulations on pesticide use, community outreach on invasive species). Coordination of activities at small-scales may produce more immediate effects.

Sensitivity analysis on the effect of starting population size on population viability highlights the risks associated with delaying recovery actions; recovery is expected to become more difficult if

abundance continues to decline, as is expected for these populations with the continued passage of time. Recovery actions should be initiated as soon as possible.

Mitigation and alternatives for freshwater, marine and estuarine threats were not addressed in detail at this meeting.

Assessment of Recovery Potential

The PVA described in the Population Dynamics section was also used to evaluate how the probability of extinction and probability of meeting the recovery target would be expected to vary with increased freshwater productivity and increased at-sea survival. Twenty-four scenarios were evaluated for both the St. Mary's River (West Branch) and LaHave River (above Morgan Falls) salmon populations. At-sea survival values considered in the analyses used the 1980s and 2000s dynamics as upper and lower estimates respectively, with the two intermediate scenarios evenly spaced between these (i.e. at one-third and two-thirds the difference between past and present values).

Increased freshwater production was modeled by increasing smolt production by factors of 1.0 (no increase), 1.2 (20% increase), 1.5 (50% increase) and 2.0 (double or 100% increase). This is the same as changing the parr mortality parameter by equivalent amounts. For example, the annual mortality of parr older than age-1 was estimated to be 0.72 for the LaHave River (above Morgan Falls) population. This is a survival of 28% annually. The increased freshwater productivity scenario of 1.5 equates to a survival of 42% annually.

Each combination of increased freshwater productivity and at-sea survival was modeled for a total of 16 scenarios (Table 6). In addition, eight other scenarios are presented to investigate the effects of extreme events. In these, freshwater productivity was increased by a factor of 1.5 and simulations were carried out for all four at-sea survival values. For each scenario, the probabilities of extinction and recovery were evaluated using 2000 simulated population trajectories.

Abundance trajectories, extinction probabilities and recovery probabilities for each scenario are provided in Figures 19, 20 and 21 and Table 6 for the LaHave River (above Morgan Falls) population. The results of these analyses clearly indicate how close SU Atlantic salmon are to the threshold between becoming extinct and being viable. Panel "A" in each figure shows the results using the current dynamics; as previously described, both populations will extirpate in the absence of human intervention or a change in vital rates for some other reason. Panel "B" shows the effect of increasing freshwater productivity by 20%. This improvement is not large, but it does markedly reduce extinction risk, even if marine mortality rates remain unchanged (Figure 20). For the LaHave River (above Morgan Falls) population, the probability of extinction within 30 years drops from 31% to 3% with this increase in survival. Increases of 50% (Panel C) drop the extinction probability to 0% for more than 50 years for both populations. Although small, numerically-viable populations are produced, none of the simulated population trajectories reached the recovery targets (Figures 19, 21). Small increases in marine survival (Panels G to J) have a similar effect. None of the simulated populations extirpated in the third increase scenarios and a small proportion reached their recovery targets for both populations. The proportion reaching the recovery target increases as freshwater productivity increases (Figure 21; compare Panels G to J). Recovery probabilities exceed 50% in 50 years for all scenarios that include a two-thirds increase in at-sea survival (Panels M to X) and extinction probabilities are zero. Within limits, these conclusions are robust to how the frequency of extreme events is modeled (Panels E, K, Q, W, F, L, R, X). When the frequency of the extreme events is reduced, the probability of recovery increases and extinction probability is reduced

(e.g. compare Panels H and K). Results for the St. Mary's River (West Branch) salmon population are similar.

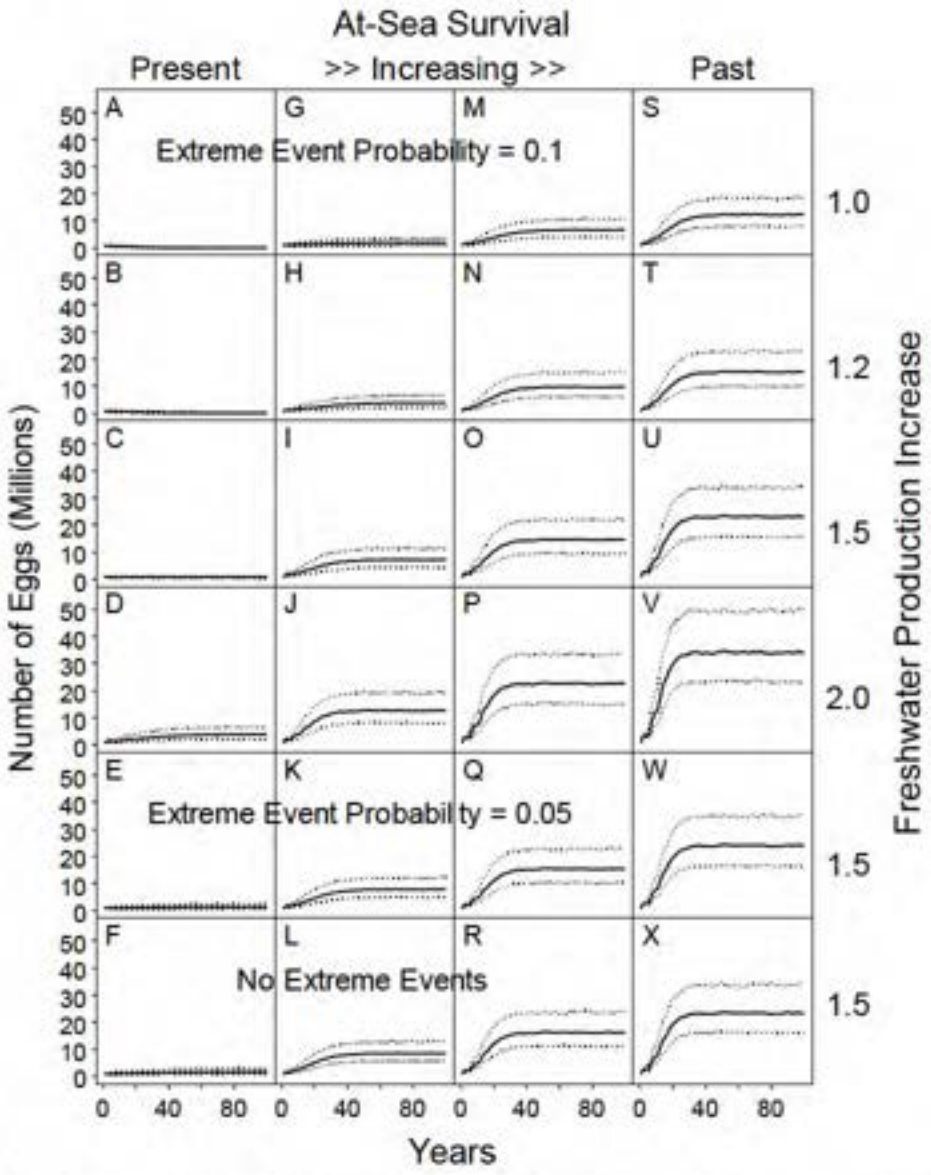

Figure 19. The effects of increasing at-sea survival and freshwater productivity on the simulated abundance of eggs for the LaHave River (above Morgan Falls) Atlantic salmon population. The graphs summarize 2000 simulations for each scenario. The median abundance (solid line), and the 10th and 90th percentiles (dashed lines) are shown. Panels on the right and the left are based on the 1980s and 2000s at-sea survival respectively, and the middle panels show scenarios using survivals increased by 1/3 and 2/3's of the difference in these values. The return rates of 1SW and 2SW salmon and survival between repeat spawning events are increased. The 2000's freshwater production is used in all scenarios. The top four rows show the effect of increasing freshwater productivity by factors of 1 (no change), 1.2 (20% increase), 1.5 (50% increase) and 2.0 (100% increase). The bottom two rows show the effect of changing the frequency of event events to an average of 1 every 20 years (5th row) and to no extreme events (bottom row).

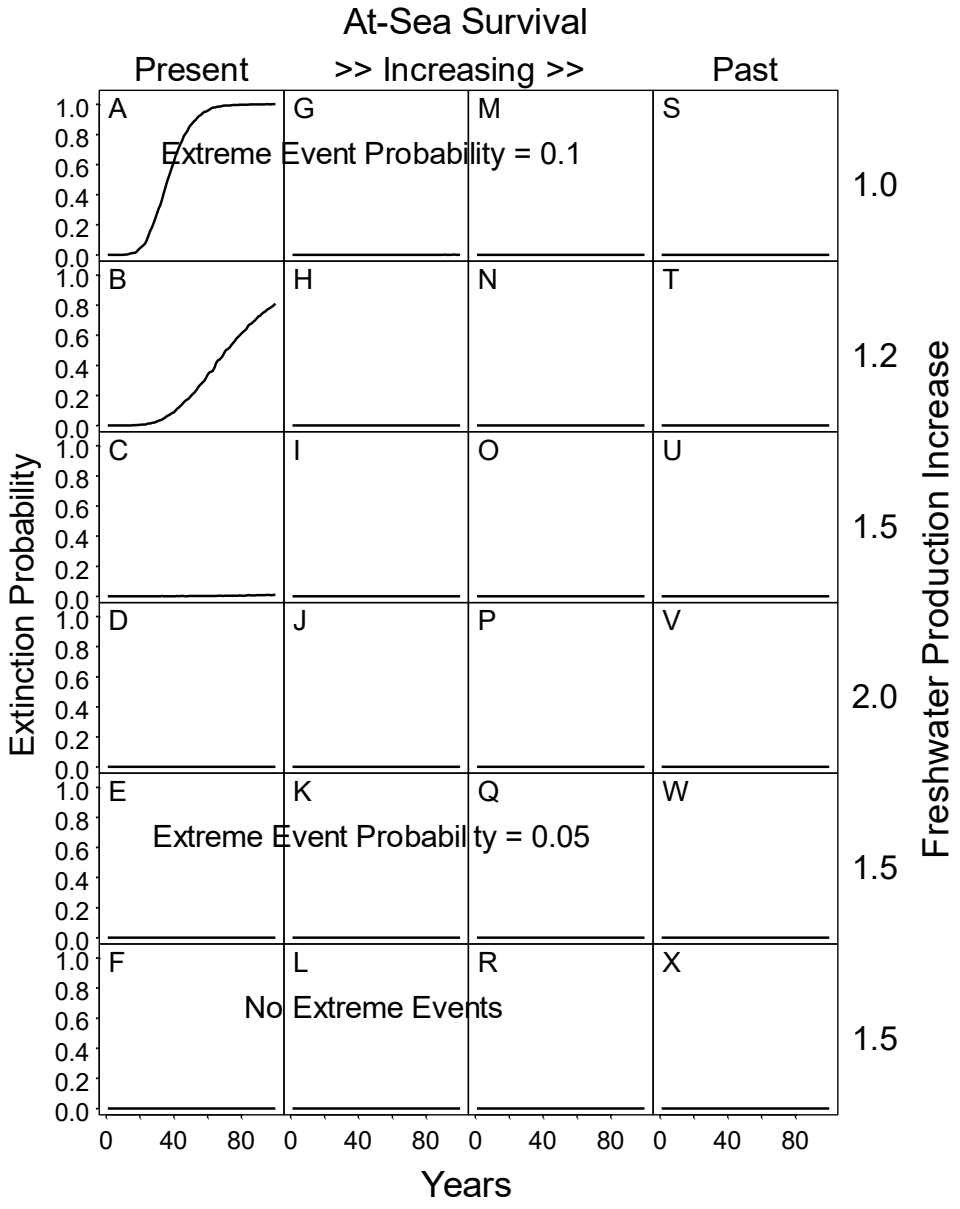

Figure 20. The effects of increasing at-sea survival and freshwater productivity on the probability of extinction for the LaHave River (above Morgan Falls) Atlantic salmon population. Panels are described in the caption for Figure 19.

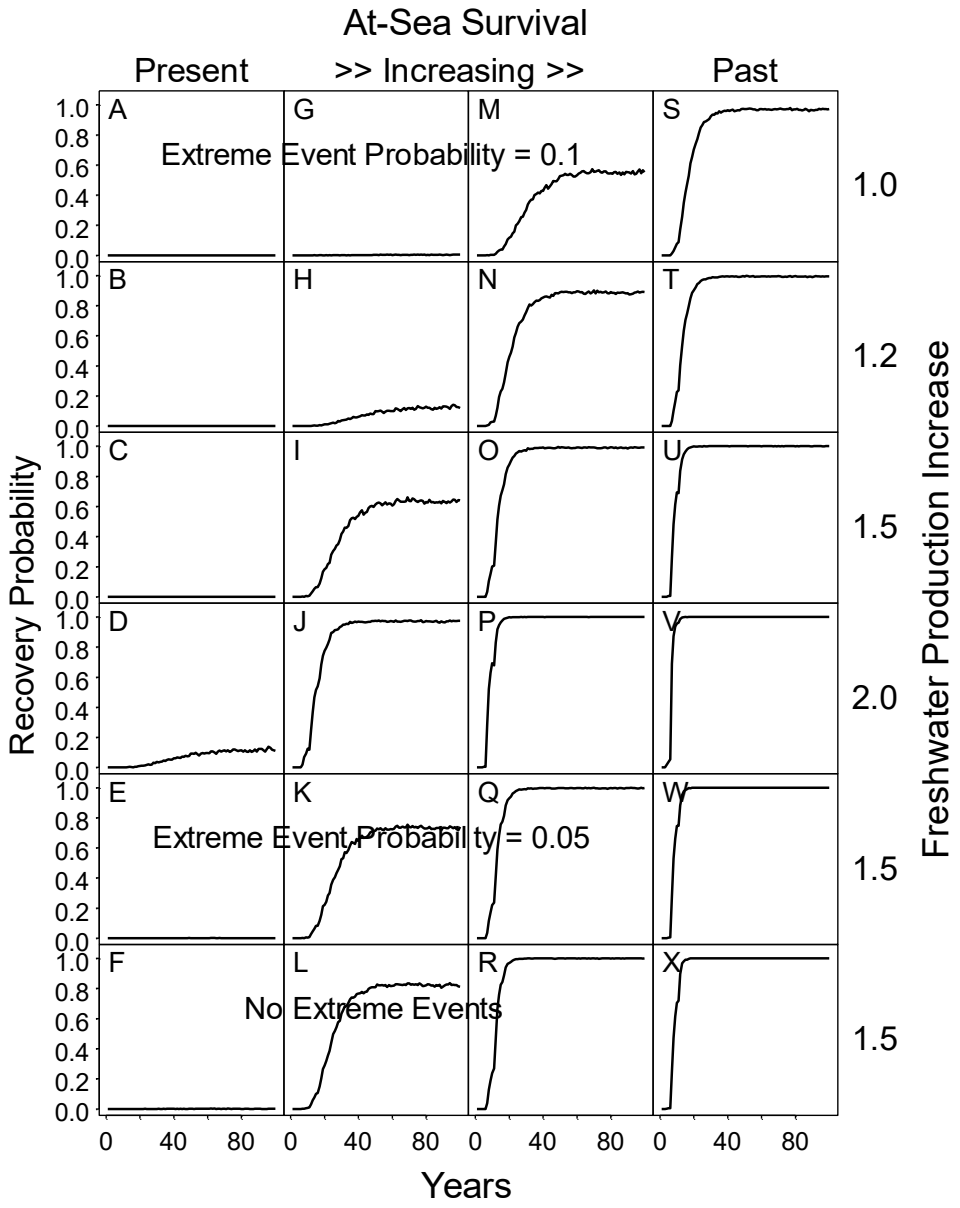

Figure 21. The effects of increasing at-sea survival and freshwater productivity on the probability of meeting the recovery target for the LaHave River (above Morgan Falls) Atlantic salmon population. Panels are described in the caption for Figure 19.

Table 6. Proportions of 2000 simulated population trajectories that either go extinct or meet the recovery target within 10, 20, 30 and 50 year time horizons based on recovery scenarios for the LaHave River (above Morgan Falls) Atlantic salmon population. The marine scenarios reflect changes from the present levels (2000s) of at-sea survival to those in the past (1980s). The freshwater scenarios reflect increases in freshwater productivity from the present level (1) to 2 times the present level. The lettering for the runs corresponds to those in Figures 19-21. Extreme event scenarios are the average frequency of extreme events and the reduction in egg to fry survival corresponding to the event.

	Marine	Freshwater	Extreme Event	Proportion Extinct				Proportion Recovered			
Run	Scenario	Scenario	Scenario	10 yr	20 yr	30 yr	50 yr	10 yr	20 yr	30 yr	50 yr
a	present	1	10 yr; 0.2	0.00	0.05	0.31	0.87	0.00	0.00	0.00	0.00
b	present	1.2	10 yr; 0.2	0.00	0.01	0.03	0.21	0.00	0.00	0.00	0.00
c	present	1.5	10 yr; 0.2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
d	present	2	10 yr; 0.2	0.00	0.00	0.00	0.00	0.00	0.01	0.03	0.09
e	present	1.5	20 yr; 0.1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
f	present	1.5	none	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
g	intermediate 1/3	1	10 yr; 0.2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
h	intermediate 1/3	1.2	10 yr; 0.2	0.00	0.00	0.00	0.00	0.00	0.01	0.04	0.10
i	intermediate 1/3	1.5	10 yr; 0.2	0.00	0.00	0.00	0.00	0.01	0.19	0.43	0.62
j	intermediate 1/3	2	10 yr; 0.2	0.00	0.00	0.00	0.00	0.12	0.80	0.95	0.97
k	intermediate 1/3	1.5	20 yr; 0.1	0.00	0.00	0.00	0.00	0.01	0.24	0.53	0.73
l	intermediate 1/3	1.5	none	0.00	0.00	0.00	0.00	0.01	0.32	0.66	0.83
m	intermediate 2/3	1	10 yr; 0.2	0.00	0.00	0.00	0.00	0.00	0.12	0.34	0.53
n	intermediate 2/3	1.2	10 yr; 0.2	0.00	0.00	0.00	0.00	0.03	0.49	0.78	0.89
o	intermediate 2/3	1.5	10 yr; 0.2	0.00	0.00	0.00	0.00	0.21	0.90	0.99	0.99
p	intermediate 2/3	2	10 yr; 0.2	0.00	0.00	0.00	0.00	0.68	1.00	1.00	1.00
q	intermediate 2/3	1.5	20 yr; 0.1	0.00	0.00	0.00	0.00	0.24	0.94	1.00	1.00
r	intermediate 2/3	1.5	none	0.00	0.00	0.00	0.00	0.27	0.98	1.00	1.00
s	past	1	10 yr; 0.2	0.00	0.00	0.00	0.00	0.09	0.74	0.94	0.97
t	past	1.2	10 yr; 0.2	0.00	0.00	0.00	0.00	0.24	0.92	0.99	1.00
u	past	1.5	10 yr; 0.2	0.00	0.00	0.00	0.00	0.69	1.00	1.00	1.00
v	past	2	10 yr; 0.2	0.00	0.00	0.00	0.00	0.96	1.00	1.00	1.00
w	past	1.5	20 yr; 0.1	0.00	0.00	0.00	0.00	0.75	1.00	1.00	1.00
x	past	1.5	none	0.00	0.00	0.00	0.00	0.72	1.00	1.00	1.00

In conclusion, population viability analyses indicate that relatively small increases in either freshwater productivity or at-sea survival are expected to decrease extinction probabilities. For example, for the LaHave River (above Morgan Falls) population increasing freshwater productivity by 20% decreases the probability of extinction within 50 years from 87% to 21%, while a freshwater productivity increase of 50% decreases the probability of extinction within 50 years to near zero. These must be accompanied by increases in at-sea survival in order to restore populations to levels above their conservation requirements.

In contrast with inner Bay of Fundy salmon populations, for which at-sea survival is so low that recovery actions in fresh water are expected to have little effect on overall viability, recovery actions focused on improving freshwater productivity are expected to reduce extinction risk for SU salmon.

These must be accompanied by larger (value) changes in at-sea survival in order to restore populations to levels above their conservation requirements, although at present the contributing factors limiting marine survival are not known.

Sensitivity to Starting Population Size

The effect of delaying recovery activities was examined by running the PVA (base model) for the LaHave River (above Morgan Falls) population starting at 100%, 50%, 25% and 10% of the 2010 abundance estimates (300 small salmon and 53 large salmon). Using the present dynamics, further reductions in population size have the effect of shortening time to extinction. A reduction in starting population size of 50% reduced the time to which 50% of the simulated populations are extinct by about 10 years, whereas a reduction in size of 75% reduced the time to which 50% of the simulated populations are extinct to about 15 years. Similarly using the 1980s dynamics, time to recovery was similarly increased. The effects of further reductions in population size prior to the initiation of recovery are most evident in scenarios where populations are on the edge of recovery. For example, with an increase in freshwater production of 1.2 times, the probability of extinction within 25 years is 1% when the starting population size equals the 2010 abundance. This value increases to 10%, 45% and 97% for reductions in the starting population size of 50%, 25% and 10% of the 2010 abundance. The effect is not so great for an increase in at-sea survival of one third because the increase in overall survival (i.e. survival from egg to adult) is greater than for an increase in freshwater production. Additional details of this analysis are provided in Gibson and Bowlby (2013).

Sources of Uncertainty

Detecting the presence of juveniles at very low abundance levels can be difficult; therefore, rivers in which salmon were not observed do not necessarily represent complete extirpation.

As described in Gibson and Bowlby (2013) the electrofishing catchability coefficient used in the freshwater production model was for the St. Mary’s River (West Branch) population could not be estimated and a value based on LaHave River (above Morgan Falls) production model was assumed. Had a different value been assumed, it is expected that the age- and stage-specific survival rates would change but the overall freshwater productivity curve would remain the same.

The dynamics of future, recovered SU salmon populations is unknown, and as a result, the sizes of those populations are unknown. Therefore, there is uncertainty about whether the proposed recovery targets for abundance are sufficient to ensure long-term population viability, but they are not considered to be unrealistically high given past abundance.

The importance of migration among rivers for ensuring numerical stability and genetic integrity within the DU is unknown; therefore, the number of populations that need to be included in the distribution component of the recovery target is also unknown.

The landscape cluster analysis used as a basis for developing distribution recovery targets is dependent on the data inputs and using additional or different environmental variables, as well as more or fewer feature classes within a variable, would affect the particular watersheds contained in the predicted number of clusters. Therefore, the watershed groupings should not be considered fixed in the sense that no other groupings are possible. However, the cluster analysis is a meaningful way of grouping landscape level patterns and demonstrates that all watersheds in the SU region cannot be considered equivalent in terms of protecting the biological diversity of Atlantic salmon populations. Diversity could also be characterized using the Eco-Districts present within the SU or using a lower level in the dendrogram presented in the Recovery Target section (e.g. the six clusters in the next tier).

PVA is a powerful and widely used technique in conservation biology to explore current conditions, assess risks and simulate how future management actions could affect a population in decline. They are known not to provide accurate estimates of the true probability of extinction or recovery, but they are useful for the relative evaluation of management actions.

The PVA models were set up with the assumption that the populations were at equilibrium abundances and age structure for the given scenario being modeled. This leads to starting abundances that can be higher than those recently observed. Short-term extinction risk would be higher if recent abundances were used for the starting values.

The PVAs were developed using a quasi-extinction threshold of 15 female salmon. Population viability analyses are known to be sensitive to the assumed threshold. This value is very low relative to the past abundances of salmon in these rivers. If depensatory dynamics exist, populations may not be able to recover from low abundances, even ones that are higher than this threshold. When scenarios were run using the 2000s dynamics, times to extinction decreased when the threshold was increased. However, this threshold has nearly no effect on time to recovery when the 1980s dynamics are used.

The PVA models were constructed such that the freshwater dynamics were independent of the marine dynamics. Marine survival rates may be improved by changes in the freshwater environment or in the freshwater population dynamics. For example, improved pH conditions may result in better marine survival of smolts as short-term exposure of smolts to low pH has been inferred to reduce early marine survival. Increased smolt production resulting in larger schools of smolts may improve early marine survival rates through prey-swamping effects when migrating through predator fields. As such, improved productivity in freshwater may directly affet marine return rates, the benefits of which will be reduced probabilities of extinction and improved probabilities of recovery. These dynamics are poorly understood in Atlantic salmon populations.

Marine distribution patterns for SU Atlantic salmon were assessed from historical tagging programs of smolts and adults combined with reported recaptures by commercial and recreational fisheries. Release data span the years from 1966 to 1998 and only include information from fish that were individually tagged (generally with numbered carlin or floy tags) and subsequently recaptured (i.e. releases with zero recaptures are not considered). Tags recovered in fisheries (or by people associated with the fishing industry such as fish plant workers) were returned voluntarily for a monetary reward. When interpreting these data, it is important to remember that sampling effort in the marine environment was non-random over space and time (i.e. the distribution of tag returns depends on the distribution of fishing effort as well as the distribution of the fish). In the Maritime Provinces and much of Newfoundland,

commercial trap nets for salmon were often at fixed locations accessible from shore. For the high-seas fisheries in Labrador and West Greenland, few of the tag recaptures were assigned a latitude and longitude when recovered; therefore, recaptures were ascribed to the mid-point of each West Greenland fishing district or to locations or communities along the coast of Labrador. Therefore, it is not possible to determine how far off shore Atlantic salmon may frequent from these data and it is similarly difficult to correlate recapture locations with environmental or oceanographic variables. Furthermore, the scarcity of tag recaptures during specific months (e.g. December to March) is largely due to the lack of sampling effort and may not reflect actual distribution patterns.

Watershed characteristics and human activities within watersheds were derived using geo-spatial data, some of which is becoming outdated. While the data used are the most current, specific information may require validation.

Although home stones potentially meet the criteria to be a residence, practically there is no way to identify whether a stone in a river is being used as a home stone.

SOURCES OF INFORMATION

This Science Advisory Report is from the May 22-25, 2012, Recovery Potential Assessment for Southern Upland Atlantic salmon. Additional publications from this meeting will be posted on the *Fisheries and Oceans Canada (DFO) Science Advisory Schedule* as they become available.

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APPENDIX A

Threats tables for the freshwater, estuarine and marine environments, summarizing human activities or sources of environmental change that either negatively impact Atlantic salmon populations (i.e. cause reduced abundance) or cause reduced quality and/or quantity of habitat in the SU region.

Definition of Table Headings and Column Values

Threat Category: The general activity or process (natural and anthropogenic) that has caused, is causing, or may cause harm, death, or behavioural changes to a species at risk; or the destruction, degradation, and/or impairment of its habitat to the extent that population-level effects occur.

Specific Threat: The specific activity or process causing stress to Atlantic salmon populations in the Southern Upland DU, where stress is defined as changes to ecological, demographic, or behavioural attributes of populations leading to reduced viability.

Level of Concern: Signifies the level of concern for species persistence if a threat remains unmitigated; where a High level of concern reflects threats that are likely to lead to substantial declines in abundance or loss of populations in the absence of mitigation, a Medium level of concern reflects threats that are likely to limit populations to low abundance and thus increase extinction risk, while a Low level of concern reflects threats that might lead to slightly increased mortality but are expected to have a relatively small impact on overall population viability. This criterion is based on the evaluation of all other information in the table with an emphasis on the extent of the threat in the DU and the number of populations likely to be affected at each level of Severity (see definition below).

Location or Extent: The description of the spatial extent of the threat in the SU was largely based on the criteria developed for the Conservation Status Report Part II (DFO and MRNF 2009), where Low corresponds to < 5% of populations affected, Medium is 5-30%, High is 30-70% and Very High is > 70%. Where possible, the actual proportion of SU Atlantic salmon populations affected by a specific threat is given in brackets.

Occurrence and Frequency: Occurrence: Description of the time frame that the threat has affected (H - historical), is (C - current) or may be (A - anticipatory) affecting Atlantic salmon populations in the Southern Upland DU. Historical – a threat that is known or is thought to have impacted salmon populations in the past where the activity is not ongoing; Current – a threat that is known or thought to be impacting populations where the activity is ongoing (this includes situations in which the threat is no longer occurring but the population-level impacts of the historical threat are still impacting the populations); Anticipatory – a threat that is not presently impacting salmon populations but may have impacts in the future (this includes situations where a current threat may increase in scope). Frequency: Description of the temporal extent of the threat over the course of a year (seasonal, recurrent, continuous).

Severity: Describes the degree of impact a given threat may have or is having on individual Atlantic salmon populations subjected to the threat given the nature and possible magnitude of population-level change. See Table A1 for definitions/examples of how severity has been evaluated.

Table A1. Definitions/examples of how severity has been evaluated.

Category	Definition/Examples
Negligible	• Habitat alteration within acceptable guidelines that does not lead to a reduction in habitat quality or quantity. • No change in population productivity.
Low	• Minor or easily recoverable changes to fish habitat (e.g. seasonal or changes <1 year). • Little change in population productivity (< 5% decline in spawner abundance)
Medium	• Moderate impact to fish habitat with medium term for habitat recovery (3-5 years). • Moderate loss of population productivity (5-30% decline in spawner abundance)
High	• Substantial damage to fish habitat such that the habitat will not recover for more than 5 years. • Substantial loss of population productivity (> 30% decline in spawner abundance)
Extreme	• Permanent and spatially significant loss of fish habitat • Severe population decline with the potential for extirpation.

Causal Certainty: Two-part definition. Part 1: Reflects the strength of the evidence linking the threat (i.e. the particular activity) to the stresses (e.g. changes in mortality rates) affecting populations of Atlantic salmon in general. As such, evidence can come from studies on any Atlantic salmon population. Part 2: Reflects the strength of the evidence linking the threat to changes in productivity for populations in the Southern Upland DU specifically. See Table A2 for definitions/examples of how causal certainty has been evaluated. Note: Does not apply to threats that are anticipatory.

Table A2. Definitions/examples of how causal certainty has been evaluated.

Causal certainty	Description
Negligible	Hypothesized.
Very Low	< 5%: Unsubstantiated but plausible link between the threat and stresses to salmon populations.
Low	5% - 24%: Plausible link with limited evidence that the threat has stressed salmon populations.
Medium	25% - 75%: There is scientific evidence linking the threat to stresses to salmon populations.
High	76% - 95%: Substantial scientific evidence of a causal link where the impact to populations is understood qualitatively.
Very High	> 95%: Very strong scientific evidence that stresses will occur and the magnitude of the impact to populations can be quantified.

Table A3. Threats to Atlantic salmon populations in the freshwater environment of the SU DU.

Threat Category	Specific Threat	Level of Concern	Location or Extent	Occurrence and Frequency	Severity	Causal Certainty	
		for the DU as a whole	of the threat in the DU	of the threat in the DU	of population level impacts	evidence linking the threat to stresses in general	evidence for changes to viability of SU salmon populations
Freshwater Environment							
Water quality and quantity	Acidification	**High**	Very High (78% of assessed populations affected)	H, C and A Continuous and recurrent	Extreme	Very High	Very High
	Extreme temperature events	**Medium**	High to Very High (anecdotal information suggests the majority of rivers are affected)	H, C and A Seasonal	High	High	Medium
	Altered hydrology	**High**	High to Very High	H, C and A Seasonal	High	High	Medium
	Water extraction	**Low**	Low	H, C and A Recurrent	Negligible to High (dependent upon timing and magnitude of extraction/alteration)	High	Low
	Chemical contaminants	**Low**	Unknown (anecdotal information suggests the majority of populations affected)	H, C and A Seasonal	Negligible to High (dependent upon concentration (dose) and time of exposure (duration)	High	Low
	Silt and sediment	**Medium**	Very High (100%)	H and C Continuous	Negligible to High (dependent upon concentration (dose) and time of exposure (duration)	High	Low
Changes to biological communities	Invasive species (fish)	**High**	Medium (22% of assessed populations)	H, C and A Continuous	High	High	Medium

Threat Category	Specific Threat	Level of Concern	Location or Extent	Occurrence and Frequency	Severity	Causal Certainty	
		for the DU as a whole	of the threat in the DU	of the threat in the DU	of population level impacts	evidence linking the threat to stresses in general	evidence for changes to viability of SU salmon populations
Freshwater Environment							
	Invasive species (other)	**Low**	Low	A Continuous	Low to High	Medium	Very Low
	Stocking for fisheries enhancement using traditional methods	**Medium**	Very High	H and C Continuous	Medium to Extreme (dependent upon number of fish stocked and length of period of stocking)	High (rate of fitness recovery after stocking ends is unknown)	Low
	Stocking (current)	**Low**	Low (several Fish Friends projects; educational programs)	C and A Continuous	Low to High (dependent upon number of juveniles stocked and size of recipient population)	High	Low
	Other salmonid stocking (rainbow, brown, & brook trout)	**Low**	Medium	H, C and A Continuous	Low to High (dependent upon number stocked and type of recipient waterbody (lake vs. river))	Medium	Low
	Salmonid aquaculture (commercial)	**Low**	Low	H, C and A Continuous	Medium	High	Low
	Avian predators	**Medium**	High	C and A Seasonal	High	Medium	Medium
	Genetic effects of small population size	**Medium**	Medium (mostly focused in southwest area of DU)	H, C and A Continuous	Negligible to High (dependent upon length of time at small population size, stocking history, and site specific conditions)	High	None (Not evaluated)
	Allee (small population size) effects	**Medium** (abundance specific)	Very High (abundance is low in all rivers)	H, C and A Continuous	Low to High (dependent on population-specific abundance)	Medium	Low

Threat Category	Specific Threat	Level of Concern	Location or Extent	Occurrence and Frequency	Severity	Causal Certainty	
		for the DU as a whole	of the threat in the DU	of the threat in the DU	of population level impacts	evidence linking the threat to stresses in general	evidence for changes to viability of SU salmon populations
Freshwater Environment							
	Scientific activities	**Low**	Low (Two Index Rivers and occasional surveys/sampling of other rivers)	H, C, A Seasonal	Low	Low	Low
Physical obstructions	Habitat fragmentation due to dams, culverts and other permanent structures	**High**	Medium to Very High	H, C and A Continuous	Low to Extreme (Dependent upon design of structure and location within watershed)	Very High	Very High
	Reservoirs	**Medium**	Medium	H, C and A Continuous	Low to High (Dependent upon size of individual reservoirs and number in series on a system)	High	Medium
Habitat alteration	Infrastructure (roads)	**Medium**	Very High (all rivers)	H, C and A Continuous	Low to High (dependent upon road density within watershed or sub-watershed)	Medium	Low
	Pulp and paper mills	**Low**	Low (only two known pulp mills in DU)	H and C Continuous	Medium to High (Dependent upon process used and effluent discharge quality)	High	Low
	Hydro power generation	**Medium**	Medium	H, C and A Continuous	Medium to Extreme (dependent upon facility design and operating schedule)	High	Medium

Threat Category	Specific Threat	Level of Concern	Location or Extent	Occurrence and Frequency	Severity	Causal Certainty	
		for the DU as a whole	of the threat in the DU	of the threat in the DU	of population level impacts	evidence linking the threat to stresses in general	evidence for changes to viability of SU salmon populations
Freshwater Environment							
	Urbanization	**Medium**	Medium	H, C and A Continuous	Low to High (dependent upon density of urbanization and infrastructure development)	High	Medium
	Agriculture	**Medium**	High	H, C and A Seasonal	Low to High (dependent upon extent within watershed and practices used)	Medium	Low
	Forestry	**Medium**	High	H, C and A Continuous	Low to High (dependent upon extent within watershed and practices used)	Medium	Low
	Mining	**Medium**	Unknown	H, C and A Continuous	Low to High (dependent upon type of mine, processes used, and susceptibility to Acid Rock Draiange)	Medium	Low
Directed salmon fishing (current)	Aboriginal FSC fishery	**Low**	Low	H, C and A Seasonal	Negligible	Very High	High
	Recreational fishery (angling)	**Low**	Low	H and A Seasonal	Negligible	Very High	High
	Illegal fishing and poaching	**High**	Unknown (but potentially high)	H, C and A Seasonal	Low to High (dependent on number of salmon removed and size of impacted population)	High	High
By-catch in other fisheries	Aboriginal or commercial fisheries	**Low**	Low	H, C and A Seasonal	Low	High	High

Threat Category	Specific Threat	Level of Concern	Location or Extent	Occurrence and Frequency	Severity	Causal Certainty	
		for the DU as a whole	of the threat in the DU	of the threat in the DU	of population level impacts	evidence linking the threat to stresses in general	evidence for changes to viability of SU salmon populations
Freshwater Environment							
	Recreational fisheries	**Low**	High	H, C and A Seasonal	Low	High	High
	Recreational fishery: illegal targeting of Atlantic salmon while fishing under a general license	**Medium**	High	H, C and A Seasonal	Low to High (dependent upon angling pressure)	High	High

Table A4. Threats to Atlantic salmon populations in the marine or estuarine environments of the SU DU.

Threat	Specific Threat	Level of Concern	Location or Extent	Occurrence and Frequency	Severity	Causal Certainty	
		for the DU as a whole	of the threat in the DU	of the threat in the DU	of population level impacts	evidence linking the threat to stresses in general	evidence for changes to viability of SU salmon populations
Marine or Estuarine Environment							
Changes to biological communities	Invasive species	**Low**	Very High (all populations)	C and A Continuous	Low	Low	Low
	Salmonid aquaculture	**High**	Very High	H, C and A Continuous	Medium to High (dependent upon location of aquaculture facilites and operating practices)	High	Low
	Other species aquaculture	**Low**	Very High (all populations)	H, C and A Seasonal	Negligible to Medium (dependent upon species under culture, location of fsaacility, and operating practices)	Low	Low
	Diseases and parasites	**Medium**	Very High (all populations)	H, C and A Continuous	Low to High (dependent upon irruptive behavior of disease/parasites resulting in outbreaks)	Low	Low
Changes in oceanographic conditions	Marine ecosystem change (including shifts in oceano-graphic conditions and changes in predator/prey abundance)	**High**	Very High (all populations)	H, C and A Continuous	Low to Extreme (dependent upon magnitude of change and sensitivity of salmon to change)	Medium	Low

Threat	Specific Threat	Level of Concern	Location or Extent	Occurrence and Frequency	Severity	Causal Certainty	
		for the DU as a whole	of the threat in the DU	of the threat in the DU	of population level impacts	evidence linking the threat to stresses in general	evidence for changes to viability of SU salmon populations
Marine or Estuarine Environment							
Physical or abiotic change	Shipping, transport, noise, seismic activity	**Low**	Very High (all populations)	H, C and A Seasonal	Uncertain; likely Negligible to Low (dependent upon proximity of salmon to source of noise/activity)	Low	Low
	Contaminants and spills (land- or water-based)	**Low**	Very High (all populations)	H, C, A Episodic	Low to Extreme (dependent upon identity and magnitude of contamination, and efficacy of cleanup)	Low	Low
	Tidal power	**Low**	Low	C and A Seasonal	Medium to High (dependent upon facility design and operating schedule)	High	Medium
Directed salmon fisheries	Subsistence fisheries (Aboriginal and Labrador residents)	**Low**	Low	H and A Seasonal	Negligible	High	High
	International fisheries (Greenland; St. Pierre-Miquelon)	**Medium**	Very High (MSW component of all populations)	H, C and A Seasonal	Negligible to High	High	Medium
By-catch in other fisheries	Commercial fisheries	**Low**	Very High (all populations)	H, C and A Seasonal	Low	High	High
Fisheries on prey species of salmon	Commercial fisheries	**Low**	Very High (all populations)	H, C and A Seasonal	Low to High (dependent upon reduction of prey species and availability of other forage species)	Low	Low

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Original Article

Natural and anthropogenic drivers of escaped farmed salmon occurrence and introgression into wild Norwegian Atlantic salmon populations

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Marine aquaculture of Atlantic salmon (*Salmo salar*) is a relatively new industry where breeding programs have led to rapid genetic change in the captive populations that were built up alongside conspecific wild individuals. Throughout its 50-years history, marine aquaculture of Atlantic salmon has been associated with escapes, and studies have shown that escapees may enter rivers, spawn successfully, and this may lead to farmed-to-wild genetic introgression and maladaptation in wild populations. Yet, an open question is what factors can best explain the variability in the proportion of farmed escapees in wild populations, and when present, which additional factors lead to introgression. Here, we combine two large-scale data sets from monitoring escaped farmed salmon and introgression in Norwegian rivers between 2006 and 2018 to model how anthropogenic, environmental, and population factors influence proportion of escapees and level of introgression. We found that increasing farming intensity and river discharge increase the expected proportions of escaped farmed salmon in rivers, whereas a larger wild salmon population size reduces the expected proportion of escapees despite increasing the expected absolute numbers of escaped farmed salmon. On a large scale, introgression is primarily a function of proportions of escaped farmed salmon, and only to a minor extent a function of local environmental factors or salmon population characteristics. This suggests that as long as salmon aquaculture is based on technologies where non-sterile fish can escape, all anadromous wild Atlantic salmon populations are at risk. Large marine protected areas without salmon aquaculture may slow down the rate of intrusion and introgression by increasing the distance between intensive aquaculture and wild populations.

Keywords: admixture, aquaculture, Atlantic salmon, escaped farmed salmon, gene flow, *Salmo salar*.

Introduction

The rapid domestication of fish species for aquaculture means that we are in position to follow the genetic process of domestication as it happens. We are also able to follow the side-effects of domestication on wild populations as large-scale aquaculture, in some years, has produced as many escapees as there are wild conspecifics (Hindar *et al.*, 1991).

Successful domestication depends on controlling the life cycle from fertilization until market size. Artificial reproduction of salmonids was mastered on a large scale from the 1850s onwards, when unfed salmonid fry (alevins) were produced in large numbers for release into the wild (Berg, 1986). The technology to raise Atlantic salmon (*Salmo salar*), hereon referred to as salmon, to market size in marine net pens was developed in the 1960s and led to the growth of a salmon aquaculture industry both within and outside its natural distribution range (Heen *et al.*, 1993). The production of salmon in fish farms has increased from half of nominal catch of wild salmon in 1980 to outnumbering it 2000 times in 2019 (ICES, 2020).

In 1986, the first concerns about escaped farmed salmon entering rivers were published (Maitland, 1986). High proportions of farmed salmon were found in many rivers in Norway during the autumn of 1987 and 1988 (Gausen and Moen, 1991). In 1989, a nationwide monitoring system for escaped farmed salmon was implemented in Norway (Lund and Hansen, 1991; Diserud *et al.*, 2019), and is now into its second and more comprehensive generation with annual sampling of more than 200 rivers (Glover *et al.*, 2019). The accumulated number of individuals being classified as wild or farmed escapees, based on growth patterns in the scales (Lund and Hansen, 1991), amounts to more than 470 000 since 1989.

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Around 1990, methods were developed to show that escaped farmed salmon could produce offspring in the wild. The first methods were based on demonstrating that feed additives (synthetic astaxanthin and canthaxanthin) were found in salmon eggs deposited in the riverbed in Scotland and Norway (Lura and Sægrov, 1991a, b; Webb *et al.*, 1991). Moreover, *ad hoc* genetic methods based on skewed allele frequencies in allozyme markers were used to show that wild salmon juveniles in Ireland had farmed parents (Crozier, 1993; Clifford *et al.*, 1998a, b). Later, microsatellite markers were used to document temporal genetic changes in wild populations, including a reduction in wild population differentiation, that were likely a result of escaped farmed salmon interbreeding (Skaala *et al.*, 2006; Glover *et al.*, 2012).

In 2011, a SNP panel to distinguish farmed and wild salmon was developed in Norway based on screening 4514 SNP markers in 12 breeding lines of Norwegian aquaculture salmon and 13 wild Atlantic populations throughout Norway, sampled before the growth of the aquaculture industry (Karlsson *et al.*, 2011). Using this method, scale samples with a confirmed wild growth pattern (Fiske *et al.*, 2005) can be used as a source of DNA, lending themselves to genetic screening for determining the degree of farm wild admixture. More than 50 000 individuals with a wild life cycle from 239 Norwegian rivers have been analyzed to estimate their probability of belonging to a wild salmon population (Karlsson *et al.*, 2016; Diserud *et al.*, 2020) by using the methodology developed by Karlsson *et al.* (2014).

In this study, we analyze the predictors that can be associated with the occurrence of escaped farmed salmon and their introgression into wild salmon in Norway, improving preliminary models presented in reports for Norwegian authorities (Fiske *et al.*, 2013; Hindar *et al.*, 2018). Heino *et al.* (2015) found that the observed proportion of escaped farmed salmon in catches and the average annual angling catch weights for rivers could provide a predictor for cumulative introgression in 20 populations, where catch served as a proxy for current population size. Sylvester *et al.* (2018) showed that within-river distribution of hybrid parr was associated with the migration effort required to reach spawning sites; the hybrid proportion decreased with increasing elevation, geographic distance, and the presence of obstructions. Keyser *et al.* (2018) predicted the distribution of escaped farmed salmon and degree of introgression in wild populations in the Northwest Atlantic from aquaculture facility locations, production estimates, reported escape events, and in-river detections of escaped farmed salmon. Mahlum *et al.* (2021) found that aquaculture intensity, wild salmon abundance, mean yearly discharge, and the interaction between the distance from river mouth to open ocean and wild salmon abundance were important predictors of escapee abundance in western Norwegian rivers. Proximity to fish farms or other indices of farm production intensity had also been found by Gausen and Moen (1991) and Fiske *et al.* (2006) to correlate with high proportions of escapees.

In autumn 1989, Norwegian authorities established a system of 52 temporary protection zones (with 125 salmon rivers) for wild salmon populations in fjords that were attractive for further development of aquaculture. These were later formalized by the Norwegian parliament (Anon, 2006) as a system of 29 National Salmon Fjords and 52 National Salmon Rivers along the Norwegian coast intended as a general protection of the wild salmon resource. The purpose of this protection system is to give the most important salmon populations in Norway a special protection against harmful anthropogenic activities in the rivers, and in adjacent fjord and coastal areas.

Here, we combine data sets on escaped farmed salmon and introgression from c. 200 rivers along the Norwegian coast, from 58°N to 71°N, to answer the questions: (1) what determines the occurrence of escaped farmed salmon into Norwegian rivers, (2) what determines the level of introgression in Norwegian salmon populations, and (3) does the establishment of protection zones for wild salmon reduce introgression from escaped farmed salmon?

Material and methods

Materials

Data on the proportions of escaped farmed salmon in Norway come from two papers that reported the distribution of escapees in rivers from 1989 to 2013 (Diserud *et al.*, 2019) and on a more comprehensive scale from 2014 (Glover *et al.*, 2019). Scales from more than 470 000 individuals, caught during summer recreational angling, autumn pre-spawning angling surveys, and broodstock fishing, have been analysed to determine their origin (escaped farmed or wild) according to fish scale growth pattern (Fiske *et al.*, 2005; see also Diserud *et al.*, 2019). Proportions estimated from summer catches may underestimate the proportion of escapees in the wild spawning populations as escaped farmed salmon often ascend rives later in the season than wild salmon (Lund *et al.*, 1991; Crozier, 1998; Erkinaro *et al.*, 2010), while autumn samples may give uncertain proportion estimates due to small sample sizes and biased estimates due to potentially differing catchabilities or spatial distribution close to the spawning period (Moe *et al.*, 2016; Svenning *et al.*, 2017). An *Incidence index* that combined the information from summer and autumn catch samples was, therefore, developed for management purposes to give the best possible annual estimate of the proportion of escaped farmed salmon in wild salmon populations (Fiske *et al.* 2006; Diserud *et al.*, 2010).

The estimated proportions of escaped farmed salmon in the wild salmon populations were averaged over the years from 2006, when the estimates for wild population status were improved (Forseth *et al.*, 2013), to 2018. Each annual estimate were given the same weight when calculating the average. This period covers the last two to three wild salmon generations. We analysed the *Incidence index* averaged over this prolonged period rather than including the temporal variation in escape proportions. This was done because genetic introgression is accumulated over time, the frequency and quality of catch reports may vary considerably, and associations can be both time-lagged and smoothed out over several years, making "correct" temporal assignments difficult. Models were fitted to 129 wild salmon populations with a minimum of 4 years of *Incidence index* estimates (Figure 1b). With a lower limit at 4 years of data, we focus on the more permanent characteristics of a population and its environment that may influence the proportion of escaped farmed salmon.

Data on introgression from escaped farmed to wild salmon in Norway was obtained from Karlsson *et al.* (2016) and the report by Diserud *et al.* (2020), which present information on introgression in 239 wild salmon populations and more than 50 000 genetically analyzed individuals with a wild

Figure 1. Maps of Norway showing: (a) locations with sea water net pens in 2015–2016, (b) rivers with incidence indices estimates ($n = 129$), and (c) rivers with genetic introgression estimates ($n = 239$). Grey areas along the coast indicate the National Salmon Fjord protection zones.

growth-scale pattern confirming that individuals were hatched in the wild (Figure 1c). Historical samples collected before significant farmed salmon introgression (c. 1990) have been analyzed for 59 of the 239 wild salmon populations, to serve as wild origin references.

The underlying estimate of introgression (or lack thereof) is the probability an individual belongs to a reference of wild salmon (*P(Wild)*), using the SNP panel developed by Karlsson *et al.* (2011) and a statistical method developed by Karlsson *et al.* (2014). *P(Wild)* is, thus the unscaled proportion of wild origin and not the estimate of introgression. Introgression is a population property accumulated over time, expected to vary among cohorts depending on escape episodes and stochastic environmental variation. We have, therefore, used the population mean *P(Wild)* as the model response variable, estimated from a contemporary sample pooled over the last salmon generation with sufficient total sample size (Diserud *et al.*, 2020). During model fitting, we only include populations with a genetic sample size of 20 fish or more. Most populations are represented by recent samples; 75% of the populations are from 2014 or later, while the oldest are from 2005.

Variables that were assumed *a priori* to be potential predictors for occurrence of escaped farmed salmon or extent of introgression, or both, are listed in Table 1. The predictors can be divided into three categories: population, environmental, and anthropogenic. Population predictors include variables like the phylogenetic group of the wild salmon, number and density of spawners, adult body size, and juvenile growth rate in fresh water. Environmental predictors include variables such as river size (discharge), migration obstacles, and the river's location along the coast. Anthropogenic predictors include factors affecting the number of escapees along the coast and in-river human activities such as hydropower regulation, release of hatchery fish, or liming. Farming intensity was estimated based on January and June biomass (or numbers) in seawater net pens for c. 1000 locations along the Norwegian coast 2006–2016 (Data courtesy of the Norwegian Directorate of Fisheries) and on measurement of the distances between river mouths to all farming locations (Figure

Table 1. List of population, environmental, and anthropogenic predictor variables that may be associated with occurrence of escaped farmed salmon in wild salmon rivers (*Incidence index*) and/or genetic introgression of farmed salmon in wild populations. The table presents a short name for each variable, variable type, data quantity (number of rivers or populations and the variable range), and *a priori* assumed effect on escapee proportion or genetic introgression. The number of populations or rivers listed in the data column refers to the 224 populations we have either escape proportions or genetic data from, or both. All predictors that vary temporally are averaged over the period from 2006 to 2018. Variables indicated with an asterisk (*) in the *Short name* column are included in one or more of the final models.

				Potential effect on …		
Predictor variable	**Short name**	**Type and unit**	**Data**	**Incidence index**	**Genetic introgression**	**References**
			Population variables			
Phylogenetic group	*Phyl**	Factor [North-East Atlantic—NEA, Barents Sea—BS, and Transition zone—TZ]	224 populations ($n_{NEA} = 181$, $n_{BS} = 27$, and $n_{TZ} = 16$)		Farmed salmon originates from NEA group so represent an endemic farmed fish in South Norway and an exotic one in Barents Sea rivers in North Norway; Introgression has larger ecological consequences where farmed salmon are exotic	Bourret *et al.* (2013), Karlsson *et al.* (2014), Bolstad *et al.* (2017), Wacker *et al.* (2021)
Population size—estimated both as pre-fishery abundance and spawner abundance.	*PopSize.PFA** *PopSize.SA**	Count	146 populations, (range PFA 119–38 822; range SA 71–16 962)	Escapees follow wild salmon back to rivers; more farmed escapees but lower proportion with larger wild salmon population size	Spawner density affects spawning success of escapees negatively through competition; juvenile density affects relative survival of farmed offspring negatively	Mahlum *et al.* (2021), Lura (1995), Fleming *et al.* (1997), Skaala *et al.* (2019), Anon (2021)
Spawning target—spawning stock conservation limit that ensure an acceptable smolt production and population viability	*SpawnTarget.no** *SpawnTarget.att*	Number of female spawners (no.), or ratio between realized spawner abundance and spawning target (attainment)	214 populations, (range 9–11 483 females)		Wild spawner abundance lower than target may indicate vacant spawning territory and increased success of escapees; potential for establishing feral populations	Fleming *et al.* (1996, 2000), Youngson *et al.* (1998), Naylor *et al.* (2005), Hindar *et al.* (2007), Forseth *et al.* (2013), Pulg *et al.* (2021)

Table 1. Continued

Predictor variable	Short name	Type and unit	Data	Potential effect on … Incidence index	Genetic introgression	References
Spawning target relative to sum of all spawning targets in proximity of river (< 60 km by water)	*RelTarget**	Proportion	153 rivers, (range 0.0025–1)	A population may "compete" with neighbouring populations for escapees as relatively large populations are more attractive? Spawning targets used instead of actual spawner abundances since we lack abundance estimates for many populations.	Neighbouring populations may serve as a source of introgressed strayers.	Hindar (1992), Jonsson and Jonsson (2017)
Growth rate in fresh water—proxy mean smolt age	*SmoltAge*	Continuous [years]	176 populations, (range 2.0–5.1 years)		Younger age at smoltification indicates good juvenile growth opportunities; farmed offspring may outgrow and displace wild offspring in favorable growing conditions	Symons (1979), McGinnity *et al.* (2003), Fleming *et al.* (2000)
Adult body size—mean catch weight	*BodySize*	Continuous, [kg]	131 populations, (range 1.2–6.0 kg)		Large body size is associated with high fecundity (females) and dominant access to females (males); less strong relationship in farmed escapees than in wild salmon	Fleming *et al.* (1996, 1997)
			Environmental variables			
Water discharge—mean annual water discharge	*WaterDis**	Continuous, [m^3 s^{-1}]	223 rivers, (range 0.4–705 m^3 s^{-1})	High waterflow is attractive to salmon		Hindar (1992), Diserud *et al.* (2019), Mahlum *et al.* (2021), NVE Atlas (https://atlas.nve.no/Html5Viewer/index.html?viewer=nveatlas#)

Table 1. Continued

Predictor variable	Short name	Type and unit	Data	Potential effect on … Incidence index	Genetic introgression	References
Length of anadromous stretch	*AnadrStr*	Continuous, [km]	169 rivers, (range 1–1 100 km)		Longer rivers may be harder to ascend to reach spawning grounds	Schaffer and Elson (1975)
Migration obstacles—proportion of anadromous section above first migration obstacle (rapid or fish ladder) encountered.	*Obstacle**	Proportion	167 rivers, (range 0–1)	Distribution of escaped farmed spawners limited because of poor swimming abilities through rapids/waterfalls/fish ladder that are passable only by wild salmon	Obstacles may limit access of escapees to spawning grounds and degree of interbreeding.	Svenning *et al.* (2021), Sylvester *et al.* (2018)
Distance to outer coast	*CoastDist*	Continuous [km]	213 rivers, (range 0.6–213 km)	Straying escaped farmed salmon may choose the first river encountered when approaching coast from the ocean		Hansen *et al.* (1993)
River discharge relative to all rivers in proximity (< 60 km by water)	*RelDis*	Proportion	175 rivers, (range 0.006–1)	Large neighbouring rivers can "compete" for escapees as relatively high discharge is more attractive		Hindar (1992), Jonsson *et al.* (2003), Kuparinen *et al.* (2010)
Lakes present or not in anadromous stretch of river.	*Lake*	Factor [No = 0, Yes = 1]	196 rivers, ($n_{No} = 87$, $n_{Yes} = 109$)	Can affect success of up-river migration and overwintering of escapes	Higher vulnerability of farmed offspring to predation from lake-dwelling species	Huitfeldt-Kaas (1923), Solberg *et al.* (2020)
			Anthropogenic variables			
Distance from river mouth to closest fish farm	*FarmDist*	Continuous [km]	212 rivers, (range 0.6–191 km)	Higher proportion of escaped farmed spawners in rivers < 20 km from fish farm; Increased distance means reduced propagule pressure		Norwegian Directorate of Fisheries data, Gausen and Moen (1991), Fiske *et al.* (2006), Bradbury *et al.* (2020), Mahlum *et al.* (2021)

Table 1. Continued

Predictor variable	Short name	Type and unit	Data	Potential effect on … Incidence index	Potential effect on … Genetic introgression	References
Farming intensity (numbers or biomass), distance weighted by a gaussian distribution.	*FarmIntens .no = Numbers* .bm = biomass*	Continuous	212 rivers and 1 377 potential farming locations	Farm production in region better predictor for proportion of escapes in river than reported escapes in region		Derived from semiannual data on standing biomass on each location (Norwegian Directorate of Fisheries data), Ford and Myers (2008), Hindar *et al.* (2018), Keyser *et al.* (2018), Mahlum *et al.* (2021)
Populations that were main sources to farmed strains.	*FarmSource**	Factor [No = 0, Yes = 1]	8 populations		Source populations of farmed salmon different P(Wild) levels from other wild populations?	Karlsson *et al.* (2014)
Proportion cultivated smolt of total smolt migration	*CultSmolt*	Proportion	165 populations, (range 0 –0.85)	May lead to errors in estimation of proportion of escaped farmed salmon, as cultivated released smolt are similar to farmed salmon escaping as smolt	Risk that escaped farmed salmon are taken as broodstock; high cultivated proportion may also be a proxy for wild population vulnerability	Hagen *et al.* (2019)
Hydropower regulation of river	*RivReg*	Factor [0 = no to 3 = heavy impact]	165 rivers, ($n_0 = 110$, $n_1 = 17$, $n_2 = 22$, and $n_3 = 16$)	Changed water flow regime may affect attractiveness of river to farmed escapees—some hydropower regulations reduce waterflow, others increase it	Hydropower regulation may reduce wetted area and wild population size, leaving easier access for escaped farmed salmon	Forseth *et al.* (2013, 2017)
Liming of acidified river	*Liming*	Factor [No = 0, Yes = 1]	224 rivers, 19 of them limed		Younger history of introgression in recently limed rivers; proxy for population vulnerability?	Hesthagen *et al.* (2011)
National salmon fjords and National salmon rivers with special protection status	*NSF* and *NSR*	Factor [No = 0, Yes = 1]	224 rivers, $n_{NSF} = 33$, $n_{NSR} = 52$	NSF are areas without net pens and increase distance between rivers and fish farms	The protection status may entail actions that make populations more robust	Anon (2006), Hindar *et al.* (2018)

1a). The contribution from the standing stock in each fish farm was weighted by a decreasing Gaussian function with a *SD* of 60 km. This resembles the calculation of "propagule pressure" for each river by Keyser *et al.* (2018). It was inspired by early reports of escaped farmed salmon in rivers in relation to regional fish farms (Gausen and Moen, 1991; Fiske *et al.*, 2006) and the dispersion of smolt and later stages of farmed salmon from known release localities (Jonsson *et al.*, 2003; Hansen, 2006; Skilbrei *et al.*, 2015). We also tested other alternatives for quantifying the accumulated influence from surrounding farms on wild salmon populations but found none that explained incidence of escaped farmed salmon better (see Hindar *et al.*, 2018). Table 1 presents a short name for each variable, variable type, data quantity (number of rivers; variable range), and an *a priori* assumed effect on escapee proportion or introgression. It is acknowledged that there are other variables that could be included in this analysis, but those in Table 1 are the ones that we identified as biologically relevant and that we have been able to quantify with sufficient precision.

All variables were averaged over the same period as the escape proportions, i.e. from 2006 to 2018, giving each annual observation the same weight. Some variables are constant, some are already given as temporal averages (e.g. mean annual discharge), some may have large uncertainty due to small annual sample sizes, and some may reflect properties accumulated or lagged over longer periods, which makes it difficult to allocate them to appropriate years or cohorts.

Methods

Here, we logit-transformed the responses, i.e. proportions of escaped farmed salmon in wild salmon populations and introgression as proportional wild ancestry, to stabilize the variance, arguing that the resulting error distributions becomes approximately normal so that traditional multiple linear regression models can be used for the transformed responses. We could not fit generalized linear models (GLMs) with binomial error distributions because neither of the responses are direct results of binomial experiments (i.e. they cannot be expressed as ratios of two integers). To validate our assumptions when applying the logit-transform, residuals are checked for constancy of variance and normality of errors.

The wild salmon population's mean *P(Wild)* is partly a result of natural genetic variation, i.e. the estimated mean levels from historical samples will vary among populations (Diserud *et al.*, 2020) and between phylogenetic groups [North-East Atlantic (NEA), Barents Sea (BS), and a transition zone (TZ) between them; Bourret *et al.*, 2013; Wennevik *et al.*, 2019]. A model predicting the variation in historical *P(Wild)* population means from phylogenetic group and other predictors is presented in the Supplementary material (S1). These associations among pre-introgression *P(Wild)* levels and predictors need to be accounted for before studying factors that affect introgression from escaped farmed salmon.

Some predictors may affect both the presence of escapees in salmon rivers and subsequent introgression (Table 1). To separate these two effects, we first modelled the proportion of escaped farmed salmon to identify predictors associated with presence of escapees. Finally, we modelled contemporary mean population *P(Wild)* and aimed to sort contributions from natural variation, presence of escapees in rivers, and potential predictors that may modify introgression, given that escaped farmed salmon were present in the spawning population.

Our variable selection procedure was initially based on residual deviance and ΔAIC, but as most predictors have missing observations for some populations, two models' AIC values may not be directly comparable. Data collection for some factors were initiated by a specific event (anthropogenic intervention), so samples may be far from random. In addition, as we wanted to predict an outcome based on multiple predictors where some may covary, the variable selection procedure had to consider this correlation structure. Therefore, model selection, and interpretation of individual predictor contributions, had to be made with caution, and should, where possible, be guided by supportive information to augment confidence in the results. Some predictors could also be considered as proxies for factors hard to quantify directly.

A sizeable proportion of the variation in predictor variables may be caused by measurement and sampling uncertainty. The slope of the regression is expected to be underestimated even with unbiased measurement and sampling uncertainty, and this underestimation increases as uncertainty increases (Carroll *et al.*, 2006). Therefore, we strived for functional simplicity and chose, among correlated variables, those with best accuracy.

Predictions based on models are often used by managers to guide mitigation of anthropogenic pressures. It is, therefore, important to validate models and evaluate their predictive performance. Model selection can be viewed as a trade-off between minimizing bias and variance for predicted values. Predictions will be biased when explanatory variables with true non-zero regression coefficient are not included in the model. To minimize prediction bias, the best strategy will be to include as many variables as possible. But as we include more variables, the prediction variance will increase. The optimal model complexity is, therefore, a model with a moderate number of parameters so that the sum of the bias and the variance (mean square prediction error) is minimized. Minimizing the AIC is in accordance with this line of thought; it strives to improve model fit (log-likelihood) and reduce model complexity (number of parameters). A model with large prediction variance can be termed "overfitted" and will be poor at predicting observations outside the calibration data set. Here, we evaluated the prediction variance by a leave-one-out cross-validation procedure, i.e. we fitted the model to all observations except one and then used this model to predict the left-out observation. By comparing the coefficient of determination R^2_{Cal} for the model calibrated to the complete data set to R^2_{Val}calculated from the observed response and the corresponding leave-one-out predictions, we could evaluate the prediction variance. For an overfitted complex model, the R^2_{Val} will be much lower than the R^2_{Cal}. An illustration of this validation approach can be found in the Supplementary material (S2).

Interaction terms were evaluated, but none were found to improve model performance. All calculations and modelling were performed using the statistical software R, version 4.0.3 (R Core Team, 2020).

Results

We started by investigating associations between the *Incidence index*, i.e. the estimated mean annual proportion of escapees

Table 2. Results from the regression model used to identify predictors associated with logit(*Incidence Index*). Predictors were farming intensity (numbers weighted with distance), estimated wild population spawner abundance, mean annual water discharge (m^3 s^{-1}), and population spawning target relative to sum of all spawning targets in near proximity (< 60 km by water). Note that the log() function refers to the natural logarithm. ** $p < 0.01$, *** $p < 0.001$.

	Estimate	Std. Err	*t*-value
Intercept	−3.87	0.874	−4.43***
log(*FarmIntens.no*)	0.21	0.043	4.88***
log(*PopSize.SA*)	−0.67	0.092	−7.25***
log(*WaterDis*)	0.59	0.085	6.95***
RelTarget	0.69	0.261	2.63**

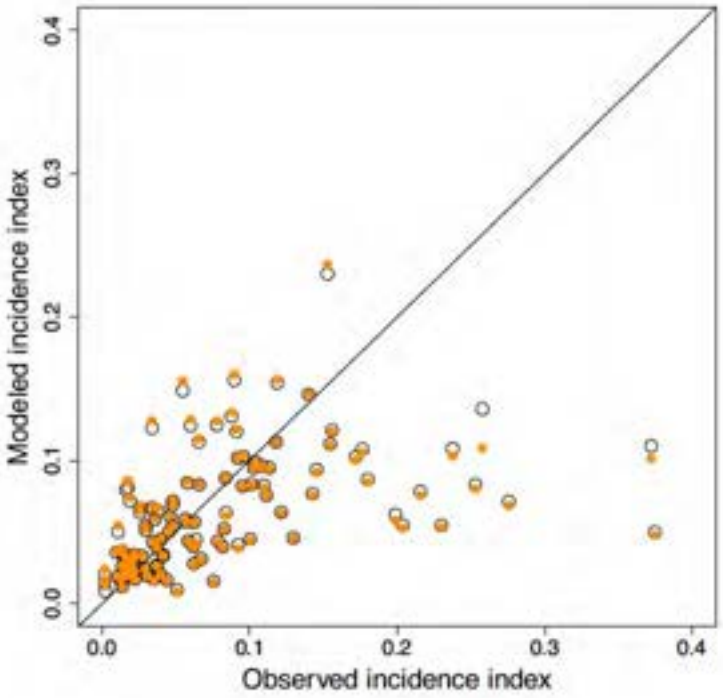

Figure 2. Model predictions from the calibration model presented in Table 2 (black open circles) and predictions from the corresponding leave-one-out validation models (orange filled circles) for the same observations.

in rivers, and relevant predictors (Table 1). The model for *Incidence index* was primarily applied as an intermediate step in the process of separating escapee attraction and introgression sensitivity, but this model could also be used as a prediction model for the proportion of escaped farmed salmon in a wild salmon population lacking data of sufficient quality. Next, we wanted to understand drivers of introgression observed in contemporary samples. A key question was if we could detect predictor variables explaining variation in the population sample mean probability of belonging to a wild salmon population *P(Wild)* in addition to those associated with historical variation and escapee proportions, indicating populations sensitive or robust to introgression.

Modelling the proportion of escaped farmed salmon in wild salmon spawner populations

An increase in expected *Incidence index* was associated with higher farming intensity *FarmInten.no*, smaller population size *PopSize.SA*, a larger water discharge *WaterDis*, and the population having a relatively large spawning target compared to the other rivers in the vicinity *RelTarget* (Table 2).

The full model with all potential predictors included (ref. Table 1) was strongly overfitted (28 out of 129 populations excluded due to missing observations: $R^2_{Cal} = 0.51$ and $R^2_{Val} = 0.24$). After variable reduction from the full model, we got the more parsimonious model for logit-transformed *Incidence index* presented in Table 2 (17 populations missing, $R^2_{Cal} = 0.43$ and $R^2_{Val} = 0.37$). Figure 2 illustrates the model fit and validation. Note that the model underestimate all the incidence indices larger than c. 0.2.

As an illustration of the back-transformed association between farming intensity and the *Incidence index*, we calculated the model predictions when varying the farming intensity from observed minimum to maximum, while keeping the other predictors fixed at their averages (Figure 3a). The expected proportion of escaped farmed salmon for an "average" population and river increased from below 0.01 to 0.08 over the range of farming intensities. Figure 3(b) shows the distributions of *Farm Intensity* for rivers within (upper violin plot) and outside (lower) the National salmon fjord protection zones.

We also modelled the number of escaped farmed salmon in wild salmon populations because numbers, rather than proportions, may be more directly related to mechanisms for the spread of escaped farmed salmon from fish farms into rivers. Moreover, the number of immigrants into wild populations is interesting for analyses of the balance between immigration and genetic drift (Ryman *et al.*, 1995). Note that the numbers

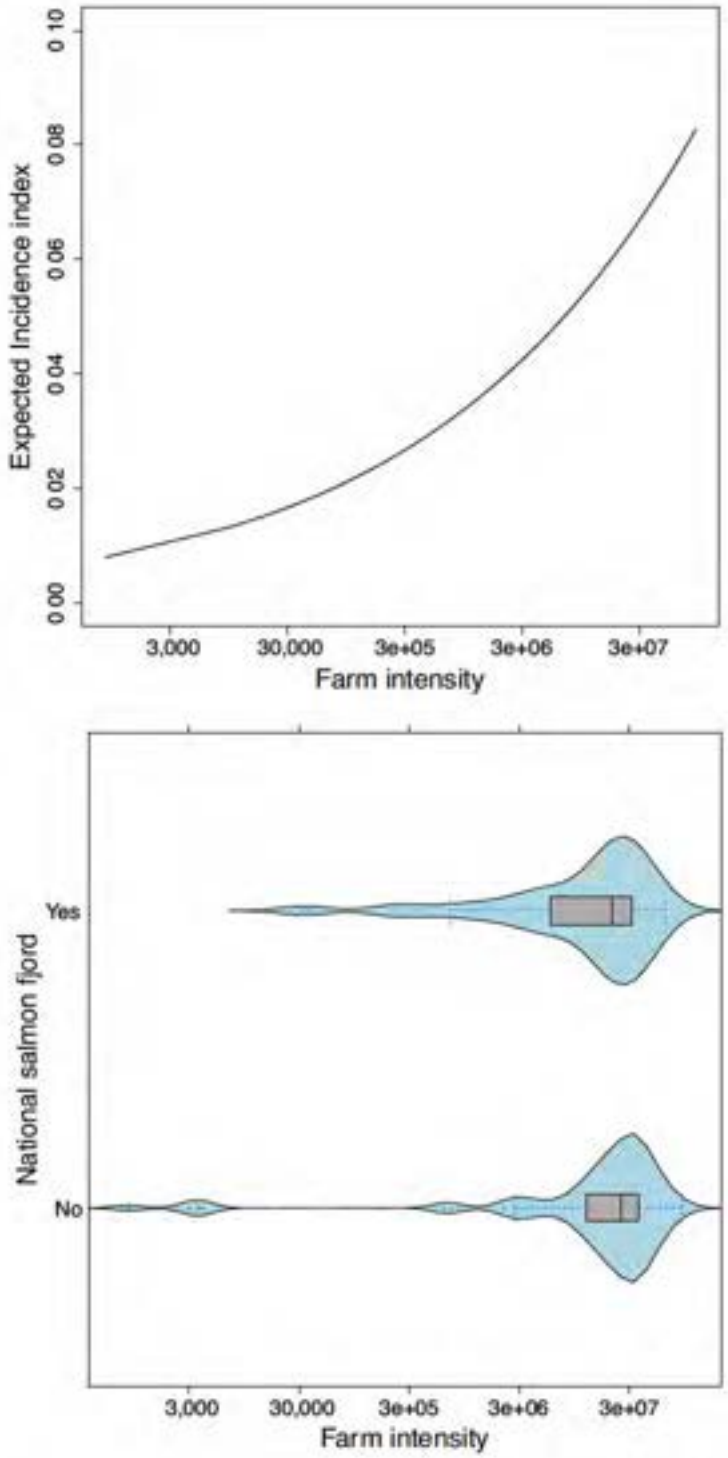

Figure 3. (a) An illustration of the association between farming intensity and expected *Incidence Index* with the other predictors fixed at their averages. (b) The distributions of *Farm Intensity* (natural logarithmic scale) for rivers within vs. outside National salmon fjords shown by violin-plots.

of escapees in wild populations were calculated as the products of the two estimates *Incidence index* and population size, so the accumulated estimation uncertainty and potential bias may be large. The model for the *number* of escapees (Supplementary Table S3-1; Supplement 3) included the same predictors as the model for the *proportion* of escapees, although the sign of the estimated coefficient for population size changed. The expected number of escapees increases with the population's spawning target, while a dilution effect ensures that the proportion of escapees decreases with increasing population size.

Modelling population mean *P(Wild)* for contemporary samples

We started by fitting a model for population mean *P(Wild)* based on *predicted* historical baseline levels (Supplementary Table S1-1) and *predicted* incidence indices (Table 2). Thereby,

Table 3. Linear regression model for logit(*P(Wild)*), where the populations' variance in predicted pre-introgression *P(Wild)* level and *Incidence Index* are accounted for. ** $p < 0.01$, *** $p < 0.001$.

	Estimate	Std. Err	*t*-value
Intercept	−0.068	0.291	−0.23
Predicted logit(*Incidence Index*)	−0.341	0.061	−5.58***
Predicted historical logit(*P(Wild)*)	0.573	0.096	5.98***

Table 4. Model used to identify predictors associated with the residuals from the logit(*P(Wild)*) model (Table 3). Predictors were upriver migration obstacles (proportion of anadromous section above first migration obstacle) and phylogenetic group. * $p < 0.05$, *** $p < 0.001$.

	Estimate	Std. Err	*t*-value
Intercept	0.012	0.044	0.28
logit(*Obstacle*)	0.025	0.012	2.05*
Phyl-BS	0.254	0.117	2.18*
Phyl-TZ	−0.598	0.174	−3.44***

we were not dependent on, or limited to, the actual historical samples or escapee observations as long as the relevant predictors were observed. Table 3 presents the linear model for logit(*P(Wild)*) where pre-introgression level and expected *Incidence index* are accounted for (133 populations used to fit the model, 91 missing; $R^2_{Cal} = 0.45$, $R^2_{Val} = 0.41$).

Next, the residuals from this model, i.e. the variation in *P(Wild)* not explained by historical levels or presence of escaped farmed salmon, were modelled by the predictor variables assumed to be relevant for introgression (Table 1). We found that phylogenetic group *Phyl* and upriver migration obstacles *Obstacle* could be associated with susceptibility for introgression, after the expected *Incidence index* had been accounted for (Table 4; 123 populations used to fit the model, 101 missing; $R^2_{Cal} = 0.16$, $R^2_{Val} = 0.08$). However, the proportion of variance explained was minor. The positive association between *Obstacle* and *P(Wild)*-residuals indicated that a large proportion of the anadromous section above first migration obstacle reduces the expected introgression. Populations from the BS phylogenetic group were expected to have positive residuals and more robust against introgression compared to the NEA group, while populations from the TZ had lower *P(Wild)* levels, i.e. more susceptible to introgression.

The fact that a river has status as a protected National salmon river or is discharging in a National salmon fjord (Marine Protected Area) did not influence the expected *P(Wild)* level of a wild salmon population beyond what could be attributed to protection-relevant predictors from the *Incidence index* model, primarily farming intensity and population size.

Discussion

This study demonstrates that genetic introgression is primarily determined by the proportions of escaped farmed salmon in rivers, and those proportions are primarily determined by farming intensity and wild population size. The main implication of these results is clear. There are currently no other sustainable mitigations than preventing farmed salmon from escaping or using sterile fish to stop further negative genetic impact on wild Atlantic salmon populations, given the present magnitude of farmed salmon production and high straying rate of escapees.

We analyzed several potential predictors (Table 1) that could modify the number and distribution of escaped farmed salmon and the introgression from escaped farmed to wild salmon. The effect of many predictors on the proportions of escapees and resulting introgression can only be identified by large data sets including many rivers and populations, and over a long period of time. Strengths of the present study are the large amount of data on proportion of escaped farmed salmon and the level of introgression in wild salmon populations as well as the large number of potential predictors that may be associated with introgression. These aspects allowed us to explore generic factors across a large geographical scale and over an extended period, which is essential to be able to establish robust guidelines to prevent further introgression of genetic material from escaped farmed salmon into wild populations.

Scale of analysis

A large spatial scale is necessary because of the wide distribution of fish farms and the far-reaching dispersal of farmed salmon after escapes. Escapees are found in major feeding areas near the Faroe Islands (Hansen *et al.*, 1999) and in the Arctic Ocean at Spitsbergen, more than 1000 km from the nearest fish farm (Jensen *et al.*, 2013). Recaptures of tagged farmed salmon released on the coast of Norway have been documented in rivers as far away as the Swedish west coast and the northern Kola Peninsula spanning a coastal distance of 3000 km (Hansen, 2006). Most escapees, however, end up in rivers in the same area as they escaped from, particularly if escaping as smolts or close to spawning time (Hansen, 2006; Skilbrei *et al.*, 2015; Jonsson and Jonsson, 2017).

Data sets covering a large temporal scale are necessary because introgression is a population property that represents a cumulative impact over time and is expected to vary among cohorts depending on escape episodes and stochastic environmental variation. The currently observed introgression is the result of more than three decades of spawning of escaped farmed salmon in rivers (Gausen and Moen, 1991). Thus, what we study here is the effect of introgression from escapees and their first- and later-generation offspring on a wide range of wild salmon populations. Salmon hatched in the wild are physically more fit and have a higher reproductive success than hatchery-produced salmon and farmed escapees (Jonsson *et al.*, 1990; Fleming *et al.*, 1996, 1997); hence, wild-born offspring of farmed escapees may disperse introgression beyond physical obstacles for farmed escapees, such as difficult-to-pass waterfalls. Furthermore, first-generation off-

spring of farmed salmon showed higher straying rates than native salmon when released as smolts in the river (Jonsson and Jonsson, 2017), and may, thus spread introgression to rivers where the proportion of direct farm escapees is very low.

Another temporal component to consider is the genetic change that takes place in the farmed salmon across generations. Farmed salmon are changing genetically over time because of selective breeding for economically important traits (Gjedrem and Baranski, 2009), because of the general process of domestication, i.e. adaptation to the captive environment, and genetic drift. One might argue that selective breeding and adaptation to the captive environment will eventually lead to farmed salmon being unable to complete a life cycle in the natural environment. Theoretical models suggest that the highest impact of escaped farmed individuals on the viability of wild salmon populations is at intermediate levels of genetic difference between them (Baskett and Waples, 2012; Huisman and Tufto, 2012). Despite the reduced fitness of farmed individuals in the wild, the most recent data suggest that escaped farmed salmon are still able to enter salmon rivers and successfully reproduce (Diserud *et al.*, 2020; Pulg *et al.*, 2021; Karlsson *et al.*, 2021).

We identified *a priori* a list of variables (Table 1) that might be important for determining the occurrence of escaped farmed salmon and level of introgression. For several reasons, not all of these variables were included in our final models. First, some were applicable to only a single or few rivers or populations and were, therefore, not suitable for modelling generic factors at the national scale but might be interesting to study in detail for a better understanding of underlying mechanisms. One example is seasonal environmental variation in rivers, such as long winters, that may affect juveniles of varying pedigree differentially as they grow older (Wacker *et al.*, 2021). Second, other variables were excluded due to limited data quality. One example is predation pressure on juvenile salmon, as predation is one mechanism by which offspring of escaped farmed salmon may show higher mortality than offspring of wild salmon (Solberg *et al.*, 2020), but which we cannot so far sufficiently quantify. Third, some variables are highly intercorrelated and could, thus be interchanged in the models without much change in the explanatory power of the models.

Predictors for proportions of escaped farmed salmon

We found that the *Incidence index* of escaped farmed salmon in rivers was associated with farming intensity as well as river and population specific features, with population size, water discharge, and the relative spawning target being the most important predictors (Table 2). This model explained 43% of the variance in the *Incidence index*. Farming intensity is associated with escapees during post-smolt to adult stage from ocean farms (Thorstad *et al.*, 2008). Norway's statistics on escapes from aquaculture, based on mandatory reporting of escape events and numbers by fish farmers (http://www.fiskeridirektoratet.no/Akvakultur/Tall-og-analyse/Roemmingsstatistikk), was not used as input in the models for escaped farmed salmon in rivers. There are at least two reasons for this. First, it was shown that for the years 1989–2004 the regional (county) number of farmed fish in net pens was a better predictor for escaped farmed salmon in rivers than the reported escapes in the same regions (Fiske *et al.*, 2006), a result later supported by Mahlum *et al.* (2021). Second, studies have shown that the reported number of escapees may be an underestimation of the actual number of escapees; Skilbrei *et al.* (2015) found the actual number of escapees to be two to four times larger than reported during the period 2005–2011. Underestimation of the reported numbers is supported by the fact that high numbers of farmed escapees can be found where no escape event has been reported (Quintela *et al.*, 2016), and furthermore, that DNA methods to trace the source of unreported escapees have been used by the Norwegian authorities on multiple occasions (Glover *et al.*, 2008; Glover, 2010). The Norwegian Directorate of Fisheries states on their home page that the escape statistics must be viewed as estimates and that numbers are uncertain even when based on counting fish left in the net after escape (http://www.fiskeridirektoratet.no/Akvakultur/Tall-og-analyse/Roemmingsstatistikk).

Estimates from the years 2010–2018 suggested that escapes from land-based facilities made up 7% and net pens 92% of the number of escapes in Norway (Føre and Thorvaldsen, 2021). Escapes from freshwater facilities may be more common in Scotland and Ireland, where more juveniles are reared to the smolt stage in net pens in lakes and where they have been shown to contribute to introgression (Clifford *et al.*, 1998a; Gilbey *et al.*, 2021), if not to the escape statistics.

The *Incidence index* of escaped farmed salmon increases with increasing average river discharge. This result was also found in an analysis of escaped farmed salmon in western Norway based on counts of escaped and farmed salmon in rivers (Mahlum *et al.*, 2021), and in reports with preliminary modelling of the all-of-Norway analyses presented here (Fiske *et al.*, 2013; Hindar *et al.*, 2018). Also, Johnsen and Jensen (1994) found when studying the spread of furunculosis from an outbreak in fish farms that the disease spread faster with escaped farmed salmon to large rivers than to nearby small rivers.

The main reason for the positive association with river discharge is likely that higher discharge is an increasingly stronger signal for escaped farmed salmon, which are essentially homeless when escaping from net pens in the sea (Hansen, 2006), although most end up in rivers in the same region they escaped from. Discharge is also positively correlated with wild salmon body size (Jonsson *et al.*, 1991) and late-escaping farmed females can be c. 40% bigger than co-occurring wild females (Hindar *et al.*, 2006). On the other hand, offspring of escaped farmed salmon have also been found in smaller rivers than those analyzed in the present study, including those primarily dominated by sea trout *Salmo trutta* (Pulg *et al.*, 2021).

Population size had a positive effect on the number of escapees ascending rivers (Supplement S3) and a negative effect on the proportion of escapees in the river (Table 2). Because population size may vary among years, the general effect on variation among rivers will only become apparent over many years. In western Norway, Mahlum *et al.* (2021) showed that wild salmon spawner abundance was an important predictor of escapee abundance and suggested that escaped farmed salmon, without a native river (Hansen, 2006), might follow wild migrants from the coast to the river. While this is possible, it cannot be the only explanation because some escaped farmed salmon may often enter rivers after the wild salmon run. More importantly, our model showed that population size has a "thinning effect" on the *Incidence index* of escaped

farmed salmon, i.e. the proportion decreases with increasing population size, and this should not be the case if escaped farmed salmon followed maturing wild salmon at random. Also, Hesthagen *et al.* (2011) showed that in formerly acidified rivers, salmon populations recovered more rapidly after liming in rivers with releases of juvenile salmon than in rivers with only natural colonization. The smell of salmon may, therefore, be an attractant (Jonsson *et al.*, 2003).

The relative spawning target enters as a factor in our model by increasing the expected *Incidence index* in rivers that have a high spawning target relative to neighbouring populations that may compete for the same pool of escapees in a fjord or a coastal region. Whereas escaped farmed salmon may be attracted to large rivers with abundant salmon populations, they may also seek a smaller population when there are no larger populations around, i.e. the relatively largest population in the region.

Predictors for level of introgression

We found that the level of introgression was strongly related to proportion of escaped farmed salmon in the rivers and that a model for contemporary logit(*P(Wild)*), where pre-introgression level and expected *Incidence index* were accounted for, explained 45% of the variance in introgression (Table 3). This means that long term introgression can be modelled from the small number of predictors.

Still, a considerable amount of the variation in introgression remains unexplained. We modelled the residuals from the logit(*P(Wild)*) model (Table 3) to see which predictors that could potentially shed light on the unexplained variation and found that phylogenetic group and upriver migration obstacles could be associated with susceptibility for introgression (Table 4). They were both significant but only accounted for 16% of the residual variance. Although potentially important for some rivers, these predictors may have a low influence on a large-scale model if they vary little for most of the populations.

The association between *Obstacle* and *P(Wild)*-residuals was positive, suggesting that wild salmon populations having to pass obstacles close to the river mouth are less susceptible to introgression. The behaviour of escaped farmed salmon within rivers differs from wild salmon both in spatial distribution and within-river migration (Moe *et al.*, 2016). Farmed escapees are known to accumulate below migration obstacles, likely because they lack a "stop signal" in the river that native salmon may recognize as a home area (Thorstad *et al.*, 1998). Obstacles in the rivers, such as waterfalls and fish ladders, appear to prevent escaped farmed salmon from entering the upper parts (Anon, 2020). Although obstacles in the present study are pragmatically defined as proportion of anadromous section above first migration obstacle encountered, an obstacle for an escaped farmed salmon is likely to be very different between farmed salmon that escaped early and have spent a long time at sea and newly escaped one. In the River Målselva, northern Norway, fewer escaped farmed salmon have been observed above compared to below a fish ladder, but this has not translated into a lower level of introgression in adult salmon in the upper part of the river (Svenning *et al.*, 2021). This result is somewhat different from Sylvester *et al.* (2018), who found that migratory challenges may restrict the introgression of escaped farmed salmon in upstream spawning sites and from Bradbury *et al.* (2020) who found that, in a model-based approach, waterfalls far down in the river could play a major role in observed introgression and numbers of escapees. The most likely explanation for this difference is that, even though the functional role of obstacles for the *Incidence index* of escaped farmed salmon seems similar in Norwegian and Newfoundland rivers, the longer history of introgression by farmed escapees in Norway results in accumulated introgression, which spreads into the whole population and to all spawning areas in the river.

The TZ between NEA and BS salmon in Norway is very sharp (Wennevik *et al.*, 2019; Diserud *et al.*, 2020). Wennevik *et al.* (2019) suggested that local environmental conditions in the TZ, with no obvious barriers to gene flow, are strong enough to maintain the genetic differentiation between them. If so, farmed salmon that originate from the NEA group (Karlsson *et al.*, 2016) should also be less successful in the BS group, which they are (Table 4). In contrast, phylogenetically admixed populations from the TZ seem more susceptible to introgression, after escapee incidence has been accounted for. Populations from the BS group have been demonstrated to migrate further east in the ocean than populations from the NEA group (Rikardsen *et al.*, 2021), and the two phylogenetic lineages could, thereby, differ in ecology (Kjærner-Semb *et al.*, 2016). This is supported by the finding that the marine life history changes more, or differently, with introgression in BS salmon than it does in NEA salmon (Bolstad *et al.*, 2017).

The higher susceptibility to introgression in the TZ is harder to explain. However, the small number of population samples in the TZ means that this result should be interpreted with caution.

Limits to analysis of predictors

The limited number of factors determining introgression at the broad national scale, modelled in this study, means that we might have missed factors that are important in limiting introgression in some specific rivers and populations. This possibility is supported by the fact that our models show poor predictive ability for the lower *P(wild)* levels and the higher escapee proportion observations (Figure 2).

Some of the unexplained variation might be found in haphazard combinations of river and population specific predictors, and the magnitude, timing, and type of escapees (Hamoutene *et al.*, 2018). A considerable, and variable, fraction of the escapees may be immature, affecting river migration behaviour, catchability, and reproduction. Factors like escapee acclimatization (time since escape), timing of spawning of wild salmon (Lura and Sægrov, 1991b), and spatial distribution of spawning grounds relative to migration obstacles may have to coincide to determine escaped farmed salmon spawning success. Aronsen *et al.* (2020) found that catches of escaped farmed salmon on the coast and in fjords came from several escape events over many years, and about half had one or more winter zones after escape. Madhun *et al.* (2017) showed, using fatty acid profiling and genetics, that escapees from multiple sources and ages entered a river in a single year. Some of the introgression may come from strayers from other rivers; Jonsson and Jonsson (2017) found that hybrids between wild and farmed salmon had a higher straying rate than pure wild salmon. In contrast, Skaala *et al.* (2019) found no difference in straying rate among offspring types. In addition to the rivers defined as salmon rivers, there are many small streams where spawning of salmon occasionally occur

and in some of these, escaped farmed salmon can be very successful and produce many offspring (Pulg *et al.*, 2021) that may stray to larger rivers.

The regression coefficients are expected to be underestimated due to measurement and sampling uncertainty (Carroll *et al.*, 2006), so better accuracy for presumed important variables is expected to improve model predictions. Some potential predictors were excluded from Table 1 due to limited data quality and will require more and improved collection efforts to become applicable, while other variables may be regarded as proxies for unmeasurable factors, and therefore, only partly describe the functional relationships. Populations that are excluded from the model calibration due to missing observations are on average much smaller than those included, so models may also be biased towards the situation in larger populations.

Marine protected areas

The protection of wild salmon populations in Norway was suggested in the Norwegian Official Report (NOU, 1999) to consist of general measures to protect the most important wild salmon populations combined with actions in all aspects of society that affected wild salmon negatively. The general measures were the establishment of 52 National Salmon Rivers (out of Norway's c. 450 salmon rivers) and 29 National Salmon Fjords by the Norwegian parliament in 2006 (Anon., 2006).

Karlsson *et al.* (2016) found that when all populations were given equal weight, average introgression levels were the same in populations within National Salmon Fjords as in rivers outside these protection areas. When averages were weighted with population size, the introgression level was almost doubled outside the protection areas. The effect of National Salmon Fjords on introgression, therefore, works through the major predictor variables listed in Table 1. The conclusion of a Norwegian report that evaluated National Salmon Rivers and National Salmon Fjords after 10 years (Hindar *et al.*, 2018) was that the protective measures taken by the Norwegian parliament could delay the negative effects of escaped farmed salmon on wild populations but not prevent them.

In the present model, farming intensity was an important predictor for proportion of escaped farmed salmon in the rivers. We found only minor differences in the distribution of farming intensity between salmon rivers inside and outside National Salmon Fjords (Figure 3b). This is not surprising as the 29 National Salmon Fjords vary in area from 16 to 1526 km^2 (Serra-Llinares *et al.*, 2014). In conclusion, we believe that in order to further delay introgression into wild salmon populations, many protected areas should be increased in size, such that they could sufficiently reduce the number of escaped farmed salmon in rivers in these areas.

Supplementary data

Supplementary material is available at the *ICESJMS* online version of the manuscript.

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Data availability statement

No new data were collected in the course of this study. The data underlying this article can be accessed from sources in the public domain. The proportions of escaped farmed salmon in wild salmon populations (*Incidence index*) are described in Diserud *et al.* (2019), Glover *et al.* (2019), and in annual reports from the Institute of Marine Research on escaped farmed salmon in rivers (Reports | Institute of Marine Research (https://www.hi.no/en/hi/nettrapporter)). Data on genetic introgression are described in Karlsson *et al.* (2016) and Diserud *et al.* (2020). Data on salmon aquaculture locations and production are courtesy of the Norwegian Directorate of Fisheries. Additional data references can be found in Table 1. The data may be shared on reasonable request to the corresponding author, with permission of the Norwegian Directorate of Fisheries.

Authors' contributions

OHD, KH, PF, SK, and KAG conceived the ideas and designed the methodology and analyses. All co-authors participated to the data collection. OHD, PF, SK, and KH analyzed the data. OHD, KH, PF, and SK drafted the manuscript. OHD, KH, PF, SK, KAG, and TFN revised the manuscript with additional contributions from the remaining authors. This manuscript is submitted with the approval of all the authors.

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Handling Editor: William Grant

Status of wild Atlantic salmon in Norway 2023

Photo: Eva B. Thorstad

Norwegian Scientific Advisory Committee for Atlantic Salmon

The status of Norwegian wild Atlantic salmon is evaluated annually by the Norwegian Scientific Advisory Committee for Atlantic Salmon. This is an English summary of the 2023 report.

The committee is appointed by the Norwegian Environment Agency to evaluate status of salmon and importance of different threats, and to give science-based catch advice and advice on other issues related to wild salmon management.

Thirteen scientists from seven institutions serve on the committee: Torbjørn Forseth (leader), Sigurd Einum, Peder Fiske, Morten Falkegård, Øyvind A. Garmo, Åse Helen Garseth, Helge Skoglund, Monica F. Solberg, Eva B. Thorstad, Kjell Rong Utne, Asbjørn Vøllestad, Knut Wiik Vollset and Vidar Wennevik. The committee is an independent body, and the members do not represent the institutions where they are employed when serving on the committee.

Contact: Torbjørn Forseth (torbjorn.forseth@nina.no), Eva B. Thorstad (eva.thorstad@nina.no), Peder Fiske (peder.fiske@nina.no), or any other member of the committe. www.vitenskapsradet.no

Status of Atlantic salmon - short summary

Both the number of Atlantic salmon returning from the ocean to Norway for spawning, and the Atlantic salmon catches were among the lowest ever recorded in 2022 (based on a time series starting in 1980), but slightly higher than the record-low in 2021. The number of salmon returning from the ocean to Norway each year is now less than half of the level recorded in the 1980s. Still, the number of salmon spawning in the rivers has increased. The increased number of spawners despite reduced numbers returning from the ocean is due to reduced fisheries in the sea and rivers.

The reasons for the decline of Atlantic salmon are impacts of human activities in combination with a large-scale decline in the sea survival. The largest population declines are seen in western and middle Norway, and negative impacts of salmon farming have contributed to this. Salmon lice, escaped farmed salmon, and infections related to salmon farming are the greatest anthropogenic threats to Norwegian wild salmon. The present mitigation measures are insufficient to stabilize and reduce these threats. The knowledge on infections related to salmon farming is poor.

Hydropower production and other habitat alterations are also threats to salmon. There is an underexploited potential for improving conditions for salmon in regulated rivers. Invasive pink salmon is a new threat. In 2023, traps are installed in many rivers in Northern Norway, to hinder pink salmon in entering the rivers, but there is lack of knowledge on the effects of pink salmon on native salmonids, and on the efficiency of the implemented measures.

Climate change impacts Atlantic salmon populations negatively. Climate change increases the need to reduce the impacts of other threats to support the ability of Atlantic salmon to adapt to changing environments.

The 2023 annual report is published in Norwegian: https://brage.nina.no/nina-xmlui/handle/11250/3074251

Spilderelva. Photo: Eva B. Thorstad

Extended summary

Major threats to Norwegian wild salmon

The committee has developed a classification system to rank different anthropogenic impacts to Norwegian Atlantic salmon (**figure 1**, Forseth et al. 2017). Assessments according to this system are updated annually by the committee.

Salmon farming

Salmon lice and escaped farmed salmon were identified as the largest threats to wild salmon (**figure 1**), to a large extent impacting wild populations negatively. Salmon lice and escaped farmed salmon are regarded as expanding population threats, which means they affect populations to the extent that populations may be critically endangered or lost in nature, and that there is a high likelihood they will cause even further reductions. Current mitigation measures are insufficient to hinder expansion of negative impacts in the future.

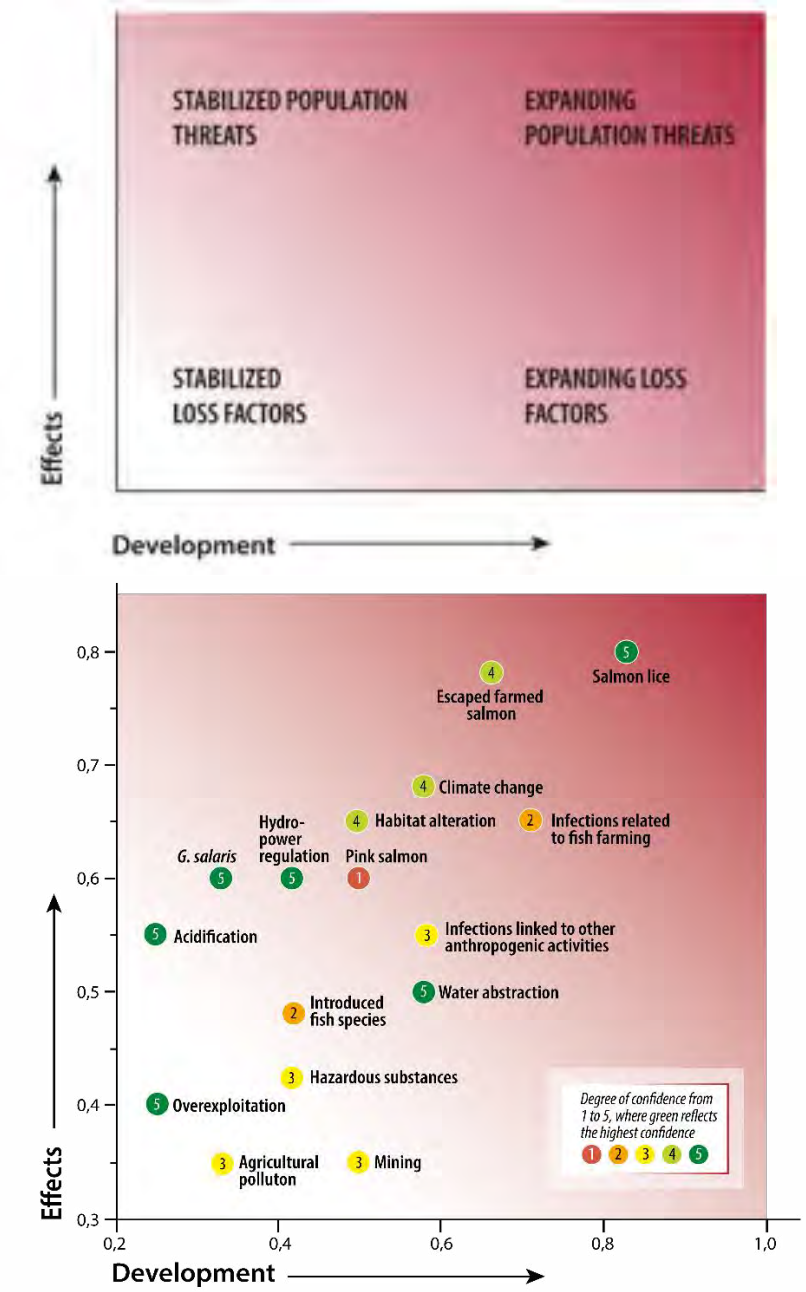

Figure 1.

Upper graph: The classification system developed to rank different anthropogenic impacts to Norwegian Atlantic salmon populations along the effect and development axes. *The four major impact categories are indicated, but the system is continuous. Dark background colour indicates the most severe impacts. The effect axis describes the effect of each impact factor on the populations, and ranges from factors that cause loss in adult returns, to factors that cause such a high loss that they threaten population viability and genetic integrity. The development axis describes the likelihood for further reductions in population size or loss of additional populations in the future.*

Lower graph: Ranking of 16 impact factors considered in 2022, according to their effects on wild Atlantic salmon populations, and the likelihood of a further negative development. *Confidence for the assessment of effect by each threat is indicated by the color of the markers, where green indicates the highest confidence level and red the lowest.*

Salmon lice have the greatest impact on Norwegian wild salmon, and by far the greatest risk of causing further losses in the future. The number of salmon returning to the rivers each year is reduced due to post-smolt mortality caused by salmon lice. This reduction threatens salmon populations in the most impacted areas and has significantly reduced the harvestable surplus for river and marine fisheries over large parts of the country. The impact of salmon lice is most severe in western and middle Norway. The areas severely impacted have increased during the last five years. Many wild salmon populations in these areas have been heavily impacted by salmon lice for many years and are now in a very poor state. Several threats impact these populations, including escaped farmed salmon, but heavy salmon lice burdens are likely the reason that they are not able to recover. Sufficient mitigation measures to improve the situation are not implemented, and the production of farmed salmon is increasing.

According to reports from fish farmers, 56 000 salmon escaped from aquaculture farms in 2021. The actual number is uncertain, but higher than the reported numbers. Due to a reduced occurrence of escaped farmed salmon recorded in rivers, the threat is adjusted slightly down compared to previous years. There is widespread genetic introgression of escaped farmed salmon in Norwegian wild salmon. In two thirds of the screened rivers, there were indications of genetic introgression from escaped farmed salmon in the wild population (150 of 239 rivers), of which 68 populations were severely impacted (28% of the screened populations). The scientific evidence that incidence of escaped farmed salmon will negatively affect Norwegian wild salmon, both ecologically and genetically, is strengthened during recent years. In addition to changing the populations genetically, hybridization between wild and escaped farmed salmon is also shown to reduce salmon production and survival.

Infections related to fish farming were also identified as a threat that can significantly impact salmon, and with a large likelihood of causing further reductions and losses in the future. However, knowledge of the impacts of infections related to fish farming is poor, and the uncertainty of the projected development of this impact factor is high. More knowledge on this impact factor is needed. There is a risk that this threat is underestimated due to lack of knowledge.

Hydropower production and other habitat alterations

Hydropower production and other habitat alterations, together with climate change and pink salmon, were also identified as threats to wild salmon, but with a lower risk of causing further loss of wild salmon in the future than the threats related to salmon farming (**figure 1**). Hydropower production and other habitat alterations have reduced many salmon populations and caused the loss of salmon in some rivers. The potential for more extensive mitigation measures related to hydropower production and other habitat alterations is large.

Climate change

Climate change is a global threat, which is already impacting salmon populations, and will impact salmon populations to a great extent in the future. Climate change impacts Atlantic salmon at all life stages, through changes in water temperature, precipitation, water quality and other environmental factors. Climate change amplifies the negative effects of other threats to Atlantic salmon populations. Threats like escaped farmed salmon, salmon lice, other infections related to salmon farming, habitat alterations, negative impacts of invasive species, pollution and others become even larger when occurring in a changing climate. This is also the case for river regulation for hydropower production, but such regulation can also in some cases be adapted to help reducing the impacts of climate change. Climate change is a threat that increases the importance of having large and genetically variable populations to enable them to meet the rapid changes in the best possible way. Hence, it is important to protect and preserve the size and genetic variation and integrity of salmon populations, and thereby the abilities of populations to adapt to new and

changing conditions. Climate change increases the needs to reduce the impacts of other threats to Atlantic salmon.

Invasive pink salmon

Pink salmon is a new threat, and the occurrence of invasive pink salmon in Norwegian rivers increased significantly in 2017, 2019 and 2021 compared to earlier years. Pink salmon were recorded in 271 rivers, and 205 000 pink salmon were caught in rivers and coastal fisheries in 2021. The highest abundance of pink salmon was recorded in Northern Norway. In 2023, traps are installed in more than 30 rivers in the most affected areas to remove pink salmon and reduce the negative impacts on native salmonids. The knowledge on the impacts on native salmonids and the effect of the mitigation measures is limited, because the area with high abundance of pink salmon may increase faster than the implementation of measures. It should be noted that this risk assessment was performed before the return of pink salmon during the 2023 season.

The invasive parasite Gyrodactylus salaris

The threat to wild salmon from the introduced parasite *Gyrodactylus salaris* is greatly reduced, because successful eradication programs have strongly reduced the number of rivers infected with the parasite, and the salmon populations have been re-established from live gene banks. Number of rivers with known occurrence of the parasite has been reduced from fifty-one to eight, due to the eradication measures. Measures are ongoing in four of the remaining eight infected rivers.

Acid rain

Due to large-scale liming of rivers and reduced emissions, the risk of increased negative impacts due to acid rain is low. Salmon populations in southern Norway have increased due to the comprehensive liming programs.

Overfishing and other impacts

Overfishing and other impacts were identified as less influential, either as stabilized or expanding factors that cause loss in terms of number of returning adults, but not to the extent that populations become threatened. Overexploitation is defined as a reduction in number of spawning females in a population to levels below the spawning target due to fishing in rivers and at sea. This means that if fisheries in rivers and the sea harvest more than the harvestable surplus of a population, and fishing is the reason for a reduced smolt output from a river, this is regarded as overexploitation.

Overexploitation is no longer regarded an important impact factor. Management based on population specific reference points (conservation limits) from 2009 has reduced exploitation in rivers and at sea. Harvest of populations with low or no harvestable surplus has been strongly reduced or closed, and salmon fishing was closed in 183 rivers in 2022.

Predation

This threat assessment covers the threats from human activities, and predation is not regarded as an anthropogenic threat *per se*. However, several human activities may lead to elevated predation at life stages where this may reduce salmon populations. Examples may be the introduction of northern pike *Esox lucius* to new watercourses, slower smolt migration in combination with improved habitats for predators in hydropower reservoirs, and elevated predation of post-smolts at sea because they are weakened due to salmon lice or freshwater acidification. This type of predation is assessed under the different human activities that are the ultimate case for the elevated mortality.

A salmon population that is reduced to very low levels due to human activities can be much more difficult to rebuild that it was to reduce it, because of predation mechanisms. This is covered in a new publication (Falkegård et al. 2023). In this publication, we conclude that there is little evidence that predation alone has been an underlying mechanism for driving salmon populations

below conservation limits. However, depending on the predator's response to salmon abundance, predation may keep decimated populations from recovering, even when the actual causes of decline have been removed.

Catches and pre-fishery abundance

In 2023, the total reported catch in sea and river fisheries was 109 000 Atlantic salmon, equaling 389 metric tons. In addition, 27 000 salmon (124 metric tons) were reported caught and released (24% of the river catches).

The number of wild Atlantic salmon returning from the ocean to Norway each year (pre-fishery abundance) is significantly reduced since the 1980s (**figure 2**). The pre-fishery abundance was more than halved from 1983-1986 to 2019-2022. The pre-fishery abundance was estimated at about 458 000 wild salmon in 2022. Both the pre-fishery abundance and catches were among the lowest ever recorded in 2022 (based on the time series starting in 1980), but higher than the year before.

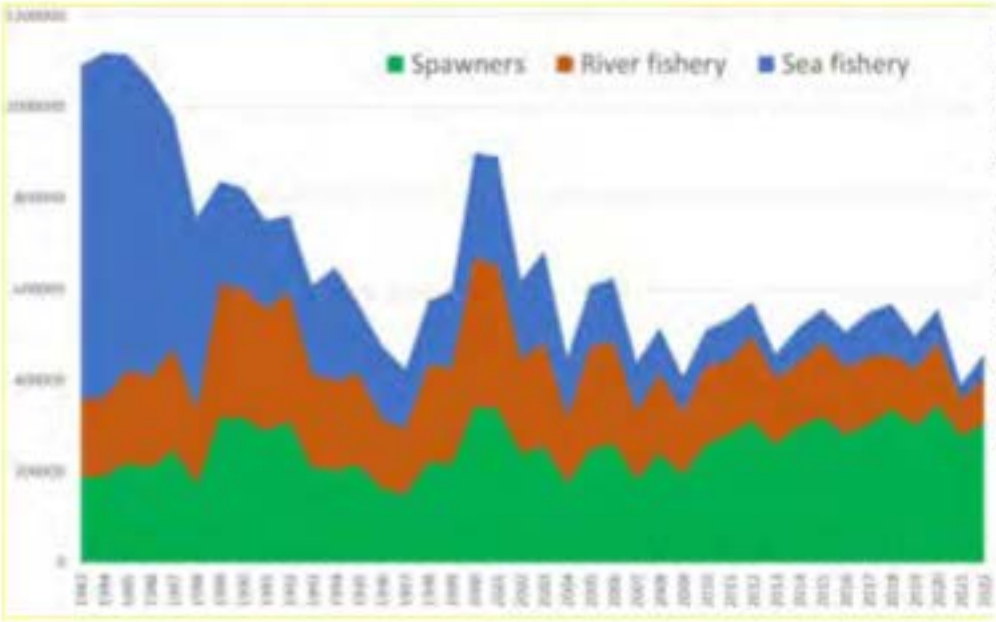

Figure 2. *Estimated number of wild salmon returning from the ocean towards Norwegian rivers each year, divided in number of fish caught in the sea fisheries, number of fish caught in the rivers during angling, and the number of fish left for spawning in the rivers during the period 1983-2022.*

The overall decline is mainly due to a decline of small salmon (body mass < 3 kg). The pre-fishery abundance of small salmon has declined from high levels in the mid-1980s and remained at a low level during the last years, except a temporal increase around year 2000. For Norway as a whole, the abundance of larger salmon (body mass > 3 kg) has not changed after the late 1980s, but there were more large salmon during the mid-1980s.

The temporal changes in numbers of salmon returning from the ocean each year differ among regions. Since 1989, when the offshore drift net fishery was banned, the abundance including all size classes has declined in middle and western Norway, and slightly increased in southern and northern Norway (when the Tana watercourse is excluded). The abundance of small salmon has declined in all parts of the country (compared to the period 1989-1993), but to the greatest extent in middle and western Norway. The pre-fishery abundance of salmon larger than 3 kg has decreased in middle Norway but increased in the rest of the country.

The large Tana watercourse has had a marked decline in the pre-fishery abundance, in contrast to the rest of Northern Norway, with a 75% reduction in the pre-fishery abundance since 1989. Both

small and large salmon have been reduced. This watercourse is shared between Norway and Finland, and overexploitation is the only known human impact factor.

Marine survival

Monitoring in the River Imsa shows that the marine survival of Atlantic salmon has been low during the last 20-25 years compared to in the 1970s and 1980s, in agreement with data from other international monitoring rivers. The smolts leaving the river during 2006-2008 had a particularly low survival. The marine survival of the smolts that left the river after 2008 increased compared to these poorest years, but remained relatively low, with a survival of only 1-4% for salmon that left the River Imsa during 2009-2020. However, for the salmon that left in 2021, the survival increased (7% until return as one-sea-winter fish in 2022) and this was the highest survival recorded in more than 20 years. Knowledge of variation in sea survival for salmon from different regions has been poor. Efforts to map sea survival are increasing by the establishment of new monitoring rivers, and so far, results show that sea survival vary significantly among rivers and years.

Attainment of spawning targets

Attainment of spawning targets (conservation limits) and exploitation were evaluated for 244 salmon rivers for the period 2019-2022. The management target of a population is attained when the average probability of reaching the spawning target over a four-year period is 75% or higher. The scientific foundation for management according to spawning targets and management targets for Norwegian rivers is described by Forseth et al. (2013). For each river, the harvestable surplus was also estimated - as the pre-fishery female abundance minus the spawning target - expressed as percentage of the spawning targets.

The management targets for the period 2019-2022 were attained, or likely attained, for 91% of the populations (**figure 3**). This is among the best results regarding attainment of the management targets since the first evaluation was done in 2009 (**figure 3**). The number and proportion of populations reaching their management targets have increased markedly from 2006-2009 to 2019-2022 (**figure 3**). The increase in proportion of populations reaching their spawning targets is largely due to stricter regulations of fisheries causing reduced exploitation rates.

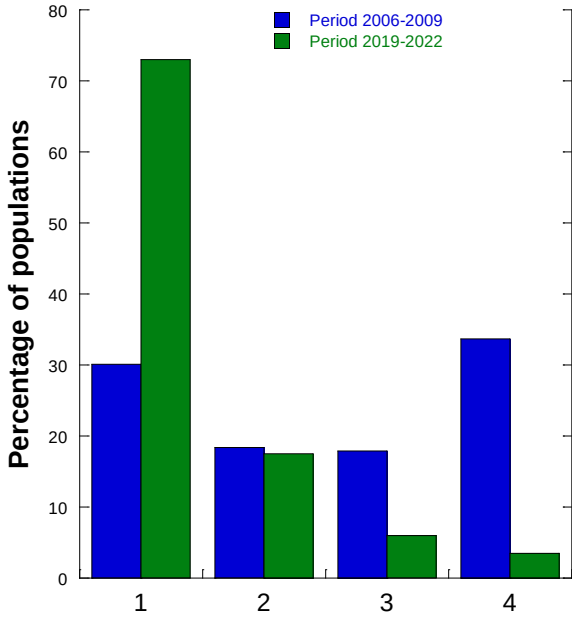

***Figure 3**. Proportion (%) of the evaluated salmon rivers in category 1: the management target is attained, category 2: there is a risk that the management target is not attained, category 3: the management target is likely not attained, and category 4: the management target is far from being attained. Data are given for the periods 2006-2009 (first assessment) and 2019-2022.*

Exploitation

An important principle in Norwegian legislation, which forms the basis for salmon management, is that both conservation and harvestable surplus of salmon should be ensured. The aim of the Salmon and Freshwater Fish Act is to ensure that populations and their habitats are managed such that diversity and productivity are conserved. Further, populations should be managed to ensure increased yields, to the benefit of fisheries stakeholders and recreational fishers. Similar principles are embedded in the Nature Diversity Act.

Annual nominal catches in the sea and rivers have been reduced from about 1500 metric tons during the early 1980s to 500-600 metric tons during recent years. In 1983-1988, more than 60% of the salmon returning from the ocean to the Norwegian coast (pre-fishery abundance) were caught in the sea (**figure 4**). When the drift net fishery was banned from 1989, the exploitation was reduced. The sea fisheries have been further reduced after the 1990s. In 2022, 12% of the salmon returning to the coast were caught in the sea.

The proportion of the salmon returning from the ocean each year that are caught in the rivers has been reduced from 2011. In 2022, 22% of the returning salmon were caught in the rivers. Of those salmon entering the rivers (after marine exploitation), exploitation has been markedly reduced from 1983-1988 to 2022 (**figure 4**). On average, 47% of the salmon entering the rivers were killed in fisheries until 2005, whereas in 2022, 25% were killed. However, exploitation rates vary among rivers, and many rivers now have very low exploitation rates, and the fishing has been closed in many rivers due to reduced populations.

Reduced exploitation has resulted in an increased number of salmon spawning in the rivers during the last years (**figure 2**). The proportion of salmon that were not killed in fisheries but allowed to become a part of the spawning populations, was less than 20% when the drift net fisheries took place (1983-88). This proportion increased to more than 30% during 1989-99, to around 60% from 2018 and onwards, and 67% in 2021.

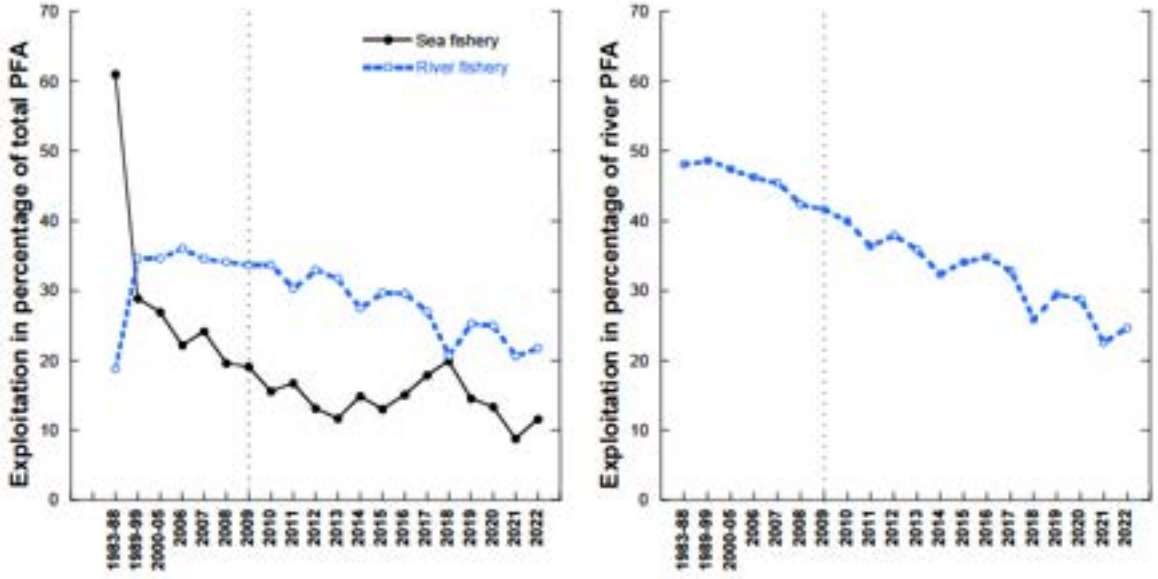

Figure 4. *Left graph: Exploitation of salmon given as percentage of the pre-fishery abundance (Total PFA, in numbers) for the periods 1983-88, 1989-99 and 2000-05 (averages) and thereafter as annual values. Right graph: Exploitation of salmon in the rivers given as the proportion of salmon entering the rivers (those left after exploitation in sea fisheries, River PFA) for the same periods and years. Hatched vertical line indicates the year when management based on spawning targets was introduced.*

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A Global Assessment of Salmon Aquaculture Impacts on Wild Salmonids

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Since the late 1980s, wild salmon catch and abundance have declined dramatically in the North Atlantic and in much of the northeastern Pacific south of Alaska. In these areas, there has been a concomitant increase in the production of farmed salmon. Previous studies have shown negative impacts on wild salmonids, but these results have been difficult to translate into predictions of change in wild population survival and abundance. We compared marine survival of salmonids in areas with salmon farming to adjacent areas without farms in Scotland, Ireland, Atlantic Canada, and Pacific Canada to estimate changes in marine survival concurrent with the growth of salmon aquaculture. Through a meta-analysis of existing data, we show a reduction in survival or abundance of Atlantic salmon; sea trout; and pink, chum, and coho salmon in association with increased production of farmed salmon. In many cases, these reductions in survival or abundance are greater than 50%. Meta-analytic estimates of the mean effect are significant and negative, suggesting that salmon farming has reduced survival of wild salmon and trout in many populations and countries.

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Introduction

Since the late 1970s, salmon aquaculture has grown into a global industry, producing over 1 million tonnes of salmon per year [1]. The majority of this biomass is held in open net pens in coastal areas through which wild salmon migrate on their way to and from the ocean. A number of studies have predicted or evaluated the impacts of salmon farming on wild salmon through a single mechanism, in a given area. It is clear that some salmonids are infected and killed by sea lice originating from salmon farms [2–5], that other diseases have been spread to wild populations from salmonid farming activities [6,7], and there is evidence that salmon parr are at lower density in areas of Scotland where there is salmon aquaculture [8]. In addition, farmed salmon escape in all areas where salmon aquaculture is practiced, and although their breeding success may be low on average, competition for mates and hybridization with wild salmon are likely to reduce survival of wild populations [9,10].

It is well established that wild salmonids can be negatively affected by salmon farming [11], however, the importance of these interactions at the population level has rarely been determined [2]. To determine population level impacts, we examined temporal trends in the abundance and survival of wild salmonids (Figure 1 and Figure S1). Our study contrasted trends in wild populations exposed to potential aquaculture impacts with those of populations not exposed. Populations in which juvenile salmonids pass by salmon farms during their migration were considered to be exposed to impacts of salmon farming. Exposed populations were carefully paired with control populations in the same region whose migrations did not lead past farms, but which otherwise experienced similar climate and anthropogenic disturbances. Use of such paired comparisons allowed us to control for confounding factors such as climate to detect population level impacts. Using the Ricker stock recruit model [12], we performed 11 comparisons, involving many stocks from both sides of the Atlantic and from British Columbia in the Pacific (Table 1, Data section of Materials and Methods).

Results

All estimates of the effect of aquaculture on survival or returns were negative. Both random effects estimates of the mean effect were negative and highly significant (Figure 2), indicating a very large reduction in survival and returns in populations exposed to aquaculture. Under the dynamics of Equation 1 (see Materials and Methods), percent change in survival or returns is represented by $(1 - \exp(\bar{\gamma}_k) * P^{1/2} * 100)$ where γ is the coefficient of aquaculture production (P) for region k. For example, the estimated change in survival per tonne of salmon farming (γ_k) for Bay d'Espoir in Newfoundland was estimated to be 0.026 (Figure 2). In 2003, the farmed salmon harvest from this area was 1,450 tonnes (t), so the estimated decrease in survival is $(1 - \exp(-0.026 * 1450^{1/2})) * 100 = 63\%$ (95% CI: 44%–80%), relative to what it would be in the absence of farms. Survival and total returns of many stocks were found to be reduced by more than 50% (Figure 2), for each generation. If all exposed populations were passing by farms with a total annual harvest of 15,000 t, the mean estimated total reduction in survival would be 73% (95% CI: 29%–90%) (Figure 2). Many regions now have farmed salmon production in excess of 20,000 t/y.

Generally, Atlantic salmon populations were depressed more than Pacific salmon populations, particularly Atlantic

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Abbreviations: AIC, Akaike information criteria; BC, British Columbia; DFO, Fisheries and Oceans Canada; SA, statistical area

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Author Summary

The impact of salmon farming on wild salmon and trout is a hotly debated issue in all countries where salmon farms and wild salmon coexist. Studies have clearly shown that escaped farm salmon breed with wild populations to the detriment of the wild stocks, and that diseases and parasites are passed from farm to wild salmon. An understanding of the importance of these impacts at the population level, however, has been lacking. In this study, we used existing data on salmon populations to compare survival of salmon and trout that swim past salmon farms early in their life cycle with the survival of nearby populations that are not exposed to salmon farms. We have detected a significant decline in survival of populations that are exposed to salmon farms, correlated with the increase in farmed salmon production in five regions. Combining the regional estimates statistically, we find a reduction in survival or abundance of wild populations of more than 50% per generation on average, associated with salmon farming. Many of the salmon populations we investigated are at dramatically reduced abundance, and reducing threats to them is necessary for their survival. Reducing impacts of salmon farming on wild salmon should be a high priority.

salmon in Atlantic Canada. Irish sea trout were also estimated to have been very strongly reduced by impacts of salmon farming, whereas estimated impacts on Atlantic salmon in Scotland depended on the data used. In British Columbia (Pacific Canada), only pink salmon showed significant declines correlated with salmon aquaculture.

Results are reported for a model including autocorrelated errors and with λ set at 0.5, rather than 1 or 2, because this minimized the Akaike information criteria (AIC) for most regions [13]. The parameter λ allows for the impacts of salmon farming to change nonlinearly with the aquaculture production. A λ of 0.5 indicates that relatively small amounts of aquaculture will depress wild populations, but the effect does not increase proportionally to aquaculture production. See Tables S1 and S2 for results of alternative models.

For the New Brunswick comparison, the outer Bay of Fundy rivers are located much closer to salmon farms than the other exposed rivers. If only these outer Bay of Fundy rivers are considered exposed to salmon farming, and other Bay of Fundy rivers (inner Bay of Fundy and Saint John River) are included among the controls, the overall estimates (i.e., meta analytic means) are still significant and negative in both versions of the analysis.

Discussion

We have estimated a significant increase in mortality of wild salmonids exposed to salmon farming across many regions. However, estimates for individual regions are dependent on assumptions detailed in the Materials and

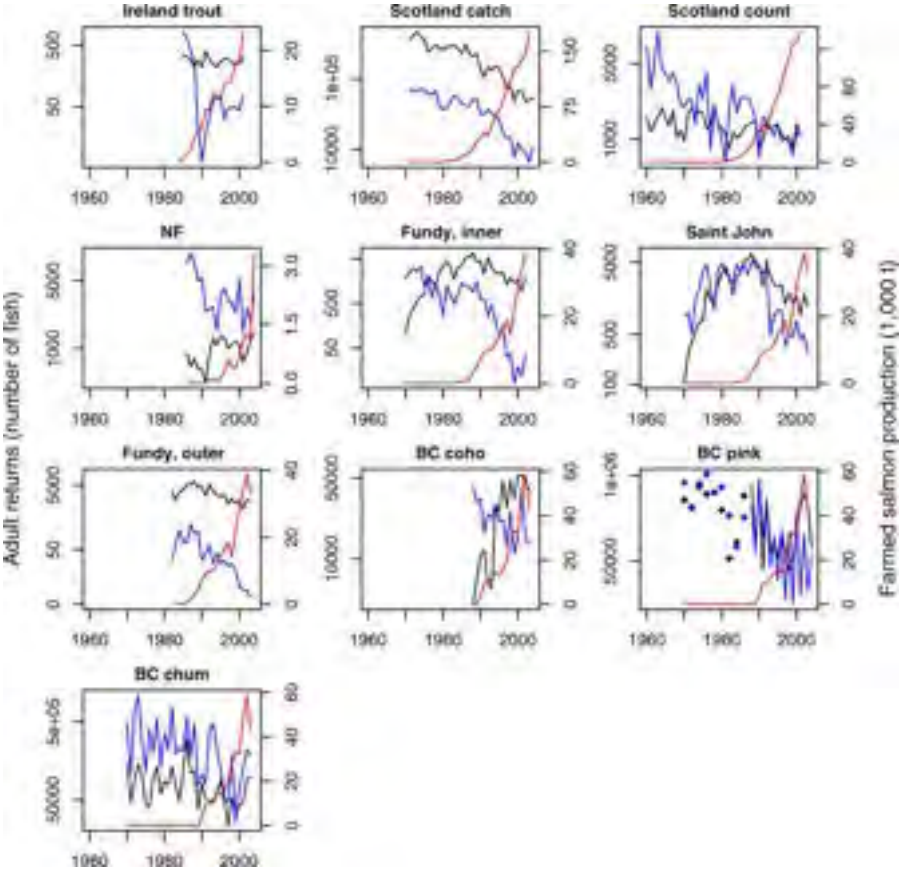

Figure 1. Adult Returns of Wild Salmonids in Control (Black) and Exposed (Blue) Stocks, with Aquaculture Production (Red)

For plotting only, the returns to controls and exposed stocks have been separately summarized by a multiplicative model ($\log(Returns_{i,y}) \quad a_i + d_y + e_{i,y}$; variables are the same as in Equation 1). The mean returns across stocks for each year are shown. Note that left hand axes are on a log scale. Only even year values are available for pink salmon prior to 1989. Irish salmon are not included because only marine survivals (not returns) are available.

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Table 1. Summary of Populations Included

ID	Species	Country	Exposed		Control		Type[a]	Reference
			Region	n[b]	Region	n[b]		
1	Sea trout	Ireland/UK	Ireland (Western Region)[c]	16	Wales	32	C	[26,16,17]
2	Atlantic salmon	Scotland	West Coast[c]	1	East Coast	1	C	[e]
3		Scotland	West Coast[c]	2	East Coast	10	T	[29]
4		Ireland	Western Region[d]	4	Rest of Ireland	9	T,S	[28]
5		Canada	Bay d'Espoir[d]	1	Rest of Newfoundland	4	T	[31]
		Canada	Bay d'Espoir[c]	1	Rest of Newfoundland	21	T,S	[31]
6		Canada	Fundy, Inner	2	Gulf of St Lawrence, Atlantic Coast	4	T,S	[28,35,36,39]
7		Canada	St John River	2	Gulf of St Lawrence, Atlantic Coast	4	T,S	[28,37,39,40]
8		Canada	Fundy, Outer	2	Gulf of St Lawrence, Atlantic Coast	4	T,S	[28,37,39,40]
9	Coho salmon	Canada	Johnstone Strait	2	BC Central Coast	4	S	[f]
10	Pink salmon	Canada	Johnstone Strait	2	BC Central Coast	4	S	[f]
11	Chum salmon	Canada	Johnstone Strait	2	BC Central Coast	4	S	49

[a] Type C refers to catches, T refers to scientific traps, and S refers to other scientific surveys.
[b] n is the number of populations; i.e., rivers, or SAs in BC.
[c] Used in returns analysis only.
[d] Used in survival analysis only.
[e] J. MacLean, FRS Scotland, unpublished data.
[f] NuSEDS database, DFO Pacific, unpublished data.
doi:10.1371/journal.pbio.0060033.t001

Methods section, and the estimates often have large confidence intervals. Given that the data analysed are affected by considerable noise including changes in fishing and environmental factors the important result of this study is that we are nonetheless able to detect a large, statistically significant effect correlated with trends in farmed salmon production. The significant increase in mortality related to salmon farming that we have estimated in almost all cases is in addition to mortality that is also acting on the control populations. In most cases, control populations were also experiencing decreases in marine (and sometimes freshwater) survival, for reasons that are only partially understood. At the same time, fishing mortality has been reduced or eliminated in many areas, which may have partially masked high mortalities associated with aquaculture.

A key assumption in this study is that exposed and control areas do not differ in a systematic way across regions. We have identified three possible ways that exposed and control sites could differ systematically: first, salmon farms could be established only in areas where wild stocks have already collapsed; second, salmon farms could be established in areas where habitat is more disturbed by human activities; or, third, climate factors could differ between the exposed areas and the controls in a systematic way.

Declines in control and exposed salmonid populations preceded the growth of the salmon aquaculture industry in some regions, but inspection of the data used do not indicate that salmon populations in the majority of our regions had declined dramatically in the exposed areas only, before the start of salmon farming (averaged returns data are shown in Figure 1). In regions such as Scotland, where declines precede the start of salmon farming, the strong aquaculture effect estimated reflects a faster decline in exposed populations concurrent with the growth of salmon farming.

Areas that we consider exposed do not seem to be more developed than control areas in general. In the Atlantic, most areas have been highly altered by human activities for hundreds of years, but there is no obvious difference between the control and exposed groups in this regard. In British Columbia, all areas considered are very remote, and the main type of anthropogenic disturbance in rivers would be forestry. Comprehensive forestry records at the watershed scale are not easily available, but logging in British Columbia's Central Coast is extensive, both historically and recently [14]. It should be noted that the comparisons in British Columbia include large numbers of rivers (> 80 rivers in each case), so differences in anthropogenic effects would have to hold over many watersheds to explain the effects we estimate.

Finally, it is also very unlikely that our results are due to a climate driven trend in which more southerly populations show stronger declines than populations to the north. Although our exposed populations are to the south of control populations in three of five regions, differences in latitude are small. In New Brunswick, the control populations are to the north of the exposed populations, but by less than 200 km, and the headwaters of some of the exposed populations are adjacent to those of the controls. In Newfoundland, the difference in latitude between exposed and control populations is similarly small. In British Columbia, the control populations are also to the north, but by less than 300 km. Also, Mueter et al. [15] found that pink and coho salmon from all of the British Columbia populations we have examined respond similarly to large scale climate trends. Thus, the pattern we found in this study does not seem attributable to a systemic difference between the control and exposed areas.

We estimated higher impacts on populations in the Atlantic than those in British Columbia, possibly because Atlantic salmon populations are conspecific with farmed salmon, and therefore susceptible to genetic effects from

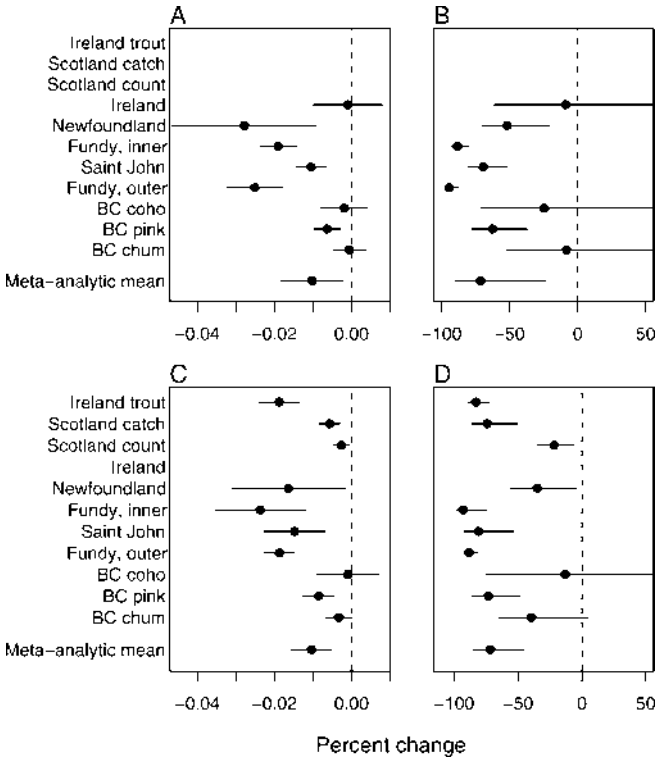

Figure 2. Estimated Effects of Salmon Farming

All estimates are for Atlantic salmon unless otherwise noted.
(A) Estimated percent change in survival of wild salmonids associated with salmon farming, per generation per tonne of farmed salmon production.
(B) Estimated percent change in survival of wild salmonids associated with salmon farming, per generation, at the mean tonnage of farmed salmon harvested in each region, during the study period. The meta analytic mean has been scaled to show mean reduction in survival when harvest of farmed salmon in the region is 15,000 t.
(C and D) As for (A) and (B), but representing the change in returns to each stock (rather than survival). The bars represent 95% confidence intervals.
doi:10.1371/journal.pbio.0060033.g002

interbreeding with escaped farm salmon, in addition to disease or other impacts. Estimated impacts in British Columbia may also be lower because we aggregated over large numbers of populations for pink, chum, and coho salmon, because estimates of fishing mortality were only available at a very coarse scale. The individual populations may vary in their exposure to salmon farms.

The large apparent impact of Atlantic salmon farming on Irish sea trout, in contrast, can not be explained by interbreeding. In the mid western region of Ireland (the exposed region), the total rod catch decreased from almost 19,000 sea trout in 1985 to 461 in 1990 [16]. In the few rivers where data were available, catch declines could not be explained by reduced effort [16]. Welsh sea trout catches (the controls) have remained relatively constant during the same time period, whereas fishing effort has decreased considerably [17]. Sea trout (anadromous brown trout) might be expected to experience higher mortalities, because they spend lengthy periods in coastal areas near salmon farms, relative to Atlantic salmon, thus being exposed to disease or parasites for a longer time [18].

The time period over which we are estimating impacts of aquaculture includes the establishment of the industry in each region. Improvements in management as industries mature may explain our finding that impacts of salmon farming on wild salmon do not increase linearly with the tonnage of farmed salmon. Better management should decrease the impact of salmon farming on a per tonne basis, although such improvements may not be able to keep pace with the growth of the salmon farming industry. The estimated reduction in survival of wild salmonids is large, and would be expected to increase if aquaculture production increases.

Materials and Methods

We modeled survival and, in a separate analysis, total returns to each stock, using a general linear mixed effects model for each region. To model survival, we used a Ricker model extended to include the production of farmed salmon in the area through which exposed juvenile salmon migrated, with random effects for each stock and year [19].

Let $S_{i,y}$ be an index of the number of fish that smolted, i.e., migrated to sea in the spring, in year y from stock i, let $R_{i,y}$ be the estimated number of those fish that would subsequently return to spawn in the absence of fishing, and let $P_{i,y}$ be the aquaculture production that those smolts were exposed to (in tonnes). The dynamics are assumed to be given by

$$\log\left(\frac{R_{i,y}}{S_{i,y}}\right) \quad \beta_0 + a_i + d_y + \beta_i S_{i,y} + \gamma(P_{i,y})^{\lambda} + e_{i,y} \qquad (1)$$

where β_0 is the fixed intercept for the average stock and year with no aquaculture production, a_i is the random deviation of the i^{th} stock intercept from β_0, d_y is the random deviation of the y^{th} year, β_i is the fixed slope of mortality (the density dependence parameter) that will vary with each stock i, and γ is the coefficient of aquaculture mortality that is assumed to scale with a possibly nonlinear function of aquaculture production, $(P_{i,y})^{\lambda}$. The random error, $e_{i,y}$, is assumed to be first order autocorrelated. We assume the a_i's and d_y's come from normal distributions with zero mean. The autocorrelation and the random year effect are included to account for established temporal and spatial correlations (respectively) in environmental effects [20].

The effects of aquaculture are summarized by the coefficient γ for each region. The regional coefficients were combined using meta analysis to obtain an overall estimate of the change in wild salmonid survival related to aquaculture. Because the best functional form for the aquaculture term in the model $(P_{i,y})^{\lambda}$ was not known, we investigated a linear increase in impacts with aquaculture, a square relationship, and a square root relationship. We selected models by AIC, and we tested our results under alternative formulations.

To test the robustness of the conclusions, and because only returns data were available for some regions, we repeated the analysis with number of returning adults as the response variable. This analysis used Equation 1 but dropped the $S_{i,y}$ and β_i terms. The response variables for this analysis included rod catches, rod plus marine catches, counts of salmon returning to rivers, and estimates of returns to rivers in the absence of fishing (see Data sources and treatment, below).

Outer Bay of Fundy salmon in New Brunswick, Canada, have been reduced to zero in one river and to a handful in another river. For this region only, we assumed negative binomial errors.

For the meta analysis, we added a subscript, k, to identify each region, to γ, which summarizes the effect of aquaculture for each region. For a fixed assumption about λ, the γ_k's are in the same units and can be directly compared. We modeled the effects of aquaculture as a mixed effects model,

$$\hat{\gamma}_k \sim N(\alpha_0, \sigma^2 + s_k^2) \qquad (2)$$

here $\hat{\gamma}_k$ is the estimated value of γ_k, α_0 is the intercept, σ^2 is the among region variance, and s_k^2 is the variance of the kth estimate (which is taken from the analysis in Equation 1, and is held fixed). A fixed effects meta analysis is obtained by constraining σ to be zero. We used maximum likelihood estimation and selected models by AIC.

For robustness, we considered five classes of models: different regions used as controls, different mixed model assumptions, differ

ent error assumptions, different functional forms for the aquaculture effect, and different autocorrelational structures, as well as perform ing a Bayesian meta analysis. Overall, the results were very similar for all models. (See Tables S1 and S2 for results of alternative models and Text S1 for details of the Bayesian analysis.)

Data sources and treatment. We analysed data for five species of wild salmonid in five regions: Ireland and Wales, Scotland, New foundland (Canada), New Brunswick (Canada), and British Columbia (Canada). There are three further regions with both wild salmonids and salmon aquaculture for which we could not carry out analyses: Norway, the west coast of Vancouver Island (Canada), and Maine (United States). We were unable to carry out analyses for Norway for three reasons. First, salmon farming in Norway is so widespread [21] that it was difficult to establish controls. Second, the adult population in many rivers has been found to contain over 50% aquaculture escapees [22], making trends in returns to rivers difficult to interpret. Third, there are confounding effects from acidification and disease [23, 24]. For the west coast of Vancouver Island, it was not possible to obtain aquaculture production data by region over time, and Maine was not included because of a lack of nearby wild populations to serve as controls.

Most populations that we considered to be exposed breed in rivers that discharge into bays or channels containing at least one salmon farm. Others breed in rivers flowing into bays without salmon farms very close to areas containing many farms. Salmon from control rivers are very unlikely to pass by salmon farms early in their life cycle, due to the direction of their migration. However, some controls may be relative, in the sense that salmon may pass by farms from a considerable distance, later during their migrations. This would tend to be conservative with respect to our study, since we would then have to detect local effects that are additional to any impacts from distant farms. Data from scientific surveys, e.g., counting fences, were used if possible; for Scottish salmon and Irish and Welsh sea trout, only catch data were available, so results are given for only the impacts on returns (not survival).

Ireland sea trout. We compared rod catches of sea trout in Ireland's Western Region to rod plus in river fixed engine catches in Wales, from 1985 to 2001 (there are no fixed engine fisheries directed at sea trout in Ireland). Salmon farming is concentrated in the Western Region (Connemara area) of Ireland, but does occur in other parts of the country [25]. Based on farm locations [25], it was estimated that all rivers considered exposed are located less than 50 km from a salmon farm, but most will enter the ocean less than 30 km from a salmon farm. There is no salmon farming in Wales. There were 16 rivers in Western Ireland considered exposed: Athry, Bhinch (Lower), Bhinch (Middle), Bhinch (Upper), Burrishoole, Costello, Crumlin, Delphi, Erriff, Gowla, Inagh, Inverbeg, Invermore, Kyle more, Newport, and Screebe [16]. The following 32 Welsh rivers served as controls: Aeron, Afan, Arto, Cleddau, Clwyd, Conwy, Dee, Dwyfawr, Dwyryd, Dyfi, Dysynni, Glaslyn, Gwendreath, Gwyrfai, Llyfni, Lougher, Mawddach, Neath, Nevern, Ogmore, Ogwen, Rheidol, Rhymney, Seiont, Taf, Taff, Tawe, Teifi, Tywi, Usk, Wye, and Ystwyth [26,27]. Trout caught and released are included in catch data from both countries. Only catch estimates were available for most of these rivers. Recruitment could not be derived, because anadromous brown trout interbreed with freshwater resident trout, about which very few data are available, so this stock was only included in the returns modeling (not survival). Farmed salmon production for all of Ireland was used in modeling [28], because the majority of farms are in the region where the exposed populations breed. This will tend to have a conservative effect, resulting in a lower estimate of the impact of aquaculture, per tonne of salmon farming.

Scotland catch data. We compared marine plus rod catches of Atlantic salmon from the east coast of Scotland to catches from the west coast of Scotland for the years 1971 to 2004. Salmon farms appear to be located in the majority of bays on the west coast of Scotland in well over 300 sites (http://www.marlab.ac.uk/Uploads/Documents/fishprodv9.pdf), so all salmon from rivers on this coast were considered exposed. There is no salmon farming on the east coast, so salmon from east coast rivers were controls. For each coast, a single time series of total catch was used in modeling. Marine catch records were from the International Council for the Exploration of the Sea (ICES) Working Group on North Atlantic Salmon [28] and rod catch records were from Fisheries Research Services of Scotland (J. MacLean, personal communication). Rod catches included salmon caught and released. These data were only used in modeling returns. Farmed salmon production for all of Scotland was used in modeling [28], because regional production data were not available.

Scotland count data. We also used counts of Atlantic salmon of all ages returning to rivers from 1960 2001 in Scotland from Thorley et al (2005) [29]. The fish counters are maintained by Fisheries Research Services or by Scottish and Southern Energy plc. There were two exposed populations. One is from the Awe Barrage, which empties into a bay with numerous salmon farms. The other is from the Morar River, which is less than 20 km from the nearest salmon farm, in an area of the coast with many farms [8]. Salmon from the control rivers (on the east coast) do not pass by salmon farms in Scotland because of the direction of their migration routes [30], unless they approach the Norwegian coast. There were ten control populations from the following rivers: Aigas, Beanna, Torr Achilty, Dundreggan, Inver garry, Logie, Westwater, Cluni, Erich, and Pitlo. Farmed salmon production for all of Scotland was used in modeling [28] because regional production data were not available.

Ireland Atlantic salmon. Estimates of marine survival to one sea winter for hatchery (and two wild) Atlantic salmon populations from Ireland and Northern Ireland (1980 2004) were collected and reported by the ICES Working Group on North Atlantic Salmon [28]. Because only survival estimates are provided, these data were only used in the survival analysis. Salmon from hatcheries on the Screebe, Burrishoole, Delphi, and Bunowen Rivers were considered exposed. Populations from hatcheries on the Shannon, Erne, Lee, Bush, and Corrib Rivers, plus wild populations from the Bush and Corrib Rivers were used as controls.

Production data were not available on a regional basis, so national values [28] were apportioned to bays into which exposed rivers empty by assuming that 30% of national production is in the Kilkieren Bay, 10% is in Clew Bay, 5% is in each of Killary Harbour and Ballinakill Bay. These proportions are based on maps of salmon farm locations from the Irish Marine Institute [25], and they approximately match stock numbers collected by the Central Fisheries Board in the years for which stock numbers are available (P. Gargan, personal communication). Years in which each bay was fallowed were obtained from the Central Fisheries Board (P. Gargan, personal communica tion), and in these years, the fallowed bays are assigned a production of zero. All exposed rivers empty into bays with salmon farms [25], while control rivers are at least 55 km away from the nearest farm.

Newfoundland, Canada. Two data sets from Newfoundland were examined marine survival estimates of wild Atlantic salmon from four rivers from 1987 to 2004 were used in the survival analysis, and grilse returns to 21 rivers from 1986 to 2004 were used in the returns modeling [31]. Salmon farming in Newfoundland is confined to Bay d'Espoir on the south coast [32] (http://www.fishaq.gov.nl.ca/aquaculture/pdf/aqua sites.pdf). Only the Conne River (in Bay d'Espoir) was considered exposed; the Little River (also in Bay d'Espoir) was excluded because it has been regularly stocked [31]. The Exploits and Rocky Rivers were also removed from the analysis because of stocking [33]. This left three control rivers for the survival analysis: the Campbellton River, the Northeast Brook (Trepassey), and Western Arm Brook. For the returns analysis, there were 18 control rivers: Campbellton, Crabbes, Fischells, Flat Bay Brook, Highlands, Humber, Lomond, Middle Brook, Middle Barachois, Northeast Brook (Trepassey), Northeast (Placentia), Northwest, Pinchgut Brook, Robinsons, Salmon, Terra Nova (upper and lower), Torrent, and Western Arm Brook. Salmon from control rivers are very unlikely to pass salmon farms because of the direction of their migrations [34]. Farmed salmon production data are from Fisheries and Oceans Canada (DFO) Statistical Services [32].

New Brunswick and Nova Scotia, Canada. We compared Atlantic salmon returns to six rivers in the Bay of Fundy (New Brunswick and Nova Scotia, Canada) to returns to four rivers from other areas of New Brunswick and Nova Scotia. We grouped the six exposed rivers into three groups and estimated the impact of aquaculture on each group separately, because salmon from these three groups have different degrees of exposure to salmon farming. The three groups of exposed rivers are the inner Bay of Fundy group (Stewiacke and Big Salmon Rivers), the Saint John River group (Saint John and Nashwaak Rivers), and the outer Bay of Fundy group (St. Croix and Magaguadavic Rivers). Salmon farming in New Brunswick is highly concentrated in the Quoddy region of the outer Bay of Fundy (http://www.gnb.ca/0177/10/Fundy.pdf), although some farms are also found along the Nova Scotia coast of the Bay of Fundy. Salmon from control rivers enter into the Atlantic directly (LaHave River) or into the Gulf of St. Lawrence (Restigouche River, Miramichi River, Catamaran Brook) and do not pass by farms during their migrations. The same controls are used for all comparisons in New Brunswick and Nova Scotia. The estimates of returns to the rivers are published by DFO [28,35 40]. Outer Bay of Fundy salmon must pass through an area containing many salmon farms early during their migrations [41]. Although Saint John River salmon enter the ocean in an area without

salmon farms, they are known to pass through the region containing many farms early during their migrations [41]. Salmon from inner Bay of Fundy rivers are considered exposed to salmon farming despite being up to 260 km away because of historical information indicating that juvenile salmon from these populations are found during the summer and fall in the area where salmon farms are currently located [42]. However, the evidence that this region is important habitat for inner Bay of Fundy and Saint John River populations is mixed [43]. For this reason, we ran an alternative model with only outer Bay of Fundy populations considered exposed, and all other New Brunswick and Nova Scotia rivers as controls.

For all New Brunswick rivers, an estimate of egg deposition was used as an index of spawners, to account for a significant increase in the age of spawners in many rivers over the study period. The number of grilse (salmon maturing after one winter at sea) and large spawners (repeat spawners or salmon maturing after two or three winters at sea) in each year was multiplied by a river specific estimate of fecundity for a salmon of that size. Then, the index of spawners in a given year was derived by adding up all the eggs that could produce smolts in a year y, using river specific ages at smolting from the literature. Returning hatchery origin spawners are also added to the "spawners" but not to "returns." "Recruits" is the number of grilse that return to each river in year $y+1$, so that $\frac{R_{i,y}}{S_{i,y}}$ (in Equation 1) is the number of grilse returning per egg that would have smolted in year y. Estimates of returns to rivers from traps and other surveys were used in the returns analysis. No corrections were made to account for marine fisheries, but marine exploitation has been quite limited since the late 1980s, when salmon farming became a substantial industry [44]. Farmed salmon production data are from DFO Statistical Services [32].

British Columbia, Canada, coho salmon. For coho salmon in British Columbia (BC), spawner estimates are based on DFO's escapement database (NuSEDS), which includes estimates of spawning salmon of all species for hundreds of rivers and streams on the BC coast since 1950 (P. VanWill, DFO Pacific, unpublished data). We considered rivers on the east side of the Queen Charlotte and Johnstone Straits to be exposed (all rivers from Wakeman Sound to Bute Inlet, DFO Statistical Areas [SAs] 12 and 13). All rivers on the BC Central Coast from Finlayson Channel to Smith Inlet (SAs 7, 8, 9, and 10) were included as controls. In the regions considered exposed in BC, all salmon must pass by farms to get into the open ocean, although in some cases, the farms are at the end of long channels down which the salmon migrate (as far as 90 km in the most extreme case). Control populations to the north do not pass by farms, because of the direction of their migration routes [45].

Coverage in the NuSEDS database varies considerably in time and space, as does the quality of the estimates. We changed all indicators of unknown values (including "none observed" and "adults present") to a common missing value indicator. To reduce effects of inconsistent monitoring procedures, only data since 1970 were included in the analysis. All rivers known to be regularly stocked with hatchery salmon or to contain constructed spawning channels were also removed from exposed and control areas, leaving 49 exposed and 70 control rivers. Estimates were combined for each SA, the smallest areas for which catch rates are estimated. This was done by modeling returns to each SA and year, using a generalized linear model with negative binomial errors. The predicted returns for each SA were then used as spawner estimates ($S_{i,y}$ in Equation 1). To derive recruitment estimates, we followed Simpson et al. (2004) [46], applying exploitation rate estimates from Toboggan Creek (J. Sawada, DFO Pacific, personal communication) to the controls, and the average of the exploitation rates for Quinsam Hatchery, Big Qualicum Hatchery, and the Black Creek wild indicator population to the exposed stocks. After 1998, only the estimates from Black Creek were used for exposed stocks. Recruitment estimates for coho were based on the assumption that coho follow a fixed 3 y life cycle.

For pink, chum, and coho salmon, aquaculture production estimates include all salmon species farmed in SAs 12 and 13 (the Queen Charlotte and Johnstone Straits) from 1990 to 2003 (H. Russell, BC Ministry of Agriculture, Food, and Fisheries, unpublished data). In years when two or fewer companies were raising salmon in either area, estimates were not available. BC salmon farm locations are made available at http://www.al.gov.bc.ca/fisheries/licences/MFF Sites Current.htm.

British Columbia, Canada, pink salmon. Estimates of pink salmon spawner abundance were derived in the same manner as described above for coho salmon. "Returns" are spawners plus catch for a given year, assuming a fixed two year life cycle. The same regions were considered exposed, but because enumeration varies by species, there were only 36 exposed rivers from SAs 12 and 13 (from Wakeman Sound to Bute Inlet) included. Wood et al. (1999) [47] consider the pink salmon catches in SAs 8, 9, and 10 to consist mainly of salmon returning to those areas (respectively), so catch data from DFO [48] were used in each of these SAs. Area 7 was excluded from the survival analysis because catches for SA 7 are difficult to estimate due to the adjacent regions being much larger [47], leaving 47 control rivers from Burke Channel to Smith Inlet.

For Queen Charlotte and Johnstone Straits (the exposed areas), DFO does not estimate catches at the level of individual SA. To obtain approximate returns to each exposed SA, we found the proportion of total escapement to the Straits that was in our dataset (i.e., regularly enumerated rivers on the east side of the Straits without a major hatchery or constructed spawning channel) and assumed the same proportion of the total catch would be returning to those rivers (i.e., assumed equal catchability across stocks). For odd years, we used estimates from the Pacific Salmon Commission (B. White, unpublished data) of the catch of pink salmon in Johnstone and Georgia Straits that were not returning to the Fraser River. In even years, there is no pink salmon run on the Fraser River, so total returns to the Straits could be used.

British Columbia, Canada, chum salmon. For chum salmon, we used estimates of returns (i.e., before exploitation) and spawners to large coastal areas [49]. Chum from the east side of Queen Charlotte and Johnstone Straits, from Wakeman Sound to Bute Inlet (SAs 12 and 13) were considered exposed to salmon farming, while chum from the Central Coast from Bute Channel to Seymour Inlet (SAs 8–11) were considered controls. Estimates were available as a single time series for the exposed area, and a time series for each SA for the controls. An index of recruits per spawner was generated by lining up returns with spawners according to age distributions given in Ryall et al. (1999) [50], to 1998, and then the average values from 1988–1998 for the subsequent years, to 2003.

Supporting Information

Figure S1. Survivals of Salmonids in Control (Black) and Exposed (Blue) Stocks, along with Aquaculture Production (Red)

The returns have been summarized by a multiplicative model ($\log\left(\frac{R_{i,y}}{S_{i,y}}\right) \quad a_i + d_y + e_{i,y}$); the mean survival across stocks for each year is plotted. Survivals for exposed Saint John River stocks have been multiplied by 10 for clarity (dashed line). Survival is estimated across different portions of the life cycle in different regions; from smolt to adult for Irish salmon and Newfoundland, from egg to adult for Bay of Fundy and Saint John River stocks, and from adult to adult in BC stocks.

Found at doi:10.1371/journal.pbio.0060033.sg001 (15 KB PDF).

Table S1. Results of Alternative Models for the Survival Analysis

Effect size estimates (y's) and their standard errors have been multiplied by 10^3, 10^4, or 10^8 (as labeled), to make numbers easier to read.

Found at doi:10.1371/journal.pbio.0060033.st001 (22 KB PDF).

Table S2. Results of Alternative Models for the Returns Analysis

Effect size estimates (y's) and their standard errors have been multiplied by 10^3, 10^4, or 10^8 (as labeled), to make numbers easier to read.

Found at doi:10.1371/journal.pbio.0060033.st002 (23 KB PDF).

Text S1. Alternative Model Formulations, Including the Bayesian Analysis

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guidance from RAM, with the exception of the SAS code for performing the meta analysis and the Bayesian section of the Text S1, which were written by RAM.

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REVIEW PAPER

Genetic variation for tolerance to acidic water in salmonids

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Water pH is an important factor affecting the general water quality as well as quality traits in fishes, and the magnitude of the effect varies among species. The massive and negative effect of acidification of rivers and lakes became evident during the 1960s and 1970s and caused the depletion of fish stocks in several countries in the northern hemisphere. Significant variation in tolerance to acidic water has been documented among salmonid species, and large genetic variation has been identified among strains of brown trout *Salmo trutta*, brook trout *Salvelinus fontinalis* and Atlantic salmon *Salmo salar*. For *S. trutta*, *S. fontinalis* and *S. salar*, there is considerable additive genetic variation in tolerance to acidic water, with heritabilities (h^2) ranging from 0·09 to 0·27 for dead eyed-eggs (the period most sensitive to low pH). The main reasons for depletion of freshwater fish stocks are discussed.

Key words: fresh water; heritability; low pH; salmonids; tolerance.

INTRODUCTION

During the 1900s, a gradual decrease in pH took place in the rivers and lakes in southern Norway. Losses of fish populations started as early as the 1920s. A catastrophic situation in 1948 was described by Rosseland (1953), where a mass mortality occurred after mild weather with snow melting in the mountain districts which constitute part of an Atlantic salmon *Salmo salar* L. 1758 river catchment area, and *S. salar* died from this acid water with pH values from 3·9 to 4·2. The most rapid losses occurred during the 1960–1970s (Rosseland *et al*., 1986; Henriksen *et al*., 1989). In Sweden, several *S. salar* populations along the western coast were lost due to acidification with no positive trends reported in the 1980s. In Finland, an increase in acidic deposition during the 1970–1980s lead to acidification in the most sensitive freshwater systems and the loss of stocks in some freshwater lakes (Rosseland *et al*., 1986). Muniz & Leivestad (1980) reported that the effect of acidification was

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evident in a 33 000 km^2 area, and in a 13 000 km^2 zone within this, fish populations were virtually extinct. By 1999, an area of 84 000 km^2 was affected, resulting in the loss of 18 *S. salar* populations and 9630 inland fish populations, with another 5400 populations affected (Hesthagen *et al.*, 1999). The problem occurred also in eastern North America, and the best documented biological consequence of acidification was the loss of all fish populations in some 200 lakes in the Adirondack Mountains, NY, and 200 lakes in Ontario, Canada (Harvey, 1980). The mechanism of extinction is species dependent. While failure of recruitment of new age classes caused by mortality on eggs and alevins is the dominant mechanism in brown trout *Salmo trutta* L. 1758 populations (Jensen & Snekvik, 1972; Rosseland *et al.*, 1980), the smolt stage represent the most sensitive life-history stage of *S. salar* resulting in lack of spawners returning from sea (Rosseland & Skogheim, 1984; Leivestad *et al.*, 1987; Rosseland & Staurnes, 1994; Kroglund *et al.*, 2008).

The increased acidity of the Norwegian waters was due to acid precipitation caused by atmospheric sulphur carried by wind to Norway from the European continent and the U.K. (Førland, 1973; Mylona, 1993), together with pollution from Norwegian industry. At first, it was thought that low pH in the water was the main reason for the depletion of fish stocks, but it became clear that part of the problem was caused by the pH dependant mobilization of aluminium (Schofield, 1977) in its different ionic forms (Driscoll *et al.*, 1980; Muniz & Leivestad, 1980).

This paper is a review of available information about genetic variation for tolerance to acidic water in salmonids, in particular discussing results from extensive studies in Norway and Canada.

SPECIES DIFFERENCES

A genetic component of tolerance to acidic water was evident early, and Muniz & Grande (1974) ranked the following salmonid species in increasing order of tolerance to low water pH: rainbow trout *Oncorhynchus mykiss*, (Walbaum 1792), *S. salar*, *S. trutta* and brook trout *Salvelinus fontinalis*, (Mitchill 1814), while Parry (1960) had the following ranking: S. Salar, *O. mykiss* and *S. trutta*. In addition, Jensen & Snekvik (1972) and Rosseland & Skogheim (1984) state that sea trout (anadromous *S. trutta*) are more susceptible than fresh water *S. trutta* but less sensitive than *S. salar*. Large variation in tolerance to acidic water has also been reported for amphibians (Pierce, 1985).

SALMO TRUTTA

To investigate the effect of acid precipitation in Norway, a joint research project 'Acid precipitation – Effects on Forest and Fish' (SNSF) was initiated by the Agricultural Research Council of Norway and Norwegian Council for Scientific and Industrial Research in 1972 (Drabløs & Tollan, 1980). One part of this national project investigated genetic variation for tolerance of fish to acidic water. Results from a three-year study were only published as project reports by Gjedrem (1976) and Edwards & Gjedrem (1979). *Salmo trutta* was selected among the salmonid species for the study since it was considered to be the most tolerant salmonid species to acidic water present in Norway.

Description of data

In a period of 3 years, 201 strains of *S. trutta* were sampled from areas in southern Norway with acidic water in order to capture as much genetic variation as possible and find some strains with extreme tolerance to acidic water. From these strains, >1900 full-sib families were produced and held in Finså klekkeri, a hatchery in Marnardal. The large River Mandalselva, passing close by the hatchery, had a reasonably stable pH around 4·7. It was decided to maintain two pH levels in the hatchery, pH = 4·7 and pH = 5·2 for year classes 1974–1975 and 1975–1976, and pH = 5·2 and pH = 6·2 for year class 1976–1977, which made it possible to also investigate possible interactions between pH levels and fish strains. At that time, aluminium was not recognized as an important factor and was not analysed. As the hatchery used natural acidic water, however, aluminium was the major stressor in these experiments. During the hatching period, a stable pH level was regulated by means of adding a solution of either NaOH or H_2SO_4.

The broodstock were caught in September to October and held in their respective districts until they were subsequently ripe in October to November. At maturity, the eggs from each female and the milt from each male were dry stripped into plastic containers and transported to the hatchery in Marnardal within 12–18 h. A nested mating design was used, sperm from one male fertilized eggs from four females. Two of the half-sib groups were held at pH level 4·7 and two at 5·2. Each ful-sib group was held in a separate box in the hatchery with flow-through water of equal temperature and quality.

Dead eggs and alevins were removed from the egg box and counted every day. Each year the experiment was terminated in early June when the alevins had absorbed two-thirds of the yolk sac and became ready to start feeding.

In the years 1976–1977 and 1977–1978, a total of 120 families from 40 cross-bred strains were tested (Edwards & Gjedrem, 1979).

The traits studied were defined as follows: dead eggs, mortalities between fertilization and eyed-egg stage; dead eyed-eggs, death rate from first eyed-egg to hatching, dead eggs at hatching included; dead alevins, death rate of alevins to two-thirds of the yolk sac was absorbed; alevines alive, frequency of alevins at close of the experiment.

Genetic analysis of these data was complicated by the fact that the data were not orthogonal with regard to number of families per strain and number of eggs per family. In addition the use of per cent mortality and survival as a trait resulted in a large and significant difference in variance from one strain to another. In order to reduce the influence of these problems, a method presented by Bogyo & Beker (1965), where percentages are transformed by arc sine $\sqrt{x}$, was used for the genetic analysis of the data. For a second method these traits were considered as all or none traits and the observations coded with 1 and 0. The models used are described by Gjedrem (1976).

Additive genetic variance was estimated by ANOVA, between sires within strains and for total estimates between sires and strains and years. The heritability was estimated by multiplying the sire component by four since the half-sib correlation represents only a quarter of the additive genetic variance (Falconer & Mackay, 1996).

Results obtained

As expected, there was a highly significant difference in death rates between pH levels for all traits studied, and with the exception of eyed-eggs, the death rates were

TABLE I. Per cent mortality and survival frequencies for eggs and alevins of *Salmo trutta* hatched in acidic water (Gjedrem, 1976; Edwards & Gjedrem, 1979)

	1974–1975*		1975–1976†		Average 1974–1976		1976–1977†	
	pH		pH		pH		pH	
Trait	4·7	5·2	4·7	5·2	4·7	5·2	5·2	6·4
Dead eggs	28·0	18·4	42·4	28·6	35·2	23·5	35·2	24·8
Dead eyed-eggs	43·6	48·7	39·7	53·3	41·7	51·0	48·1	1·0
Dead alevins	20·8	9·1	11·5	7·2	16·2	8·2	2·2	0·4
Live alevins	7·6	24·1	6·5	10·9	7·1	17·5	14·3	73·8

*Seven hundred and seventy full-sib families from 77 strains.

†One thousand one hundred and sixty-eight full-sib families from 124 strains.

highest at the low pH level. The survival rate was more than two times higher at high pH compared with low pH.

Under normal hatching conditions, with water quality close to the neutral pH level, mortality usually occurred before the eyed-egg stages, as can be seen for pH 6·4 in Table I. In acidic water, the most critical point seemed to be just as the eggshell broke during hatching (Gjedrem, 1976), a period found to be very critical due to sensitive enzymes being inhibited by low pH (Rosseland &Staurnes, 1994).

The strain variance is very large and represents >40% of the total variation for dead eggs, dead eyed-eggs and dead alevins and 33% for live alevins (Gjedrem, 1976), and this is a combination of genetic and environmental variation which could not be separated.

Possible environmental difference between strains relates back to the environmental conditions that the broodstock was subject to. After fertilization of eggs, the environmental differences between strains were negligible. It is therefore quite likely that the greater part of the strain variance is genetic. The variation in survival among strains ranged from 0 to 58% (Fig. 1). This is a relatively large difference, and it is possible that the complete range could be even wider than this.

The heritabilities given in Table II ranged from 0·09 to 0·33. Negative estimates are meaningless, but it shows that the estimates have associated errors and are given to show the range. Dead eyed-eggs have the highest heritabilities. According to Gjedrem (1976), the estimates of heritability obtained show that tolerance to acidic water is a heritable trait with a higher heritability compared to what is usually found for fitness traits in fishes.

The crossbreeding experiment showed that crosses on average had much higher survival than the mean of their parental strains hatched at pH 5·2. Of a total of 40 strain-crosses tested, 30 had a higher average survival than the mean of their two parental strains. Furthermore, of these 30, 25 showed higher mean survival than either of the parental groups. The average per cent survival of all the strain crosses combined was 36% compared with an average of 18% for all their parental groups.

Survival time of 11 *S. trutta* strains held in water with low pH was studied by Edwards & Gjedrem (1979). This fish was <1 year averaging 5·8 g. Average survival time was 107 min when held in water with pH = 2·5 compared with 300 min for fish held at pH = 3·0. The rank correlation between strains held at these low pH levels

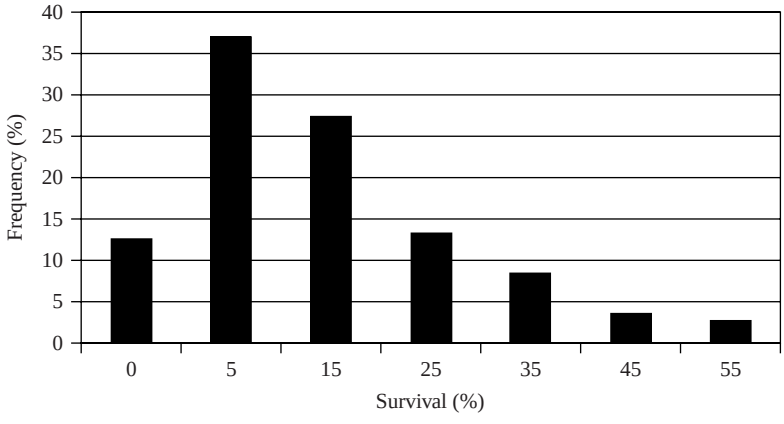

FIG. 1. Per cent strains of *Salmo trutta* with varying rate of survival in acidic water. A total of 770 full-sib groups from 77 strains was tested (Gjedrem, 1976).

was as high as 0·89. Survival time of three strains of fish at different size classes was also compared. Large fish (10·2 g) survived longer in water pH 3 compared with smaller fish (3·6 g). Furthermore, survival time was significantly longer in water with lower temperature which held true for the four temperatures studied: 1·5, 5·0, 7·0 and 11·2° C (Edwards & Gjedrem, 1979).

A study comprising both laboratory and field studies to assess acid tolerance among indigenous Norwegian strains of *S. trutta* involved restocking and subsequent test fishing of 13 lakes with five *S. trutta* strains (Dalziel *et al*., 1995). There was considerable variation in the ability of individual lakes to support adult fish. One strain, Bygland, was found to be relatively acid tolerant, accounting for >60% of all fish recaptured by test fishing over a 5 year period. This was consistent with better survival of young life stages of the Bygland strain, compared with that of the other strains, in laboratory experiments employing acidic conditions.

Dalziel *et al*. (1995) found that the Bygland strain always had less skeletal calcification than that of the other strains at a defined developing stage, irrespective of pH level, although the total body calcium (Ca) was similar. Conversely, Tunhovd

TABLE II. Heritabilities for mortality and survival frequencies of *Salmo trutta* eggs and alevins in acidic water (Gjedrem, 1976; Edwards & Gjedrem, 1979). Values are mean ± S.E.

	1974–1975*		1975–1977†
Trait	Coding	Arc sine $\sqrt{x}$	Arc sine $\sqrt{x}$
Dead eggs	0·04 ± 0·06	0·02 ± 0·05	0·33
Dead eyed-eggs	0·14 ± 0·05	0·09 ± 0·02	0·27
Dead alevins	0·14 ± 0·06	0·00 ± 0·01	0·00
Live alevins	−0·06 ± 0·05	−0·09 ± 0·01	0·10

*Seven hundred and seventy full-sib families from 77 strains.

†One thousand one hundred and sixty-eight full-sib families from 124 strains.

strain fry consistently showed the most advanced skeletal calcification of any strain and had very poor survival in acidic water. Thus, the internal household of the Ca storage might have an important role in the tolerance to acidic water.

SALVELINUS FONTINALIS

Swarts *et al.* (1978) studied 14 strains and strain combinations of hatchery reared *S. fontinalis* in addition to wild strains exposed to low pH water in the laboratory and in the field. There was a pronounced difference between strains in the per cent egg hatch at pH as low as 4·4 with a variation between strains ranging from 0 to 50%. Strain differences were also demonstrated in juvenile and adult stages. A single selection with low selection pressure of one strain for high resistance to sulphuric acid solution did not yield F1 progeny of greater resistance. Significant differences in survival time of different strains of juvenile *S. fontinalis* at lethal pH values have also been demonstrated by Robinson *et al.* (1976) and Falk & Dunson (1977). In an acidic stream, they found a similar ranking in tolerance to low pH of some of the strains to that found by Swarts *et al.* (1978).

Lachance *et al.* (2000) compared *S. fontinalis* from three different lakes. The strain from Lake Arsenault was presumed acidic tolerant which could survive and reproduce in an acidified environment where pH was low (<5·2), Al was high (>200 μg l^{-1}) and Ca^{2+} low (<2 mg l^{-1}). The two other strains were from lakes with non-limiting physicochemical conditions for *S. fontinalis*. When these three strains were tested in acidic conditions of anthropogenic origin, they found no differences in egg and fingerling survival.

SALMO SALAR

For *S. salar*, the critical pH below which no recruitment occurs was according to Watt *et al.* (1983) for Nova Scotia, Canada, rivers pH <4·7, and rivers in the range of pH 4·7–5·0 show a decline in *S. salar* return. Rivers with pH > 5·0 in general had normal density of *S. salar*. Jensen & Snekvik (1972) reported, however, the critical pH for recruitments in Norwegian rivers to be 5·0–5·5, but today, a minimum pH of 6·2 is considered to be a safeguard for normal recruitment in Norwegian *S. salar* rivers (Kroglund *et al.*, 2002; Rosseland & Kroglund, 2011). Schom *et al.* (1984) argued that this difference between Canadian and Norwegian stocks' tolerance to acidic waters could be genetic. The differences in organic content [humic or total organic carbon (TOC)] which can bind Al and reduce the toxic inorganic Al species is thought to be the main explanation for the high tolerance to the TOC-rich waters with low pH found in Canada (Rosseland & Kroglund, 2011). Acid precipitation has killed the fish populations of 14 rivers in Nova Scotia's southern upland region, 20 rivers have only 10% of their *S. salar* left and another 30 are threatened (Amiro & Gibson, 2006). In the north-eastern U.S.A., 14 out of 25 rivers known to have *S. salar* have lost their populations (Fay *et al.*, 2006) and in toxicity studies, *S. salar* of the north-eastern U.S.A. seems as sensitive to inorganic Al as the Norwegian *S. salar* (McCormick *et al.*, 2009).

To study the genetic variation in *S. salar* for tolerance to acidic waters, Schom (1986) performed a four-year study (1980–1983) based on fish from Big Salmon River in New Brunswick, Canada, where he selected for high and low tolerance to

TABLE III. Heritability (h^2) for size (h^2_S) and dam (h^2_D) of survival time in each year class of *Salmo salar* calculated using the nested ANOVA at the indicated age post-hatch (Schom, 1986). Values are mean ± S.E.

Brood year	Months post-hatch	h^2_D*	h^2_S*
1980	1	0·54 ± 0·30	0·18 ± 0·08
1981	4	0·62 ± 0·26	0·19 ± 0·06
1982	5	0·57 ± 0·30	0·22 ± 0·09

*Total 96 full-sib families tested and 27 300 fish challenged.

acidic water. To reduce the generation interval, mature parr was used as males. The experimental design used in all but one trial was nested with two or three males within a female, that is each female's eggs were split in two or three lots with a different male used to fertilize each lot. The exception in one year had both a nested component and three sets of a 3×4 factorial design. To get a measurement of tolerance to acidic water, the fish were challenged in recirculated water with low pH (pH varied from 3·2 to 4·3 between year classes). For each year class, all fish from the different families were kept in one tank and time till death was recorded.

Schom (1986) found considerable genetic variation in survival time of *S. salar* salmon in the acid water. The estimates of heritabilities based on the sire component (h^2_S, mean ± S.E.) varied from 0·18 ± 0·08 to 0·22 ± 0·09 (Table III) which is similar to estimates obtained by Gjedrem (1976) for *S. trutta* and Rahel (1983) for yellow perch *Perca flarescens* (Mitchill 1814). In all year classes, the dam component (h^2_D, mean ± S.E.) was much higher than the sire component of heritability which indicates considerable non-additive genetic variance in survival time (Schom, 1986). This is in agreement with the finding of Edwards & Gjedrem (1979) who also found higher heritability estimates of dam component compared with sire component for survival of eggs and alevins. The sire-offspring regression and the realized heritability were of approximately the same magnitude as the sire component (Schom, 1986). The ranking of survival time for families in one trial did not change markedly in the next trial, the rank correlation varied from 0·61 to 0·80. Mature parr were more resistant than the non-mature parr. Further, the genetic gain from selection was much higher in the down direction compared with the up direction.

Rosseland *et al*. (2001) studied the tolerance to acidic water among five strains of *S. salar*, three strains from the acidic Rivers Bjerkreim, Ogna and Vikedal (pH < 5·6, Al > 40 μg l^{-1}) and two from non-acidic Rivers Lone and Imsa (pH > 6, Al < 10 μg l^{-1}). The experiment started with fry (1·9 cm in fork length, L_F) and continued until they reached the smolt stage (13·3–16·6 cm L_F). The fish were kept in water with a low pH as well as with high aluminium concentration. The results suggested that genetic variation for tolerance to acidic water exists between *S. salar* populations, but unexpectedly, it was concluded that strains originating from rivers undergoing acidification were not more tolerant to acidic water than populations originating from non-acidified rivers (Staurnes *et al*., 1995; Rosseland *et al*., 2001). This is contrary to the finding of Schom (1985) who reported that those fish coming from river systems with a history of deteriorating pH outperformed, in acute trials, those fish from more pristine systems.

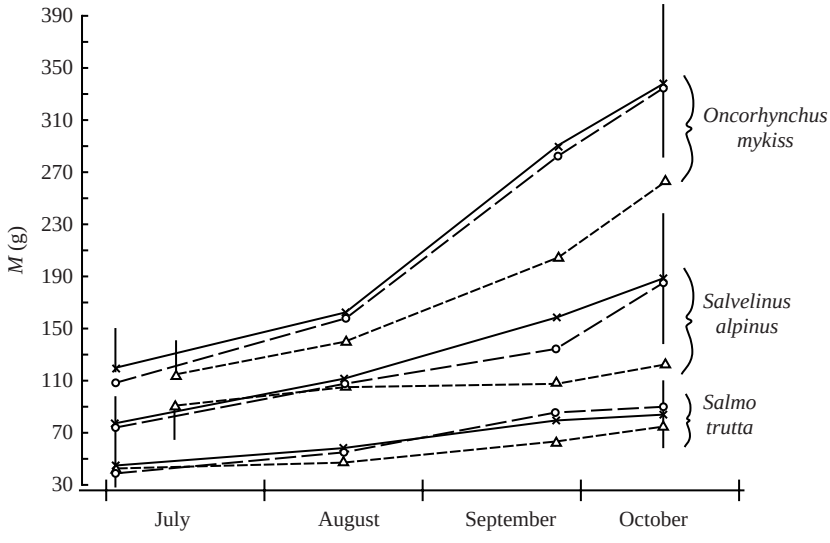

FIG. 2. Mean mass of salmonids held in water at each of three pH levels (×, control; ○, pH 5·5; △, pH 4·8) during a 3·5 month growth experiment. S.D. are shown where possible (Edwards & Hjeldnes, 1977).

For all strains, parr were most sensitive to low pH, whilst pre-smolts and smolts were most sensitive to aluminium (Rosseland *et al*., 2001). Furthermore, it is well documented that for anadromous fish, the smolt stage is more sensitive to acidification than the parr stage (Rosseland & Skogheim, 1984; Rosseland & Staurnes, 1994).

EFFECT ON GROWTH RATE AND SURVIVAL TIME

The effect of acidic water on growth rate and time to survival of 1 year-old *S. trutta*, Arctic charr *Salvelinus alpinus* (L. 1758) and *O. mykiss* was reported by Edwards & Hjeldnes (1977). Each species were reared in 6 2 m^2 tanks with 75 fish in each for *S. trutta* and *S. alpinus*, and 50 for *O. mykiss*. Growth rate and L_F was recorded during a 3·5 month period for each of three pH levels: 4·8–5·0, 5·5 and 6·1–6·2, with a water temperature of 12·2° C. The average L_F was different between species from start of the experiment, *O. mykiss* 208 cm, *S. alpinus* 179 cm and *S. trutta* 145 cm. After 3·5 month of growth, no significant difference in mass or L_F was found between fish of the same species kept at pH 5·5 and 6·1–6·2 (Fig. 2). For *O. mykiss* and *S. alpinus*, however, those groups of fishes kept at pH 4·8–5·0 were significantly smaller than those in the other two groups, which indicate that pH can exert a direct depressing effect on fish growth. *Salmo trutta* kept at pH 4·8 also had a lower body mass and L_F compared with fish at higher pH. *Salmo trutta*, however, had a low growth rate during the experimental period. Also in amphibians, low pH inhibits larval growth rate (Pierce & Wooten, 1992).

Edwards & Hjeldnes (1977) also tested the three species for survival time in water of very low pH (pH = 2·55, 2·64 and 3·01). Thirty-six fish of each species from the growth experiment were used. Survival time was always longest for *S. trutta*, intermediate for *S. alpinus* and shortest for *O. mykiss*. All fishes used were of the

same age (1+ year), and there was no correlation between fish size and survival time in low pH water. This is in agreement with Loyd & Jordan (1964) who studied the correlation between size of *O. mykiss* and their sensitivity to low pH, but they demonstrated a significant correlation between pH susceptibility and fish age.

RECOVERY FROM ACIDIFICATION

Due to international agreements, a reduction in sulphuric rainfall has taken place. In Norway, acid deposition reached its peak in the late 1970s, and Aas *et al*. (2004) report a decline by *c*. 70% (sulphur) and 20% (nitrogen) since the mid 1980s. This is one example that major environmental problems to some degree can be reduced by international agreements. To reduce the still ongoing acidification problems in Norway, the Norwegian Government annually invests in liming of lakes and rivers. Up to now, 2500 lake populations have been saved or restored and 21 *S. salar* rivers have been continuously treated, bringing fish back to former barren regions (Kroglund *et al*. 2001; Sandøy & Langåker, 2001; Hesthagen *et al*., 2011*a*; Rosseland & Kroglund, 2011). A proposal to start a breeding programme for *S. trutta* to increase tolerance to acidic water was not supported.

During this period, acidified lakes have shown substantial chemical recovery, with increasing pH, acid neutralizing capacity (ANC) and lower concentrations of labile aluminium (Skjelkvåle *et al*., 2001, 2003). A report from 2009 based on interviews of lake owners concludes with a reduction in affected catchment areas by near 38%, although little improvement has been observed in the historically most affected areas of southern Norway (Hesthagen & Østborg, 2008). Models based on observations of fish populations in several lakes undergoing the process of recovery from acidification show that the chemistry has reached a 'non-toxic level' and it will take at least 10–15 years before fish populations in lakes 'fully' recover (Rosseland *et al*., 2005; Hesthagen *et al*., 2011*b*), or 20 years for *S. salar* populations (Hesthagen *et al*., 2011*a*). A successful recovery means that all year classes including postspawners must be found in the lakes. It is interesting that postspawners seems to be the most critical stage in the recovery phase, as that life-history stage also was found to be most sensitive during the process of acidification (Rosseland *et al*., 1980). This indicates that both male and female *S. trutta* are particularly sensitive to acidic waters after their first spawning (Rosseland *et al*., 1980, 2005; Rosseland & Skogheim, 1987; Hesthagen *et al*. 2011*b*).

DISCUSSION AND CONCLUSION

The acidification of rivers and lakes became a serious threat to freshwater fishes and occurred on a particularly large scale during 1900s in parts of Scandinavia and North America, and the problem was particularly serious in waters with low mineral content (Muniz & Leivestad, 1980). The resulting loss of fishes in thousands of lakes and rivers became particularly evident during the1960s and 1970s (Muniz & Leivestad, 1980; Henriksen *et al*., 1989). The reason for the loss of salmonide species in several countries is considered to be caused by acidification leading to low pH conditions and subsequent increased aluminium concentration in

low Ca waters (Rosseland & Staurnes, 1994; Gensemer & Playle, 1999; Rosseland & Kroglund, 2011).

So far, the acidification of oceans does not seem to affect anadromeous salmonid species directly, but indirectly they may be affected since they feed on calcifying organism such as shrimps (*Pandalus borealis*, *Calanus finmarchicus* and *Euphausia superba*) which are affected (Orr *et al*., 2005).

Changes in climatic and environmental conditions are usually gradual and take place over a long period of time, allowing plants and animals to adapt by means of natural selection to survive and reproduce under new conditions. The response to natural as well as artificial selection (ΔG) depends on three factors (Falconer and Mackay, 1996): $\Delta G = ih^2\sigma_P$ where i is selection intensity, h^2 is heritability and σ_P is phenotypic S.D. Generally, natural selection is inefficient compared with artificial selection because wild animals live under varying environmental conditions which masks the genetic variation and causes the heritability to become low. Selection intensity is also low because nature is not accurate and systematic in its selection while S.D. is not so much affected. This is the main reasons why adaptation to changes in environmental conditions takes a very long time. When the environmental conditions exceed the level of tolerance for a strain or species, however, they will be eradicated. So why did natural selection not enable adaptation of fish species to these changes in water quality? After all, acidification of rivers and lakes went on for many years. Was it a lack of genetic variation in tolerance to low water pH or was the acidification too rapid for natural selection to adapt the fishes to the new conditions?

In early 1970s, the knowledge about the magnitude of genetic variation for tolerance to acidic water was limited, and therefore, several investigations were initiated to study this question. The first observations showed that there were species-specific differences in tolerance to acidity water in salmonid species, and Muniz &Grande (1974) ranked the species as follows in increasing order of tolerance: *O. mykiss*, *S. salar*, anadromous *S. trutta*, *S. alpinus*, *S. trutta* and *S. fontinalis*.

In the extensive study of 201 Norwegian strains and >1900 families of *S. trutta*, Gjedrem (1976) and Edwards & Gjedrem (1979) clearly documented that there was large variation among strains (Fig. 1) and relatively large additive genetic variation for survival in water of pH 4·7 and 5·2. For dead eyed-eggs, the heritability h^2 ranged from 0·09 to 0·27 (Table II). The time until hatching seemed to be the most critical period for survival in these experiments. Similar heritability estimates were obtained for tolerance to acidic water for *S. salar*, $h^2 = 0{\cdot}18–0{\cdot}22$ (Schom, 1986). Besides affecting survival, the water pH also affects growth rate. This was demonstrated by Edwards & Hjeldnes (1977), who compared growth rate in water of pH 4·8, 5·5 and 6·1. The growth rate was significantly lower at pH 4·8 compared with the higher levels (Fig. 2). From these reports including several salmonid species, it is concluded that there is considerable genetic variation in tolerance to acidic water.

There are varying results about adaptation to lower pH in fresh water. In *S. salar*, Schom (1985) found that fish coming from rivers with lowering of pH had higher rate of survival compared with fish from unaffected rivers, while Staurnes *et al*. (1995) and Rosseland *et al*. (2001) did not find such effect.

From a study of moor frog *Rana arvalis*, Andren *et al*. (1989) conclude that an adaptation to acid condition had taken place during a period of 15 generations since

all premetamorphic stages of the frog from the acid locality had higher acid tolerance than the population from a neutral environment.

On this background, it is most likely that the acidification events occurred too rapidly for natural selection to adapt the fish populations to the new water quality. To try to repopulate, rivers and lakes in southern Norway financed a national liming project. By looking back, the liming and the international reductions in especially SO_2 emissions have had a very positive effect on restoring fish populations in several areas, which in the coming future hopefully will have succeeded in the process of fully recovery from acidification.

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MANAGEMENT BRIEF

Populations on the Brink: Low Abundance of Southern Upland Atlantic Salmon in Nova Scotia, Canada

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Abstract

Populations of Atlantic salmon *Salmo salar* in the Southern Upland region of Nova Scotia, Canada, have declined to very low abundances. Sixty-three rivers in this region—9% of the total number of Canadian rivers that contain Atlantic salmon—are known to have supported populations in the recent past. Annual adult abundance data from four rivers show declines of 83–99% from peak levels in the 1980s; this pattern is consistent with trends in recreational catch within the region. Regionwide comparisons of juvenile density data from more than 50 other rivers indicate significant ongoing declines and provide evidence for river-specific extirpations. Based on surveys conducted in 2000 and again in 2008–2009, total juvenile density decreased substantially at the majority of locations; during the 2008–2009 survey, juveniles were not found at nine sites (four rivers) where they were present in 2000. Although river acidification has significantly contributed to the deterioration or extirpation of Atlantic salmon populations from many Southern Upland rivers during the last century, contemporary declines occurring in nonacidified rivers indicate that other factors are now affecting these populations. Several lines of evidence demonstrate that Southern Upland Atlantic salmon are biologically unique and that their extinction would constitute an irreplaceable loss of Atlantic salmon biodiversity.

Historically, wild populations of Atlantic salmon *Salmo salar* were distributed throughout rivers flowing into the North Atlantic Ocean and Baltic Sea. In recent years, extensive population declines and river-specific extirpations of Atlantic salmon have been documented in several countries (WWF 2001; ICES 2010); currently, only Norway, Ireland, Iceland, and Scotland have apparently healthy populations in the majority of rivers. Canada is second only to Norway in the number of rivers containing Atlantic salmon, thus representing a significant proportion of the species' range. Canadian Atlantic salmon populations reportedly declined by at least 75% from 1970 to 2000 (WWF 2001). Despite closures (1985, 1992, and 2000) of commercial fisheries for Atlantic salmon and the implementation of restrictive recreational fishing regulations since 1983, populations in many rivers continue to decline (DFO and MNRF 2009). Currently, the conservation status of specific Canadian Atlantic salmon populations ranges broadly from relatively stable or increasing (i.e., in some parts of Quebec and Newfoundland) to extirpated (i.e., in Lake Ontario; COSEWIC 2006a).

Nova Scotia contains 147 of the 728 Canadian rivers in which Atlantic salmon are or were present within the last half-century (DFO and MNRF 2008). Populations in Nova Scotia are thought to be comprised of five distinct groups: the inner Bay of Fundy population assemblage (22 rivers), the Southern Upland (SU) populations (63 rivers), two population assemblages (Lowland and Highland) in eastern Cape Breton (29 rivers), and a population assemblage inhabiting rivers that flow into the Gulf of St. Lawrence (33 rivers; DFO and MRNF 2008; Figure 1). Of these groupings, the Gulf of St. Lawrence populations appear to be much healthier than populations in other regions (DFO and MRNF 2008), whereas the inner Bay of Fundy population assemblage is endangered (COSEWIC 2006b) and is expected to rapidly become extinct without a captive rearing program designed to maintain genetic diversity in support of population recovery in the event that conditions improve (Gibson et al. 2008a).

Here, we review the current status of Atlantic salmon populations in the SU region, where abundance in 2009 was lower than abundances observed at any time during the last 35 years, and we present evidence for multiple river-specific declines and potential extirpations. Additionally, we summarize several lines of evidence to suggest that SU Atlantic salmon are distinct from other Atlantic salmon population assemblages. Finally, we discuss the some of the major threats that must be addressed for recovery of SU populations.

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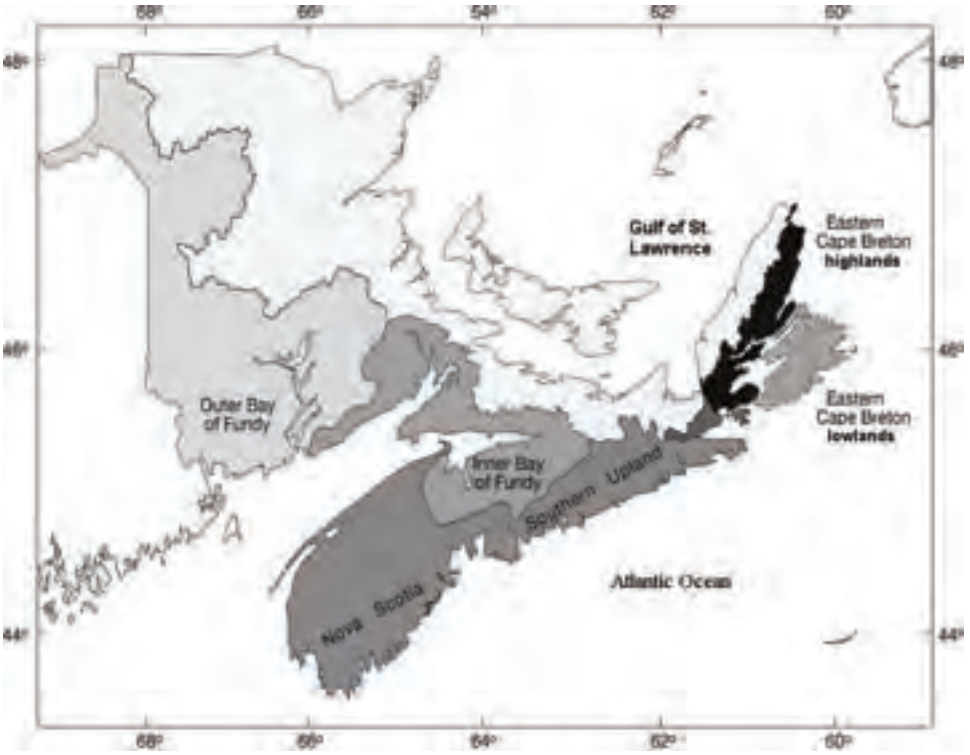

FIGURE 1. Map showing the major population assemblages of Atlantic salmon in Nova Scotia, Canada, including the Southern Upland region.

UNIQUENESS OF SOUTHERN UPLAND ATLANTIC SALMON

It is generally accepted that the identification and protection of biological diversity below the species level are essential for effective species conservation. The Committee on the Status of Endangered Wildlife in Canada (COSEWIC 2006a, 2006b) examines four factors in its determination of appropriate population groupings that can be considered for listing under Canada's endangered species legislation: (1) established taxonomy, (2) genetic evidence, (3) range disjunction, and (4) biogeographic distinction. Genetic evidence, regional geography, and differences in life history characteristics all support the distinctiveness of SU Atlantic salmon relative to other population assemblages (DFO and MNRF 2008). The SU Atlantic salmon constituted one of six regional groupings that were identified by Verspoor (2005) based on analyses of allozyme variation in Atlantic salmon throughout North America. A unique mitochondrial DNA haplotype found at high frequencies in Atlantic salmon from most SU rivers has not been observed in other regions; furthermore, Atlantic salmon in the adjacent inner Bay of Fundy region possess a unique mitochondrial DNA haplotype that has not been found in SU populations (Verspoor et al. 2002, 2005).

In addition to genetic differences, local phenotypic adaptations have been demonstrated for SU Atlantic salmon. The SU region is characterized by shallow soils that are often underlain by acidic slates; thus, many rivers have poor buffering capacity and organic acid-stained water. Recent experiments have demonstrated the existence of genetically based differences in low pH tolerance between Atlantic salmon alevins from the Tusket River in the SU region and alevins from well-buffered Bay of Fundy rivers (Fraser et al. 2008). This local adaptation may one day be important for reestablishing Atlantic salmon in acidified and lower-pH rivers within the SU region and in other acid-impacted rivers throughout North America. Differences in marine migration patterns between Atlantic salmon in the SU region and those in the neighboring inner Bay of Fundy region have been documented. Historical tag returns (Amiro et al. 2003) and acoustic telemetry studies (Lacroix et al. 2005) suggest that inner Bay of Fundy Atlantic salmon have a prolonged local residency in the Bay of Fundy, whereas historical tag returns indicate that SU Atlantic salmon are long-distance migrants that travel to feeding areas off the coast of western Greenland (Ritter 1989). Fraser et al. (2010) compared the growth and body shape of Atlantic salmon from a single inner Bay of Fundy population with those of Atlantic salmon from a single SU population in "common garden" experiments. Fraser et al. (2010) found that the SU population displayed faster growth rates and a more streamlined body shape consistent with the migration patterns of that population (larger, more streamlined body forms presumably improve swimming speed and energetics for longer migrations).

Historical tagging data (1964–2002) from regions surrounding the SU region support the hypothesis that straying is limited among regions. Overall, tag return rates were less than 2%, and

recaptures other than in the river of origin tended to occur in nearshore or estuarine environments, which would not necessarily be indicative of individuals entering rivers to spawn. Of the 18,717 recaptures of Atlantic salmon that were tagged in the outer Bay of Fundy, 111 were recaptured in nearshore or estuarine locations in the SU region, while 110 (0.6%) were recaptured within freshwater at fishways on three SU rivers: the LaHave River (6 tagged as adults; 69 tagged as smolts), East River (Sheet Harbour; 1 tagged as an adult), and Liscomb River (34 tagged as smolts). Similarly, of the 270 recaptures of Atlantic salmon that were tagged in eastern Cape Breton, only 16 were recaptured in the SU region and all were in coastal or estuarine locations. Furthermore, the timing of the recaptures suggests that these fish were not entering rivers to spawn, as 14 fish were recaptured within 3 months of release. If we assume that the individuals captured in the SU region during the year after release in Cape Breton were going to spawn, the estimated straying rate would be 0.7%; among these Atlantic salmon, all of the fish that were tagged as smolts were of hatchery origin and all of the fish that were tagged as adults were of unknown origin. Straying rates have been shown to be higher among salmon of hatchery origin (Quinn 1993), so we would expect straying rates for individuals of wild origin to be even lower than the rates reported here.

Although there is intraregional variation among SU populations (see Chaput et al. 2006), there are more general differences in run timing and maturation schedules between populations in the SU region and those in surrounding areas (DFO and MNRF 2008). For example, counts of Atlantic salmon ascending a fish ladder in the LaHave River (SU region) demonstrate the spring and summer run timing of this population. On average, 50.3% (range = 12.0–79.1%) of the run returns to the LaHave River before July 6, a pattern that is considered representative of other larger rivers in the region based on comparisons with recreational catch data. In contrast, recreational catch data indicate an autumn Atlantic salmon run in the Middle and Baddeck rivers, which are the best-studied populations of the Cape Breton Lowland and Highland regions. Over 90% of these annual runs occur during September and October (Canada Department of Fisheries and Oceans, unpublished data). Atlantic salmon in the inner Bay of Fundy region return to rivers almost exclusively in the fall (Amiro 2003), and Atlantic salmon in the nearby outer Bay of Fundy region (Figure 1) follow an intermediate strategy characterized by significant early and late-season runs. In contrast to the SU region, Atlantic salmon populations in the outer Bay of Fundy region typically have a lower rate of maturity after one winter at sea as well as a lower proportion of females in this sea-age class (Chaput et al. 2006).

In summary, the view that SU Atlantic salmon are a distinct and important component of the species' biodiversity is supported by the genetic and phylogenetic characteristics of SU Atlantic salmon, the minimal historical gene flow between populations in the SU region and populations in the surrounding regions, and the evidence for local adaptation to environmental conditions in the SU region. If SU populations become extirpated, subsequent recolonization of isolated rivers would be possible (Perrier et al. 2010), but the immigrants would differ genetically and phenotypically from the Atlantic salmon that are presently found in the SU region. As such, extirpation of SU Atlantic salmon would constitute an irreplaceable loss of biodiversity even if Atlantic salmon are able to recolonize the SU rivers. Additionally, recolonization in the foreseeable future appears to be unlikely given the current low abundance of Atlantic salmon in the surrounding regions (COSEWIC 2006b; Gibson and Bowlby 2009; Jones et al. 2010) and given the very low inter-regional straying rates calculated from the tagging data.

STATUS OF SOUTHERN UPLAND ATLANTIC SALMON: METHODS

Adult abundance trends for SU Atlantic salmon were evaluated by using count data from four populations—LaHave River, St. Mary's River, East River (Sheet Harbour), and Liscomb River—as well as recreational catch data from all rivers in the region. For the East (1970–2004, 2008), Liscomb (1979–1999), and LaHave (1970–2009) rivers, the data were counts of Atlantic salmon ascending fish ladders. The counts on the East and Liscomb rivers were conducted near the river mouths, whereas the LaHave River counts were made at the fishway at Morgans Falls, which provides access to 52% of the habitat in the watershed. The fish ladder counts reflect the total returns to each facility given that (1) the fish ladders provide access to habitat that is upstream of otherwise impassable barriers, (2) the facilities are operated for the entire duration of the spawning migrations (mid-spring to late fall), and (3) all Atlantic salmon ascending the ladders are captured in traps and are sampled to determine sex, fork length, weight, and age before being released upstream. The fish are marked prior to release to ensure that they are not double-counted if they move downstream and then ascend the ladder for a second time.

Abundance estimates for the St. Mary's River were obtained from mark–recapture seining experiments (Gibson et al. 2009). The St. Mary's River has two main branches; since 1997, escapement estimates have been calculated for the West Branch only. The experiments took place during the fall, when nearly all Atlantic salmon are thought to be in the river (those that recently entered the river could be identified by color and were rarely encountered during the experiments). The experiments were carried out by seining a set of pools, waiting 2 weeks or longer to allow for the mixing of marked and unmarked fish, and then seining a second time to determine the marked proportion of the population. A corrected Peterson estimate of adult abundance was then calculated from the mark–recapture data (Gibson et al. 2009). This population estimate is considered to be representative of spawner escapement in the West Branch of the St. Mary's River because the experiments were conducted after the recreational fisheries were closed for the year.

Abundance trends were analyzed by using least-squares linear regression after log transformation of the abundance data. Models were fitted to data for two time periods: (1) the most recent three generations (15 years), consistent with the time period used by COSEWIC when evaluating status; and (2) from the year with the maximum observed abundance to the present (i.e., 2008 or 2009). If data collections were terminated in an earlier year, the analyses were based only on the years for which data were available (i.e., declines were not extrapolated to the present).

Abundance in other rivers was assessed primarily by electrofishing and by monitoring the recreational fisheries. Catch and effort data from the annual recreational Atlantic salmon fishery have been collected since 1983 by using a fishing license stub return program. After the close of the fishing season, anglers return their license stubs, on which they have recorded their Atlantic salmon fishing dates, locations, and catch. Large (≥63 cm fork length) and small (<63 cm fork length) Atlantic salmon are recorded separately. Small Atlantic salmon typically have matured after one winter at sea, whereas large fish typically have matured after two or more winters at sea or have spawned previously. The catch was corrected for nonreporting by using a regression developed from the change in reported catch resulting from sending multiple reminder letters to license holders (i.e., to increase the number of returned license stubs). Recreational fishing seasons for Atlantic salmon in the SU region are managed on a river-specific basis; thus, depending on population status, fishing may be open on some rivers but closed on others. Catch-and-release regulations were implemented throughout the region: first for large Atlantic salmon in nearly all rivers by 1984, and then for both large and small Atlantic salmon in nearly all rivers by 1999. In 2010, all rivers in the SU region were closed to Atlantic salmon fishing. In this paper, the corrected catch of large and small Atlantic salmon, instead of catch per unit effort (CPUE), is presented as an abundance index because very little effort occurs in rivers where abundance is very low and because these fish are quite easily targeted when they occupy staging pools in the river, creating the potential for hyperstability in the CPUE index (Hilborn and Walters 1992). Our approach is consistent with the results of Dempson et al. (2004), who found statistically significant relationships between the catch of Atlantic salmon and fishery-independent abundance indices—but not between CPUE and the same abundance indices—for rivers in Newfoundland, Canada, after the closure of the commercial fisheries.

Regionwide electrofishing surveys of SU Atlantic salmon juvenile abundance were conducted in 2000 and again in 2008–2009. In the latter survey, all but three of the rivers were sampled in 2008. Marginally more sites were completed in 2008–2009 than in 2000 (151 versus 128), and two more rivers were visited (54 in 2008–2009; 52 in 2000). Site size averaged 914 m^2 (range = 65–4,600 m^2), and the shape of the site varied depending on the size of the river and the site's location within the river. The mean length and width of the sites were 89 and 10 m, respectively. Total effort was greater in 2008–2009 than in 2000 (electroshocking duration: 150,827 s versus 104,331 s), but the total area surveyed was lower (107,639 m^2 in 2008–2009; 128,841 m^2 in 2000). When possible, the same sites were electrofished in both surveys ($n = 81$), and the average electroshocking duration at each site was similar (966 s in 2000 versus 1,052 s in 2008–2009). Abundance was low enough at most sites to preclude site-specific abundance estimation by either mark–recapture or depletion methods, so a mean catchability (0.428) was used to estimate fish abundance at all sites based on a meta-analysis of electrofishing catchability (Gibson et al. 2003).

To provide a basis for interpreting the juvenile density estimates, we compared the juvenile densities reported herein with reference values known as "Elson's norm." Elson (1967) studied the effects of DDT spraying on wild Atlantic salmon and reported the typical densities of Atlantic salmon in unsprayed rivers. These Elson's norm values have subsequently been used as reference levels against which juvenile densities have been compared (e.g., COSEWIC 2006b; DFO and MRNF 2008). Elson (1967) reported "normal" densities of 24 fry/100 yd^2 and 32 parr/100 yd^2; we converted these values to 29 fry/100 m^2 and 38 parr/100 m^2 for consistency with the units of area used in the present study.

RESULTS

All of the time series of adult Atlantic salmon abundance showed marked declines (Figure 2). Counts of Atlantic salmon in the LaHave River (above Morgans Falls) peaked at 3,969 fish in 1988, compared with 221 fish in 2009—the lowest value since 1973, the fourth year of operation of the fishway. The LaHave River population was estimated to have declined by 68% over the last 15 years and by 88% from maximum estimated abundance (Figure 2). During the most recent 5 years for which data were available, the abundance of Atlantic salmon in the Liscomb River was estimated to have declined by 98% (Figure 2). At peak abundance, over 1,600 wild Atlantic salmon returned to this river (Gibson et al. 2009), and abundance has since declined by more than 99%. Abundances in the LaHave and Liscomb rivers initially increased due to colonization of upstream habitat after fish passage facilities were constructed in conjunction with population enhancement activities, including stocking. These initial population increases represent the colonization of new habitat at a time when marine survival and acidity conditions were favorable for population growth (see Discussion). For the East River, abundance was estimated to have declined 93% over the last three generations and 99% from the maximum observed abundance (Figure 2). In the West Branch of the St. Mary's River, the adult population was estimated to have declined by 83% during the last 15 years (Figure 2). Abundance is thought to have been higher in the mid-1980s than in 1997, when the West Branch time series began. For example, based on an analysis of the recreational catch for 1986, Atlantic salmon abundance in the entire river (West Branch contains 55% of the

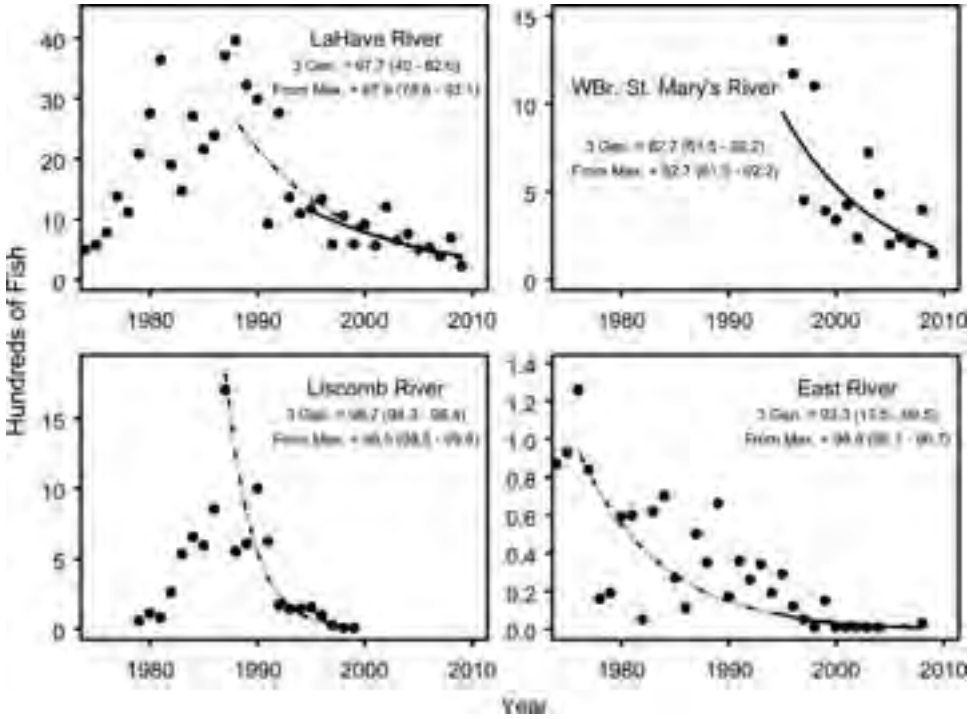

FIGURE 2. Abundance trends for Atlantic salmon populations in four rivers within the Southern Upland region of Nova Scotia. Data points show spawning escapement estimates based on counts of adults ascending fish ladders (LaHave, Liscomb, and East rivers) or based on mark–recapture experiments (West Branch [WBr.] of the St. Mary's River). Percent decline in abundance (with 95% confidence interval in parentheses) estimated with a log-linear model is provided for two time periods: the last three generations (15 years; solid line); and from the maximum observed abundance to the most recent year of data collection (dashed line). For data series that did not extend to the present, percent decline is calculated only based on the time period for which data are available (e.g., 5 years for the Liscomb River over the most recent three generations).

available habitat) was estimated at more than 8,000 fish (O'Neil et al. 1998).

Higher past abundances and subsequent abundance declines during the 1980s and 1990s are evident in the Atlantic salmon recreational catch data for 48 rivers in the SU region. During 1983–2008 (years with estimates from license stub returns), the total recreational catch peaked in 1986 at 9,534 fish (6,324 small fish; 3,210 large fish). By 1995, total recreational catch had dropped to 2,496 fish (1,846 small fish; 650 large fish); in 2008, the recreational catch was 342 fish (251 small fish; 91 large fish). However, the 2008 value is not directly comparable with the other estimates because the fisheries in most rivers were closed by 2008. Nevertheless, the declining trends in recreational catch for the 16 SU rivers with the largest catches (Figure 3) occurred while the fisheries were open, thus indicating that the closure of river-specific fisheries was not solely responsible for the decline in catch.

The regionwide electrofishing surveys in 2000 and 2008–2009 allowed for a comparison of juvenile Atlantic salmon abundance. At a regional level, the number of juveniles captured during the 2008–2009 survey (1,019 fish) was just over one-quarter of the number captured in 2000 (3,733 fish). During the 2000 survey, juvenile Atlantic salmon were found in 54% of the rivers (28 of 52), but during 2008–2009 juveniles were found in only 39% of the rivers (21 of 54; Figure 4). There were no instances in which Atlantic salmon were found in a river during 2008–2009 where they had not also been found during 2000. Of the sites surveyed during both sampling periods ($n = 81$), total juvenile density decreased in 44% ($n = 36$) and increased in 7% ($n = 6$) between 2000 and 2008–2009. The remainder of the sites ($n = 39$) had densities of zero in both sampling periods. Any increase in age-0 fry density was relatively small, whereas the declines were significantly larger. A one-tailed Wilcoxon's signed rank test on paired site data (including only sites at which Atlantic salmon had been found during at least one of the surveys) indicated that there was a near-zero probability that juvenile densities were the same in both surveys. During the 2008–2009 survey, juvenile Atlantic salmon were not found at nine sites (four rivers) where they were present in 2000. In general, mean juvenile densities in both surveys were much lower than the Elson's norm values of 29 fry/100 m^2 and 38 parr/100 m^2 (Figure 4).

DISCUSSION

The available data strongly support the view that some populations of SU Atlantic salmon are extirpated and that the largest populations are at very low abundance levels and continue to

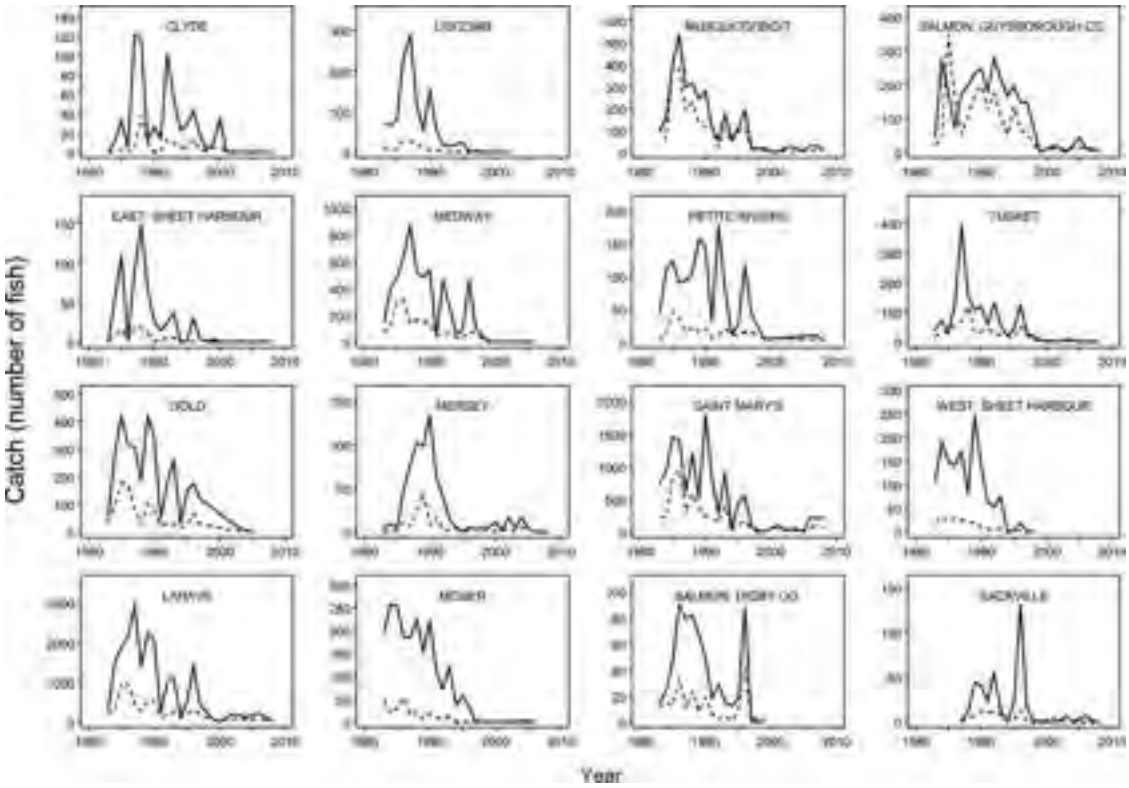

FIGURE 3. Estimated recreational catch of small (<63 cm fork length; solid line) and large (≥63 cm; dashed line) Atlantic salmon from 1983 to 2008 in the 16 Southern Upland rivers with the largest catches. Catch was estimated from fishing license stub returns corrected for nonreporting and is shown for each river and year with an open recreational fishery season.

decline. This conclusion is consistent with adult abundance trends from adult monitoring and recreational catch data and with the regionwide assessments of juvenile density. Given that juvenile density is significantly below reference values for Atlantic salmon populations in productive habitat (DFO and MRNF 2008), the low density is likely indicative of low total abundance. Such small populations are vulnerable to environmental and demographic stochasticity as well as to declining fitness caused by genetic effects, including inbreeding and loss of genetic variation (Frankham 2008).

Annual time series of juvenile abundance were available for the St. Mary's River and LaHave River Atlantic salmon populations. These series indicated that despite large declines in adult abundance, juvenile densities in the St. Mary's and LaHave rivers have remained relatively stable over the previous 15 years (nonsignificant estimated declines of 4% and 1%, respectively), although juvenile abundance was higher in the more distant past (Gibson et al. 2009). However, because of the stabilizing effect of density dependence in freshwater, large changes in adult abundance can take place before juvenile density begins to decline (Gibson et al. 2008b). During the 2008–2009 electrofishing survey, mean juvenile densities in the St. Mary's and LaHave rivers were the second- and third-highest values recorded (Figure 4), which suggests that these populations are among the largest (on a per-unit-area basis) in the region. Given that juvenile abundance has remained relatively stable, spawner abundance in the St. Mary's and LaHave River populations is apparently sufficient to maintain juvenile production. In contrast, juvenile densities in the majority of rivers were lower in 2008–2009 than in 2000, indicating that adult abundance in most rivers is low enough to impair juvenile production.

Within the SU region, some threats to persistence are relatively well understood, whereas others are less so. Sulfate deposition in the form of acid rain has lowered the pH of many SU rivers to the point that they may no longer be able to support viable Atlantic salmon populations. Watt (1987) classified 60 rivers in the SU region based on mean annual pH and suggested that the 13 rivers with pH levels below 4.7 would not be able to support Atlantic salmon populations. The 18 rivers with pH levels between 4.7 and 5.0 were considered to be partially impacted and able to support remnant populations, primarily in localized tributaries with higher pH (Watt 1987). The impact of acidification on 13 rivers with pH between 5.1 and 5.4 was considered to be low, and 16 rivers were considered to have no impacts from acidification. A stochastic, life-history-based population model designed to assess the impacts of acidification on Atlantic salmon populations (Korman et al. 1994) was used

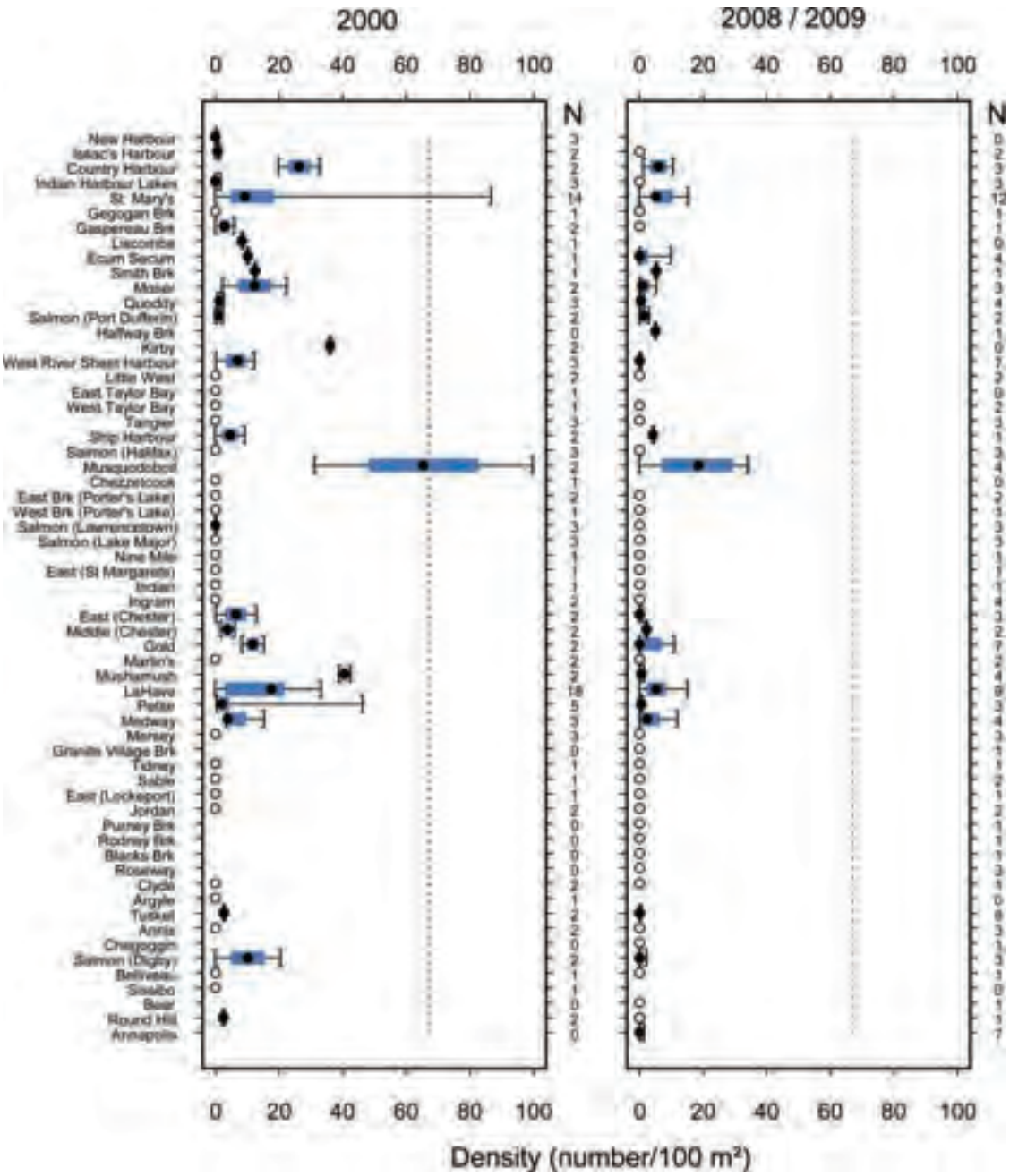

FIGURE 4. Box-and-whisker plots showing the combined density of Atlantic salmon fry and parr in Southern Upland rivers based on electrofishing surveys conducted during 2000 and 2008–2009 (black shaded circle = median density; box = interquartile range; open circle = zero density at sites on the indicated watershed; whiskers = minimum and maximum; N = number of sites that were electrofished in each river; Brk = Brook). For the 2008–2009 survey, all but three rivers (Indian Harbour Lakes, Country Harbour, and Isaac's Harbour rivers) were surveyed in 2008. The dotted line indicates Elson's norm (Elson 1967), a reference level used for assessing the status of juvenile salmon populations. [Figure available in color online.]

by Amiro (2000) to evaluate population viability in the SU region by considering the effects of both acidification and low at-sea survival. Amiro (2000) concluded that Atlantic salmon populations in 40 of the 47 rivers included in his analyses would become extirpated if at-sea survival rates were less than 5%. The adult trends and juvenile surveys presented here are consistent with the conclusions made by both Watt (1987) and Amiro (2000). Although sulfate deposition was significantly reduced in the 1990s, the low buffering capacity of rivers in the SU region prevents the natural restoration of higher pH levels (Clair et al. 2004); therefore, acidification remains an ongoing threat, lowering the productive potential of affected rivers. The continuous

addition of lime (with a doser) can increase the pH of an acid-impacted river downstream of the lime doser installation site and has successfully increased juvenile Atlantic salmon production in European and North American rivers (Clair and Hindar 2005).

Less well understood are the factors that have led to a reduction in at-sea survival, which is one of the most serious issues affecting Atlantic salmon populations in eastern North America (DFO and MNRF 2008). Low at-sea survival can limit population growth rates and the effectiveness of recovery actions that are focused only on the freshwater environment. At-sea survival is typically monitored by calculating a return rate, which is the ratio of returning adults to emigrating smolts for a given river. In inner Bay of Fundy Atlantic salmon populations, for which return rates are about 0.2%, survival at sea is so low that freshwater productivity cannot be increased to a point where populations would become viable (Gibson et al. 2008a). In contrast, return rates in the SU region have also declined but remain roughly an order of magnitude higher than return rates for inner Bay of Fundy populations, such that small equilibrium population sizes are possible in productive habitat within the SU region. Thus, recovery actions that focus on freshwater habitat in the SU region (e.g., lime addition or habitat restoration) are expected to increase population viability but not to restore populations to sizes above their conservation reference levels (Gibson et al. 2008b).

Many of the SU rivers have been stocked with Atlantic salmon at least intermittently since the late 1800s, and three of the four rivers we used in the analysis of adult counts were stocked for fisheries enhancement during the 1980s and 1990s. The question of whether stocking programs are beneficial or whether they may actually harm the populations remains a controversial issue in salmonid conservation (Myers et al. 2004; Fraser 2008). Stocking has the potential to mask declines in wild abundance as well as the effects of habitat degradation, and may lead to fitness losses in the wild (Araki et al. 2007; Thériault et al. 2011). Most of the fishery enhancement programs in the SU region were phased out in the late 1990s as populations continued to decline and as the effects of stocking—particularly for small, declining populations—became better understood. However, at present, the overall effect of these stocking programs on the abundance trajectories of SU Atlantic salmon is not known.

Currently, inner Bay of Fundy Atlantic salmon are the only anadromous Atlantic salmon population assemblage that is listed for protection (i.e., Schedule 1) under Canada's Species at Risk Act. Actions to prevent the extinction of the inner Bay of Fundy assemblage are primarily focused on a captive rearing program for maintaining a few small populations, thereby preserving the potential for population rebuilding if at-sea survival improves. If current abundance trends for SU Atlantic salmon continue, timely recovery actions will be required to avoid the need for a similar life support system and to prevent the extinction of these unique populations.

ACKNOWLEDGMENTS

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RESEARCH ARTICLE Open Access

Atlantic salmon populations invaded by farmed escapees: quantifying genetic introgression with a Bayesian approach and SNPs

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Abstract

Background: Many native Atlantic salmon populations have been invaded by domesticated escapees for three decades or longer. However, thus far, the cumulative level of gene flow that has occurred from farmed to wild salmon has not been reported for any native Atlantic salmon population. The aim of the present study was to investigate temporal genetic stability in native populations, and, quantify gene flow from farmed salmon that caused genetic changes where they were observed. This was achieved by genotyping historical and contemporary samples from 20 populations covering all of Norway with recently identified single nucleotide polymorphism markers that are collectively diagnostic for farmed and wild salmon. These analyses were combined with analysis of farmed salmon and implementation of Approximate Bayesian computation based simulations.

Results: Five of the populations displayed statistically significant temporal genetic changes. All five of these populations became more similar to a pool of farmed fish with time, strongly suggesting introgression of farmed fish as the primary cause. The remaining 15 populations displayed weak or non significant temporal genetic changes. Estimated introgression of farmed fish ranged from 2 47% per population using approximate Bayesian computation. Thus, some populations exhibited high degrees of farmed salmon introgression while others were more or less unaffected. The observed frequency of escapees in each population was moderately correlated with estimated introgression per population $R^2 = 0.47$ $P < 0.001$. Genetic isolation by distance existed within the historical and contemporary data sets, however, the among population level of divergence decreased with time.

Conclusions: This is the first study to quantify cumulative introgression of farmed salmon in any native Atlantic salmon population. The estimations demonstrate that the level of introgression has been population specific, and that the level of introgression is not solely predicted by the frequency of escapees observed in the population. However, some populations have been strongly admixed with farmed salmon, and these data provide policy makers with unique information to address this situation.

Keywords: Admixture, Aquaculture, Environmental impact, Escapees, Simulation, ABC, Fish farming, Migration

Background

Aquaculture production of Atlantic salmon (*Salmo salar* L.) was started in Norway in the early 1970's, and now represents a globally significant industry. Each year, hundreds of thousands of farmed salmon escape into the wild [1]. Some of these escapees enter rivers inhabited by native populations [2-4], outnumbering wild conspecifics on the spawning grounds of some rivers in some years [5]. The Atlantic salmon is characterized by highly significant population genetic structuring [6,7]. This reflects evolutionary relationships among populations [8-10], including the potential for adaptive differences [11,12]. Consequently, the large-scale invasion of Atlantic salmon populations by domesticated farmed escapees represents one of the most striking examples of human-mediated increased straying rates for any organism. This has raised global concerns for the fitness of native populations [13-15].

Genetic changes in native Atlantic salmon populations as a result of introgression of farmed escapees have been observed in populations in Ireland [16-19], North America

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[20], and Norway [21,22]. In the most extensive of these studies, six of 21 Norwegian populations investigated displayed significant temporal genetic changes. Based upon several genetic parameters, the authors concluded that the observed changes were primarily driven by introgression of escapees. However, in none of the above-mentioned studies has the accumulated level of introgression, i.e., "admixture", been quantified in a native population. From a management perspective, this is important, if not essential, in order to understand the extent of the problems, and ultimately implement guidelines via the process of risk assessment [23].

Where gene flow arises from a single and definable population or hatchery strain, statistical parameters such as individual-based admixture can be computed to estimate the level of introgression and degree of remaining wild population e.g., [24,25]. Even in cases of low numbers of populations, it is also possible to infer admixture using a combination of molecular genetic data on real samples in addition to simulations [26,27]. However, the quantification of genetic introgression of farmed Atlantic salmon into native Norwegian populations represents a more complicated situation than one in which a single or a low number of populations are exchanging genes among themselves [28]. This is because of several factors which are addressed briefly below.

The commercial production of Atlantic salmon in Norway is based upon rearing fish from multiple domesticated strains that were initially founded on wild salmon from more than 40 Norwegian rivers in the 1970´s [29]. These domesticated strains have remained genetically isolated from wild salmon since. As a result of founder effects and genetic drift, there are highly significant differences in microsatellite allele frequencies among these farmed strains [30]. Thus, microsatellites provide enough information to distinguish some farmed strains and wild populations in a pair-wise manner [30]. However, the allele frequencies of microsatellites [30] and SNPs [31] display overlap between the farmed strains and wild populations when looking across multiple strains and populations simultaneously. Over time, escapees originate from multiple farms. As a result, the accumulated genetic change in the native population, due to introgression of this pool of farmed salmon, becomes very complicated to quantify, and is potentially underestimated [28]. Adding further to the complexity of this situation is the fact that the domesticated strains have changed greatly over time. Some strains have been terminated, while others have been mixed. Thus, when these points are taken together with the fact that there is non-random distribution of genetic material from the breeding companies to the production farms [32] where the majority of the farmed salmon are held and thus the majority of escapees originate from, it is impossible to accurately reconstruct the allele frequencies of the farmed strains used in Norway in the three to four decade period in which escapees have been observed on the spawning grounds.

A recent genome scan using a 7 k chip identified a set of SNPs that are collectively diagnostic in identifying Norwegian wild and farmed salmon, regardless of their population of origin [31]. These collectively diagnostic markers have the potential to circumvent some of the challenges described above to quantify introgression of farmed salmon in native Norwegian populations. At the same time, statistical approaches such as Approximate Bayesian Computation (ABC) [33] have been used to quantify complex models in population and evolutionary biology [34-36]. The present study aimed to take advantage of these recently discovered genetic markers and genotype a set of historical and contemporary samples from 20 Norwegian salmon populations that have displayed varying level of farmed escapees on the spawning grounds over the past 2–3 decades. In addition, a pool of farmed salmon was genotyped in order to investigate the direction of any observed temporal genetic changes in the wild populations. Finally, ABC and fixed migration simulation methods were implemented in order to attempt to quantify the level of cumulative introgression of farmed salmon in native populations for the first time.

Methods

Wild salmon samples

Samples of wild salmon were collected from a total of 20 rivers spanning the entire coastline of Norway (Figure 1). Each river was represented by a historical sample that was collected prior to or in the early to moderate stages of the development of the commercial aquaculture industry in Norway, in addition to a contemporary sample that was collected in the period 2000–2009. Most of these samples were based upon fish scales taken from adult fish captured within each river (Table 1). The historical adult samples were primarily collected by the Norwegian Atlantic salmon gene bank. These samples were taken from multiple locations and years within each river to ensure a representative sample of each population. Of the contemporary adult samples, the majority were taken in association with recreational angling. These samples form the basis of the national monitoring program for estimating the frequencies of escapees within Norwegian rivers [1,4,5]. Where angling was the primary sampling method, samples were collected from multiple years and locations within each river in order to ensure the samples were as representative for the populations as possible. A few of the river samples were represented by juveniles (parr) collected by electrofishing (Table 1). These samples were collected in multiple locations within each

Figure 1 Location of the 20 rivers upon which the study is based.

river, and consisted of fish of varying age in order to ensure representative sampling. Due to the fact that the samples upon which this project were based were captured by recreational angling or in association with other previous research projects and monitoring programs (and subsequently donated to this study), no specific licenses were required for this specific study.

Prior to isolation of DNA, all scale samples were first examined for growth patterns in order to exclude any potential farmed salmon that had escaped from a commercial fish farm, using established methods [37]. The habitat and demographic data for the populations included in the present study, including numbers of escapees observed, are presented online (Additional file 1: Table S1).

Farmed salmon samples

A total of 375 farmed salmon were analysed in this study. These fish were collected in the period 2005–2010 from 49 separate sources. These included 48 marine cages (approx. 8 salmon per cage) located on 35 commercial farms spanning from the south to the far north of Norway, in addition to eight escapees captured in the sea. These samples were picked from approximately 6000 farmed fish that had been previously genotyped with microsatellites in association with a forensic service conducted by the Institute of Marine Research to identify the farm of origin for escaped salmon for the legal authorities [32,38-41]. The aim of this sampling strategy was to generate a pool of farmed fish representing the genetic diversity of farmed fish in Norway.

Genotyping

DNA was extracted in 96-well format using Qiagen DNeasy blood and tissue kit. Each plate contained two or more negative control wells. DNA aliquots of these samples were sent to the Centre for Integrative Genetics (CIGENE) in Norway for SNP (n = 99) analysis using a Sequenom platform. A list of the markers, their NCBI assay details and linkage map positions are available (Additional file 2: Table S2).

Seventy of the SNP markers genotyped here were selected from the panel of SNPs that have been suggested to be collectively diagnostic for farmed and wild Norwegian salmon [31]. These 70 SNPs include the ten top ranking loci, 54 of the 60 top ranking loci, and a further 16 selected from the top 200 ranking loci (these were selected in order to create working Sequenom assays Additional file 2: Table S2). As the collectively diagnostic loci identified by Karlsson et al. (2011) were ranked based upon their F_{ST} between a pool of farmed and pool of wild Atlantic salmon, and the sequential

Table 1 Numbers and types of samples, including some population genetics summary statistics for the 20 Atlantic salmon rivers

Population	N	uH_E	HWE	LD	Sample type	Population	N	uH_E	HWE	LD	Sample type
Neiden H (1979 82)	70	0.35	2	130	AD	Ørsta H (1986 89)	38	0.38	2	98	AD
Neiden C (2009)	77	0.36	5	112	AD	Ørsta C (2006 08)	31	0.36	4	130	AD
V. Jakobselva H (1989 91)	92	0.35	5	243	AD	GaulaSF H (1987 93)	35	0.36	0	120	AD
V. Jakobselva C (2007 08)	96	0.37	2	183	AD	GaulaSF C (2006 08)	82	0.36	2	131	AD
Alta H (1988 90)	39	0.34	1	85	AD	Lærdalselva H (1973)	90	0.36	1	125	AD
Alta C (2005 2007)	63	0.34	2	102	P	Lærdalselva C (2005 08)	45	0.36	1	120	AD
Reisa H (1986 91)	44	0.35	4	101	AD	Vosso H (1980)	45	0.34	0	98	AD
Reisa C (2006)	55	0.35	1	136	P	Vosso C (2007 08)	43	0.36	0	138	SM
Målselva H (1986 88)	39	0.35	3	102	AD	Loneelva H (1986 93)	59	0.34	0	136	AD
Målselva C (2008)	30	0.36	1	111	P	Loneelva C (2001 07)	50	0.36	3	134	AD
Roksdalsvassdraget H (1987 93)	31	0.37	0	110	AD	Opo H (1971 73)	60	0.35	3	116	AD
Roksdalsvassdraget C (2008)	89	0.37	3	128	AD	Opo C (2010)	61	0.36	3	180	P
Namsen H (1977)	74	0.36	0	129	AD	Etne H (1983)	72	0.35	1	121	AD
Namsen C (2008)	89	0.37	1	140	AD	Etne C (2006 2008)	83	0.36	1	122	AD
Surna H (1986 89)	23	0.36	2	90	AD	Figgjo H (1972 75)	51	0.35	1	118	AD
Surna C (2005 08)	45	0.37	4	122	AD	Figgjo C (2006)	71	0.36	1	119	AD
Eira H (1986 94)	31	0.36	2	108	AD	Numedalslågen H (1989 93)	42	0.35	1	89	AD
Eira C (2005 2008)	40	0.35	1	123	AD	Numedalslågen C (2007 08)	68	0.36	3	132	AD
Bondalselva H (1986 88)	39	0.37	3	103	AD	Berbyelva H (1988 93)	44	0.33	1	132	AD
Bondalselva C (2007)	13	0.36	1	70	P	Berbyelva C (2007 08)	87	0.33	5	139	AD
						Farmed pool (2005 2010)	375	0.37	17	491	FA

Rivers are sorted north to south.
Population = name of river with postscript letter H = historical sample, C = contemporary sample. N = number of fish included in the genetic analyses, uHE = unbiased heterozygosity, HWE = number of deviations from Hardy Weinberg Equilibrium $P < 0.05$ (over the total of 72 loci), LD = number of times that linkage disequilibrium was observed between pairs of loci within any single sample at $P < 0.05$ (from a total of 72 loci = 2556 pair wise combinations), Sample type = AD adults captured within river by angling, P parr, SM smolt, FA = taken from farm. Methods used to compute these summary statistics are detailed in the Methods section, while more extensive population genetic summary statistics per population are presented in Additional file 3: Table S3.

difference in F_{ST} between each locus was very small, it is not expected there is any specific combination of loci required to create the genetic signal permitting identification of farmed and wild salmon. However, the ability of the collectively diagnostic markers used in the present study to differentiate farmed and wild salmon has been empirically evaluated here (see Results). It is furthermore important to note that seven of the wild populations used in the marker identification study conducted by Karlsson et al. (2011) overlap with populations in the present study (Alta, Namsen, Surna, Lærdalselva, Vosso, Figgjo, Numedalslågen). While this can potentially cause ascertainment bias, this has been considered when interpreting results. In addition to the 70 diagnostic SNPs, a further 29 SNPs were also genotyped. These were selected as putatively neutral SNPs that are known to be polymorphic in Norwegian salmon.

Statistical data analysis

In order to investigate temporal genetic stability in the 20 populations, and the direction of any potential changes, the data set was organized into the historical and contemporary samples. The pool of farmed salmon was only used for specific tests and the simulations to quantify gene flow (see below). For all computations, the data sets were divided into the loci that are collectively diagnostic between farmed and wild Atlantic salmon and the randomly selected loci.

Genotypic data was first organized in the program MSA, coding the nucleotides A, C, G and T as alleles 1–4 [42]. MSA was used to compute F_{ST} values (global and pair-wise) and compute significance levels associated with these tests using the Fisher's exact method as implemented in the program. F_{ST} values were all computed using the Weir and Cockerham estimator [43]. Confidence intervals associated with the global F_{ST} values for the historical and contemporary sets of samples were computed from the distribution of 1000 F_{ST} values calculated from 1000 bootstraps where 35% of the individuals from each population were randomly re-sampled. This latter test was computed in the program R (R development team).

Population-specific summary statistics, i.e., numbers of alleles, heterozygosities, numbers of deviations from Hardy Weinberg Equilibrium (HWE), and the numbers of times that linkage disequilibrium (LD) was observed between pairs of loci within each sample were computed in the program GENALEX v6. [44] using program default parameters for these tests. Where appropriate, statistical significance levels were tested against $P < 0.05$, $P < 0.001$ and Bonferroni corrected threshold levels.

GENALEX v6. was also used to create principal component analysis plots (PCA) based upon a matrix of F_{ST} values. This was conducted using the program's default values in order to investigate spatio-temporal population genetic structure in addition to the direction of any observed temporal changes in relation to the pool of farmed fish. Isolation by Distance (IBD) analyses on the historical and contemporary set of samples was conducted using the Mantel test as implemented in the R package "Vegan". The test was computed with input data from a matrix of pair-wise F_{ST} values, and, the pair-wise distances between river mouths in kilometers.

Genetic assignment tests, using direct assignment and exclusion and different combinations of samples and loci sets, were conducted in the program GENECLASS2 [45], using a specific algorithm for the computations [46] and probabilities of $P < 0.05$ and $P < 0.001$ for exclusion. Bayesian clustering analysis was computed in the program STRUCTURE [47,48]. This was used to look at temporal genetic changes within each wild population one at a time by including the historical and contemporary sample. Analysis parameters included an admixture model, correlated allele frequencies, and assuming no population prior. Each analysis with this program consisted of 5 replicate runs for K = 1-5, each with a burn-in of 250 000 replications, and a run length of 500 000 Markov chain Monte Carlo (MCMC) iterations.

In order to investigate the statistical power of the different sets of genetic markers implemented here, several tests were computed. First, the distribution of pair-wise F_{ST} between two groups of 100 randomly selected fish from within each of the groups (wild and farmed) was plotted following 1000 bootstraps. This was conducted for farmed vs. wild, wild vs. wild and farmed vs. farmed using the diagnostic markers and the randomly selected markers. Next, the assignment power of these sets of loci was examined in STRUCTURE. Due to the fact that there were more diagnostic loci than randomly selected loci, and that assignment power is influenced by the number of loci, a sub-set of the diagnostic loci (equal to the number of randomly selected loci) was also used to compute these assignment tests. The following routine was repeated 1000 times for each set of SNPs: 100 farmed fish were sampled as the learning farmed sample, 100 wild fish among all populations were sampled as the learning wild samples, then one fish was sampled randomly to constitute the unknown individual to identify. STRUCTURE was then run with 50 000 burn-in and 500 000 iterations, at K = 2, using population information and population flag, so that population information is only used for the learning samples, but not for the unknown individual. The accuracy of assignment of this individual to either the farmed or the wild salmon groups was compared for the different sets of SNPs using the threshold probability of 0.6. The re-sampling simulations were performed in R, and the STRUCTURE runs with ParallelStructure R package (https://r-forge.r-project.org/projects/parallstructure/) [49].

Simulations to quantify gene flow

Two alternative methods were employed to quantify the amount of gene flow that would be required from an alien population to cause the observed temporal genetic change within each wild population, computed as F_{ST} between each wild population's historical and contemporary sample. The simulations were coded and executed in R using previously published scripts to simulate realistic genetic introgression using gametes sampled randomly from the donating and recipient populations [28]. Average generation time for each wild population was set to five years, thus, the number of generations used to simulate gene flow was set as the number of years between the historical and contemporary sample for each population, divided by five. An effective population size (N_E) of 200 for all populations except those displaying an N_E less than this in which case the observed N_E, as reported previously [21], using a one sample linkage disequilibrium based method implemented in LDNE 1.31 software [50], was used.

First, the posterior point estimate of migration rate (M) was inferred by an ABC algorithm [33]. The routine to quantify this gene flow consisted of the following steps: 1. Determine a prior distribution for migration rate M (e.g., $M \sim N(0.1,0.1)$). 2. Simulate n scenario of introgression where the value of M is sampled from the prior distribution, and compute the F_{ST} between historical and each simulated population. 3. Calculate the vector s of the n differences between simulated and observed F_{ST}: s: $(s_1, s_2, \ldots s_n)$ where $s_i = (F_{STO} - F_{STi})$. 4. Solve the linear regression: $M = \alpha + \beta s + \varepsilon$ (Where ε is a vector of residuals, s is the vector estimated in step 3, and α is a constant). The estimate of α gives the expected value of M when $F_{STO} - F_{STi} = 0$. 5. Update the prior distribution of M with estimated distribution of α. 6. Repeat step 2–5 until α converges to a stable estimate.

To account for the standard deviation of the observed F_{ST} between historical and contemporary sample in the estimation of M, the posterior distribution of M was also estimated with an alternative "fixed migration rate" approach that consisted of the following steps: 1. Simulating genetic introgression from alien population with a fixed

migration rate M per generation. Tested values of M were from 1 to 80%, every 1% between 1 and 20, and every 5% between 20 and 80%. 2. From the set of simulated populations (each corresponding to a value of $M = 1, 2, 3, 4\ldots$ 80% migration), selecting 1000 among those that gave an F_{ST} that fit within the 95% confidence interval of the observed F_{ST}. 3. Mean and standard deviation of migration rate from the 1000 selected scenario provide a posterior distribution of (M).

For both ABC and fixed migration rate approach, two alternative scenarios were tested. First, the alien population considered for the simulations was a pool of farmed salmon, and second, to account for possible straying, the alien population was the historical population of the geographically nearest neighbour river. The R package ADEgenet [51] was used to generate PCA plots of historical, contemporary and simulated populations from each scenario based on individual genotypes.

Results

Data quality

Loci that displayed technically unreliable genotype clustering, or a genotyping coverage <90% in the entire data set, were excluded prior to all statistical analyses. This stringent quality control reduced the total number of loci from 99 to 72. Among the remaining loci were 47 SNPs selected from the collectively diagnostic panel for farmed and wild salmon (including the top ten ranked loci, and 35 of the 60 highest ranking loci) [31], and a set of 25 random SNPs. These panels are hereafter referred to as 47d (diagnostic) and 25r (random). Individual fish displaying genotyping coverage <75% over the remaining 72 loci were also removed from the data set (301 fish removed from 2912 samples). Thus, the final data set for analysis consisted of 2611 individuals genotyped for 72 loci. Within this, a total of 364 178 alleles were successfully scored, giving an overall genotyping coverage of 97%.

Within population summary statistics

A range of population genetics parameters are summarized for all wild populations and the pool of farmed salmon (Table 1; Additional file 3: Table S3). The unbiased expected heterozygosity (UH_E) over all 72 loci was very even among wild populations, ranging from a low of 0.33 for both the historical and contemporary samples for Berbyelva, to a high of 0.38 for the historical sample representing the river Ørstadelva. None of the populations displayed clear increases or decreases in this parameter between their historical and contemporary samples.

The parameters HWE and LD have the ability to indicate disturbances in populations due to introgression of genetically distinct fish. At the significance level $P < 0.05$, no wild salmon sample displayed more than five deviations from HWE across the 72 loci. This is the number of observations that are more or less expected by chance at this significance level. From a total of 2556 comparisons within each sample (72 loci pair-wise) at $P < 0.05$, LD was observed from a low of 70 times (2.6%) in Bondalselva contemporary sample, to a high of 243 (9.2%) in the historical Vestre Jakobselva sample. The pool of farmed salmon displayed similar summary statistic values to the wild populations, although increased frequency of HWE and LD was observed in this sample due to it being a mixture of fish from multiple sources (Table 1; Additional file 3: Table S3).

The effective population size for each wild population is presented (Additional file 4: Table S4). Most of the samples and populations displayed N_E 100-1000+. Notable examples of low N_E were Vestre Jakobselva (98), Bondalselva (25), Ørstaelva (94), and Opo (57) for the historical samples, and Vestre Jakobselva (75) and Berbyelva (67) for the contemporary samples. The upper and lower 95% confidence intervals surrounding all estimates were large however, with the upper boundary often reaching infinity.

Comparison between 47d and 25r

In order to test the statistical characteristics of the putatively diagnostic loci 47d vs. the randomly selected loci 25r, several comparisons were conducted. The number of loci displaying non-overlapping allele frequencies between the pool of farmed salmon and all 20 historical wild salmon samples separately was 15 for 47d (32%) and 3 for 25r (12%) (Additional file 3: Table S3). Thus, while 32% of the loci from 47d displayed non-overlapping allele frequencies, the majority did not. Of these non-overlapping loci, some displayed moderately strong differentiating frequencies but not all. Based upon the distribution of the F_{ST} values between 100 farmed and 100 wild salmon (historical samples) randomly re-sampled from the data set 1000 times, 47d displayed approximately double F_{ST} than 25r (Additional file 5: Figure S1).

Genetic assignment values as computed in STRUCTURE revealed that assignment to correct source (farmed pool or wild pool) was higher for 47d than 25 randomly re-sampled loci from 47d ("25resam"), and from 25r (Correctly assigned wild: 69, 63, and 23%; correctly assigned farmed: 82, 58, and 30% when using 47d, "25resam" and 25r respectively). Together, these tests demonstrate that the diagnostic loci contain far greater statistical power to differentiate farmed and wild salmon than the randomly selected loci.

Temporal F_{ST} changes

Over the full set of 72 loci, the number of populations displaying significant temporal genetic changes at $P < 0.05$ and $P < 0.001$ was 11 and five respectively. For either 47d or 25r at $P < 0.05$ (Table 2), the number of populations displaying temporal changes was 12. At the more stringent threshold $P < 0.001$, this number dropped to five. Looking specifically at 47d, the three rivers

displaying the largest temporal genetic changes were all located on the west of Norway; Opo, Vosso and Loneelva respectively. In all three of these rivers, temporal genetic changes, and measured by F_{ST}, were greater for 47d than 25r, a trend observed in a total of 13 of the 20 populations in the entire data set.

A statistically significant correlation was observed between the degree of within-river temporal genetic change (as revealed by F_{ST}) using 47d and 25r (R^2 = 0.36 P = 0.0049) (Additional file 6: Figure S2). However, the degree of within-river change was more strongly related between 47d and previously published results for these populations based upon 22 microsatellites [21] (R^2 = 0.63 $P < 0.0001$) (Additional file 6: Figure S2).

Genetic assignment tests were used to investigate the probability of excluding the composite genotype for each individual fish taken from the contemporary sample from the historical genetic profile of the population. This compliments temporal F_{ST} analyses as it also reflects distribution of genotypes among individual fish in the contemporary sample. At $P < 0.001$, the percentage of fish from the contemporary sample that could be excluded from the historical sample ranged from a low of 0% for the river Neiden, to a high of 12% for the river Vosso. Using 47d or 25r only, the percentage of fish from the contemporary sample that could be excluded from the historical profile were generally lower than with the 72 loci (Table 2). In comparison with previous data on these populations using 22 microsatellites [21], the exclusion levels achieved with even the full set of 72 SNPs were strikingly lower (Table 2).

Bayesian clustering analysis for each population separately revealed clear temporal genetic changes in the rivers Opo and Vosso when computed using data from all 72 loci (Additional file 7: Table S5). The results of admixture analysis for the remaining rivers, using either all 72 loci or 47d was either very subtle, or non-existent (Additional file 7: Table S5).

Comparisons of wild salmon to the farmed salmon

Computed pair-wise, all historical and contemporary samples representing the wild populations displayed statistically significant differences to the pool of farmed salmon, using both sets of SNPs separately, and following adjustment for multiple testing (all $P < 0.001$). Several trends can be

Table 2 Temporal genetic stability in the 20 rivers ordered north to south

Population	Pair wise F_{ST} historical vs Contemporary				Exclusion of contemporary sample from historical $P < 0.001$			
	72 SNPs	47d SNPs	25r SNPs	22 Micros	72 SNPs	47d SNPs	25r SNPs	22 Micros
Neiden	0.0002	0.0017	0.0025	0.0009	0%	0%	0%	6%
V. Jakobselv	0.0054**	0.0067**	0.0029	0.0064**	7%	5%	1%	16%
Alta	0.0040*	0.0059*	0.0003	0.0002	2%	3%	0%	2%
Reisa	0.0023	0.0036	0.0004	0.0041*	6%	4%	0%	15%
Målselv	0.0038	0.0082*	0.0043	0.0026	7%	10%	0%	13%
Roksdalsvass.	0.0037*	0.0066*	0.0016	0.0014	1%	1%	0%	20%
Namsen	0.0042**	0.0027*	0.0068*	0.0013*	1%	2%	0%	9%
Surna	0.0093	0.0010	0.0055	0.0025	2%	2%	0%	12%
Eira	0.0039	0.0051	0.0016	0.0005	5%	0%	0%	34%
Bondalselva	0.0034	0.0041	0.0022	0.0043	8%	8%	8%	14%
Ørstaelva	0.0006	0.0005	0.0008	0.0003	6%	3%	0%	6%
GaulaSF	0.0011	0.0025	0.0015	0.0001	0%	2%	1%	0%
Lærdalselva	0.0005	0.0001	0.0011	0.0015	2%	4%	0%	17%
Vosso	0.0125**	0.0168**	0.0049	0.0070**	12%	16%	2%	58%
Loneelva	0.0071**	0.0105**	0.0003	0.012**	4%	2%	0%	52%
Opo	0.0200**	0.0216**	0.0172**	0.0258**	8%	2%	8%	100%
Etne	0.0038*	0.0062**	0.0007	0.0006	1%	0%	1%	5%
Figgjo	0.0050*	0.0040*	0.0071*	0.0048**	3%	0%	0%	38%
Numedalslågen	0.0036*	0.0023	0.0058*	0.0032*	4%	0%	0%	29%
Berbyelva	0.0032*	0.0042*	0.0015	0.0053**	1%	3%	0%	16%

Stability is quantified by pair wise F_{ST} and percent exclusion of the contemporary sample from the historical sample. Both parameters measured using sub sets of SNPs and previously published data using microsatellites.
Micros = microsatellite data taken from [21]. Note, the contemporary Vosso sample is not the same as the contemporary Vosso sample used in Glover et al., 2012b. * = significant $P < 0.05$, ** = significant $P < 0.001$.

extrapolated from these comparisons (Figure 2). First, all F_{ST} values computed pair-wise between each historical wild sample and the farmed sample was higher for 47d compared to 25r (Anova DF $_{1,38}$, $F = 12.1$, $P < 0.001$). This is consistent with the results of the section above dedicated to comparing the statistical properties of 47d and 25r. Second, a geographic trend to the pair-wise difference between each wild population's historical sample and the pooled farmed sample was present. Populations from the far north and south of Norway displayed much greater pair-wise F_{ST} estimates to the farmed sample than populations from mid- and western Norway displayed to the farmed sample. This was detected in 47d and 25r, but more pronounced in the former. These observations suggest that a population in northern Norway is likely to display a greater genetic change compared to a population from mid Norway for the same level of farmed salmon introgression.

Looking specifically at 47d, the pair-wise difference between the wild sample and the pool of farmed salmon decreased with time in 14 of the 20 populations. This was most noticeable for Vosso, Opo and Vestre Jakobselva (Figure 2). For example, the pair-wise F_{ST} between Vosso and the farmed sample dropped from 0.085 to 0.038. Furthermore, a significant positive relationship between temporal genetic change within each river, and the change in pair-wise F_{ST} between that specific rivers historical sample and the farmed salmon sample, and that rivers contemporary sample and the farmed sample, was observed for 47d ($R^2 = 0.41$, $P = 0.0022$), but not for 25r ($R^2 = 0.07$, $P = 0.25$) (Figure 3). Thus, analyses based upon 47d demonstrate that where rivers display temporal genetic change, it is largely in the direction of the farm sample.

Assignment tests were also used to exclude the 375 genotyped farmed fish from each wild population (Additional file 8: Figure S3). For both the historical and contemporary samples, the exclusion percentages followed a similar geographic pattern as observed for the pair-wise F_{ST} between each population and the farmed sample (Figure 2). The percentage of farmed fish that could be excluded from each wild fish sample

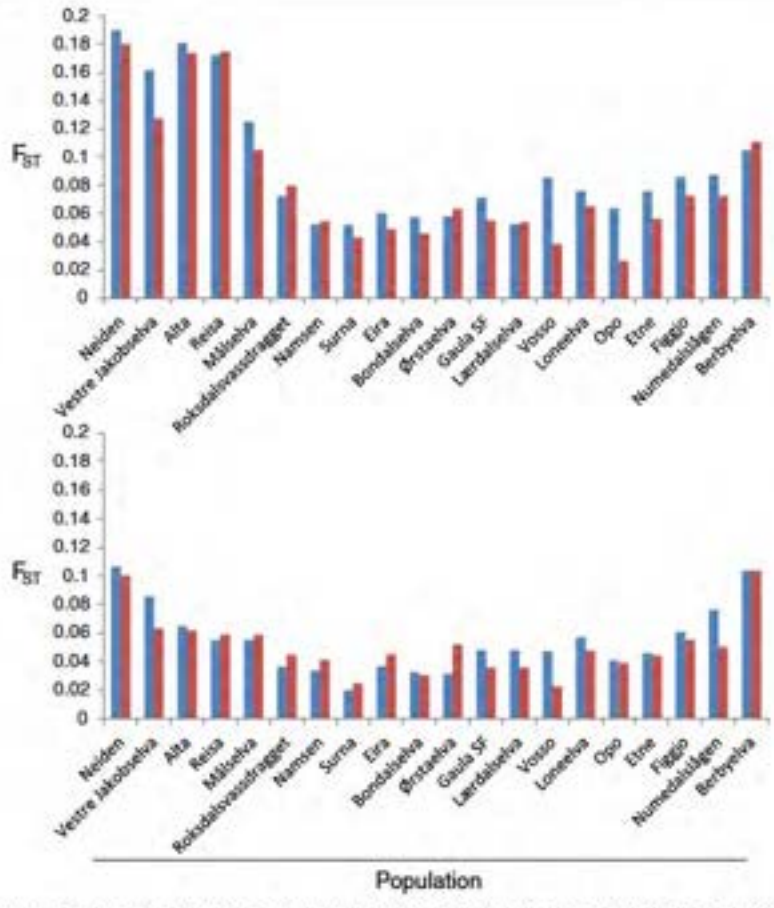

Figure 2 Pair wise F_{ST} between the historic sample for each wild population and the farm sample (blue left bar), and the contemporary sample for each wild population and the farm sample (red right bar), computed using 47 diagnostic SNPs (top), and 25 randomly selected SNPs (bottom). Populations are ordered north to south.

decreased between the full set of 72 loci to 47d and 25r. The relative change for any given population, i.e., the drop in exclusion of the farmed fish between the historical sample and contemporary sample (which is more interesting in this specific context than the absolute level of exclusion) was still very noticeable for 47d.

An alternative way of investigating the direction of the observed temporal genetic changes in the wild populations is to conduct PCA analysis. A PCA plot for the seven populations located in the west of Norway, which includes the three rivers displaying the highest temporal genetic changes in the complete data set for 47d, revealed that six of the seven populations displayed genetic changes in the direction of the farm sample (Figure 4). Notably, none of them displayed temporal genetic changes away from the farm sample. While some other populations located in the other regions of Norway displayed temporal genetic changes in the approximate direction of the farm sample, this was not observed for all samples displaying temporal genetic changes (Additional file 9: Figure S4).

Quantification of gene flow required to cause observed changes

The estimated level of farmed salmon introgression required to cause the observed temporal genetic changes in the 20 populations ranged from 2-47% and 7-41% using the ABC and fixed migration methods respectively (Table 3). For the two populations displaying the greatest temporal genetic change (Opo and Vosso), the estimated level of farmed salmon introgression ranged from 36-41% and 33-47%. In contrast, the simulated level of gene flow required from the nearest neighbor population, i.e., straying,

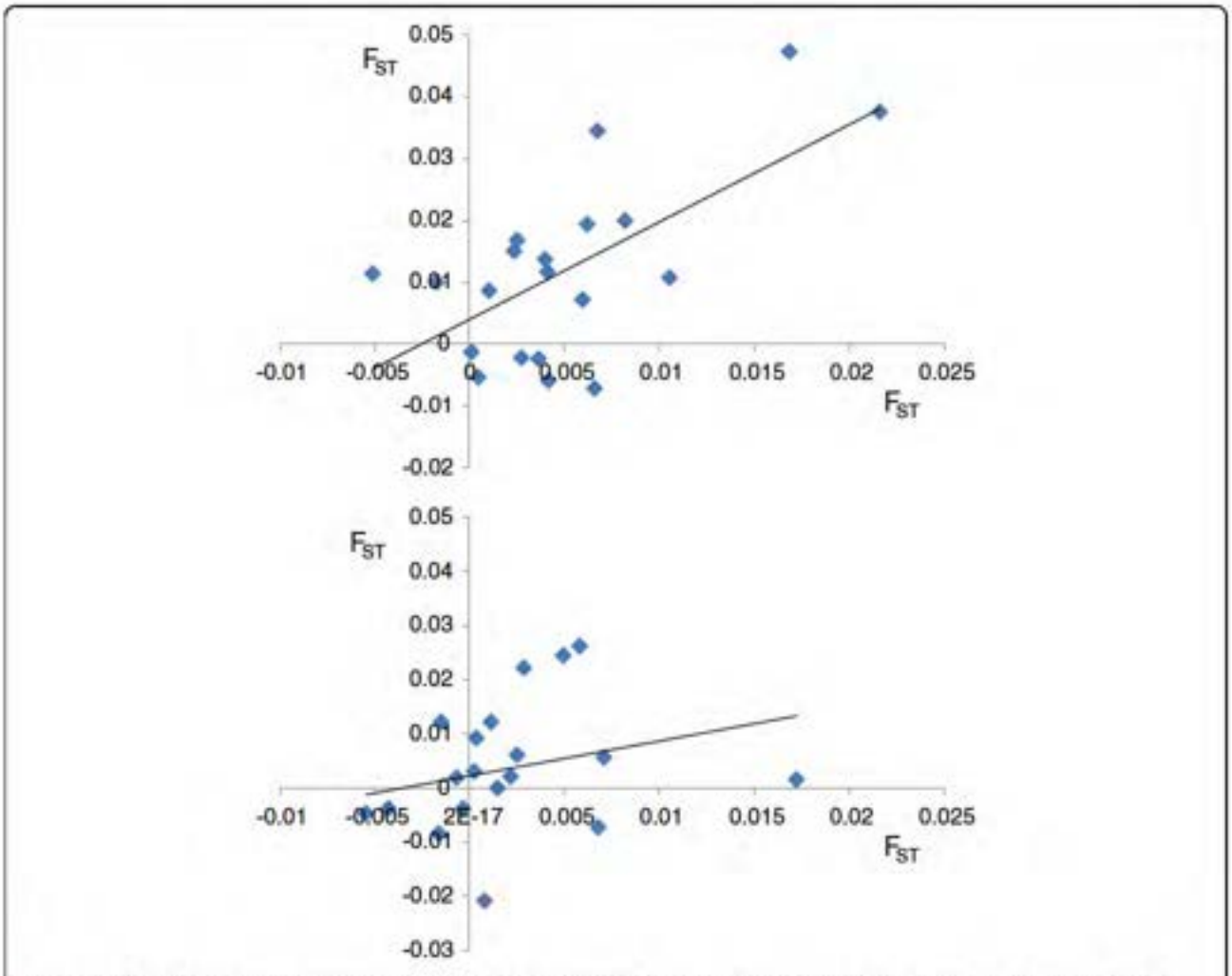

Figure 3 Relationship between the temporal genetic change observed within any given population (i.e., pair wise F_{ST} between the historical and ceontemporay sample) on the X axis, and the difference in pair wise F_{ST} between each populations historical sample and the farm sample, and that same populations contemporary sample and the farm sample, placed on the Y axis. These relationships are computed with 47 diagnostic SNPs (top) ($R^2 = 0.41$ $P = 0.0022$) and 25 randomly selected SNPs (bottom) ($R^2 = 0.07$ $P = 0.25$). Each point represents the above mentioned relationship for each of the 20 populations.

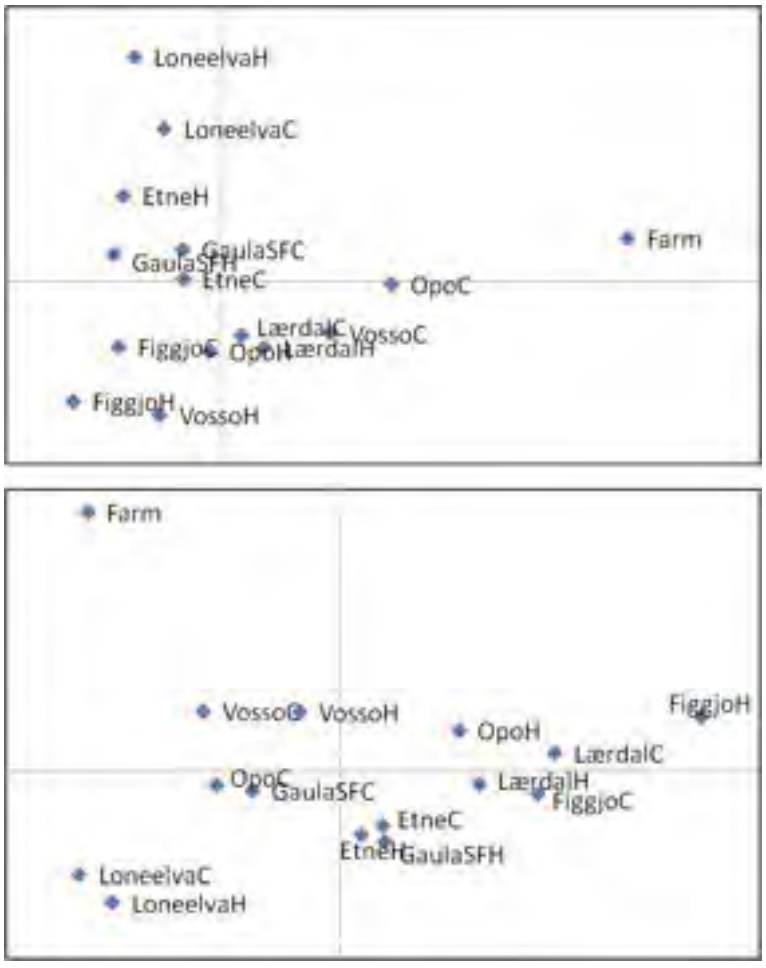

Figure 4 Principal compont analysis for the historical (H) and contemporary (C) samples for the seven rivers grouped into the west of Norway. This region includes the three rivers displaying the greatest temporal genetic changes in the entire study. Plots are based upon 47 diagnostic SNPs (top) and 25 randomly selected SNPs (bottom). X and Y axes explain 35, 23%, and 29, 23% for the top and bottom figures respectively. PCA plots for all 20 rivers arranged into four geograpahic regions are also presented online (Additional file 9: Figure S4).

to cause the observed temporal changes in these two populations was much greater, approximately 80-82% and 46-61 % for the ABC and fixed migration methods respectively. This is far greater than straying rates typical for this species. For both computation methods, the introgression rate required by the straying scenario was always far greater than introgression required by the farmed escapees scenario. This is likely to reflect the genetic similarity of the nearest neighbor population to each recipient wild population, and how distinct each wild population's historical sample was to the farmed sample, the latter of which follows a geographic trend as demonstrated earlier (Figure 2).

The observed temporal genetic changes for the rivers Opo and Vosso, in relation to the simulated genetic change by introgression of farmed fish, or the nearest neighboring population, confirmed results presented earlier that the genetic changes observed in these two populations were clearly directional to the farmed fish, and not likely to be cause by natural straying (Figure 5). Put simply, the observed temporal genetic change in both of these populations overlapped almost perfectly with the simulated genetic change caused by the pool of farmed salmon, but not by the nearest neighbor (Figure 5). For other populations that displayed more modest temporal genetic changes however, this pattern was more difficult to elucidate from the simulation-based PCA plots due to the fact that the changes were small and distributions overlapped.

Observed frequency of escapees and genetic changes

A set of correlations between the frequency of farmed escapees observed on the spawning grounds in the time-period in which the present study is conducted, and various genetic parameters produced with the present study for these populations are summarized (Table 4), and plotted graphically (Figure 6). All genetic parameters gave stronger correlations with the weighted mean frequency

Table 3 Estimated percentage introgression of farmed salmon, or the nearest wild population required to cause the observed temporal genetic changes

Population	Admixture from farm salmon				Admixture from nearest neighbor population			
	ABC		Fixed migr.		ABC		Fixed migr.	
	Pr. Gen.	Total	Pr. Gen.	Total	Pr. Gen.	Total	Pr. Gen.	Total
Neiden	0.004 ±0.009	0.022 ±0.051	0.014 ±0.006	0.073 ±0.031	0.029 ±0.035	0.139 ±0.152	0.028 ±0.023	0.142 ±0.110
V. Jakobs.	0.031 ±0.005	0.116 ±0.030	0.032 ±0.020	0.116 ±0.063	0.130 ±0.011	0.385 ±0.045	0.175 ±0.133	0.414 ±0.223
Alta	0.031 ±0.004	0.116 ±0.024	0.043 ±0.031	0.117 ±0.062	0.114 ± 0.013	0.350 ±0.042	0.136 ±0.092	0.359 ±0.198
Reisa	0.017 ±0.006	0.066 ±0.028	0.035 ±0.023	0.126 ±0.070	0.040 ±0.017	0.143 ±0.061	0.116 ±0.084	0.319 ±0.190
Målselv	0.054 ±0.006	0.190 ±0.032	0.051 ±0.041	0.167 ±0.114	0.083 ±0.004	0.273 ±0.030	0.097 ±0.071	0.280 ±0.175
Roksdalsvass	0.055 ±0.004	0.192 ± 0027	0.063 ±0.054	0.199 ±0.146	0.071 ±0.004	0.239 ±0.029	0.077 ±0.052	0.220 ±0.143
Namsen	0.01 ±0.013	0.062 ±0.074	0.033 ±0.020	0.170 ±0.090	0.056 ±0.047	0.252 ±0.210	0.027 ±0.037	0.126 ±0.162
Surna	0.010 ±0.014	0.038 ±0.057	0.083 ±0.073	0.243 ± 0179	0.039 ±0.046	0.137 ±0.138	0.255 ±0.188	0.507 ±0.253
Eira	0.016 ±0.031	0.053 ±0.100	0.042 ±0.032	0.145 ±0.097	0.033 ±0.035	0.116 ±0.141	0.092 ±0.069	0.268 ±0.168
Bondalselva	0.026 ±0.015	0.098 ±0.055	0.092 ±0.081	0.263 ± 0193	0.122 ±0.029	0.363 ±0.074	0.301 ±0.223	0.547 ±0.270
Ørstaelva	0.014 ±0.019	0.050 ±0.068	0.070 ±0.060	0.217 ±0.154	0.050 ±0.042	0.165 ±0.129	0.304 ±0.219	0.554 ±0.273
GaulaSF	0.022 ±0.009	0.085 ±0.038	0.050 ±0.041	0.165 ± 0115	0.028 ±0.008	0.105 ±0.034	0.097 ±0.070	0.280 ±0.172
Lærdalselva	0.015 ±0.027	0.088 ±0.142	0.027 ±0.013	0.169 ±0.077	0.019 ±0.028	0.115 ±0.144	0.026 ±0.021	0.154 ±0.112
Vosso	0.077 ±0.003	0.360 ±0.032	0.102 ±0.015	0.410 ±0.200	0.107 ±0.005	0.459 ±0.033	0.283 ±0.140	0.605 ±0.145
Loneelva	0.094 ±0.010	0.307 ±0.029	0.075 ±0.069	0.226 ±0.166	0.124 ±0.006	0.375 ±0.031	0.109 ±0.071	0.311 ±0.172
Opo	0.084 ±0.004	0.474 ±0.044	0.061 ±0.053	0.331 ±0.209	0.238 ±0.020	0.817 ±0.029	0.338 ±0.205	0.804 ±0.197
Etne	0.044 ±0.005	0.197 ±0.033	0.040 ±0.030	0.170 ±0.107	0.069 ±0.033	0.274 ±0.117	0.098 ±0.067	0.337 ±0.189
Figgjo	0.009 ±0.010	0.060 ±0.069	0.029 ±0.013	0.178 ±0.077	0.018 ±0.023	0.120 ±0.125	0.044 ±0.035	0.236 ±0.162
Numedals.	0.007 ±0.006	0.030 ±0.026	0.040 ±0.030	0.143 ±0.089	0.021 ±0.010	0.078 ±0.041	0.060 ±0.046	0.191 ±0.130
Berbyelva	0.025 ±0.012	0.093 ±0.049	0.040 ±0.029	0.138 ±0.089	0.045 ±0.005	0.163 ±0.030	0.070 ±0.050	0.219 ±0.138

Estimations of introgression are based upon approximate Bayesian computation (ABC), or a fixed migration rate based simulation (Fixed migr.). Populations ordered north to south. ABC = estimation of introgression using approximate Bayesian computation, Fixed migr. = estimation of introgression using a fixed migration rate estimator (see Methods).

of escaped salmon than the un-weighted mean frequency of farmed escaped salmon. This suggests that the weighted estimate more accurately reflects the true numbers of escapees in the populations as it corrects for noise in the estimations (i.e., small sample sizes in some years etc.).

Spatio-temporal variation

The pair-wise F_{ST} values among all samples included in this study, including associated *P* values, are presented online (Additional file 10: Table S6). Using all sets of markers, global F_{ST} estimates among the 20 wild populations decreased significantly with time (Table 5). Nevertheless, a geographic pattern to the genetic structure was still evident with both 47d and 25r (Additional file 11: Figure S5), and there was no detectable change in IBD (Table 5). This means that while overall variation among the wild samples decreased with time, this happened "evenly" which did not influence the relationship between genetic and physical distance.

Discussion

Norway is the world's largest producer of farmed Atlantic salmon, and has over 200 rivers supporting native Atlantic salmon populations. Many Norwegian populations have displayed moderate to high frequencies of domesticated farmed escapees on the spawning grounds for two decades or more [1,4,54]. At the same time, Norwegian farmed salmon originated from native Norwegian populations approximately ten generations ago. Thus, it follows that Norway is not only the country where the potential genetic interaction between farmed escaped salmon and wild conspecifics is the most extensive, it represents the country in which the statistical challenges to quantify genetic introgression of farmed escapees are the most demanding.

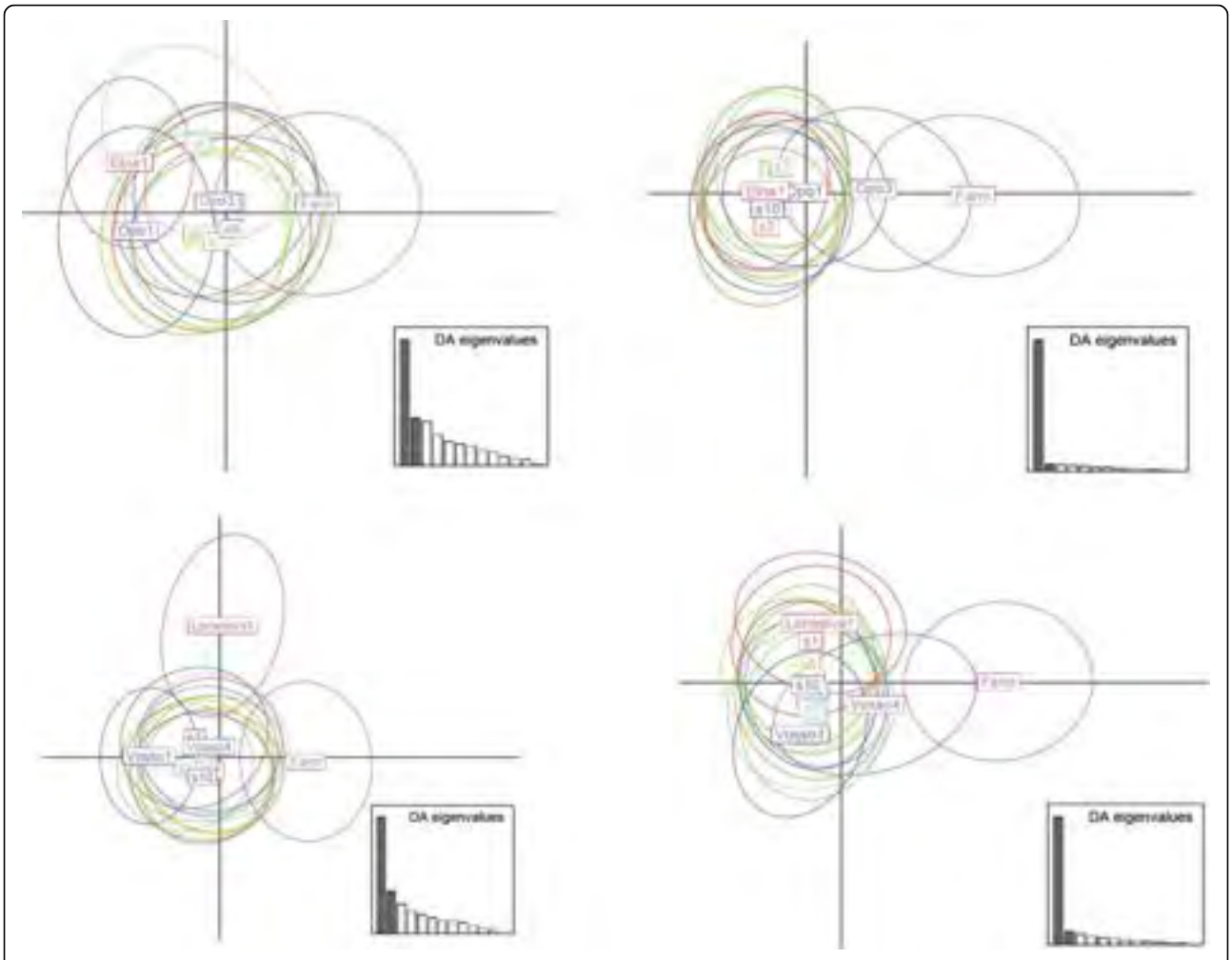

Figure 5 Principal component analysis depicting observed historical and contemporary samples for the river Opo (top panels), and Vosso (bottom panels), including the farmed sample and nearest wild population, following simulated introgression from the farmed sample (left panels), and simulated introgression from the nearest neighbor (right panels). In each case, the results of ten independent simulations, S1 S10 are presented. The text box represents the center point of the observations with the 95% confidence interval represented by the ellipse.

Table 4 Relationships between the frequency of farmed escaped salmon in the spawning population, and observed changes in various genetic parameters

Statistic	Un weighted mean	Weighted mean
Pair wise F_{ST} 72	0.19 (0.052)	0.52 (0.0003)
Pair wise F_{ST} 47d	0.17 (0.067)	0.44 (0.0014)
Pair wise F_{ST} 25r	0.06 (0.30)	0.41 (0.0022)
F_{ST} to farm 72	0.13 (0.11)	0.22 (0.039)
F_{ST} to farm 47d	0.25 (0.024)	0.37 (0.0045)
F_{ST} to farm 25r	<0.01 (0.72)	<0.01 (0.99)
ABC introgression	0.16 (0.08)	0.47 (0.0007)
Fixed migration rate introgression	0.25 (0.025	0.36 (0.005)

Values computed are R^2 (*P* value). Number behind statistics refers to number of SNP loci used, introgession computations only compared for 47d.
Pair wise F_{ST} = observed value between historical and contemporary sample for each river; F_{ST} to farm = absolute difference in pair wise F_{ST} between the populations historical sample and the farm sample, and between the populations contemporary sample and the farm sample, ABC introgression = estimated introgression of farmed salmon (Table 3) using ABC method, Fixed migration rate introgression = estimated introgression of farmed salmon (Table 3) using the fixed migration rate method. Un weighted mean = mean% of farmed salmon observed on the spawning grounds for these rivers in the period 1989 2009 [5,52], weighted mean = weighted average percentage of farmed salmon in the population combining data from both sports fishing and spawning population samples [53].

The present study addressed this situation by genotyping a sub-set of SNP markers (47d) that have been reported to be collectively diagnostic for farmed and wild salmon [31], and by implementing ABC simulations to quantify introgression of farmed salmon.

The most important results of this study can be summarized as follows: 1. All populations displaying significant temporal genetic changes with 47d became more similar to the pooled of farmed salmon. Furthermore, the stronger the temporal genetic change with 47d, the more similar it became to the pool. This strongly suggests that where populations displayed clear temporal genetic changes, introgression of farmed fish has been the primary cause 2. This is the first study to estimate cumulative introgression of farmed salmon in any native Atlantic salmon population. Estimations ranged between 2–47% and 7–41% per population using the ABC and fixed migration simulation methods respectively. It is concluded that while the level of introgression has been population specific, farmed salmon have heavily introgressed in some wild Norwegian populations.

Are the diagnostic SNPs universally informative

The panel of diagnostic SNPs used here (47d) represents a sub-set of the markers recently identified as collectively diagnostic for farmed and wild Norwegian salmon [31]. The panel 47d included 35 of the top 60 loci ranked by Karlsson et al. (2011), including the top 10 ranking loci, and a further set of 12 loci taken from the ranks 60–200. While this study has not used the exact combination of markers reported to be collectively diagnostic, which is in large part due to poor genotyping quality for many of those markers, 47d still provides similar characteristics of the panel reported to be collectively diagnostic. This is based upon the comparisons reported here showing the greater level of signal for 47d vs. 25r for a variety of statistic parameters (e.g., Figures 2, 3, Additional file 5: Figure S1, Table 2).

Seven of the populations investigated here overlap with some of the populations used to identify the diagnostic SNPs in Karlsson et al. [31]. However, there were no sign that ascertainment bias influenced the results of the present study. This is based upon the following observations: 1. The population displaying the greatest temporal change with 47d was not included in the ascertainment panel, 2. Pair-wise F_{ST} between each wild population and the pool of farmed salmon showed a clear geographic trend (Figure 2), with populations in the north of Norway displaying the greatest difference to the farmed pool. This is likely to reflect a combination of the fact that there is a distinct evolutionary divide between Atlantic salmon populations in the north and rest of Norway [21], and that Norwegian farmed strains were largely sourced from wild populations south of the observed evolutionary divide [29,55]. Thus, it follows that detection of introgression in native populations in Northern Norway should be easier to detect with this set of markers than in populations for example from mid- and western Norway. Consequently, the present study serves to validate the usefulness of the collectively diagnostic markers in populations other than those in which the marker identification was conducted. Given that Atlantic salmon populations display highly significant population genetic structuring throughout the Atlantic [6,7], it is likely that these markers will also serve useful to identify introgression of Norwegian farmed salmon in native populations outside Norway.

Assignment tests using 47d provided less statistical power to reject individual salmon from the historical baseline than with microsatellites for all 20 populations (Table 2). This is probably due to the fact that assignment power is strongly influenced by total number of alleles in the data set [39,56], and the fact that a microsatellite data set based upon 22 markers [21] has approximately 3–4 times more alleles than 47 SNPs. Inclusion of more of the collectively diagnostic SNPs identified by Karlsson et al. (2011) would increase these assignment statistics. However, in order to accurately identify the ancestry of hybrids beyond the second admixed generation, it has been suggested that 50 or more ancestry diagnostic markers (i.e., fixed allele differences) are required [57,58]. Only 15 markers from the panel 47d displayed non-overlapping allele frequencies between the pool of farm salmon and all historical samples for each wild population. Furthermore, the allele frequency

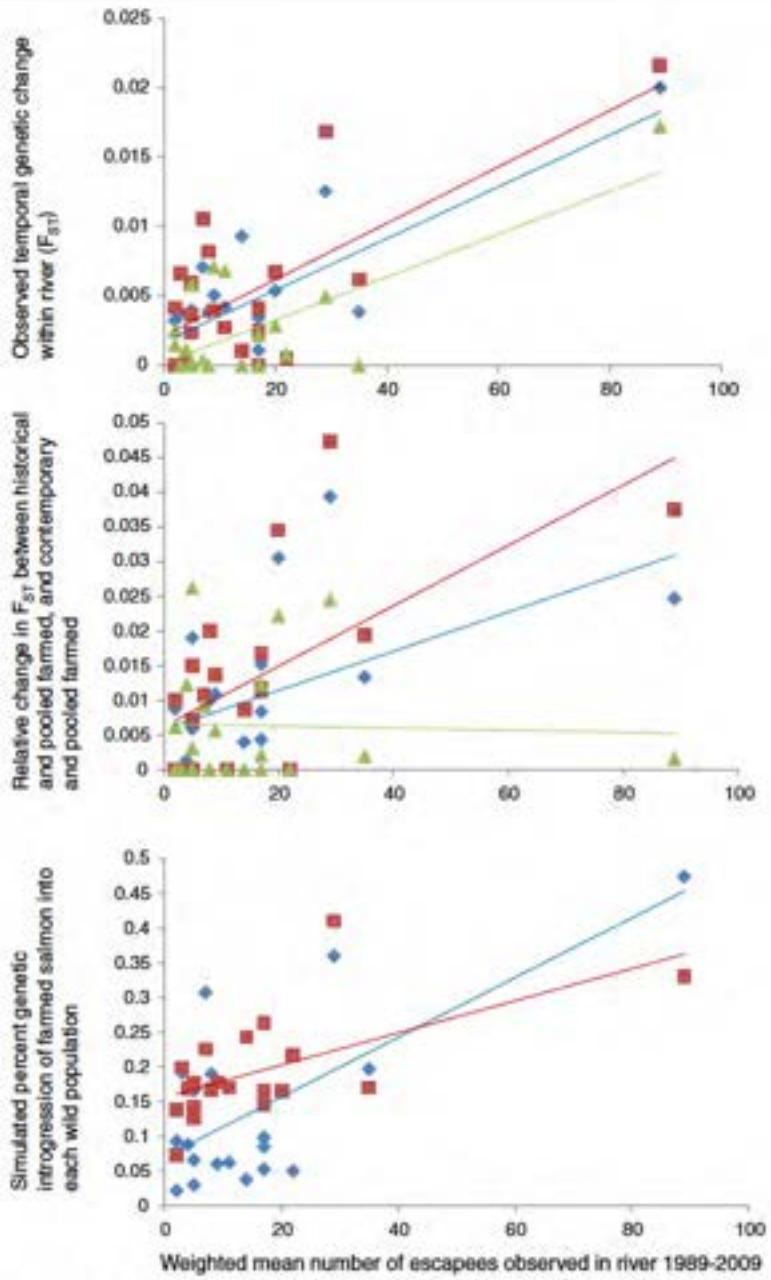

Figure 6 Relationship between the weighted mean number of escapees observed in each of the rivers in the period 1989 2009 (X axis), and the following three sets of estimators of genetic change in the 20 populations (Y axes). Top graph Y axis = the observed within river temporal genetic change as measured by pair wise F_{ST} using all 72 loci (blue diamonds), 47d (red squares) and 25r (green triangles). Middle graph Y axis = the relative change (%) in pair wise F_{ST} between a population's historical sample and the pooled sample of farmed salmon, and the same populations contemporary sample and the pooled sample of farmed salmon using all 72 loci (blue diamonds), 47d (red squares) and 25r (green triangles). Bottom graph Y axis = the simulated level of farmed salmon genetic introgression required in each population to cause the observed temporal genetic changes as based upon the ABC method (blue triangles) and the fixed migration method (red squares), both of these simulations computed using 47d. Significance levels and R^2 values for all relationships are provided in Table 4.

differences for these non-overlapping markers were not close to fixation between groups (Additional file 3: Table S3). Thus, while the markers identified by Karlsson et al. (2011) provide more information to differentiate farmed and wild salmon than randomly selected markers, the identified markers are more correctly regarded as "collectively informative" than diagnostic. Clearly, there is a need to identify more informative genetic markers on the

Table 5 Spatio-temporal analysis of population genetic structure (global F_{ST}) including isolation by distance (IBD)

Loci	Historical samples				Contemporary samples				Global F_{ST} change?
	IBD	*P*	Global F_{ST} (SD)	*P*	IBD	*P*	Global F_{ST} (SD)	*P*	*P*
72	0.79	<0.001	0.055 (0.004)	<0.001	0.77	<0.001	0.046 (0.003)	<0.001	≤0.003
47d	0.79	<0.001	0.061 (0.004)	<0.001	0.76	<0.001	0.051 (0.004)	<0.001	≤0.001
25r	0.60	<0.001	0.043 (0.006)	<0.001	0.66	<0.001	0.036 (0.004)	<0.001	0.046

IBD = isolation by distance with associated *P* value, Global F_{ST} = global F_{ST} among the wild populations with standard error in brackets and associated *P* value. Hist. Vs. Cont. = statistical significance whether or not the change in global F_{ST} with time was significant or not.

domesticated/wild interface. As farmed salmon outgrow wild salmon approximately 2–3 times under hatchery conditions [59,60], it is suggested that there is significant potential to identify markers tightly linked with this trait that has been selected for in all farmed strains [29,61].

ABC and fixed migration estimations of introgression

Admixture between hatchery fish released deliberately into the wild and native populations has been computed in other species and systems, for example brown trout (*Salmo trutta*) in Danish rivers [25]. Often, admixture has been estimated using Bayesian clustering implemented for example in the program STRUCTURE [47,48]. Here, clustering analysis was able to reveal temporal changes in the populations Opo and Vosso (the two populations displaying the largest temporal changes), however, in other rivers, also those displaying statistically temporal genetic changes, this analysis did not reveal changes. This is consistent with previous results using microsatellites [21] and is likely to be caused in part by the fact that the observed genetic changes for most of the rivers were low to modest, and therefore under the detection potential for STRUCTURE.

Introgression of farmed salmon was estimated here using the simulation approach. As it is implemented, the ABC routine finds the point estimate of migration rate *M* that best explains the observed F_{ST} between historical and contemporary population. However this approach does not take into account the possibly large confidence intervals around the observed F_{ST}. In many of the populations studied here, the genetic distance between historical and contemporary samples was not significantly different from zero. Therefore an alternative approach was also employed to better account for the uncertainty around the observed F_{ST}. By choosing a set of scenarios where the genetic distance between historical and simulated population fitted within the 95% confidence interval of the observed F_{ST}, the second approach reflects the range of variation around the posterior mean of *M*, accounting for the uncertainty around the observed F_{ST}. The standard deviation of *M* given by ABC estimation only accounts for the variation induced by random gamete sampling of our simulations and is thus lower than the standard deviation of *M* given by fix migration rate approach. This last approach explores the possible values of *M* stepwise with predefined steps (1,2…20%) and is deemed to be less accurate to estimate the posterior mean of *M* than the ABC routine that converges gradually to an optimum value. We therefore present the results from the two methods as complementary. Other alternative approaches could also have provided reliable estimates of posterior distribution of *M*, such as rejection based Bayesian Inference [33,62], that would have estimated both posterior mean and standard deviation of *M*, taking into account the uncertainty around the observed F_{ST}. However, algorithms that estimate the value of a parameter based on its posterior likelihood are sensitive to the shape of the likelihood curve or "likelihood landscape"; a leptocurtic curve would result the algorithm to converging rapidly to the optimum value of the estimated parameter, whereas a platicurtic curve would result in the algorithm converging slowly. In the present data, F_{ST} between historical and contemporary samples were close to zero with large standard deviations for some populations. Such combination of small mean value and large standard deviation represents a flat likelihood landscape where the algorithm is slow to converge, and often lacks precision.

Challenges and alternative approaches to quantify introgression

Some of the challenges to quantify introgression of farmed salmon in native populations have been described in detail in the introduction. Briefly, these include the complicated logistics associated with the distribution of genetic material within the aquaculture industry [38], the fact that there are multiple farmed strains which have and continue to change significantly over time due to splitting and mixing, the fact that over time, gene-flow arises from multiple farmed sources which partially conceals the degree of genetic change in wild populations when studying non-diagnostic markers [28], and finally, the fact that most loci display overlapping allele frequencies between groups of farmed and wild salmon. It is likely that many of these challenges will exist in other countries where farmed and wild salmon coexist, and, in other aquaculture species such as marine fish where interactions between escapees and wild conspecifics have already been registered [63-65].

In order to investigate the direction of genetic change in wild populations and quantify introgression, a pool of 375 farmed salmon, sampled over a five year period from 48 cages located on 35 farms spanning the Norwegian coastline were included in the present study. While this pool of

farmed salmon does not necessarily accurately represent the allele frequencies of escapees entering all of these rivers for the entire study period, they clearly permitted elucidation of the direction of the genetic changes in wild populations (Figures 3 and 4). Furthermore, in the populations Opo and Vosso, which showed the highest estimated introgression rates, the observed direction of genetic change in relation to this pool of farmed salmon, and the simulated direction in relation to gene-flow from the pool of farmed salmon displayed almost perfect overlap (Figure 5). Thus, these results strongly indicate that this approach is valid. Nevertheless, it is suggested that a more accurate estimation of introgression of farmed salmon could be achieved if samples of farmed fish entering each specific river were collected together with samples of adult wild fish. If this was conducted yearly, together with sampling offspring from the subsequent generation at different life history stages (Figure 7), this would provide the most robust estimations of the allele frequencies for the native and intruding farmed fish. This cohort-based sampling could then be combined with the ABC and fixed migration simulations presented here to quantify introgression per year and thereafter per generation.

The offspring of farmed salmon display genetically-based lower survival in the natural environment compared to wild salmon [66-69]. Thus, it is likely that the frequency of farmed and hybrid salmon in a cohort will decrease with time. While this was not tested here, it is noteworthy that the two populations displaying the greatest introgression levels, i.e., Opo and Vosso, were represented by juvenile as opposed to adult samples. It is therefore possible that the level of farmed salmon introgression in these two populations would have been less if the contemporary samples for each of these rivers were adults. The cohort-based design (Figure 7) would permit addressing this issue, and potentially provide estimations in strength of selection against farmed fish in the different rivers, for the different life-history stages.

Implications for management

The direct translation of molecular genetic data into aquaculture or fisheries management and regulation has not been without its challenges [70,71]. Good examples include identification of the farm of origin for escapees [38,41], poaching from protected populations [72], fishing competition fraud [73], and regulation of harvest at the individual [74] and population level [75]. Nevertheless, there is still a need to improve the translation of molecular genetic data into a "currency" that governing bodies can implement if these techniques are to find a routine place in the management of aquaculture and fisheries resources. While it is acknowledged that the methods for quantification of introgression presented here are still in need of further refinement, they provide management authorities with the first multiple-population estimations of introgression of farmed salmon, an essential early step in risk assessment [23]. Nevertheless, other important management issues, such as what early warning indicators for introgression exist, and what are the biological consequences of introgression, remain.

One of the limitations in using genetics techniques to quantify introgression of farmed salmon in native populations is that fact that it can only be used to validate introgression. While this is important, the obvious management target for mitigation prior to introgression is the frequency of farmed salmon in the native population. However, the correlation between some of the genetic parameters investigated here and the frequency of farmed salmon observed in these populations was at best modest. This is consistent with observations from a previous study [21], and is likely to be associated with the fact that there are large gaps in the data reporting the numbers of escapees in rivers, and that the density of the native population appears linked with resilience of the population to introgression. The implications of this for managers is that monitoring the frequency of farmed salmon in wild populations, or modeling genetic changes in the populations based upon the observed frequency of

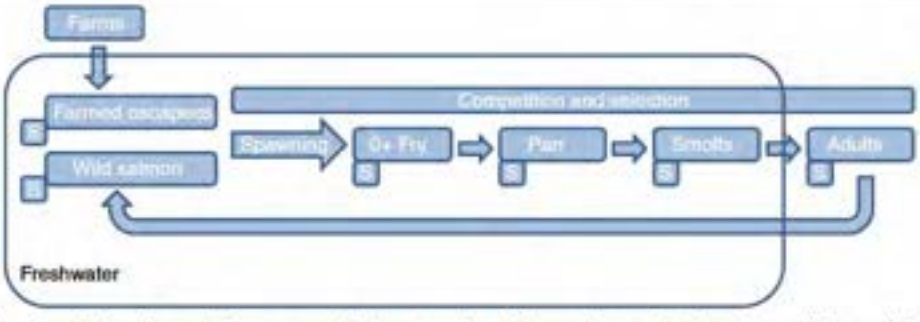

Figure 7 Illustration of a single cohort design to quantify introgression of farmed escaped salmon in a wild population, and estimate selection at the different life history stages. S = sampling for genetic analysis.

escapees [53,76], will not provide an accurate estimation of introgression for all populations.

There are widespread concerns regarding the genetic integrity and long-term fitness of native populations where large numbers of escapees have been observed [13-15]. However, while introgression of farmed escaped salmon has been documented in rivers in several countries, biological consequences of introgression has thus far not been reported for any wild Atlantic salmon population. This provides several challenges for managers. At what level should acceptable thresholds be set in the absence of fitness-consequence data in wild populations? And if a very low management threshold is set (for example <5% introgression), will the analytical methods and genetic markers available today, or in the near future, be able to accurately quantify introgression at such low levels? Given that there have been major advances in the use of sterile triploid Atlantic salmon for the commercial aquaculture, and 100% containment of aquaculture fish is ultimately unrealistic, management authorities should consider increasing efforts to convert the industry over to the use of sterile fish.

Conclusion

This study is the first to quantify cumulative introgression of farmed salmon in any native Atlantic salmon population. Based upon ABC and fixed migration estimations, it has been demonstrated that introgression of farmed salmon in wild Norwegian Atlantic salmon populations has been population-specific, ranging from no detectable impact in some populations to strong introgression in others. Furthermore, where populations displayed clear temporal genetic changes, they all became more similar to a pool of farmed salmon. While the level of farmed salmon introgression was partially correlated with the frequency of escapees observed in the population, it is concluded that other mechanisms, such as the density of the recipient native population, is likely to influence the relative success of farmed fish. These data provide policy makers with unique information to address the influence of farmed escaped salmon on native populations.

Additional files

Additional file 1: Table S1. Characteristics of the 20 Atlantic salmon rivers including catch statistics and observed numbers of escapees.

Additional file 2: Table S2. Technical details for the genetic markers used in the present study.

Additional file 3: Table S3. Population genetic summary statistics for all wild samples and the pool of farmed salmon.

Additional file 4: Table S4. Effective population size for all samples.

Additional file 5: Figure S1. Distribution of pair wise F_{ST} values between a 100 randomly selected farmed salmon vs. 100 randomly selected wild salmon using 47d and 25r.

Additional file 6: Figure S2. Relationships between within river genetic change as measured by 47d, 25r and 22 microsatellelites from previously published data.

Additional file 7: Table S5. Bayesian clustering analysis for each population.

Additional file 8: Figure S3. Percent exclusion of the 375 farmed salmon from each populations's historic (blue left bar) and contemporary (red right bar) sample.

Additional file 9: Figure S4. Principal component analysis for each geographic region based upon 47d and 25r.

Additional file 10: Table S6. Matrix of pair wise F_{ST} values among all samples included in the present study.

Additional file 11: Figure S5. Principal compont analysis for the historical and contemporary samples for all 20 rivers and the group of farmed salmon based upon 47d and 25r.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

KAG, ØS, FB and VW conceived and designed the study. MK conducted SNP genotyping. KAG, CP, FB and VW conducted statistic analyses. KAG and FB conceived and conducted the ABC and fixed migration computations to quantify introgression respectively. KAG wrote the first draft of the paper and coordinated the study. All authors contributed to and approved the final version of the manuscript.

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Survival and behaviour of migrating Atlantic salmon (*Salmo salar* L.) kelts in river, estuarine, and coastal habitat

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Hubley, P. B., Amiro, P. G., Gibson, A. J. F., Lacroix, G. L., and Redden, A. M. 2008. Survival and behaviour of migrating Atlantic salmon (*Salmo salar* L.) kelts in river, estuarine, and coastal habitat. ICES Journal of Marine Science, 65: 1626 1634.

The downstream migration of 30 Atlantic salmon (*Salmo salar*) kelts tagged with acoustic transmitters was monitored using 26 underwater receivers at eight locations from April to October 2006 in the LaHave River and Estuary. In all, 27 tags were detected as they left the coastal environment by the middle of May, 5 weeks after release, indicating a possible 90% kelt survival to coastal departure. Two missing tags and one dropped tag were assumed to be attributable to natural mortality in the estuary. Migration time from release to the outermost coastal receivers 24 km below the tide limit took an average of 14 d, but varied from 3 to 32 d. Some 40% of the kelts lingered and were active in the lower estuary. Five kelts monitored with depth transmitters migrated mostly at the surface in all habitats, with occasional brief descent to the bottom. A consecutive spawning salmon returned after 79 d outside the outermost array. The low rate of returns is consistent with the historical repeat spawning schedule for this river, and more precisely documents the temporal and spatial habitat use of migrating kelts.

Keywords: acoustic telemetry, Atlantic salmon, downstream migration, estuary, kelts, *Salmo salar*.

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Introduction

Returns of Atlantic salmon (*Salmo salar*) to most rivers in North America have declined since the 1990s, especially in the southern range of their distribution (Chaput and Prevost, 1999; ICES, 2005; Gibson *et al*., 2006). Many factors have been suggested for these declines (Cairns, 2001), and low survival during marine stages of both immature (smolts, post smolts, and immature one sea winter) and previously spawned adult salmon (kelts) has been identified as a particular concern in some rivers of the Maritime Provinces of Canada (Department of Fisheries and Oceans, 2003; Trzcinski *et al*., 2004; Amiro *et al*., 2006). Migration and early marine mortality of smolts have been investigated across a range of stocks (Dutil and Coutu, 1988; Holm *et al*., 2000; Lacroix *et al*., 2004b, 2005; Lacroix and Knox, 2005), but similar information for migrating kelts is limited (Ritter, 1989).

Atlantic salmon have a high fidelity to their natal river and may survive to repeat spawn as many as six times (Jones and King, 1949; Ducharme, 1969). In some populations, kelt survival is high, and in some instances increasing (Ducharme, 1969; Jessop, 1976; Chaput *et al*., 2001; Dempson and O'Connell, 2004). Iteroparity can help maintain populations at times when survival to first spawning (recruitment) is low (Niemela *et al*., 2006). However, in an increasing number of salmon populations, neither recruitment nor repeat spawning is currently sufficient to maintain population persistence, and many populations are declining or extirpated (Amiro, 2003; Amiro *et al*., 2006).

Depending on the characteristics of a river, most kelts overwinter in pools and descend in spring (Bardonnet and Baglinière, 2000), but if suitable overwinter habitat is limited, kelts may exit a river in autumn, after spawning (Lévesque *et al*., 1985), or overwinter in an estuary (Cunjak *et al*., 1998). Repeat spawning salmon from the Maritime Provinces of Canada generally have two distinct life history strategies: (i) consecutive spawners which return the same year as their kelt migration after a short ocean residence; (ii) alternate spawners which return the following year and are known from tagging studies to travel as far north as West Greenland (Ritter, 1989). For some rivers, particularly where there is an extended estuary, it has been suggested that kelts may remain in marine habitat near the river plume while reconditioning, before they return as consecutive spawners (Huntsman, 1938; Mills, 1971).

Here, we used acoustic telemetry to examine the early migration success, behaviour, and habitat use of Atlantic salmon kelts from the LaHave River, Nova Scotia. The research focused on kelts because of knowledge gaps concerning movement and survival during emigration and their potential for significant contribution to population persistence. In the LaHave River, adult and smolt monitoring indicates that survival of repeat spawning salmon returning in both alternate (80% of kelts) and

consecutive years has fallen from 6 to 3% between the periods 1980 1992 and 1993 2005 (Department of Fisheries and Oceans, unpublished data). Determination of migration timing, habitat use, and location of mortality between successive spawning could be important for management actions that affect recovery of this and other Atlantic salmon populations. Therefore, the objec tives of our research were (i) to document kelt behaviour during outward migration, (ii) to evaluate the possibility of extended kelt residence within the estuary and coastal habitat, and (iii) to estimate the mortality of kelts in different habitats as they emigrate from the river and estuary.

Methods

Study site and capture of kelts

The LaHave River flows southwest into the Atlantic Ocean at the town of Bridgewater, NS (44°23′54′N 64°32′34′W; Figure 1). It has a drainage basin of ~1668 km^2 and an estuary that is up to 600 m wide and 17.5 km long from Bridgewater to Riverport (Gray *et al.*, 1989). Salmon spawn throughout the watershed, and there are many suitable areas for kelts to overwinter (lakes and still waters; Figure 1). Upstream migrating salmon have been monitored since 1974 at the Morgans Falls fishway and trap, a complete barrier 25 km upriver from the tidal limit. Downstream migrating smolts have been monitored since 1996 at a low head run of the river hydroelectric power generation facility at Morgans Falls Power Company, on the opposite bank to the fishway (Amiro and Jansen, 2000).

Monitoring and local knowledge of the timing and locations for the overwinter residence of kelts were used to initiate capture efforts in April 2006 (Figure 1). In all, 34 kelts were captured by three methods: (i) 28 were angled by volunteers from the inlet and outlet of New Germany Lake ~1.5 km above the fishway; (ii) two were captured by seine above New Germany Lake; (iii)

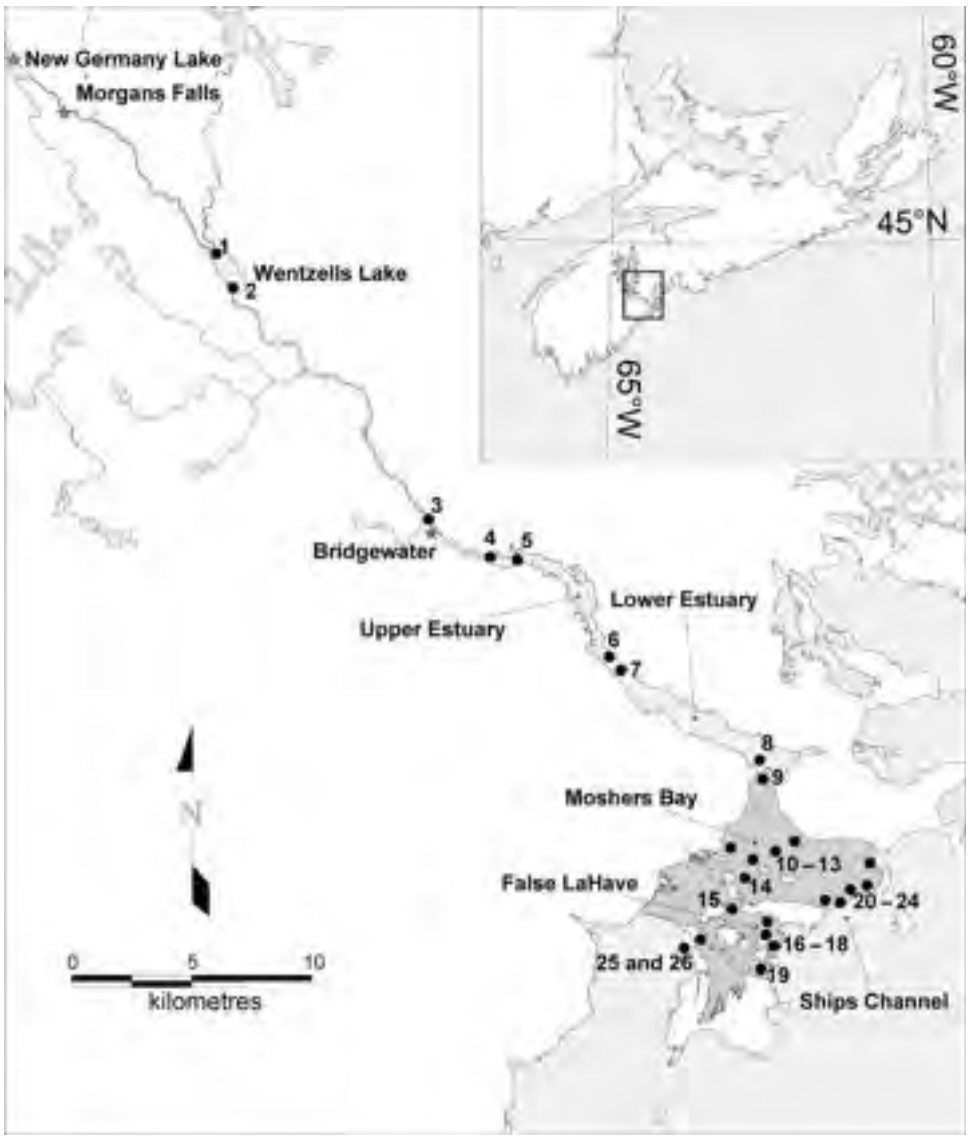

Figure 1. Location and map of the LaHave River and Estuary, Nova Scotia, Canada, indicating the capture site of Atlantic salmon kelts at New Germany Lake, the release site at Morgans Falls, the tidal limit at the town of Bridgewater (stars), and the location of acoustic receivers 1 26 (dots). The dark shaded region represents the coastal area monitored in this study.

four were captured as they passed through the downstream assess ment facility at the Morgans Falls Power Station. Once captured, all fish were transported and held in low flows at the Morgans Falls fishway for 2 11 d, which allowed for recovery from the effects of capture before acoustic transmitters were applied, No mortality or unusual behaviour was observed in the captured kelts before application of the transmitters.

Application of transmitters

In all, 30 kelts had non buoyant acoustic transmitters implanted on 8 or 12 April 2006. Biological characteristics (sea age, sex, origin, length, and weight) were recorded and are summarized in Table 1. A small blue carlin type tag, with an identification number and mailing address, was applied using monofilament line tied subcutaneously beneath the dorsal fin (Carlin, 1955). Carlin tags provide identification if the salmon is captured in an area where there are no receivers or returns to the fishway after the expected battery life of 250 d. The transmitters (Vemco Limited, Halifax, Nova Scotia, Canada) consisted of 25 V13s (36×13 mm; 6 g) and 5 V13Ps (44×13 mm; 6.6 g), with pressure sensors to record depth. The average tag to body mass ratio for this study was 0.74% (range 0.26 1.11%) and was con sidered low enough to avoid effects on survival and behaviour (Thorstad *et al.*, 2000; Lacroix *et al.*, 2004a).

Surgical procedures and equipment were as described by Lacroix *et al.* (2004a, b). Following the insertion of transmitters, kelts were held in a recovery tank treated with Stress Coat® until they regained swimming equilibrium, then returned to a tank of 3 m diameter containing flow through river water. Kelts were released into the pool below Morgan Falls (Figure 1) on three separate days (10 per day on the 11, 12, and 14 April 2006) at the intervals from 09:00 to 16:00, to reduce the probability of multiple code collisions and possible loss of detection at the receivers. The transmitters had a code repeat interval of 20 40 s to further reduce multiple code collisions, to allow for a high probability of detection while passing a receiver at maximum swimming speed, and to maximize battery life (Lacroix and Voegeli, 2000).

Deployment of receivers

Acoustic tags were monitored continuously from May to October 2006. Receivers were placed at strategic river, estuary, and coastal locations to minimize the number of receivers and maximize the probability of detection (Figure 1). In all, 26 VR2 underwater receivers (Vemco Limited) capable of decoding signals up to 500 m away (Lacroix and Voegeli, 2000) were deployed. Receivers were paired upstream and downstream in the river and upper estuary, to allow determination of the direction of movement of recorded signals.

The receivers were anchored with weights of 20 50 kg using 3 m of 8 mm polypropylene rope to which the receiver was attached midway to a float that orientated the receiver upright and above the bottom. A weighted 8 mm rope 60 90 m long was attached to the anchor and laid along the bottom to a second anchor, such that it could be grappled using a towed hook to retrieve the receiver. The positions of weights at both ends of the grapple line were recorded using a hand held Global Positioning System (GPS). The distance between receivers and the shore was usually <300 m (range 100 350 m).

All receivers were retrieved and their electronic information downloaded via an acoustic coupler using Vemco vr2pc software. Receivers were downloaded on 5 May at Wentzells Lake, 13 June at Moshers Bay, 7 July at False LaHave and Ships Channel, and on 14 and 20 July in the upper estuary. Receivers were also downloaded at the completion of the experiment on 21 September in the upper estuary, 6 October at Wentzells Lake and site 2, 11 October at False LaHave, and on 25 October at Moshers Bay and Ships Channel.

Data analysis

Raw data were collected from all receivers. Tags that remained stationary for long periods of time or were lost within the study area were assumed to be mortalities. All mobile tags were assumed to have remained implanted in live kelts. Distances between receivers were measured following the mid course of the river or estuary from the GPS recorded coordinates for recei vers, as mapped on 1:50 000 digital topographic maps using a GIS package (MapInfo®). The rates of migration between receivers were calculated as the distance between two receivers minus 1 km (to account for detection range), divided by the time between the last detection of the first receiver and the first detec tion of the second. ANOVA and Mann Whitney U tests were preformed to test for effects of sex, origin, and date of release on migration rates. χ^2 tests were performed to test the influence of diurnal and tide cycles on kelt movement.

Results

Kelts were detected over a significant proportion of their migration, revealing behavioural variation in the speed and nature of migration (Figure 2). Two tags were detected at down stream receivers without previously being detected at the tidal limit (location 3: Figure 1) without previously being detected by any upstream receiver. Six kelts reached the open ocean within a week, and another three took >4 weeks (Table 2). The rate of migration ranged from 1.61 to 16.2 km d ^{1} with a median rate of 3.7 km d ^{1}. Males migrated slightly faster than females, but the difference was not significant at a confidence level of 95% (Mann Whitney test, $p = 0.085$). The effect of release date on the rate of

Table 1. Biological characteristics of 30 kelts selected as subjects for this study.

Sea age	Origin[a]	Number tagged	Sex ratio (M:F)	Length (cm)				Weight (kg)			
				Mean	Minimum	Maximum	s.d.	Mean	Minimum	Maximum	s.d.
1	H	10	3:2	58.0	54.1	63.5	3.4	1.5	1.3	1.9	0.2
	W	18	4:5	55.3	48.5	60.8	2.7	1.4	1.0	1.8	0.2
2	H	1	0:1	76.7				4.1			
	W	1	0:1	69.3				2.9			

[a]H, Hatchery; W, Wild.

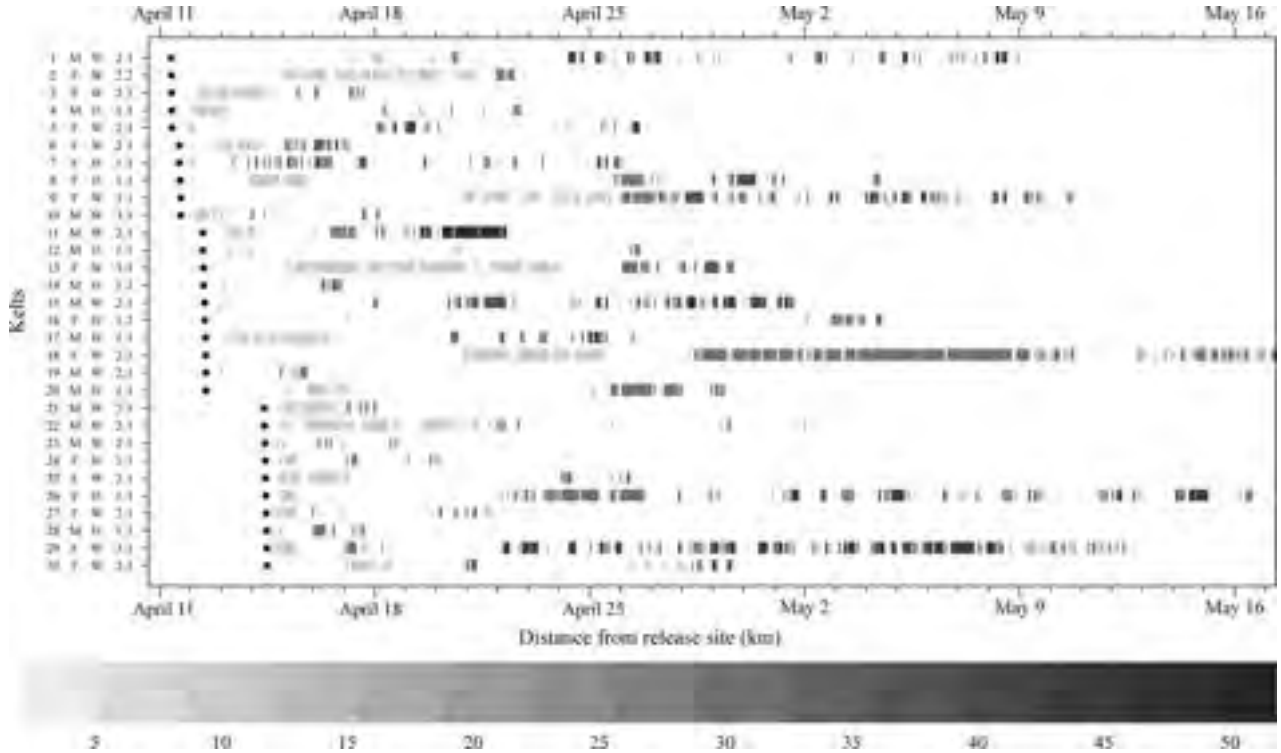

Figure 2. Detections of 30 kelts implanted with acoustic transmitters during their migration out of the LaHave River. The shade of the bands indicates the distance from the release site at Morgans Falls. Dots indicate the timing of the releases and the *y*-axis labels show kelt number, sex, origin, and age (river.sea).

migration was not significant (ANOVA, $p = 0.11$ and 0.56, respectively). There was no significant difference in the rates of migration between fish of hatchery or wild origin (Mann Whitney test, $p = 0.77$). Up to 2005, the LaHave River was stocked with smolts since identifiable as adults, because the adipose fin was removed. There was also no significant difference between the rates of migration in the river and estuary (Mann Whitney test, $p = 0.69$), but for the estuary portion of the migration, four rates of migration >25 km d^{-1} were recorded (Figure 3).

Depth data showed that migrating kelts were most frequently recorded near or at the surface, and less in the water column or near the bottom; in 90% of detections, a kelt was located within 1 m of the surface. However, there were occasional descents to $\sim$3 m consistently throughout the area (Figure 4). There were only four detections of deep dives, up to 15 m, all of which were in Ships Channel, the deepest part of the study area. Overall, the depths of the occasional dives approached the depths at the receiver location, suggesting that the dives could have been to or near the bottom.

The pattern of migration was not consistent for all kelts, except that none showed extended residence in the river, estuary, or inside the LaHave Islands in the marine environment. Observed patterns included: (i) short residence periods in Wentzells Lake and the estuary; (ii) backtracking movements up and down the estuary; (iii) direct migration through the estuary (Figure 2).

Diurnal patterns were not revealed in our analysis of kelt movement downstream. The difference between the expected and observed frequency of kelts arriving at and departing from receivers during daylight was not significant ($\chi^2 = 0.0057$, $p = 0.94$). River discharge increased then decreased during the period of

Table 2. Number of tagged kelts present by location each week after their release.

Location (receivers)	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Morgans Falls to Wentzells Lake (release to 1)	30	2	0	0	0	0
Wentzells Lake (1 to 2)	28	7	1	0	0	0
Wentzells Lake to Bridgewater (2 to 3)	23	13	6	0	0	0
Bridgewater (3 to 4)	15	10	7	1	0	0
Upper estuary (5 to 6)	13	14	6	1	0	0
Lower estuary (7 to 9)	9	13	13	5	3	1
Moshers Bay (10 to 14)	7	7	12	5	3	1
False LaHave (15 to 19)	0	2	0	1	0	0
Ships Channel (20 to 24)	6	8	10	2	2	1

A kelt may be present in more than one location each week, and may reside in any given location for more than 1 week.

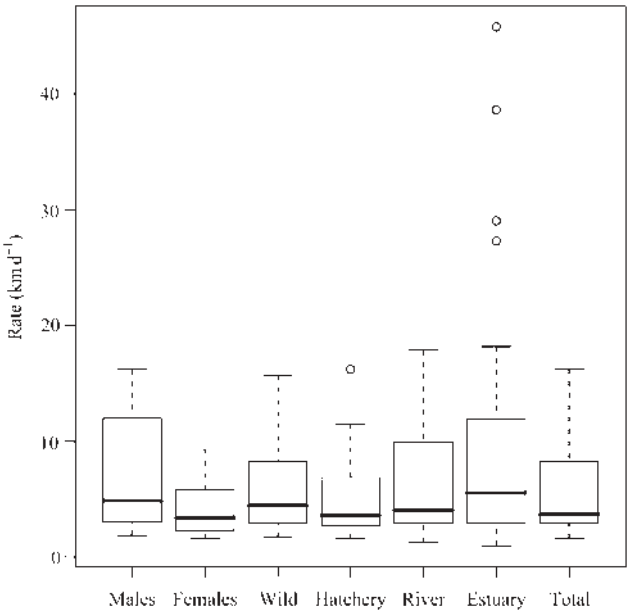

Figure 3. Comparison of the rates of migration for 30 Atlantic salmon kelts during their downstream migration in spring 2006, by sex, origin, and location. River is from release to tidal limit (25 km), estuary is from tidal limit to last detection (25 km), and total is from release to last detection (50 km). Outliers for the estuary are four kelts that passed through this area relatively quickly.

kelt migration downstream. However, the greatest frequency of kelts departing the river was before the rise in water levels (Figure 5), leading us to infer that the negative correlation between river discharge ($m^3 s^{-1}$) and migration rate ($km d^{-1}$) was not credible. There appear to be fewer observations of kelts exiting the river and estuary during a low rising tide (Figure 6). Tidal cycle had a significant influence on the movement patterns of the kelts observed exiting the estuary ($\chi^2 = 71.0$, $p < 0.001$), but not the river ($\chi^2 = 3.71$, $p = 0.29$).

Residence times were variable in both fresh water and estuarine habitat. On average, kelts spent ~2 d in the relatively small (2 km × 800 m) Wentzells Lake, although a few kelts spent up to a week in the lake. Kelts were detected at both ends of the lake, with an approximate 60/40 split between upper and lower halves. The area with the longest average residence was the lower estuary, between receivers 7 and 8 (Table 3, Figure 1), with a mean residence of nearly 5 d. Six kelts were present within the lower estuary for >10 d. Most of the residence time was spent around the narrow passage between the estuary and Moshers Bay, ~45 km from the release site (Figure 2). It was not uncommon for a kelt to travel back and forth from the inner ring of receivers in Moshers Bay to the receivers in the lower estuary. Although only two kelts left through False LaHave, one spent nearly 3 d there. The passage time of kelts moving through Ships Channel tended to be brief (<2 h, Table 3).

Overall mortality for the downstream migration was estimated to be 10%, and all mortalities were below the tidal limit. In all, 27 kelts passed all 26 receivers by 17 May 2006, 5 weeks after their releases (Figure 1). Except the receiver at the tidal limit (location 3), no tags were detected farther downstream after being missed by an upstream receiver, suggesting that these tags were removed from the estuary. Therefore, two kelts (numbers 1 and 15) were presumed to have died in the estuary, because the implanted tags were not detected leaving the coastal area (Figure 1). Those kelts were last detected in the lower estuary and in Moshers Bay on 8 May 2006 (28 d after release) and 1 May 2006 (19 d after release), respectively. The other presumed mortality (number 18) was first detected at the fourth receiver just downstream of Bridgewater on 27 April 2006 (15 d after release), and was detected there continuously until the receiver was removed in September (Figure 1). If the kelts had continued to experience a similar survival rate beyond the study area, then we would expect 5 of 26 tagged kelts to return as alternate spawners. However, no tagged fish returned as alternate spawners in 2007, and given the return rate of repeat spawners at Morgans Falls (3%, Department of Fisheries and Oceans, unpublished data), the mortality rate between spawning events is likely much higher.

After leaving the study area, kelts were expected to either return as consecutive or alternate spawners, or to perish in the ocean. A single two sea winter (2SW) salmon (number 2) was detected on 10 July 2006 in False LaHave, returning as a consecutive spawning salmon, and was recaptured at Morgans Falls on 22 July 2006. Its rate of downstream migration was 7.84 km d^{-1}, and its rate of upstream migration was 5.44 km d^{-1}. Of a total of 102 d from release to recapture, 79 d were spent in the ocean beyond our study area, and during that period, the fish had reconditioned, increased in weight by 50%, and was more active when it re entered the estuary than when it departed. Depth data for that fish showed that the reconditioned returning salmon made more frequent and deeper dives while returning (Figure 4) than it did as an out migrating 2SW kelt.

Discussion

A major driving force in research of anadromous fish species is in identifying the location and causes of natural mortality, to address management and conservation objectives. We found that survival of post spawning Atlantic salmon migrating through river, estuarine, and near coastal habitat was high, although the seaward migration rates of post spawning Atlantic salmon were variable. Moreover, the extent of mortality during transit through these areas was insufficient to account for the decline in the rate of repeat spawning observed in this population.

The variable rates of movement detected likely reflected different behavioural patterns of migrating kelts. The migration rates for the kelts in this study were slightly less but still within the range of migration rates reported for upstream migrations of Atlantic salmon returning to spawn (Mills, 1989; Gerlier and Roche, 1998). The fastest migration rate recorded in this study was the same as the maximum sustainable swimming velocity for kelts (0.5 m s^{-1} for 200 min) reported by Booth *et al.* (1997), indicating non stop migration for this fish. It is likely that water levels were sufficient to allow for kelt migration at the beginning of this study, because most kelts exited the river before the water level rose. The differences in the level of significance for the effect of tide cycle on the timing of kelt movement may be related to the difference in the number of observations between the tidal limit and estuary mouth. The consistency of the pattern, however, suggests that the effect may be real. Although brief periods of relatively continuous migrations were fairly common (30% were <1 week; Figure 2), the periods of residence and backtracking in the migration of many other kelts indicate that, unlike upstream migrating salmon (Baglinière *et al.*, 1990; Finstad *et al.*, 2005), there does not appear to be a typical pattern of kelt migration.

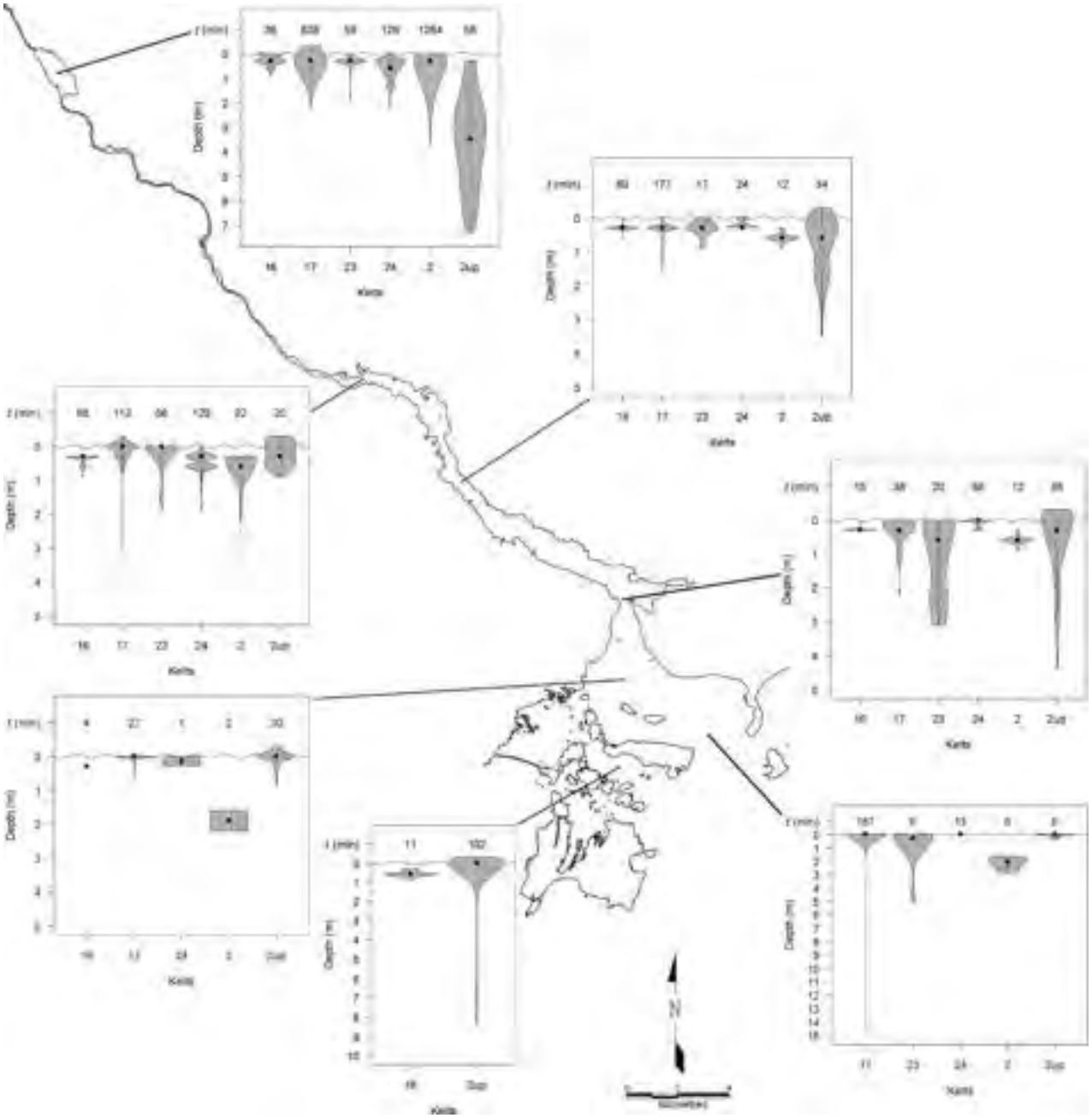

Figure 4. Depth profiles of five Atlantic salmon kelts (numbers 2, 16, 17, 23, and 24) during their downstream migration in spring 2006, plus the upstream migration of the reconditioned kelt (2up) in July 2006. The profiles represent the relative amount of time each kelt spent at a given depth. Shown above the plots is the total time (t) in min for which depth data were recorded for each kelt, at each location.

Movements and delays in the estuary could be related to active feeding or predator avoidance. Common prey species for Atlantic salmon, such as rainbow smelt and sandlance (Hislop and Shelton, 1993; Lacroix and Knox, 2005), are routinely caught in the estuary by local fishers in spring. The occasional dive observed for some kelts could have been associated with feeding or searching for prey. Kelts are voracious in fresh water, and White (1942) suggested that feeding before entering the sea may be critically important to their survival to the next spawning event. Alternatively, the behaviours may have been associated with acclimation to seawater in the halocline of the estuary, or adaptation to other physiological stresses, such as temperature regulation (Reddin *et al.*, 2004, 2006). Bendall *et al.* (2005) suggested that the variation in the time it takes individual fish to "re smoltify" may be related to the differences in the bioenergetic costs of spawning. This could have resulted in the differences we observed in migration rate, but in an associated analysis not pre sented here, we failed to find a significant correlation between migration rate and condition factor ($r^2 = 0.143$).

Although estuarine residence times for returning salmon are not well studied, it appears that kelts do not spend most of their time in the marine environment in the estuary. All surviving

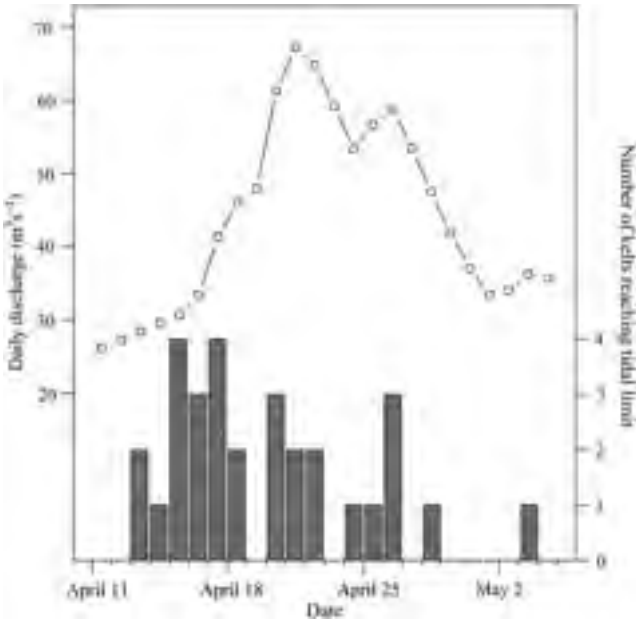

Figure 5. Number of Atlantic salmon kelts reaching the tidal limit during downstream migration (bars, right *y*-axis) relative to the daily discharge (line, left *y*-axis) of the LaHave River, NS, Canada.

kelts left the estuary within 5 weeks of their release, indicating that they were still exhibiting migrating behaviour, and just one returned as a consecutive spawning salmon. This fish left the estuary 11 d after its release, and spent 79 d in the ocean beyond the coastal array of receivers. River discharge was 22 $m^3 s^{-1}$ when it entered the estuary, and it entered the river 4 d later, when the discharge had increased to 48 $m^3 s^{-1}$. The time spent in an estuary before entering a river varies between rivers and depends on conditions. Some returning salmon arrive in an estuary in spring and stay there until they ascend the river in autumn (Brawn, 1982). Based on our observations of kelts exiting the estuary, and from information on one reconditioned kelt, we conclude that kelts do not stay within our estuary to recondition for extended periods before returning as consecutive spawning salmon.

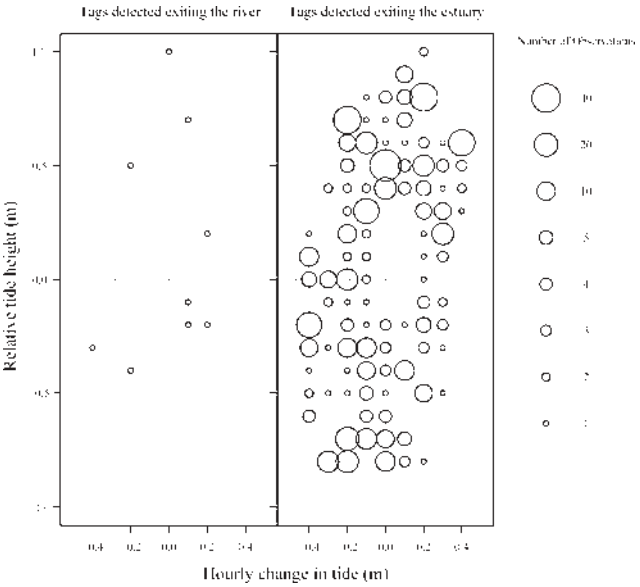

Figure 6. The number of detections for tags leaving the river (left panel) and estuary (right panel) at a given point in the tide cycle. The tide cycle is represented by relative tide height (deviations from the mean), and hourly change in tide. Negative values for relative tide height indicate a low tide, positive values a high tide. Negative values for hourly change in tide indicate an ebb tide, positive values a flood tide. The size of the points indicate the number of times a tag was detected at a given tide height.

Table 3. Summary of residence times (d) per location for 30 downstream-migrating kelts.

Location (receivers)	Mean	s.d.	Minimum	Maximum
Morgans Falls to Wentzells Lake (release to 1)	1.73	2.37	0.11	9.14
Wentzells Lake (1 to 2)	2.00	2.24	0.09	8.90
Wentzells Lake to Bridgewater (2 to 3)	3.37	3.62	0.24	15.97
Bridgewater (3 to 4)	0.68	1.00	0.02	4.45
Upper estuary (5 to 6)	1.42	2.25	0.08	10.38
Lower estuary (7 to 9)	4.79	6.44	0.09	23.56
Moshers Bay (10 to 14)	1.40	3.49	0.0003	13.14
False LaHave (15 to 19)	0.11	0.54	0.00	2.80
Ships Channel (20 to 24)	0.06	0.07	0.003	0.33
Total	13.06	8.24	3.18	32.10

All kelts in this study survived capture, holding, and surgical procedures. Kelts were retained until recovery, appeared to migrate actively, and reached salt water within 2 weeks of release, indicating that it is unlikely that tagging influenced mor tality. This statement is supported by the results of previous work, which found that kelts recover more quickly from the stres ses associated with angling than salmon that have recently entered fresh water from the ocean (Brobbel *et al.*, 1996), and that implanting dummy transmitters on Atlantic salmon of similar size had no effect on swimming performance (Thorstad *et al.*, 2000). Therefore, there is a strong possibility that missing and stationary tags were the result of natural mortality.

All tags that exited the bay within the first 5 weeks were assumed to remain intact in kelts. The rates of movements and general direction of movement to the open ocean support this assumption; however, there is still a possibility that a detection was of a tag inside a predator. Potential predators that were observed in the estuary during the course of the study, and fre quently spotted by local fishers include osprey (*Pandion haliaetus*), bald eagle (*Haliaeetus leucocephalus*), harbour seal (*Phoca vitu lina*), and grey seal (*Halichoerus grypus*). Perhaps lost or stationary tags could have been the result of predation by a predatory bird or by a seal that did not ingest the tag, because such tags would not be detected moving past the receivers. It is not known whether either seal species would ingest and retain tags from a kelt and hence give the appearance of a moving tag. However, based on the observed residence time of these predators in the estuary or bay, the observed high movement speeds of tags, and the digestion time for seals (Markussen, 1993; Thompson *et al.*, 1996), it is unlikely that observations of moving tags were from tags ingested by seals.

The level of mortality experienced by Atlantic salmon during winter after they spawn has not been well quantified and needs future study. Overwinter survival in fresh water was once

estimated at 30–46% for wild kelts from the East River Sheet Harbour, Nova Scotia, and 98.8% for kelts in captivity (Ruggles, 1980). Because few salmon are found dead in a river, Dymond (1963) suggested that mortality most likely took place in salt water. Therefore, given the results of this study, we conclude that the location of mortality for most migrating kelts was outside the estuary and bay. It is unlikely that the consecutive spawner in this study would have had sufficient time to reach coastal waters of Newfoundland or Greenland, where alternate spawners of populations from other Canadian Maritime rivers have been found (Ritter, 1989; Reddin and Lear, 1990). Much of the route for alternate spawning salmon is similar to maiden 2SW salmon (Reddin and Shearer, 1987), for which the timing and location of the increased mortality since 1990 is unknown (Chaput *et al.*, 2005). Therefore, future studies involving kelt tagging and distant oceanic receivers as well as correspondence tags may permit these and other questions regarding the migration of Atlantic salmon to be addressed. Determining the marine distribution and locations of high mortality is a primary goal in Atlantic salmon research, and is essential for implementing steps to mitigate the continuing population decline.

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ICES WKCULEF REPORT 2016

ICES ADVISORY COMMITTEE

ICES CM 2016/ACOM:42

Report of the Workshop to address the NASCO request for advice on possible effects of salmonid aquaculture on wild Atlantic salmon populations in the North Atlantic (WKCULEF)

1–3 March 2016

Charlottenlund, Denmark

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Contents

Executive summary

Workshop to address the NASCO request for advice on possible effects of salmonid aquaculture on wild Atlantic salmon populations in the North Atlantic [WKCULEF], Copenhagen, Denmark, 1–3 March 2016.

Chairs: Ian Russell (UK) and Ole Torrissen (Norway).

Number of meeting participants: 25 representing six countries: Norway (ten), Ireland (four), UK (Scotland) (four), Canada (three), UK (England & Wales) (two) and USA (one). Additional participants also attended from the ICES Secretariat.

WKCULEF met to consider a question posed to ICES by the North Atlantic Salmon Conservation Organisation (NASCO): *Advise on possible effects of salmonid aquaculture on wild Atlantic salmon populations focusing on the effects of sea lice, genetic interactions and the impact on wild salmon production*.

This question was originally included among a suite of questions developed by NASCO, and due to be addressed by the annual meeting of the Working Group on North Atlantic Salmon (WGNAS). However, given that the question was pertinent to other Expert Groups at ICES, particularly the Working Group on Aquaculture (WGAQUA), the Working Group on Pathology and Diseases of Marine Organisms (WGPDMO) and the Working Group on the Application of Genetics in Fisheries and Mariculture (WGAGFM), it was recommended that the question would be best addressed by means of a Workshop, independent of the Working Groups. WKCULEF enabled experts in aquaculture effects, wild Atlantic salmon, disease transmission and genetic interaction to share and discuss relevant information and recent findings, in order to meet the objectives and timeline of the request.

The terms of reference were addressed though a comprehensive review of the recent peer-reviewed literature. This was facilitated by a range of presentations from participants, by reviewing working documents prepared ahead of the meeting as well as the development of documents and text for the report during the meeting. The report is structured in two main sections, one focusing on the effects of sea lice and the other on genetic interactions. The third issue specified in the question from NASCO, namely the impact of salmon farming on wild salmon production, has been relatively poorly researched and most information derives from attempts to evaluate population level effects related to sea lice infestation and genetic introgression. This information has therefore been reported in the sea lice and genetics sections of the report, respectively.

WKCULEF briefly discussed microbial diseases in aquaculture and the potential impact on wild salmon. However, it was not possible to review this issue in detail and it has not been included in this report.

The key findings of the Workshop were:

Sea lice

- The sea louse (*Lepeophtheirus salmonis*) has widespread geographic distribution, is an important parasite of salmonids and has been a serious problem for the Atlantic salmon farming industry since the 1970s. Sea lice have a greater economic impact on the industry than any other parasite and control of lice levels on farms is of key importance.

- Salmon farming has been shown to increase the abundance of lice in the marine environment and the risk of infection among wild salmonid populations. However, there is considerable uncertainty, and spatial and temporal variability, about the extent of the zones of elevated risk.
- It has been shown in laboratory studies that 0.04–0.15 lice per gramme fish weight can increase stress levels. Laboratory studies have also demonstrated that infections of 0.75 lice per gramme fish weight, or approximately eleven sea lice per fish, can kill a recently emigrated wild salmon smolt of about 15 g if all the sea lice develop into pre-adult and adult stages.
- A number of studies in Norway and Ireland have estimated the relative marine survival of smolts treated to provide lice resistance and control groups. All studies have reported an overall improved return rate for treated salmon, but all showed significant spatial and temporal variability in the magnitude of the treatment effect.
- The survival of Atlantic salmon during their marine phase has fallen in recent decades. This downturn in survival is evident over a broad geographical area and is associated with large-scale oceanographic changes. Viewed against current marine mortality rates commonly at or above 95%, the 'additional' mortality attributable to sea lice has been estimated at around 1%.
- In some studies, the impact of sea lice has also been estimated as losses of returning adult salmon to rivers. These estimates indicate marked variability, with losses in individual experiments ranging from 0.6% to 39%. These results suggest that sea lice induced mortality has an impact on Atlantic salmon returns, which may influence the achievement of conservation requirements for affected stocks.
- Much of the heterogeneity among trials comparing the survival to adulthood of juvenile salmon administered sea lice medicines and control groups could be explained by the release location, time period and baseline (i.e. marine) survival. In a recent meta-analysis of Norwegian data, baseline survival was reported to be the most important predictor variable. When this was low, the effect of treatment was high. In contrast, when baseline survival was high, the effect of treatment was undetectable. However, it is unclear whether baseline survival is affected by sea lice exposure.

Genetic effects

- Each year, large numbers of domesticated salmon escape from commercial fish farms. While many of these are reported, the true number of escapees is likely to be significantly higher. Escapees are observed in rivers in all regions where farming occurs, although the numbers of escapees vary both spatially and temporally. It has been noted that in some rivers in some years, the numbers of escapees have approached 50% or more of the spawning population.
- The spawning success of escaped farmed salmon is much lower than wild salmon. Despite this, genetic studies have demonstrated that farmed salmon have displayed widespread introgression in a large number of Norwegian populations where this has been investigated. Introgression has also been shown in other countries, but the full extent of introgression remains to be investigated.

- Farmed salmon are domesticated and display significant genetic differences to wild salmon in a wide range of fitness-related traits. Whole-river experimental studies have demonstrated that the offspring of farmed and cultured salmon in general, display lower fitness than their wild counterparts in the wild.
- Juvenile escapees and the offspring of farmed salmon compete with wild salmon for territory and food. Therefore, their presence in the natural habitat will reduce the total production of wild fish. Studies have also shown this can result in a decreased overall productivity of the population.
- Where farmed salmon have successfully interbred with natural populations, it is likely that recipient populations will display changes in life-history traits. These changes are likely to be maladaptive for the wild population.
- The long-term consequences of introgression across river stocks can be expected to lead to reduced productivity and decreased resilience to future impacts such as climate change (i.e. less fish and more fragile stocks).
- The evidence from studies in the wild, and the extensive literature relating to salmonids in general, demonstrates that the offspring of farmed salmon display reduced fitness in the wild. However, the results of these studies suggest that the relative success of farmed salmon and, likewise, the relative potential negative effect on a native population, is likely to vary in time and space. Wild populations that are already under evolutionary strain from other challenges such as disease pressure, sea lice infection, over exploitation, habitat destruction and poor water quality are more likely to be sensitive to the potential negative effects of genetic introgression and loss of fitness. Therefore, such effects have to be seen in the context of other challenges.
- While recognising that there were still uncertainties, WKCULEF considered that the evidence relating to the impacts of escapees / genetic introgression provided a clear indication of impacts on wild salmon populations. A substantial reduction of escaped farmed salmon in the wild, or sterilization of farmed salmon, would be required in order to minimize effects on native populations.

In reviewing the latest evidence pertaining to sea lice and genetic interactions, WKCULEF considered where there were gaps in current knowledge and identified areas for further investigation.

1 Introduction

1.1 Workshop rationale and objectives

At its 2015 Statutory Meeting, ICES resolved (C. Res. 2015/2/ACOM10) that the Working Group on North Atlantic Salmon [WGNAS] (chaired by: Jonathan White, Ireland) would meet at ICES, Copenhagen, 30 March–8 April 2016 to consider various questions posed to ICES by the North Atlantic Salmon Conservation Organisation (NASCO). However, one of these questions, relating to the possible effects of salmonid aquaculture on wild Atlantic salmon, has a particularly broad remit and cuts across the work of a number of ICES Groups. In subsequent discussions between the ICES Secretariat and WGNAS participants, it was agreed that responding to this question required the input of experts from a range of disciplines and different Expert Groups within ICES. Given the timing of the annual meetings of these different Expert Groups and the requirement for the advice to be drafted, reviewed and made available by early May 2016, it was decided that an independent workshop needed to be convened to address this question.

ICES subsequently resolved (C. Res. 2015/2/ACOM:42) that the Workshop to address the NASCO request for advice on possible effects of salmonid aquaculture on wild Atlantic salmon populations in the North Atlantic (WKCULEF), chaired by Ole Torrissen (Norway) and Ian Russell (UK), will meet at ICES, Copenhagen 01–03 March 2016.

WKCULEF was publicised on the ICES website and members of the following relevant ICES Expert Groups were encouraged to send appropriate representation: the Working Group on Aquaculture (WGAQUA), the Working Group on North Atlantic Salmon (WGNAS), the Working Group on Pathology and Diseases of Marine Organisms (WGPDMO) and the Working Group on the Application of Genetics in Fisheries and Mariculture (WGAGFM). ICES Workshops are open to all interested parties and participants from academic and stakeholder organisations also registered to attend WKCULEF. The level of interest in the Workshop was such that numbers of participants exceeded the space originally set aside for the meeting at ICES. The workshop was therefore relocated to DTU-Aqua, located at Charlottenlund just to the north of Copenhagen.

The terms of reference for WKCULEF are to:

a) Identify the possible effects of salmonid aquaculture on wild Atlantic salmon populations, focusing on the effects of sea lice, genetic interactions and the impact on wild salmon production.

b) Based on the issues identified in (a):

  i) Update the findings of the 2005 ICES/NASCO symposium on the impacts of aquaculture.

  ii) Update the ICES advice provided to OSPAR in 2010 and 2014 (ICES, 2010; 2014).

  iii) Prepare the first draft of the advice to address the NASCO request.

WKCULEF will report by 11 March, 2016 for the attention of the ICES Advisory Committee.

WKCULEF were advised that NASCO plan to hold a Theme-based Special Session on the topic of developments in relation to minimizing the impacts of farmed salmon on wild salmon stocks at their annual meeting in June 2016, and the advice will provide a very useful input to that process. ICES are expected to provide the opening presentation at this event.

The terms of reference for WKCULEF focus on interactions between salmon farming and Atlantic salmon and supporting evidence utilised in this report primarily draws upon the scientific literature pertaining specifically to this species. Salmon farming activities can impact on other salmonid species, in particular sea trout and Arctic char, and there is an extensive literature related to these species. However, the majority of such work has not been incorporated into this report.

In addressing the terms of reference, WKCULEF felt that it was particularly difficult to disentangle the issue of the possible impact of salmon aquaculture on wild salmon production from the sea lice and genetic interaction questions. As a result, information pertaining to population level effects was integrated into both these sections and has not been included as a separate section of the report. WKCULEF sought to highlight where there were gaps in current knowledge and identified areas where further investigation was required.

WKCULEF briefly discussed microbial diseases in aquaculture and the potential impact on wild salmon. However, it was not possible to review this issue in detail and such information has not been included in the report.

In response to the Terms of Reference, the Workshop considered 14 Working Documents / presentations submitted by participants (Annex 1); other references cited in the Report are given in Annex 2. A full address list for the meeting participants is provided in Annex 3.

1.2 Participants

Member	Country
Jonathan Carr	Canada
Catherine Collins	UK (Scotland)
Anne Cooper	ICES Secretariat, Denmark
Mark Coulson	UK (Scotland)
Bengt Finstad	Norway
Kevin Glover	Norway
Paddy Gargan	Ireland
Kjetil Hindar	Norway
Dave Jackson	Ireland
Martin Jaffa	UK (England & Wales)
Simon Jones	Canada
Bjørn Olav Kvamme	Norway
Marie Lillehammer	Norway
John Martell	Canada

Philip McGinnity	Ireland
Olav Moberg	Norway
David Morris	UK (Scotland)
Kjell Emil Naas	Norway
Hans Petter Næs	Norway
Michael Pietrak (by Skype)	USA
Ian Russell (chair)	UK (England & Wales)
Terje Svåsand	Norway
Ole Torrissen (chair)	Norway
Eric Verspoor	UK (Scotland)
Jonathan White	Ireland

1.3 Background

The farming of Atlantic salmon has expanded rapidly since the early 1980s. Production of farmed salmon in the North Atlantic is now approximately 1.5 million tonnes (over 2 million tonnes worldwide) and vastly exceeds the nominal catch of wild Atlantic salmon (FishstatJ, FAO, 2013). In 2014, it was estimated that farmed Atlantic salmon production exceeded the nominal wild catch in the North Atlantic by over 1900 times (ICES, 2015).

Interactions between salmon farming and wild stocks have raised concerns, in particular related to disease, parasite, genetic and ecological interactions. Such issues have been subject to extensive research and dialogue as efforts have been made to balance the needs of industry with the requirement to safeguard wild stocks. The topic remains an area of continued intensive research interest. In seeking fresh advice from ICES on the possible effects of salmonid aquaculture on wild Atlantic salmon populations in the North Atlantic, NASCO have highlighted that this should update previous findings and advice, citing in particular the ICES/NASCO symposium on the impacts of aquaculture held in 2005 and previous ICES advice to OSPAR on aquaculture impacts. The following paragraphs provide a brief overview of these earlier information sources.

ICES/NASCO Symposium, 2005

The ICES/NASCO Symposium (*Interactions between aquaculture and wild stocks of Atlantic salmon and other diadromous fish species: Science and management, challenges and solutions*) was held in Bergen, Norway in October 2005. This, in turn, aimed to build on two earlier international symposia on the subject. In 1991, an initial symposium was convened by the Norwegian Directorate For Nature Management and NASCO in Loen, Norway (Hansen *et al*., 1991), and this was followed by an ICES/NASCO symposium in Bath, UK in 1997 (Hutchinson, 1997). This latter symposium helped to inform development of a NASCO resolution aimed at minimising impacts from aquaculture, introductions and transfers, and transgenics on wild salmon stocks (Williamsburg Resolution; NASCO, 2006).

The objectives of the 2005 ICES/NASCO symposium were:

i) to summarise available knowledge of the interactions between aquaculture and wild salmon stocks and other diadromous fish species;

ii) to identify gaps in current understanding of these interactions and to develop recommendations for future research priorities;

iii) to review progress in managing interactions, the remaining challenges, and possible solutions; and

iv) to make recommendations for additional measures to ensure that aquaculture practices are sustainable and consistent with the Precautionary Approach.

A convener's report was prepared (Hansen and Windsor, 2006) with many of the papers included in a special edition of the ICES Journal of Marine Science (Hutchinson, 2006).

The issues covered by the symposium in relation to sea lice included:

- *Gaining a better understanding of the behaviour and ecology of sea lice*. Topics covered: the impact of temperature and salinity on development, behaviour and dispersal of lice; population structure and genetic diversity of sea lice; dispersal patterns / models; evaluation of changes in lice levels relative to the farm production cycle; and the refinement of pest management strategies, including assessing risks to wild populations and possible vaccine development.
- *Evaluation of interactions / impacts*. Topics covered: the effects of lice on the physiology and osmoregulation of fish; infection pressure relative to farm proximity, site and year; the possible development of 'threshold' levels and predictors of mortality to aid management. A particular gap was the lack of information on the effects of lice on wild populations, with the hope that 'new' studies would provide such assessments.
- *Sea lice management*. Topics covered: monitoring programmes; the heavy reliance on a few key medicines and treatments; development of resistance to treatments; alternative controls measures (e.g. wrasse); and the importance of effective integrated pest management strategies.

The issues covered by the symposium in relation to genetic and ecological interactions included:

- *Escapees*. Topics covered: improvements in reporting (both successes and failures) and in understanding the causes of escapes and in management responses; dispersal investigations and variable survival / behaviour with timing of release (and other factors); indications that levels of farmed salmon in cages were a better predictor of escapees rather than reported losses (suggesting possible failure to account for 'trickle' losses / concerns about the reliability of reporting); cage design developments; escape of juveniles from freshwater hatcheries and risks posed by hatchery releases and stocking.
- *Genetic developments and interactions*. Topics covered: genetic selection in farms and 'domestication' of strains; potential for the genetic tracing of the source of escapees; clear evidence of farmed fish contributing to spawning

in rivers and of changes in genetic composition of wild stocks over time (reduced population differentiation can occur quite quickly); impacts on wild stocks related to numbers of farm-origin spawners; application of models to predict cumulative effects over generations; and meta analysis suggesting reduced productivity of wild populations in proximity of farms.

In an overview, the conveners concluded that the symposium had provided significant advances in understanding in the management of both sea lice and escapees. However, significant challenges remained and risks were not fully understood. They welcomed the recognition from industry representatives that farming can have damaging impacts on wild stocks. This was seen as a clear prerequisite to cooperative action, but needed to to be continued and enhanced if solutions to remaining challenges were to be found. Ongoing data sharing, trust and cooperation between industry, regulators and wild fish interests was seen as essential to developing effective management control strategies.

The conveners noted that numbers of escapees remained large relative to wild stocks, with risk of irreversible damage to the stock structure and diversity of wild salmon and potential consequences for the fitness and productivity of stocks and their ability to adapt to environmental change. As a result, they proposed that interactions needed to be virtually eliminated, not just reduced, and that containment measues needed to be much improved, or production shifted to the use of sterile salmon.

Priorities for further work were seen as improving understanding in:

- The dispersal and spawning success of escapees;
- Impacts on wild populations;
- Genetic techniques for tracing the origin of escapees;
- The potential for using sterile fish / triploids;
- Sea lice treatments and other emerging disease challenges;
- Cage designs and the possible increased risk from storms related to climate change.

ICES advice to OSPAR

In recent years, ICES has been asked to provide advice to OSPAR on interactions between wild and farmed fish (ICES, 2010; 2014). These requests have extended to all finfish mariculture activities, although such activities are dominated by Atlantic salmon production.

In 2010, ICES was asked to provide advice on the current state of knowledge of the interaction of finfish mariculture on the condition of wild fish populations at a local and regional scale, including from parasites, escaped fish and the use of fish feed in mariculture. Advice was also requested on how the interactions will change as a result of an expansion of mariculture activities. ICES collated available information and completed a risk analysis of interactions between mariculture and wild fish populations. The summary of the advice generated noted that the degree of interactions may be 'moderate' between finfish mariculture and wild fish populations at the scale of a river local to a salmon farm, but are lower at a broader scale.

In 2014, the request from OSPAR identified a number of potential pressures arising from mariculture on which advice was required:

i) introduction of antibiotics and other pharmaceuticals;

ii) transfer of disease and parasite interactions;

iii) release of nutrients and organic matter;

iv) introgression of foreign genes, from both hatchery-reared fish and genetically modified fish and invertebrates, in wild populations;

v) effects on small cetaceans, such as the bottlenose dolphin, due to their interaction with aquaculture cages;

vi) non-indigenous species.

ICES provided a brief update on the knowledge in each of these areas, commented on potential management solutions to mitigate pressures and outlined monitoring needs. The advice summary was similar to that in 2010 in concluding that most interactions examined in the request are expected to be localized to the vicinity of the mariculture sites. However, the advice noted that although there is reasonable evidence that interactions occur, scientific support for the significance of identified interactions is generally weak. ICES advised that formal risk assessments prior to establishing new mariculture developments may help identify issues and prevent the development of negative interactions. ICES further advised that the inclusion of genetic risks in such assessments is critical and often over-looked.

2 The effects of sea lice on Atlantic salmon

2.1 Introduction

All fish are susceptible to parasitic infections. The sea louse (*Lepeophtheirus salmonis*), also commonly called the salmon louse, has widespread geographic distribution, is an important parasite of salmonids and has been a serious problem for the Atlantic salmon farming industry since the 1970s (Thorstad *et al*., 2015). Sea lice have a greater economic impact on the industry than any other parasite (ICES, 2010) and control of lice levels on farms is of key importance. The high density of salmon in cages has provided a large number of potential hosts and promoted the transmission and population growth of the parasite (Torrissen *et al*., 2013). As a result, salmon farming has been shown to increase the abundance of lice in the marine environment. However, knowledge of parasite infection rates and resulting effects in wild populations of fish is relatively poor.

Historically, naturally occurring lice levels on wild salmonids have typically been low - a few (0–10) adult lice per returning salmon and sea trout (Torrissen *et al*., 2013; Serra-Llinares *et al*., 2014). Elevated levels of sea lice on wild salmonids collected from coastal areas in the vicinity of salmon farms has been regarded as evidence that mariculture is a main source of the infections and studies have demonstrated a link between fish-farming activity and sea lice infestations on wild salmonids (Helland *et al*., 2012; 2015; Middlemas *et al*., 2010; 2013; Serra-Llinares *et al*., 2014). Thus, the risk of infection among wild salmon populations can be elevated in areas that support salmon mariculture, although louse management activities can reduce the prevalence and intensity of infection on wild fish (Penston and Davies, 2009; Serra-Llinares *et al*., 2014). There is considerable uncertainty about the extent of the zones of elevated risk of infection and this will be subject to both spatial and temporal variability, for example as a result of changes in local hydrological processes (Amundrud and Murray, 2009; Salama *et al*., 2013; 2015; Jones *et al*., 2015; Johnsen *et al*., 2016).

The extent to which elevated infections of sea lice pose a risk to the health of wild salmon populations has been the subject of extensive research. However, there are many difficulties in quantifying effects at the population level, particularly for fish stocks that are characterised by highly variable survival linked to environmental variables, such as Atlantic salmon (Vollset *et al*., 2015; Helland *et al*., 2015). The following sections aim to summarise the current state of knowledge in relation to the impact of sea lice on Atlantic salmon.

2.2 Physiological effects

Several laboratory studies have presented the effect of sea lice on host physiology of Atlantic salmon, sea trout and Arctic charr smolts (reviewed in Finstad and Bjørn, 2011; Thorstad *et al*., 2015). Major primary (nervous, hormonal), secondary (blood parameters) and tertiary (whole body response) physiological effects, including high levels of plasma cortisol and glucose, reduced osmoregulatory ability and reduced non-specific immunity in the host occur when the lice develop from the sessile chalimus 2 stage to the mobile first pre-adult stage. Sublethal tertiary effects, such as reduced growth, reduced reproduction; reduced swimming performance and impaired immune defence have also been reported (see Finstad and Bjørn, 2011 for references). In addition, differences in genetic susceptibility to sea lice are recognised among host stocks and species.

It has been shown in laboratory studies that 0.04–0.15 lice per gramme fish weight can increase stress levels, reduce swimming ability and create disturbances in water and salt balance in Atlantic salmon. In sea trout, around 50 mobile lice are likely to give direct mortality, and 13 mobile lice, or approximately 0.35 lice per gramme fish weight might cause physiological stress in sea trout (weight range of 19–70 g). Moreover, around 0.05–0.15 lice per gramme fish weight were found to affect growth, condition and reproductive output in sexually maturing Arctic charr (Tveiten *et al*., 2010).

Laboratory studies have also indicated that infections of 0.75 lice per gramme fish weight, or approximately eleven sea lice per fish, can kill a recently emigrated wild salmon smolt of about 15 g if all the sea lice develop into pre-adult and adult stages (Finstad *et al*., 2000). Studies of naturally infested wild salmon post-smolts indicate that only those with less than ten lice survived the infection. This is consistent with field studies on sea lice infections in salmon post-smolts in the Norwegian Sea where more than 3000 post-smolts have been examined for lice, but none observed carrying more than ten adult lice. Fish with up to ten mobile lice were observed to be in poor condition with a low haematocrit level and poor growth (Holst *et al*., 2003). Further support for this threshold comes from an experimental study of naturally infected migrating salmon smolts collected during a monitoring cruise. Half of the fish were deloused as a control, and the health of the two fish groups were monitored in the laboratory. Only fish carrying eleven mobile lice or less survived (Holst *et al*., 2003). The results have been further verified in the laboratory on wild-caught Atlantic salmon post-smolts infected with sea lice and showing the same level of tolerance for sea lice infections (Karlsen *et al*., in prep.)

These results have been used in Norway to provide estimates of death rates according to lice densities on migrating salmon smolts as a management tool and have been adopted in the Norwegian risk assessment for fish farming (Taranger *et al*., 2015). The categories are: 100% mortality in the group >0.3 lice per gramme fish weight, 50% mortality in the group 0.2–0.3 lice per gramme fish weight, 20% mortality in the group 0.1–0.2 lice per gramme fish weight and 0% mortality in the group <0.1 lice per gramme fish weight. Wagner *et al*. (2008) discuss the wider factors that should be taken into account when estimating sea louse threshold levels detrimental to a host.

2.3 Evidence from monitoring programmes

Monitoring programmes have been implemented in a number of countries to assess lice levels to inform management decisions. Given the difficulties of sampling out-migrating wild salmon smolts, sea trout are commonly sampled and in some cases may be used as a proxy for potential levels on salmon (Thorstad *et al*., 2014).

In Norway, the lice infection on wild salmonid populations is estimated through a national monitoring programme (Serra-Llinares *et al*., 2014; Taranger *et al*., 2015). The aim of the sea lice monitoring programme is to evaluate the effectiveness and consequences of zone regulations in national salmon fjords (areas where salmon farming is prohibited), as well as the Norwegian strategy for an environmentally sustainable growth of aquaculture.

Monitoring is carried out during the salmon smolt migration and in summer to estimate lice levels on sea trout and Arctic charr. The fish are collected using traps, fishing nets and surface trawling (Holm *et al*., 2000; Holst *et al*., 2003; Heuch *et al*., 2005; Bjørn *et al*., 2007). Also, sentinel cages have been used to investigate infestation rates (Bjørn *et al*., 2011).

The results indicate considerable variation between years and sampling locations in the risk of lice related mortality, based on the Norwegian risk assessment criteria for detrimental lice threshold levels (low: <10%, moderate 10–30% and high: >30%). The risk for sea trout (and also Arctic charr in the Northern regions) is higher compared with Atlantic salmon post-smolts and the results show moderate-to-high risk of lice related mortality on sea trout in most counties with high salmon farming activity.

The estimated risk of lice-related mortality for Atlantic salmon varies between years and sites, and was low at most sites in 2010 and 2013, but moderate and high at several sites in 2011, 2012 and 2014.

In Scotland, analysis of wild sea trout monitored over five successive farm cycles found that lice burdens above critical levels (based on laboratory studies of sea trout) were significantly higher in the second year of the production cycle (Middlemas *et al*., 2010). In Norway, preliminary analysis of data from fallowing zones indicate that lice levels in farming areas are also correlated with farmed biomass. In years with high biomass lice epidemics are present in some zones, but such epidemics are not seen in years with low biomass (Serra-Llinares *et al*., submitted).

2.4 Population effects

Population level impacts of sea lice infestation have been estimated in Atlantic salmon post-smolts from a series of long-term studies and analyses in Ireland and Norway involving the paired release of treated and control groups of smolts (Jackson *et al*., 2011 a and b; Jackson *et al*., 2013; Gargan *et al*., 2012; Skilbrei *et al*., 2013; Krkošek *et al*., 2013; Vollset *et al*., 2014; 2015). These studies assumed that the sea louse treatments were efficacious, and that released smolts were exposed to sea lice during the period of the outmigration in which the treatment was effective. Furthermore, the studies were not designed to discriminate between lice from farm and non-farm sources.

Survival estimates have been based on a statistical analysis of differential survival to adults among release groups (Gargan *et al*., 2012; Jackson *et al*., 2011 a, b; 2013) including odds ratios (Jackson *et al*., 2013; Skilbrei *et al*., 2013; Krkošek *et al*., 2013; Torrissen *et al*., 2013; Vollset *et al*., 2015). An odds ratio is a measure of association between an exposure and an outcome and represents the odds that an outcome will occur given a particular exposure, compared to the odds of the outcome occurring in the absence of that exposure. Thus, in these studies, the odds ratio represented the probability of being recaptured in the treated group divided by the probability of being recaptured in the control group. All studies reported an overall improved return rate for treated vs. control salmon, but all showed significant spatial and temporal variability in the magnitude of the treatment effect.

Gargan *et al.* (2012) reported that the ratio of return rates of treated:control fish in individual trials ranged from 1:1 to 21.6:1, with a median ratio of 1.8:1. Similarly, odds ratios of 1.1:1 to 1.2:1 in favour of treated smolts were reported in Ireland and Norway, respectively (Torrissen *et al*., 2013). Krkošek *et al*. (2013) reported that treatment had a significant positive effect with an overall odds ratio of 1.29:1 (95% CI: 1.18–1.42). A recent meta-analysis of Norwegian data (Vollset *et al*., 2015) based on 118 release groups (3989 recaptured out of 657 624 released), reported an overall odds ratio of 1.18:1 (95% CI: 1.07–1.30) in favour of treated fish. Further analysis found that the age of returning salmon was on average higher and weight lower in untreated fish compared with treated fish (Vollset *et al.,* 2014; Skilbrei *et al.,* 2013).

The survival of Atlantic salmon during their marine phase has fallen in recent decades (Chaput, 2012; ICES, 2015). This downturn in survival is evident over a broad

geographical area and is associated with large-scale oceanographic changes (Beaugrand and Reid, 2003; Friedland *et al*., 2000; 2005; 2009; 2014). For monitored stocks around the North Atlantic, current estimates of marine survival are at historically low levels with typically fewer than 5% of out-migrating smolts returning to their home rivers for the majority of wild stocks, with lower levels for hatchery-origin fish (ICES 2015). Viewed against marine mortality rates at or above 95%, the 'additional' mortality attributable to sea lice has been estimated at around 1% (Jackson *et al*., 2013).

In some studies, the impacts of sea lice have also been estimated as losses of returning adult fish to rivers. Such estimates indicate marked variability, ranging from 0.6% to 39% in individual trials (Gargan *et al*., 2012; Krkošek *et al*., 2013; Skilbrei *et al*., 2013). These results suggest that sea lice induced mortality has an impact on Atlantic salmon returns which may influence the achievement of conservation requirements for affected stocks (Gargan *et al*., 2012).

Vollset *et al.* (2015) concluded that much of the heterogeneity among trials could be explained by the release location, time period and baseline (i.e. marine) survival. Baseline survival was reported to be the most important predictor variable. When this was low (few recaptures from the control group), the effect of treatment was relatively high (odds ratio of 1.7:1). However, when baseline survival was high, the effect of treatment was undetectable (odds ratio of ~1:1). One explanation for this finding is that the detrimental effect of lice is exacerbated when the fish are subject to other stressors; the findings of other studies support this hypothesis (Finstad *et al*., 2007; Connors *et al*., 2012; Jackson *et al*., 2013; Godwin *et al*., 2015). Vollset *et al.* (2015) concluded that their study supported the hypothesis that sea lice contribute to the mortality of salmon. However, they cautioned that the effect was not consistently present, was strongly modulated by other risk factors and suggested that population-level effects of sea lice on wild salmon stocks cannot be estimated independently of the other factors that affect marine survival.

2.5 Summary

- The sea louse (*Lepeophtheirus salmonis*) has widespread geographic distribution, is an important parasite of salmonids and has been a serious problem for the Atlantic salmon farming industry since the 1970s. Sea lice have a greater economic impact on the industry than any other parasite and control of lice levels on farms is of key importance.
- Salmon farming has been shown to increase the abundance of lice in the marine environment and the risk of infection among wild salmonid populations. However, there is considerable uncertainty, and spatial and temporal variability, about the extent of the zones of elevated risk.
- It has been shown in laboratory studies that 0.04–0.15 lice per gramme fish weight can increase stress levels. Laboratory studies have also demonstrated that infections of 0.75 lice per gramme fish weight, or approximately eleven sea lice per fish, can kill a recently emigrated wild salmon smolt of about 15 g if all the sea lice develop into pre-adult and adult stages.
- A number of studies in Norway and Ireland have estimated the relative marine survival of smolts treated to provide lice resistance and control groups. All studies have reported an overall improved return rate for treated salmon, but all showed significant spatial and temporal variability in the magnitude of the treatment effect.

- The survival of Atlantic salmon during their marine phase has fallen in recent decades. This downturn in survival is evident over a broad geographical area and is associated with large-scale oceanographic changes. Viewed against current marine mortality rates commonly at or above 95%, the 'additional' mortality attributable to sea lice has been estimated at around 1%.
- In some studies, the impact of sea lice has also been estimated as losses of returning adult salmon to rivers. These estimates indicate marked variability, with losses in individual experiments ranging from 0.6% to 39%. These results suggest that sea lice induced mortality has an impact on Atlantic salmon returns, which may influence the achievement of conservation requirements for affected stocks.
- Much of the heterogeneity among trials comparing the survival to adulthood of juvenile salmon administered sea lice medicines and control groups could be explained by the release location, time period and baseline (i.e. marine) survival. In a recent meta-analysis of Norwegian data, baseline survival was reported to be the most important predictor variable. When this was low, the effect of treatment was high. In contrast, when baseline survival was high, the effect of treatment was undetectable. However, it is unclear whether baseline survival is affected by sea lice exposure.

2.6 Knowledge gaps and research priorities

- Factors influencing marine mortality of Atlantic salmon need to be identified and quantified.
- Efficacious salmon lice management procedures need to be further developed for farmed salmon.
- Transmission dynamics of salmon lice between farmed fish and wild salmonids in time and space need to be better understood.
- Long-term effects of sea lice impact on the stability of wild salmon stocks need to be assessed, relative to the number of returning adults, their condition and age.
- Improved methods are needed to assess the risk of sea lice impacts from salmon aquaculture on wild salmon, particularly during their early marine migration.
- The impact of salmon farming on wild salmon production has been relatively poorly researched, and it is timely to increase the knowledge within this area.

3 Escapees, genetic interactions and effects on wild Atlantic salmon

3.1 Numbers of escapees and observations in rivers

Although aquaculture technology and fish-farm safety has significantly increased over the past decade or more, each year, large numbers of Atlantic salmon still escape from aquaculture installations into the wild. While many of these are reported, for example see the statistics from the Norwegian Directorate of Fisheries for reported escapes from Norwegian farms (http://www.fiskeridir.no/Akvakultur/Statistikk-akvakultur/Roemmingsstatistikk), in many circumstances, escapes go unnoticed. Therefore, the numbers of escapees are likely to be significantly higher than the reported numbers and, in Norway, the true numbers escaping from farms have been estimated to be 2–5 times higher than the official statistics (Skilbrei *et al.*, 2015). In other salmon producing countries, for example Scotland http://aquaculture.scotland.gov.uk/data/fish_escapes.aspx, eastern Canada and USA http://www.nasco.int/pdf/reports_annual/2015%20Commissions%20Report.pdf the numbers of farmed escapees are also reported. The degree of underreporting in these regions remains unquantified.

Farmed salmon may escape at both the freshwater (Clifford *et al.*, 1998a; Carr and Whoriskey, 2006; Uglem *et al.*, 2013) and marine stages of production (Clifford *et al.*, 1998b; Webb *et al.*, 1991; Carr *et al.*, 1997a). Most known escapes occur from sea cages (Jensen *et al.*, 2010). However, due to differences in rearing practices between countries and regions, the extent of freshwater escapes may differ. In some countries, such as Scotland, it is likely to be higher than, for example, in Norway. In Scotland, in the order of 20 million smolts are produced annually from freshwater pens (Franklin *et al.*, 2012). In Norway, most smolts are produced in land-based tanks from which escape is less likely.

Although the probability of surviving to adulthood and maturing vary between the different life-history stages at which the salmon escape, the great majority of salmon that escape from farms disappear never to be seen again (Skilbrei, 2010a; Skilbrei, 2010b; Hansen, 2006; Whoriskey *et al.*, 2006). Nevertheless, some of the escapees are in or enter into rivers where native salmon populations exist. While not all escapees in rivers are sexually mature (Carr *et al.*, 1997b; Madhun *et al.*, 2015) or indeed in the process of maturing, most are, and these may attempt to spawn with wild salmon (this includes both parr and adults). Farmed escaped salmon have been observed in rivers in all regions where Atlantic salmon farming occurs; Norway (Gausen and Moen, 1991; Fiske *et al.*, 2006), UK (Youngson *et al.*, 1997; Webb *et al.*, 1991; Green *et al.*, 2012), eastern Canada and USA (Morris *et al.*, 2008; Carr *et al.*, 1997a), and Chile (Sepulveda *et al.*, 2013). Furthermore, farmed salmon can migrate great distances post escape (Hansen and Jacobsen, 2003; Jensen *et al.*, 2013), and have been observed in rivers outside farming dense regions for example Iceland (Gudjonsson, 1991). Still, the incidence of farmed escaped salmon in rivers is likely to be correlated with the volume of farming within the region, as determined by a study conducted in Norway (Fiske *et al.*, 2006), and in Scotland (where there are differences between the east and west coasts) (Green *et al.*, 2012).

While the incidence of farmed escaped salmon has been investigated in a number of rivers in Norway in the period 1989 to 2013 (Fiske *et al.*, 2006), a new national monitoring programme for farmed escaped salmon was established in Norway in 2014,

and based upon data from angling catches, dedicated autumn angling and diving surveys 30 out of the 140 rivers surveyed displayed a frequency of >10% escapees (http://www.imr.no/publikasjoner/andre_publikasjoner/romt_oppdrettslaks_i_vassdrag/nb-no). These surveys demonstrate that the number of escapees within rivers varies in time and space (Gausen and Moen, 1991; Fiske *et al*., 2006).

Farmed salmon escapees may attempt to partake in spawning with wild salmon or among themselves. Several studies have reported observations of farmed salmon spawning with wild fish in rivers. This has for example been reported in rivers in Scotland (Webb *et al*., 1991; Webb *et al*., 1993; Butler *et al*., 2005), Norway (Lura and Saegrov, 1991; Saegrov *et al*., 1997) and Canada (Carr *et al*., 1997a). However, experiments demonstrate that the spawning success of farmed salmon is significantly reduced (Fleming *et al*., 1996; Fleming *et al*., 2000; Weir *et al*., 2004), perhaps just 1–3% and <30% of the success of wild males and females respectively (Fleming *et al*., 1996). However, the relative spawning success is likely to also vary with the life-stage at which the fish escaped (Fleming *et al*., 1997; Weir *et al*., 2005). Therefore, if a river has for example 10% farmed escapees observed on the spawning grounds, the genetic contribution to the next generation is likely to be significantly lower than 10%.

3.2 Identification of escapees

Farmed salmon escapees are typically identified using external morphological characteristics and growth patterns on fish scales (Fiske *et al*., 2006; Lund and Hansen, 1991). In Norway, genetic methods to identify farmed escaped salmon back to their farm(s) of origin has been developed and is routinely implemented in cases of unreported escapes (Glover *et al*., 2008; Glover, 2010). As of 01.01.2016, the method has been used in ~20 cases of unreported escape and has resulted in initiation of legal investigations successfully resulting in fines for companies found in breach of regulations (Glover, 2010). Since 2003, all aquaculture salmon in Maine must be marked before placement into marine net pens so that in the event of an escape the fish can be traced to the farm of origin (NMFS, 2005). Maine's marking programme utilises a genetic pedigree based approach to identify fish. In other countries, no formal active identification programmes are in place. There are ongoing efforts to develop other genetic and non-genetic tagging methods to permit the routine identification of escapees back to their farms of origin.

3.3 Intraspecific hybridisation and introgression

There are still just a few published studies that have addressed genetic changes in wild populations following invasion of escaped farmed salmon. This may be due to the fact that such studies are often challenging. For example, they often require representative samples of the wild populations ideally before and after invasion, and access to representative farmed samples, as well as informative set of molecular genetic markers (Besnier *et al*., 2011; Karlsson *et al*., 2011).

The first studies of introgression were conducted in Ireland (Clifford *et al*., 1998b; Clifford *et al*., 1998a) and Northern Ireland (Crozier, 1993; Crozier, 2000) demonstrating introgression of farmed salmon in rivers as a response to escapes from local farms. These escapees originated from both cage escapes in salt water, as well as escapes from freshwater smolt rearing facilities located within rivers. Later on, a set of experiments looking at genetic changes in Norwegian populations was conducted. The first of these studies demonstrated temporal genetic changes in three out of seven populations located on the west and middle parts of Norway, and concluded that

introgression of farmed salmon was the primary driver (Skaala *et al*., 2006). Later, a spatio-temporal investigation of 21 populations across Norway revealed significant temporal genetic changes in several rivers caused by introgression of farmed salmon, and importantly, observed an overall reduction in interpopulation genetic diversity (Glover *et al*., 2012). The latter observation is consistent with predictions of population homogenization as a result of farmed salmon interbreeding (Mork, 1991). Importantly, all rivers that displayed temporal genetic changes due to spawning of farmed escapees, displayed an increase in genetic variation revealed as total number of alleles observed in the population. This is consistent with introgression from fish of a non-local source. The final published study in Norway used recently developed diagnostic genetic markers for identification of farmed and wild salmon (Karlsson *et al*., 2011) to estimate cumulative introgression of farmed salmon escapees in 20 wild populations (Glover *et al*., 2013). In this study, cumulative introgression over 2–3 decades was estimated between 0–47% among rivers. Differences in introgression levels between populations was positively linked with the observed proportions of escapees in the rivers, but it was also suggested that the density of the wild population, and therefore level of competition on the spawning grounds and during juvenile stages, also influenced introgression (Glover *et al*., 2013). A recent study conducted in the Magaguadavic River in eastern Canada demonstrated introgression of farmed escapees with the native population (Bourret *et al*., 2011).

The most recent and by far the most extensive investigation of introgression of farmed salmon was recently published as a report in Norwegian by researchers from NINA and IMR (http://www.nina.no/english/News/News-article/ArticleId/3984). Here, a total of 125 Norwegian salmon populations were classified using a combination of the estimate of wild genome P(wild) (Karlsson *et al*., 2014) and the introgression estimates from the study by Glover *et al*. (2013). These authors established four categories of introgression: green = no genetic changes observed; yellow = weak genetic changes indicated but less than 4% farmed salmon introgression; orange = moderate genetic changes documented 4–10% farmed salmon introgression; red = large genetic changes demonstrated >10% farmed salmon introgression. Based upon these analyses, 44, 41, nine and 31 of the populations studied fell into categories green–red respectively. This huge volume of data therefore provides a comprehensive status for many Norwegian populations but is lacking for all other regions.

3.4 Domestication and divergence from wild salmon

From the very start of the Atlantic salmon aquaculture industry in the early 1970s, breeding programmes to select salmon for higher performance in culture were initiated (Gjedrem *et al*., 1991; Ferguson *et al*., 2007; Gjoen and Bentsen, 1997). The largest and most significant of these programmes globally are those initiated in Norway which are based upon material originating from >40 Norwegian rivers (Gjedrem *et al*., 1991). Other programmes in Norway were also established from wild salmon, and in other countries salmon breeding programmes have also been established. Farmed salmon originating from the three main breeding companies in Norway: Marine Harvest - Mowi strain, Aqua Gen AS, and SalmoBreed AS, dominate global production although this varies from country to country. For example, in eastern Canada only the St John River domesticated strain (Friars *et al*., 1995) is permitted for use in commercial aquaculture, and in Scotland some locally based strains e.g. Landcatch (Powell *et al*., 2008) are also being used.

Initially, salmon breeding programmes concentrated on increasing growth, but rapidly expanded to include other traits that are also of commercial importance, such as

flesh characteristics, age at maturation and disease resistance (Gjedrem, 2000; Gjedrem, 2010). Today, breeding programmes have advanced to 12+ generations, and genome-assisted selection is being utilised in several of the breeding programmes. QTL selected sub-strains are now commercially available displaying characteristics such as reduced sensitivity to specific diseases (Moen *et al*., 2009) and increased growth. It is likely that full utilisation of genomic selection will increase the diversity of traits that can be accurately targeted by selection for rapid gains in breeding. For example, the recently identified strong influence of the vgll3 locus on age in maturation in salmon (Ayllon *et al*., 2015; Barson *et al*., 2015) could represent an effective target to inhibit grilsing (i.e. early maturation) in aquaculture.

As a result of: (1) directional selection for commercially important traits, (2) inadvertent domestication selection (the widespread genetic changes associated with adaptation to the human-controlled environment and its associated reduction in natural selection pressure), (3) non-local origin, and (4) random genetic changes (drift), farmed salmon display a range of genetic differences to wild salmon (Ferguson *et al*., 2007). Examples of these differences include growth rate under controlled conditions (Glover *et al*., 2006; Glover *et al*., 2009; Solberg *et al*., 2013 a and b; Thodesen *et al*., 1999), gene transcription patterns (Bicskei *et al*., 2014; Roberge *et al*., 2006; Roberge *et al*., 2008), stress tolerance (Solberg *et al*., 2013a), and behavioural traits including predator avoidance and dominance (Einum and Fleming, 1997). In addition, farmed salmon strains typically display lower levels of allelic variation when compared to wild salmon strains (Norris *et al*., 1999; Skaala *et al*., 2004), although not all classes of genetic marker reveal the same trends (Karlsson *et al*., 2010). Looking at the level of genetic variation coding for phenotypic traits such as growth, some data are emerging suggesting a possibly reduced variation in farmed strains (Solberg *et al*., 2013a; Reed *et al*., 2015). The latter observation is expected given the fact that farmed fish have been selected for this trait since the early 1970s.

3.5 Fitness studies

Thus far, only three published studies have addressed survival of farmed, hybrid and wild salmon in the natural environment. Such studies are exceptionally demanding on logistics, and require experimental periods extending beyond what typical funding sources permit.

The first study was conducted in the River Burrishoole in Ireland, and involved planting eggs of farmed, hybrid and wild parentage into a natural river system (McGinnity *et al*., 1997). These fish were identified using DNA profiling and followed through a two-generation experiment. The authors concluded that the lifetime fitness of farmed fish was just 2% of wild fish, and that the relative-fitness increased along a gradient towards the offspring of a F1 hybrid survivor spawning together with a wild salmon (= back cross) that displayed a lifetime survival of 89% compared to the offspring of a wild salmon (McGinnity *et al*., 2003). The authors concluded that repeated invasions of farmed salmon in a wild population may cause the fitness of the native population to seriously decline, and potentially enter an "extinction-vortex" in extreme cases.

In Norway, a slightly different but complimentary experiment was conducted in the River Imsa (Fleming *et al*., 2000). Here, the authors permitted migrating adult salmon of farmed and wild native origin entry to the River Imsa, once they had been sampled in the upstream trap. They thereafter spawned naturally and their offspring were monitored until adulthood. This study reported a lifetime fitness of farmed salmon

(i.e. escaped adult to adult) of 16% compared with wild salmon (Fleming *et al*., 2000). Important additional data from this study was the fact that productivity of the wild salmon from the river decreased, following the permitted invasion of farmed salmon, both with respect to the total smolt production and when smolt production from native females was considered alone (Fleming *et al*., 2000). This is because the offspring of the farmed and hybrid salmon competed with wild salmon for both territory and resources, and the dynamics of this may vary across life-history stages (Sundt-Hansen *et al*., 2015).

The most recently published study to address the relative fitness of farmed and wild Atlantic salmon in a natural environment was conducted in the River Guddal in Norway (Skaala *et al*., 2012). Here, these authors used a similar design to the Irish study, releasing large numbers of farmed, hybrid and wild salmon eggs into the river and following their survival. The study included planting out eggs across three cohorts, and permitted for the first time, comparisons of family as well as group fitness (farmed hybrid and wild) in freshwater. The study did not use a local wild fish, but salmon from the Norwegian gene bank as a wild fish proxy. While these authors reported reduced genetic fitness of farmed salmon offspring compared to the non-local wild salmon, egg size was closely related to family survival in the river. Therefore, some farmed salmon families with large eggs displayed surprisingly high survival rates in freshwater (higher than some wild families), although when egg size was adjusted for, farmed salmon offspring displayed significantly lower survival in freshwater compared to the wild salmon. To illustrate this, in 15 of 17 pairwise comparisons of maternal half-sib groups, families sired with wild males performed better compared with families sired with farmed fish. The study also revealed that farmed and wild salmon overlapped in diet in the river, an observation also reported from an earlier small-scale planting study (Einum and Fleming, 1997) and from the full-generation study in the River Imsa (Fleming *et al*., 2000).

Studies cross-examining the underlying details, mechanisms, and genomics of the observed survival differences between farmed and wild salmon in natural habitats have also been published (Besnier *et al*., 2015; Reed *et al*., 2015), although the exact mechanisms still remain elusive. For example, attempts at quantifying predation in the wild (Skaala *et al*., 2014), and predation susceptibility in semi-natural contests (Solberg *et al*., 2015) have not revealed greater predation of farmed salmon offspring than wild salmon offspring, despite earlier studies suggesting reduced predation awareness caused by domestication (Einum and Fleming, 1997).

Collectively, the results of the whole-river studies outlined above are supported by the widespread literature demonstrating the reduced fitness of hatchery reared salmonids, as part of supplementation programmes, in the wild (Araki *et al*., 2007; Araki *et al*., 2009).

3.6 Short-term consequences of introgression for wild salmon populations (i.e. a few salmon generations)

In natural habitats such as rivers, territory and food resources are typically limited, and survival is often controlled by density-dependent factors, and habitats have carrying capacities (Jonsson *et al*., 1998; Bacon *et al*., 2015). Studies have demonstrated that the offspring of farmed salmon compete with wild salmon for resources such as food and space (Skaala *et al*., 2012; Fleming *et al*., 2000). Therefore, when farmed salmon manage to spawn, and their offspring constitute a component of a given river's juvenile population, the production of juveniles with a pure wild background

will be depressed though competition for these resources. In addition, data from controlled studies have indicated that the total productivity of smolts in the river following introgression of farmed salmon can decrease (Fleming *et al*., 2000; McGinnity *et al*., 1997).

As discussed in the section above, farmed salmon display a range of genetic differences to wild populations, which includes various life-history and behavioural traits. In controlled experiments with farmed and wild salmon (McGinnity *et al*., 1997; McGinnity *et al*., 2003; Fleming *et al*., 2000; Fraser *et al*., 2010 a; Skaala *et al*., 2012) differences in freshwater growth and body shape, timing of hatching and smolt migration, age of smoltification, incidence of male parr maturation, sea age-at-maturity and growth in the marine environment have been observed, with some variation across farmed–wild comparisons (Fraser *et al*., 2010 b). Therefore, where farmed salmon have introgressed in natural populations, it is likely that recipient populations will display changes in life-history traits in the direction of the farmed strains. Given that life-history traits are likely to be associated with fitness in the wild and local adaptation (Garcia de Leaniz *et al*., 2007; Taylor, 1991; Fraser *et al*., 2011; Barson *et al*., 2015), these changes in life-history characteristics are likely to be associated with a loss of fitness (which will also contribute to an overall reduction in productivity). These changes will be difficult to detect against the background of natural variability in stock abundance and require long-term studies to quantify accurately, and at the present, there is a lack of empirical data demonstrating such changes in effected wild populations.

The short-term consequences for wild populations will scale with the magnitude and frequency of interbreeding events. For example, in rivers where density of wild spawners is low, spawning success of escapees will increase compared with locations where density of wild spawners is high. Similarly, low density of wild juveniles with relaxed competition, will give farm offspring better survival opportunities than they will have in locations with high density of wild juveniles. Thus, when populations are under stress and density of individuals goes down, impact from escapees is expected to increase, which is in agreement with studies on observed introgression rates in salmon (Glover *et al*., 2012; Heino *et al*., 2015; Glover *et al*., 2013), but also supported for example by studies on brown trout supplemented by non-local hatchery fish (Hansen and Mensberg, 2009).

Atlantic salmon river stocks are characterized by widespread structuring into genetically distinct and differentiated populations (Ståhl, 1987; Verspoor *et al*., 2005). This is conditioned by the evolutionary relationships among populations (Dionne *et al*., 2008; Perrier *et al*., 2011; Dillane *et al*., 2008) and adaptive responses to historical and contemporary to environmental differences (Garcia de Leaniz *et al*., 2007; Taylor, 1991). A spatio-temporal genetic study of 21 populations in Norway revealed an overall reduction in interpopulation diversity caused by interbreeding of farmed escaped salmon (Glover *et al*., 2012). It is likely that further introgression of farmed salmon will continue to erode this diversity.

3.7 Long-term consequences of introgression for wild salmon populations (i.e. more than a few generations)

The conservation of genetic variation within and among populations (as outlined in the UN Convention on Biological Diversity, 1992) is important for the resilience of local stocks to human or natural disturbances (Ryman, 1991; Schindler *et al*., 2010), and in the long term, reduced genetic variability will affect the species' ability to cope

with a changing environment (McGinnity *et al*., 2009; Lande and Shannon, 1996). Therefore, one way gene flow, as occurs through the successful spawning of farmed escapees potentially represents a powerful evolutionary force. It erodes genetic variation among wild populations (Glover *et al*., 2012), and in the long run, may also erode the genetic variation within populations under certain situations (Tufto and Hindar, 2003) as the recipient wild populations become more similar to the less variable farmed populations.

Although evolutionary theory permits us to outline general trajectories, it remains difficult to predict and demonstrate the evolutionary fate of specific wild populations receiving farmed immigrants. The severity and nature of the effect depends on a number of factors, including the magnitude of the differences between wild and farmed populations (both historical and adaptive differences), the mechanisms underlying genetic differences between wild and farmed salmon, the frequency of intrusions of farmed fish, and the numbers of intruding farmed fish relative to wild spawning population sizes (Hutchings and Fraser, 2008). Furthermore, wild populations that are already under evolutionary strain from other challenges such as disease pressure, sea lice infection, overharvest, habitat destruction and poor water quality, etc. are more likely to be sensitive to the potential negative effects of genetic introgression and loss of fitness. Therefore, genetic introgression has to be seen in the context of other challenges also.

Taken collectively, existing understanding makes it clear that the long-term consequences of introgression across river stocks can be expected to lead to reduced productivity and decreased resilience to future impacts such as climate change (i.e. less fish and more fragile stocks). Therefore, a substantial reduction or even total elimination of escaped farmed salmon in the wild is essential in order to minimize or avoid negative effects on native populations.

3.8 Summary

- Each year, large numbers of domesticated salmon escape from commercial fish farms. While many of these are reported, the true number of escapees is likely to be significantly higher. Escapees are observed in rivers in all regions where farming occurs, although the numbers of escapees vary both spatially and temporally. It has been noted that in some rivers in some years, the numbers of escapees have approached 50% or more of the spawning population.
- The spawning success of escaped farmed salmon is much lower than wild salmon. Despite this, genetic studies have demonstrated that farmed salmon have displayed widespread introgression in a large number of Norwegian populations where this has been investigated. Introgression has also been shown in other countries, but the full extent of introgression remains to be investigated.
- Farmed salmon are domesticated and display significant genetic differences to wild salmon in a wide range of fitness related traits. Whole-river experimental studies have demonstrated that the offspring of farmed and cultured salmon in general, display lower fitness than their wild counterparts in the wild.
- Juvenile escapees and the offspring of farmed salmon compete with wild salmon for territory and food. Therefore, their presence in the natural habi-

tat will reduce the total production of wild fish. Studies have also shown this can result in a decreased overall productivity of the population.

- Where farmed salmon have successfully interbred with natural populations, it is likely that recipient populations will display changes in life-history traits. These changes are likely to be maladaptive for the wild population.
- The long-term consequences of introgression across river stocks can be expected to lead to reduced productivity and decreased resilience to future impacts such as climate change (i.e. less fish and more fragile stocks).
- The evidence from studies in the wild, and the extensive literature relating to salmonids in general, demonstrates that the offspring of farmed salmon display reduced fitness in the wild. However, the results of these studies suggest that the relative success of farmed salmon and, likewise, the relative potential negative effect on a native population, is likely to vary in time and space. Wild populations that are already under evolutionary strain from other challenges such as disease pressure, sea lice infection, over exploitation, habitat destruction and poor water quality are more likely to be sensitive to the potential negative effects of genetic introgression and loss of fitness. Therefore, such effects have to be seen in the context of other challenges.
- While recognising that there were still uncertainties, WKCULEF considered that the evidence relating to the impacts of escapees / genetic introgression provided a clear indication of impacts on wild salmon populations. A substantial reduction of escaped farmed salmon in the wild, or sterilization of farmed salmon, would be required in order to minimize effects on native populations.

3.9 Knowledge gaps and research priorities

- To increase the level of monitoring and dedicated studies looking into the numbers of escapees and their genetic introgression in native populations, especially in knowledge poor regions. This will also include further characterisation of aquaculture strains and development of monitoring tools across countries through international collaboration.
- To increase understanding of the environmental and biological factors that influence levels of farmed salmon introgression and their ecological consequences including productivity.
- To understand the genomic architecture of domestication and the underlying genetic differences between farmed and wild salmon in both the hatchery and natural environments, and how this affects fitness.
- To identify and quantify adaptive genetic changes in wild populations that have been subject to introgression of farmed escaped salmon. This includes quantification of natural selection and fitness.

Annex 1: Working documents submitted to the Workshop on possible effects of salmonid aquaculture on wild Atlantic salmon populations in the North Atlantic, 1–3 March, 2016

WP No.	Authors	Title
1	Glover, K.A., Skaala, Ø., Solberg, M., Skilbrei, O.T., Svåsand, T. and Wennevik, V.	Salmon escapees and status of knowledge.
2	Jackson, D.	Sea Lice - introduction, background and current state of knowledge.
3	Lillehammer, M.	Stochastic simulations of introgression of farmed salmon into wild populations.
4	Finstad, B. and Gargan, P.	Effects of sea lice on Atlantic salmon - from individual- to population effects.
5	Jaffa, M.	Sea lice in context.
6	Hindar, K.	Genetic introgression from farmed to wild salmon.
7	Coulson, M.	Fish-farm escapes to stay or go? Imlications for the River Polla.
8	Karlsbakk, E.	Microbial diseases in aqauculture and impact on wild salmonids.
9	McGinnity, P.	Effects of farm escapees on salmon production.
10	Svasand, T.	Risk asessment - environmental impacts of Norwegan fish farming.
11	Verspoor, E.	Assessment of interbreeding and introgression of farm genes in a small Scottish Atlantic salmon (*Salmo salar*) stock: *ad hoc* samples - *ad hoc* results?
12	Gargan, P.	Sea lice - perspectives on studies in Ireland.
13	Svasand, T.	Sea lice monitoring and modelling in Norway.
14	Kvamme, B.O.	National sea lice monitoring programme.

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Annex 4: Technical minutes from the Review Group on Possible effects of salmonid aquaculture

- RGAQUA
- Deadline: 21 April 2016
- Participants: Martin Krkošek, Robin Waples and Einar E. Nielsen (Chair)
- Expert Group: WKCULEF

Review of: Report of the Workshop to address the NASCO request for advice on possible effects of salmonid aquaculture on wild Atlantic salmon populations in the North Atlantic (WKCULEF).

The review group would like to compliment the workshop participants for a very clear, well-structured, insightful and comprehensive report. In our view only very few points have been missed and we agree with the vast majority of the conclusions presented. We still have a few suggestions for amendment in relation to issues that may be unclear, could be treated in more detail or are missing altogether in the draft report. We hope that our comments/suggestions can help to improve the report and look forward to work with you in relation to completing the final draft advice.

Similar to the report, we have split our comments and suggestions into two sections, relating to sea-lice and genetic interactions respectively. Our main comments are outlined below. However for both sections we think that the link between the main text and the sections on "Knowledge gaps and research priorities" is relatively weak. It is difficult to find a direct justification for the outlined research priorities. We suggest numbering the priorities, and subsequently provide direct appropriate reference to each of them in the main text.

There is a general bias in the published literature and available data with respect to effects on wild salmon populations from salmonid aquaculture (both sea lice and genetics) in countries and areas that have intensive salmon farming industries. This is a consequence of the importance of the parasite to management of farmed salmon and the expected magnitude of interactions. However, it also presents a challenge to understand the scale of sea lice and genetic effects on wild salmon in salmon farming areas relative to areas without salmon farms. Likewise, it is mentioned (page 19)… " the great majority of salmon that escape from farms disappear never to be seen again". That could well be true, especially given how hard it is to track escapees. But just because they are never seen again, does not mean they have no effects on wild populations in regions which are not subject to intense monitoring and/or reported in the scientific literature. Thus, a general recommendation to also investigate effects in geographic regions without intensive aquaculture could be warranted.

Sea lice

The review presents two different interpretations of % mortality caused by sea lice that are reported in the literature, but that give different representations of the effect of sea lice on salmon populations (Jackson *et al*., 2013; Krkošek *et al*., 2013). The interpretations seem incompatible, which can be confusing, and more effort is needed to clarify how the interpretations are related and how they differ. In one view (Jackson *et al*., 2013), the emphasis is placed on the absolute difference in marine mortality between fish treated with parasiticides and those that are not. The example given in the review is a difference of one percent, where mortality in treated groups is 95% compared to 96% in untreated groups. The additional one percent mortality between

groups is attributed to sea lice, which is interpreted as a small number compared to the 95% mortality from the treatment groups. The other interpretation of this same example is in terms of the percent loss of recruitment or abundance of adult salmon due to exposure to sea lice. In this interpretation, the same example corresponds to a 20% loss in adult salmon abundance due to sea lice; for every five fish that return as adults in the treated groups (95% mortality), there are four fish that return as adults in the untreated group (96% mortality). In other words, one in five fish are lost to sea lice effects. These differences in interpretation of the same data differ by 20x and reflect the nuances of interpreting survival data. It is therefore important to clarify for non-expert readers how to interpret the results. It is true that natural marine mortality of salmon is high and multiple factors are involved, but it is also true that a small incremental increase in marine mortality due to sea lice (or any other factor) can result in losses of salmon abundance that are relevant for fisheries and conservation management.

The review has an emphasis on the physiological responses to sea lice infection as well as experimental data on lethal infection loads. However, there could be more discussion and explanation of the environmental/biological stressors and ecological processes that mediate the relationship between lice and marine survival of Atlantic salmon. While laboratory estimates of lethal loads and physiological responses are attractive to predict impacts on wild populations, this is likely an over-simplified view because natural ecological processes such as predation and competition are likely to remove infected fish before the lice kill the fish directly. In this view, sublethal effects seen in the lab may increase or decrease mortality in the field (e.g. Pacific salmon) (Peacock *et al*., 2014), and so laboratory results need to be connected with behavioural changes in the fish that alter predator–prey interactions between the smolts and their predators as well as the smolts and their prey (e.g. migration behaviour) (Birkeland and Jakobsen, 1997). Also, early marine growth is important for smolts to escape predation and also access a more diverse prey field and so it is therefore particularly relevant under resource-limited or parasitized conditions. Finally, there are also abiotic stressors such as pollutants that may affect the effects of sea lice on salmon smolts. These potentially interactive effects of multiple factors are likely to be important for explaining the result from meta-analysis that the effect of sea lice on salmon survival depends on the baseline survival of untreated fish (Vollset *et al*., 2015). However, in that work, the baseline survival used is that from untreated groups, which is itself likely to be affected by louse abundance, introducing a circularity that leaves the interactive effects between lice and other factors on salmon survival poorly characterized.

There is little mention of recent difficulties in controlling sea lice on salmon farms in some areas. The difficulties are because lice have evolved resistance to the common chemical treatments. This presents a challenge to controlling lice on farms, and therefore is relevant to the wild salmon that migrate through those areas. Alternative methods and technologies are needed to provide more effective and sustainable control of sea lice on salmon farms. Work in this area includes alternative medicines, biocontrol using wrasse, and hydrogen peroxide bath treatments in specialized vessels that service farms.

The literature reviewed mixes results from Pacific salmon together with results from Atlantic salmon (as also done in this review). It is unclear to what extent the mechanisms of lice effects on wild salmon are the same between these two areas. There are key differences between Pacific and Atlantic situations, including differences in the genome of the lice themselves as well as the ecological context of the salmon. In the

Pacific, salmonids are more diverse in their life-history traits, species composition, and abundance. Also, the salmon farming industry is smaller. Thus, the extent to which the results from the Pacific on sea lice effects on wild salmon are transferable to the Atlantic situation should be at least briefly discussed.

Genetic effects

There is little reference to previous attempts to model the persistence of wild salmon populations interbreeding with farmed conspecifics. Early modelling work by Hutchings (1991) predicted that the extinction risk of native genomes is largest when interbreeding occurs and when farmed fish occur frequently and at high densities. The risk is largest in small wild populations, which is related to both demographic and genetic effects. Hindar *et al*. (2006) refined this work by using life-stage specific fitness and narrowing the modelling to realistic scenarios based on experimental data. They found that under high intrusion scenarios the recovery of the wild population is not likely under all circumstances even when interbreeding has been ceased for many decades. Baskett *et al*. (2013) used a model with coupled demographic and genetic dynamics to evaluate how genetic consequences of aquaculture escapes depend on how divergent the captive and wild populations are. They found negative genetic consequences increased with divergence of the captive population, unless strong selection removes escapes before they reproduce. Recent modelling work by Castellani *et al*. (2015) has focused on using individual based eco-genetic models, which are parameterized taking processes such as growth, mortality and maturation as well environmental and genotypic variation into account. This should allow improved power for predicting the outcome of genetic and ecological interactions between wild and farmed salmon.

"3.9 Knowledge gaps." A key issue that was not discussed involves the timing and pace of escapes. For example, given a fixed number N of escapes over a fixed time period T, is it worse for the wild population if they come in one big pulse, or gradually in small amounts of "leakage"? Hindar *et al*. (2006) concluded that large pulses of escapes are more damaging, while Baskett *et al*. (2013) reached the opposite conclusion; that constant, small-scale leakage created greater fitness losses to the wild population. The different conclusions can be largely explained by different time frames of reference: Hindar *et al*. focused on short-term effects, while Baskett *et al*. evaluated mean effects over long periods of time. However, this topic merits more detailed study. Also, Baskett *et al*. did not explicitly consider overlapping generations. So, more work is needed in order to evaluate results as a function of escapes across generations in species with age structure like Atlantic salmon. This is important to resolve; as it is convenient to ignore low-level leakage because it is very difficult to eliminate or even monitor, but some results at least suggest it can have extremely important effects on wild populations.

Regarding variable estimates of relative spawning success of escapes: Apart from natural variability and sampling error, a logical explanation for the wide range of estimates is that the lower estimates apply to escapes from aquaculture stocks that are the most strongly domesticated. If so, then those interbreeding events likely have more serious per capita consequences than interbreeding events involving less domesticated stocks. This would mean that simply focusing on the rate of interbreeding will not necessarily provide a full picture of the genetic consequences of escapes. For discussion see Basket and Waples (2013).

Regarding the text on page 23 that mentions reduced fitness of hatchery fish used in salmon supplementation, the review paper of Christie *et al*. (2014) on this topic could be cited.

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Editor's Choice

Widespread genetic introgression of escaped farmed Atlantic salmon in wild salmon populations

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Farmed Atlantic salmon (*Salmo salar*) escape from net pens and enter rivers to spawn, potentially resulting in genetic introgression and reduced fitness of wild salmon. Here, we quantify genetic introgression of farmed to wild salmon, using molecular genetic markers, in populations from 147 salmon rivers, representing three-quarters of the total wild salmon spawning population in Norway. For 109 rivers with adult modern samples and sample sizes of 20 or more, the average level of farmed genetic introgression was 6.4% (median = 2.3%), with a range between 0.0% and 42.2%. Fifty-one of these rivers showed significant farmed genetic introgression when compared with historical reference samples. We observed a highly significant correlation between estimated farmed introgression and average proportion of escaped farmed salmon. We quantify levels of introgression as unweighted averages or weighted by population sizes, to compare geographical regions and to compare levels of introgression in rivers and fjords designated as locations deserving a high level of protection. We found a generally lower level of introgression in National Salmon Rivers and National Salmon Fjords subjected to formal protection by parliament. We conclude that farmed to wild genetic introgression is high in a large proportion of Norwegian salmon rivers, with the highest levels found in the most intensive areas of salmon farming. The extensive genetic introgression documented here poses a serious challenge to the management of farmed and wild Atlantic salmon in Norway and, in all likelihood, in other regions where farmed-salmon escape events occur with regularity

Keywords: atlantic salmon, aquaculture, farmed salmon, genetic introgression, genetics, SNPs.

Introduction

Farmed Atlantic salmon differ genetically from wild salmon because of a variety of causes. Breeding programs of farmed Atlantic salmon were established in Norway in the early 1970s based on salmon collected from several populations in Central and Western Norway (Gjedrem *et al.*, 1991; Gjøen and Bentsen, 1997). The breeding program has successfully changed the genetics of farmed Atlantic salmon to improve commercially important traits, such as growth, utilization of feed, and filet quality (Thodesen *et al.*, 1999; Gjedrem and Baranski, 2009; Solberg *et al.*, 2013). These genetic improvements have undoubtedly contributed to the rapid expansion of the Atlantic salmon farming industry in Norway, with a production close to 1.3 million tons in 2015.

Farmed Atlantic salmon also differ genetically from wild salmon because of selection to captivity, and loss of genetic variation from a limited number of wild founders and subsequent genetic drift (Hutchings and Fraser, 2008). Because of the reduced fitness (Fleming *et al.*, 2000; McGinnity *et al.*, 2003; Skaala *et al.*, 2012; Reed *et al.*, 2015) and lower genetic variation in farmed salmon (Mjølnerød *et al.*, 1997; Skaala *et al.*, 2004, 2005; Karlsson *et al.*, 2010) compared with their wild conspecifics, there is a concern that genetic introgression of escaped farmed salmon to wild salmon might reduce the viability of wild Atlantic salmon. Reported numbers of escaped farmed salmon in Norway have ranged from 39 000 to 920 000 since 1993, with an average of 380 000 (Norwegian Directorate of Fisheries, http://www.fiskeridir.no/English). Inventories since 1989 have shown high proportions of

escaped farmed salmon in many Norwegian rivers, with large var iations between years (Fiske *et al.*, 2006) and rivers (Gausen and Moen, 1991; Diserud *et al.*, 2013). A similar situation has been documented in eastern North America with a large number of es caped farmed entering salmon rivers, in many rivers outnumber ing the wild spawning population and with extensive variation between rivers and years (Morris *et al.*, 2008).

Genetic introgression of escaped farmed salmon to wild sal mon populations has been modelled (Hindar *et al.*, 2006) based on relative fitness estimates (Fleming *et al.*, 2000; McGinnity *et al.*, 2003) and observed proportions of escaped farmed salmon (Fiske *et al.*, 2006). The spawning success of escaped farmed sal mon (Fleming *et al.*, 1996, 1997) and survival of their offspring (Fraser *et al.*, 2008, 2010; Skaala *et al.*, 2012; Sundt Hansen *et al.*, 2015) depend on a variety of factors in wild populations, farmed escapes and the environment in which they meet, and make it dif ficult to accurately predict farmed to wild genetic introgression. The development of improved models with important and more precise parameters requires quantification of the farmed to wild genetic introgression (Heino *et al.*, 2015).

Several molecular genetic markers for quantifying genetic in trogression of farmed escaped salmon in wild salmon popula tions have been identified (Karlsson *et al.*, 2011). These markers were used to quantify genetic introgression in 20 Norwegian sal mon populations, based on observed temporal genetic changes and Approximate Bayesian Computation (ABC) of the farmed to wild gene flow that is consistent with these changes (Glover *et al.*, 2013). The ABC method is restricted, as it relies on the ex istence of historical samples from each population to be analysed. From the generic genetic differences observed at the genetic markers identified by Karlsson *et al.* (2011), an alterna tive standardized method was developed by Karlsson *et al.* (2014). This method does not rely on historical samples from all populations, but uses the directional genetic change from farm to wild introgression, and not genetic changes stemming from genetic drift and/or gene flow between wild populations. In short, the method uses historical samples from many wild popu lations and samples from the Norwegian breeding kernels for farmed salmon, and estimates for each individual of interest the proportion of membership to these two groups, using STRUCTURE (Pritchard *et al.*, 2000).

The objective of the present study was to obtain an extensive coverage of farmed to wild Atlantic salmon genetic introgression using the new molecular genetic and analytical methods. We ana lysed 21 562 Atlantic salmon hatched in the wild in 147 Norwegian rivers, including 16 407 adults and 5155 juveniles. Here, we first present a comprehensive geographical coverage of status with respect to farmed to wild introgression. Second, we compare estimates of introgression in samples based on juveniles with samples based on returning adults from the same popula tion. Third, we assess the relationship between long term propor tions of escaped farmed salmon and genetic introgression. Finally, we assess to what extent a major conservation policy deci sion in Norway, designating 52 rivers as National Salmon Rivers and 29 fjords as National Salmon Fjords where important salmon populations receive extra protection (e.g. Vøllestad *et al.*, 2014), has an effect on the levels of introgression.

Material and methods

To quantify genetic introgression resulting from spawning of escaped farmed salmon in the wild, we analysed only fish hatched in the wild. We excluded fish classified as escaped farmed salmon, or with uncertain classification, based on their growth patterns in the scales (Lund and Hansen, 1991; Fiske *et al.*, 2005). Samples of juvenile, pre smolt salmon can safely be regarded as hatched in the wild, because the escape of juve nile farmed salmon from land based facilities to rivers in this study is unlikely.

We extracted total genomic DNA from scales of adult salmon and from fin clips of juvenile salmon using DNEASY tissue kit (QIAGEN). Initially, we used the Sequenom SNP genotyping plat form for genotyping of 5897 individuals at 99 SNP loci, with PCR amplifications in 4 multiplexes. Primer extension reactions followed recommendations from Sequenom (www.sequenom. com) and fragments were separated and identified using Sequenom Mass ARRAY™ analyzer (Autoflex mass spectrome ter). We conducted genotyping in real time depending on the presence or absence of a mass peak in expected mass range for each locus (Tang *et al.*, 1999) using the MassARRAY™ RT 3.4 software. We obtained reliable genotypes from 59 SNPs de scribed as being collectively diagnostic in differentiating be tween wild and farm salmon (Karlsson *et al.*, 2011; Jensen *et al.*, 2013). For the remaining 15 293 individuals, we used the EP1™ 96.96 Dynamic array IFCs genotyping platform (Fluidigm, San Francisco, CA). Reliable genotypes were obtained for 48 of the same SNPs genotyped by the Sequenom platform (Karlsson *et al.*, 2011). The SNP genotypes from the Sequenom and the Fluidigm SNP genotypes were merged for the 48 common SNP loci (Supplementary Table S1).

As a reference for farmed salmon, we used genotypes from 503 individuals from the three leading breeding companies (Marine Harvest, Salmobreed and AquaGen) from the year clas ses 2004 2009 (MH), 2004 2007 (SB) and 1998 2001, 2008 (AG). Each yearclass represented one of four different breeding kernels from each breeding company. In 2005, AquaGen pooled the four breeding kernels into one big kernel, represented by the 2008 sample. To investigate historical genetic signatures of farmed salmon, we used 129 samples from 1982 to 1988 from the four AquaGen breeding kernels. As references for non admixed wild salmon, we used historical samples of 2187 wild individuals from 39 populations, geographically distributed in rivers from southern to northern Norway. In agreement with previous studies (Bourret *et al.*, 2013; Jensen *et al.*, 2014), the Norwegian populations clustered into an Atlantic and a Barents White Sea phylogenetic group, with the latter including populations from Finnmark County and the former including populations south of Finnmark (Figure 1). All founder popula tions for the farm strains are from the Atlantic Sea phylogenetic group, as judged from the genetic contributions from source populations in the third generation of the breeding program (Gjøen and Bentsen, 1997). Although the Atlantic and the Barents White Sea phylogenetic groups are well separated, some populations in Troms County represent a transition between them. We analysed samples from 147 Norwegian rivers, includ ing 5155 juvenile individuals and 16 407 adult individuals. From 109 of these populations, we had adult modern samples with sample sizes of >20. For the remaining 38 populations we had only juveniles, historical samples, sample sizes <20 (for detailed information, see Supplementary Table S2). Scale samples of adult salmon were obtained from sport fishing, and from catches of broodfish for stocking or during autumn monitoring, while juvenile samples were obtained by electrofishing.

Statistical analyses

We applied the method by Karlsson *et al.* (2014) to estimate the level of farmed to wild genetic introgression. This method uses the STRUCTURE program (Pritchard *et al.*, 2000) in a manner that avoids bias from the level of heterogeneity and different sample sizes as described by Kalinowski (2011) and standardizes the estimates of admixture when introgression occurs from several farmed populations. We generated an idealized wild and farmed population in Hardy Weinberg proportions from a pool of reference individuals of wild and farmed salmon using the HybridLab program (Nielsen *et al.*, 2006). For the farmed salmon, we used all modern samples from the three breeding companies. For the wild salmon, we generated one population for the Atlantic phylogenetic group and one for the Barents White Sea group. Samples used for creating these wild centre points are indicated in Supplementary Table S2, column "REF Year". These ideal populations ($n = 100$) represented centre points for the three groups to which the probability of belonging was estimated (Figure 1). In an analysis of molecular variance (AMOVA), including the farmed reference samples and the historical wild reference samples from the Barents White Sea group, 18.01% of the variance was ascribed to variations between these groups ($p < 0.001$), and 4.08% to variance among populations within the groups ($p < 0.001$). In a comparison between farmed populations and the historical wild reference population from the Atlantic group, 7.19% of the variance was ascribed to variations between these groups ($p < 0.001$) and 3.48% to variance among populations within groups ($p < 0.001$). Single individuals were analysed with the farmed centre point and the two wild centre points representing the correct phylogenetic group for that individual, using an admixed model, 50 000 repetitions as burn in and 100 000 repetitions after burn in as implemented in STRUCTURE (Pritchard *et al.*, 2000). For each fish, the probability of belonging to the wild centre point, hereafter *P(Wild)*, was recorded.

For statistical analyses of farmed introgression, we generated probability distributions of belonging to the wild centre point for historical wild salmon (all samples in column "REF Year" in Supplementary Table S2) and for modern farmed salmon. Four populations (rivers Skibotnelva [river ID 205.Z], Signaldalselva [204.Z], Målselv [196.Z], and Skipsfjordelva [202.11Z]) represented genetic transitions between the Atlantic and the Barents White Sea phylogenetic groups and could not be analysed using the Atlantic or the Barents White Sea centre points. Instead, they were analysed by generating *in silico* populations from historical samples for each of these populations, except for Signaldalselva for which we did not have historical samples. Samples from the nearby River Skibotnelva were used as the analytical centre point for the Signaldalselva population.

From the distribution of individual probabilities of belonging to the wild centre point *P(Wild)* for a given sample (population and year), we estimated genetic introgression from escaped farmed salmon into this year's wild Atlantic salmon populations (Karlsson *et al.*, 2014). *P(Wild)* was logit transformed before the statistical inference (Warton and Hui, 2011). For each contemporary sample with a historical reference from the same river, we tested whether this population was introgressed with a two sample test for comparing means, assuming random sampling and equal variances for contemporary and reference samples. Further, we assumed that all wild populations had the same variance, estimated as the weighted average of the historical wild reference sample variances. Although the distributions for logit transformed *P(Wild)* for wild reference samples are relatively symmetric (see example in Figure 2), they depart too much from normality to perform standard tests for homogeneity of variances. By resampling squared deviations from all wild references, we found that 8.6% (3 of 35) of the wild population variances were significantly different from the pooled variance with a 5% significance level and were close to what we expected under the homogeneity assumption. Several of the wild reference populations have significantly different averages, so when testing whether a population without historical reference is introgressed we needed to consider this variance in wild population average values within a phylogenetic group. For populations without a historical reference from the same river, the contemporary average was therefore

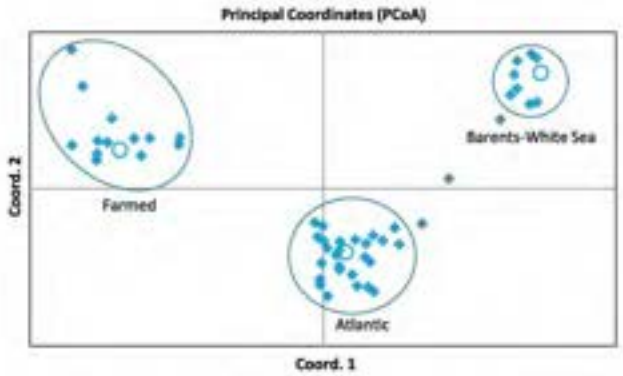

Figure 1. PCoA plot of pairwise F_{ST} estimates between historical samples from 39 Atlantic salmon populations and 13 farmed strain populations (diamonds), clustered into one farmed group (Farmed), one wild Atlantic salmon group from Finnmark (Barents White Sea), and one wild Atlantic salmon group form South of Finnmark (Atlantic). Grey diamonds are populations (River Skibotnelva, River Målselva, and River Skipsfjordelva) outside the clusters and genetic introgression is analysed by using the local historical samples. Open circles are *in silico* generated populations from a pool of the historical samples within each cluster.

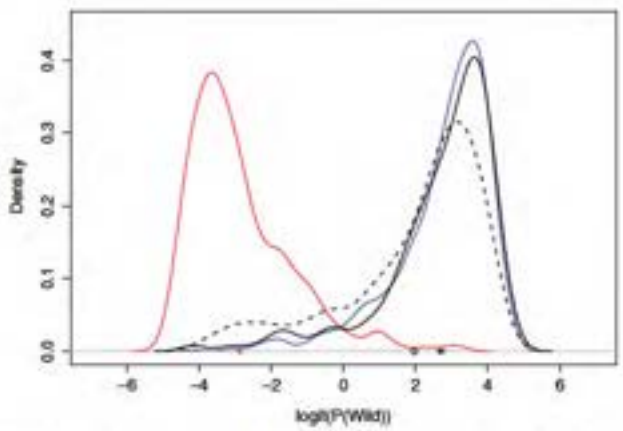

Figure 2. Distribution of logit-transformed probabilities of being of wild origin *P(Wild)*, for farmed reference (red line; mean value indicated by the red diamond), wild references for the whole Atlantic phylogenetic group (blue line and diamond), wild reference for River Eira (black line and diamond) and contemporary sample for River Eira (dashed black line and open black diamond; $n = 786$ for years 2012, 2013, 2014, 2015 pooled).

compared with the overall wild average for this phylogenetic group with this additional variance component included in the sampling distribution. For both tests, the null hypothesis states no genetic introgression; that is, mean $P(Wild)$ from the contemporary population equals the mean $P(Wild)$ from the historical reference population. The alternative hypothesis states that the contemporary mean $P(Wild)$ is smaller than the historical mean.

The distributions for individual $P(Wild)$ values for the samples are illustrated for the River Eira (104.Z) in Figure 2. Notable is the distinctiveness of the distribution for the farm references (red line). The distribution for the contemporary sample (pooled sample for the years 2012–2015; dashed black line) has a mean value significantly smaller than both the historical distribution for the River Eira population (solid black line) and the distribution for the whole Atlantic phylogenetic group (solid blue line).

For many samples, the observed change in mean value may not be significant, even if the populations show signs of genetic introgression. As in Figure 2, the contemporary distribution can indicate that a proportion of the population is introgressed by having a heavy left tail while the majority of the population is still mostly wild like. Genetic introgression into a subpopulation can be tested by, e.g. inspecting the lower 5 percentile of the distributions. Expected tail properties will be sensitive to distribution assumptions, so we opted for a randomization test approach. If the 5 percentile of a contemporary sample of a given size is much lower than expected from a sample of the same size from the historical distribution, it indicates that this sample has a too large proportion of individuals that genetically are admixed with farmed salmon. This effect was evaluated by simulating $n = 10\,000$ samples of the same size as the contemporary sample from the historical reference for the whole phylogenetic group, and registering the 5 percentiles of each simulated sample. The proportion of simulated 5 percentiles that was lower than the 5 percentile of the historical reference is the p value of the test.

Juveniles of farmed and admixed origin show lower survival to adulthood than juveniles of pure wild origin (Fleming *et al.*, 2000; McGinnity *et al.*, 2003). We expected therefore to find a higher level of introgression in juveniles than in adults in the same cohorts. To explore this in our data, we compared juvenile samples with adult samples from the same river, using a quasi cohort comparison. Specifically, we compared farmed introgression between juvenile and adult samples in 26 rivers, where sampling of juveniles occurred 3–5 years earlier than sampling of adults. Even though this is not a formal cohort analysis, at least some of the same year classes are likely represented in both the juvenile and adult samples.

Regional averages of introgression were constructed as unweighted averages and as averages weighted by spawning population size in each river studied (Forseth *et al.*, 2013). We defined regions as counties from the northernmost, Finnmark County, to the southernmost in western Norway, Rogaland County, whereas the counties from southernmost Norway to the south eastern border with Sweden, were treated as one region (Fiske *et al.*, 2006) denoted Southeast.

A major conservation policy for wild Atlantic salmon in Norway, National Salmon Rivers and National Salmon Fjords, was established by the Norwegian Parliament in 2003 (completed 2007) to increase the level of protection of Atlantic salmon, including protection from fish farming. By the final decision in 2007, 52 rivers were designated as National Salmon Rivers (of which we studied 48, cf. Vøllestad *et al.*, 2014) and 29 coastal areas were designated National Salmon Fjords (all are represented by our samples). We calculated unweighted and weighted averages for these groups of rivers in the same manner as for counties.

To study associations between group levels of introgression and average proportions of escaped farmed salmon, we used the method developed by Fiske *et al.* (2006) and Diserud *et al.* (2010) to calculate an "annual incidence" of escaped farmed salmon, by averaging proportions of escaped farmed salmon in anglers' catches in summer and in organized surveys in autumn and by calculating a weighted average by river catches. At the individual river level, Diserud *et al.* (2012, 2013) developed a long term "average annual incidence" for the years 1989–2012 for all rivers that were represented by four or more years in the time series.

Results

Based on adult modern samples from 109 salmon rivers with a sample size of 20 or more, we observed significant genetic introgression from escaped farmed salmon in 51 wild salmon populations (47%) and an estimated level of introgression >10% in 27 populations, between 4% and 10% in 19 populations and < 4% in 63 populations (Supplementary Table S2 and Figure 3). When all samples were considered, significant genetic introgression was observed in 77 of 147 rivers (Supplementary Table S2).

Comparisons in 26 rivers of juvenile samples with adult samples taken 3–5 years later, presumably representing the same cohorts, showed an average reduction of 2.5 percentage points between estimates of introgression in juvenile and adult life stages. Variation between rivers was high ranging from a 13% increase to a 17% reduction in farmed introgression from juvenile to adult samples.

Geographical distribution of farmed to wild genetic introgression

In the following, the presentation of level of introgression is based on pooled adult samples from recent sampling years in 109 rivers with a sample size of at least 20 individuals. National Salmon Rivers with special protection against anthropogenic impacts, including salmon farming, had on average lower levels of farmed genetic introgression (unweighted average, 4.5%) than salmon rivers without protection (unweighted average, 7.8%). The protecting effect of National Salmon Fjords appeared to be smaller as salmon rivers in and outside these fjords had similar (average, 6.4%) levels of farmed genetic introgression (Table 1). When considering population size (weighted averages), rivers within the National Salmon Fjords had however a lower level of introgression (1.8%) than other rivers (3.5%).

Genetic introgression has occurred in all regions of Norway, and the highest genetic introgression is found in the most intensive salmon farming regions (Figure 3). Unweighted averages of genetic introgression were largest in Troms County (14.5%) and Hordaland County (13.9%) and smallest in Nord Trøndelag County (0%) and Rogaland County (1.8%) (Table 2). However, four regional averages were based on <10 rivers, Troms and Nord Trøndelag being two of them. We also found significant introgression in samples excluded because of sample sizes <20, including adult samples (Byaelva [128.Z] and Salvassdraget [140.Z]) from Nord Trøndelag (Supplementary Table S2). Hence, no region in Norway is without farmed introgression.

Table 1. Farmed genetic introgression for Norwegian Atlantic salmon rivers with and without the protection status of being National Salmon Rivers, and for salmon rivers in and not in fjords with the a protection status of being National Salmon Fjords.

Group	*N* Ind	*N* pop	Farm introgression unweighted average/median	Farm introgression weighted average/median
National rivers	4347	47	0.045/0.016	0.016/0.000
Not national rivers	4741	62	0.078/0.028	0.048/0.014
National fjords	5337	59	0.064/0.018	0.018/0.000
Not national fjords	3751	50	0.064/0.026	0.035/0.025

Farm introgression values are given as averages and medians, both unweighted and weighted with estimated population size.

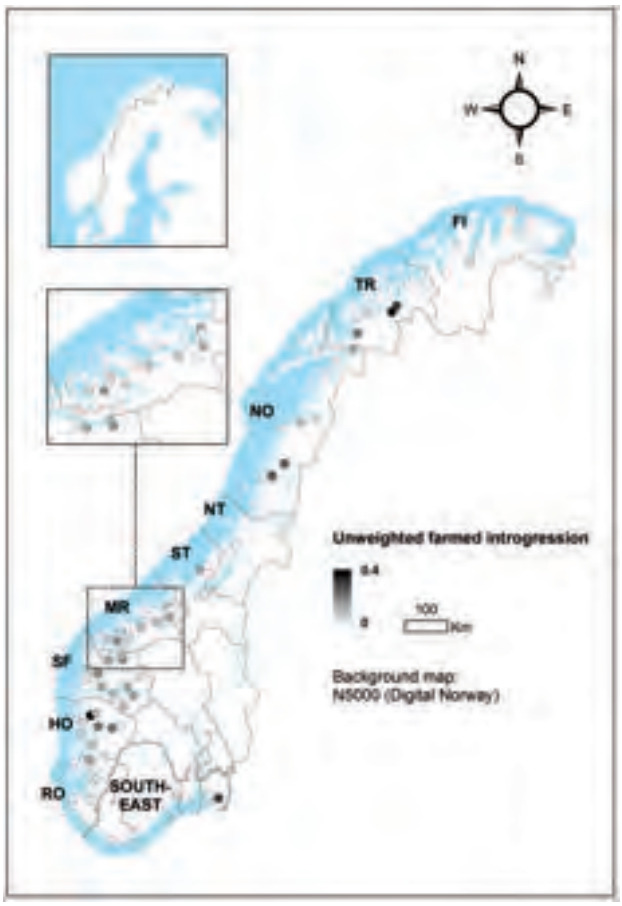

Figure 3. Map of Norway showing estimated farmed genetic introgression in 109 Norwegian salmon rivers from contemporary adult samples. Codes used for counties: FI = Finnmark, TR = Troms, NO = Nordland, NT = Nord-Trøndelag, ST = Sør-Trøndelag, MR = Møre og Romsdal, SF = Sogn og Fjordane, HO = Hordaland, RO = Rogaland, and SOUTHEAST is the southeasternmost counties pooled into one region.

Weighted averages by wild population size in the sampled rivers within each county were largely determined by status of the largest rivers and illustrate the geographical distribution of farmed introgression relative to the number of genes of farmed origin (proportion of farmed genomes). Hordaland County had the largest proportion of genomes with farmed origin (11.1%), and Nord Trøndelag County the smallest (0%). In the two phylogenetic groups of Norway, we found more introgression in the Atlantic group (unweighted average = 6.9%, weighted average = 2.6%) than in the Barents White Sea group (unweighted average = 2.6%, weighted average = 1.0%). Nationally, unweighted and weighted estimated proportions of farmed genomes were 6.4%, and 2.1%, respectively (Table 2).

Genetic introgression relative to farmed escapees

We observed a highly significant relationship between accumulated genetic introgression and average annual proportion of escaped farmed salmon, explaining 24% of the variance in introgression between rivers (Figure 4). The relationship was stronger at the region level, with proportion of escaped farmed salmon explaining 56% of the variance when weighted by population size (open diamonds in Figure 4). For populations in the Atlantic Sea phylogenetic group, the relationship was highly significant (red solid diamonds and dashed red line in Figure 4; $p < 0.01$, $R^2 = 0.19$, gradient = 0.3), while for populations in the Barents White Sea phylogenetic group the relationship was weak and not significant (blue solid diamonds and dashed line, Figure 4; $p > 0.05$, $R^2 = 0.05$, gradient = 0.05).

Temporal trends

We had samples from different periods (decades) in 27 populations, allowing us to examine temporal trends in the level of genetic introgression. Twelve of the populations showed an increase in genetic introgression, seven a decrease and six showing no introgression over time. In three populations for which we had more than two samples in time, there were increases followed by decreases in genetic introgression. Populations with downward trends had initial levels of genetic introgression between 1.8% and 6.1%, and in a more recent sample levels of introgression were between 0.0% and 3.8% (median = 0.2%). River Kinso (050.1Z) showed a decrease from a high of 24.7% in the 2000s to 12.7% in the 2010s. However, the trend in River Kinso is uncertain because there was only one sampling year representing the 2010s period and only 15 fish were analysed. A sample of juveniles from 2011 showed 29.4% introgression. A majority of the populations with an upward trend in genetic introgression had initial levels of genetic introgression between 0.0% and 7.5%, but showed large increases in genetic introgression with temporal differences in genetic introgression ranging from 1.5% to 23.7% (median = 11.2%).

Detecting early genetic introgression

We tested to what extent our set of SNP markers and the standardized method for detecting introgression (Karlsson *et al.*, 2014) worked for characterizing earlier generations of farmed salmon than those used for selecting SNPs differentiating between farmed (breeding kernel year classes 1998 2009) and historical wild salmon (Karlsson *et al.*, 2011). A comparison of distributions of *P*(*Wild*) between historical (1982 1988) and contemporary

Table 2. Farmed genetic introgression in Norwegian geographical regions.

Region	*N* Ind	*N* pop	Farm introgression, unweighted	Farm introgression, weighted
Southeast	899	11	0.038/0.000	0.015/0.000
Rogaland	1070	9	0.018/0.008	0.007/0.000
Hordaland	922	10	0.139/0.108	0.114/0.108
Sogn og Fjordane	1992	21	0.068/0.042	0.064/0.000
Møre og Romsdal	1946	16	0.062/0.044	0.039/0.014
Sør Trøndelag	365	6	0.047/0.020	0.013/0.012
Nord Trøndelag	162	4	0.000/0.000	0.000/0.000
Nordland	556	12	0.079/0.028	0.077/0.078
Troms	324	7	0.145/0.083	0.067/0.083
Finnmark	852	13	0.026/0.021	0.010/0.000
National	9088	109	0.064/0.023	0.021/0.000

Regions are set as counties (Figure 3), except for region Southeast which includes the south and the southeastern counties (Østfold, Akershus, Buskerud, Vestfold, Telemark, Aust-Agder and Vest-Agder). Regional farm introgression values are given as averages and medians, both unweighted and weighted with estimated population size.

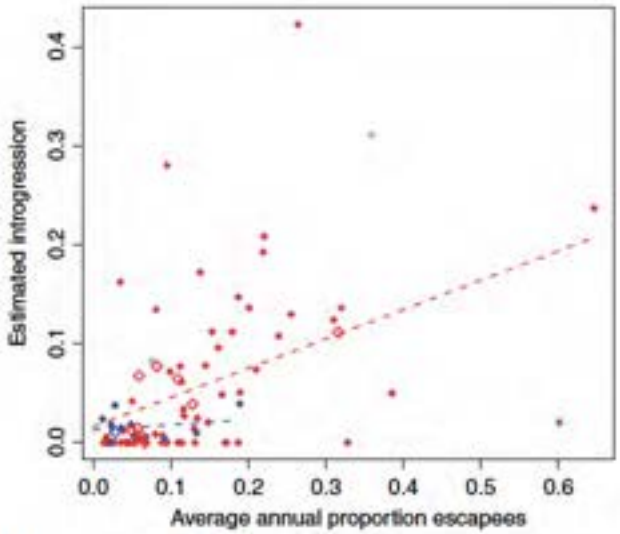

Figure 4. Relationship between mean annual proportions of escaped farmed salmon between 1989 and 2012 and estimated proportion of farmed genetic introgression from molecular genetic markers for 77 salmon populations (solid diamonds and dashed lines), and averaged for populations within geographical regions (open diamonds). Observations from the Atlantic Sea phylogenetic group are shown in red, from the Barents-White Sea phylogenetic group shown in blue, and two populations from the transition area are shown in grey.

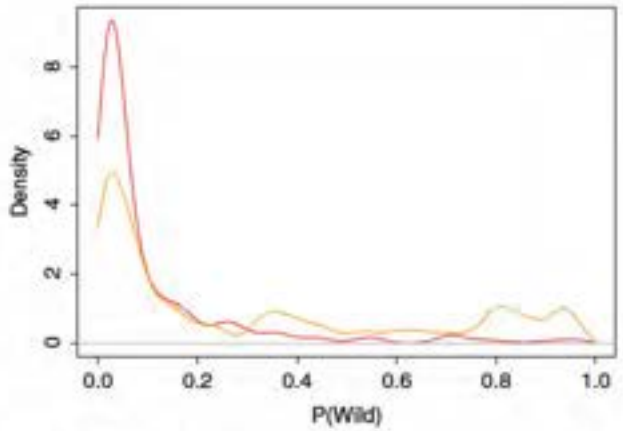

Figure 5. Distribution of STRUCTURE-generated probabilities of being of wild origin *P*(*wild*) for modern (red line) and historical (orange line) samples from the AquaGen farmed strains.

AquaGen samples indicated that the historical farmed populations appeared to be more wild than modern samples of farmed salmon (Figure 5).

Discussion

We quantified genetic introgression of farmed Atlantic salmon into 21 562 wild salmon from 147 populations. Levels of introgression >10% can now be found in any part of Norway in juveniles, as well as in adult salmon that have completed a life cycle in the wild. As expected, the level of introgression in wild populations is significantly associated with the average proportion of escaped farmed salmon in the river over the last 25 years.

Our method allows quantification of introgression from the individual level to populations, regions and the national level, and in rivers with and without a historical baseline. We found the highest levels of introgression in the counties of Norway where escaped farmed salmon have been present in highest proportions.

In Hordaland County, western Norway, one of the two cradles of fish farming, several populations show high levels of introgression, with the rivers Opo (048.Z), Granvin (052.1Z; juveniles) and Dale (061.Z) showing recent levels of introgression >40% and three other rivers [Vosso (062.Z), Kinso (050.1Z), and Etne (041.Z)] showing introgression >10%. Affected rivers are found both along the coast and within the major (Hardangerfjord) and minor fjords in the county. Highly affected rivers in this county were also found in a study of 20 Norwegian rivers by Glover *et al.* (2013).

Other rivers with high levels of introgression are found in Troms County in northern Norway, Sogn og Fjordane County and Møre og Romsdal County in western Norway. Rivers with low levels of introgression are most common in south eastern Norway, Rogaland County in the southwest and Finnmark County in the northeast. The river holding Norway's largest Atlantic salmon population, River Tana (234.Z) on the border with Finland, has a low level of introgression (0 in our Supplementary Table S2). The spawning population (or rather, populations, see Vähä *et al.*, 2008) of the Tana is so large

(> 40 000 fish), compared the other salmon rivers (average = 1900, range: 100 18 000), that it strongly affects regional weighted averages in Finnmark, the Barents White Sea phylogenetic group and even the national average.

Current levels of introgression are likely underestimated

The farmed references in the present study are representative for introgression that occurred between the 1990s and present. They cover a large part of this period (year classes hatched 1998 2009) and may be representative of more years, as a previous study showed no significant change in allele frequencies at microsatellites in two breeding kernels sampled one generation (Karlsson *et al.*, 2010).

Intrusion of farmed Atlantic salmon on the spawning grounds of wild salmon was detected on a large scale from 1986 onwards (Gausen and Moen, 1991). Introgression during this early time period is, however, likely underestimated, because we found a weaker genetic contrast between historical wild salmon and farmed salmon samples from the 1982 1988 than in the farmed salmon samples from 1998 to 2009 used as farmed references. Our statistical method has been tested against simulated data sets and has been shown to give precise estimates of introgression at the population level (Karlsson *et al.*, 2014). Precaution is therefore warranted in our evaluation of the status of populations with no or only weak levels of farmed introgression detected in the present study.

Introgression varies by farmed intrusion and phylogeographic origin

Geographical variation in levels of introgression may have several explanations, the most immediate being that the proportion of escaped farmed salmon in spawning populations also varies. On both local (river) and regional (county) levels, we found a significant, positive correlation between average annual proportions of escaped farmed salmon 1989 2012 (Diserud *et al.*, 2013) and introgression in recent samples.

The most impacted rivers, with respect to long term average proportions of escaped farmed salmon, are found in the counties of Hordaland (Opo [048.Z], Kinso [050.1Z], Eio [050.Z] and Frugardselva [044.3Z]) being highest among those studied genetically, all with average proportions 1989 2012 of escaped farmed salmon >50% according to Diserud *et al.*, 2013) and Troms (River Salangselva [191.Z] with 65%, Diserud *et al.*, 2013).

Our samples of wild Atlantic salmon are represented by two phylogenetic groups, the Atlantic group and the Barents White Sea group (Bourret *et al.*, 2013). Even though wild populations from both phylogenetic groups were represented among the source populations (Gjedrem *et al.*, 1991), only the Atlantic group was represented in the third generation of farmed salmon in the breeding programme (cf. Gjøen and Bentsen, 1997). Interestingly, we found a significant association between proportions of escaped farmed salmon and introgression for the Atlantic group and not for the Barents White Sea group, and a steeper gradient in the Atlantic group (Figure 4). This might reflect differences in genomic architecture between the two phylogenetic groups and a higher barrier to introgression in the Barents White Sea group. The barrier is not absolute, as we found significant introgression in several of the Barents White Sea populations, even in numerically strong populations like River Alta (212.Z) and Vestre Jakobselv (240.Z). The number of samples from the Barents White Sea group is however limited, and a conclusion about barriers to introgression in relation to phylogenetic origin must await further study.

Additional explanations of variation in introgression

A large proportion of the variance in the level of introgression could not be explained by proportions of escaped farmed salmon (Figure 4). This is not unexpected, as one of the main conclusions from a review of genetic effects following releases was the wide variety of outcomes, ranging from no detectable effect to complete introgression or displacement of the native population (Hindar *et al.*, 1991). Experimental studies of farmed and wild salmon, however, point to some general findings about causes of variation.

It has been shown experimentally that farmed salmon escaping early from captivity have higher reproductive success in competition with wild salmon than later escaping farmed salmon, i.e. comparing hatchery released smolts with farmed adults (Fleming *et al.*, 1996, 1997). So far, this has not been accounted for in analyses of how escaped farmed salmon leads to introgression, but will be possible in the future as scale reading advances to include the likely size at which farmed salmon escape.

The density of wild Atlantic salmon on the spawning ground may also be important. The breeding behavior of Atlantic salmon involves female to female competition for access to high quality spawning sites to excavate the nests, and male to male competition for access to females (Fleming and Einum, 2011). Lura (1995) suggested that the spawning success of escaped farmed females was density dependent because the contributions of eyed eggs, relative to their proportion among the spawners, were lower in rivers and years with high densities of spawners. Likely explanations may be that farmed females are outcompeted from the most favourable nest sites at high densities (Lura, 1995), and there may be a larger proportion of unspawned eggs in farmed than in wild salmon at high densities (Jonsson *et al.*, 1990; Fleming *et al.*, 1996, 2000). For males, Fleming *et al.* (1997) showed density dependent spawning success in an experimental study of hatchery reared vs. wild River Imsa males. In contrast, late escaping farmed males showed poor reproductive success regardless of density in the same spawning arenas (Fleming *et al.*, 1996). We do not yet know whether there are differences in reproductive success among the various selected strains of farmed salmon, but we know that farmed fish vary in their genetic relationships with wild salmon (Karlsson *et al.*, 2011, 2010, 2014).

Lower average introgression is found in National Salmon Rivers and to a lesser extent in rivers within a National Salmon Fjord. This indicates that national salmon fjords and rivers provide increased protection from farmed introgression. One common factor among these populations is that emphasis was put on the numerically strongest populations when rivers were chosen for designation as National Salmon Rivers. Population size in itself may be a protective measure from introgression (Heino *et al.*, 2015), which is also supported by the difference between unweighted and weighted averages found here (Table 2). Another type of protection is the increased distance between aquaculture operations and wild salmon rivers, which makes it less likely for a salmon river in a National Salmon Fjords to receive escaped farmed salmon, than outside of such a fjord, other things being equal (Fiske *et al.*, 2013).

However, we do not see low introgression levels in all salmon populations with this protective regime. For example, River Daleelva (061.Z), River Vosso (062.Z), River Vikja (070.Z), River Årøyelva (077.Z), River Jølstra (084.Z), River Olden (088.1Z), River Røssåga (155.Z) and River Beiarelva (161.Z) are National Salmon Rivers or are situated in a National Salmon Fjord and have > 10% farmed genetic introgression. One explanation for the variation in the protecting effect of National salmon rivers and fjords might be the size of the protected region, exemplified by the large Trondheimsfjord. The entire Trondheimsfjord is a National Salmon Fjord in a highly intensive farming region, where a high level of introgression was found in a coastal population (River Teksdalselva [134.Z]), but consistently lower levels were found in rivers inside the major Trondheimsfjord. In the Hardangerfjord system, on the other hand, only a small part (<5% of the fjord area) is designated as a National Salmon Fjord, Etnefjorden. Most rivers in the Hardangerfjord show high levels of introgression, as does River Etne (041.Z). Another explanation for high levels of introgression in salmon populations within National Salmon Fjords is that some of these rivers have occasionally had low levels of wild spawners, because of the parasite *Gyrodactylus salaris* (Vikja, Røssåga, Beiarelva; Johnsen and Jensen, 1991), or of other anthropogenic factors. A likely mechanism is easier access to spawning opportunities when wild population size is low (Sægrov *et al.*, 1997).

An explanation for the variable effect of National Salmon Rivers and Fjords not yet highlighted is the possibility that an introgressed population may impact neighbouring populations through straying of wild offspring of cultured fish (Felsenstein, 1997). An important question in this regard is a potentially weaker homing of offspring from escaped farmed salmon compared with the locally adapted wild salmon, because of different genetic (Jonsson *et al.*, 2003) or epigenetic origins (Christie *et al.*, 2016). In experiments with wild and farmed Atlantic salmon, hatchery produced smolts of farmed origin showed a higher straying rate than hatchery produced Imsa salmon released into the Imsa (Jonsson *et al.*, 2003).

Differential survival of introgressed individuals

Levels of introgression were similar between juvenile and adult salmon samples in our study. Experimental studies generally show a lower lifetime survival of farmed offspring than wild offspring, with hybrid groups being intermediate (McGinnity *et al.*, 1997, 2003; Fleming *et al.*, 2000; Skaala *et al.*, 2012). It is therefore expected that within the same cohort, a general reduction in mean *P*(*Wild*) should be observed across life stages from alevin, to parr, to smolt, to returning adults. In our material, we could not make a formal cohort analysis, but some populations could be compared between juvenile and adult samples that likely showed some year class overlap. The average reduction was estimated at 2.5 percentage points, with a large variation between populations, including some where the level of introgression was higher among adults than among juveniles. Observational studies that control for year class (cohort) are needed before the effect of viability selection on introgression can be quantified more precisely.

What do the levels of introgression found in this study mean?

This question may be discussed at several different levels: genetics, fitness and viability, ecology and life history, management, and conservation. With respect to genetics, three concerns are important: loss of genetic variation within populations, loss of genetic variation between populations and loss of fitness (Waples *et al.*, 2012). Farmed Atlantic salmon have in general lower genetic variation than wild Atlantic salmon (Mjølnerød *et al.*, 1997; Skaala *et al.*, 2004, 2005; Karlsson *et al.*, 2010), and the long term prediction from escapes is that lower genetic diversity will eventually lead to a drop in diversity in recipient wild populations (Tufto and Hindar, 2003), even though in the short term, genetic variation may increase from interbreeding with farmed salmon. Loss of genetic variation between populations as a result of introgression from farmed Atlantic salmon has been demonstrated both theoretically (Mork, 1991) and empirically (Skaala *et al.*, 2006; Glover *et al.*, 2012, 2013). Loss of fitness has been demonstrated in controlled rivers in Ireland (McGinnity *et al.*, 1997, 2003) and Norway (Fleming *et al.*, 2000; Skaala *et al.*, 2012) and in large scale experiments in Canada (Fraser *et al.*, 2010). The loss of viability is also indicated by these same studies, as well as in meta analysis of the population dynamics of salmon populations near or far from aquaculture operations (Ford and Myers, 2008; Vøllestad *et al.*, 2009). For the latter studies, however, several mechanisms in addition to introgression may be at work, such as increased mortality caused by parasites associated with fish farming activities (e.g. Krkosek *et al.*, 2013).

Ecological change in introgressed individuals was evident from experiments in controlled, natural rivers showing changes in growth rate, condition factor (length weight relationship) and age at smoltification and maturation (Fleming *et al.*, 2000; McGinnity *et al.*, 2003). This was also true in a large scale observational study that tested whether *P*(*Wild*) had an impact on ecological key traits (Geir Bolstad, NINA *et al.* in prep.). This change in ecological traits also likely has a negative effect on fitness (Tufto, 2001; Huisman and Tufto, 2012; Baskett *et al.*, 2013).

Implications for management and conservation

In a management and conservation context, a pertinent question is how much introgression can be allowed (Ryman *et al.*, 1995). While there is no simple answer to this question, it is clear that near zero limits need to be set in order not to compromise the genetic integrity of wild populations. Ryman *et al.* (1995) suggested that a defensible strategy, based on population genetic considerations, could be to allow gene flow at a rate that matched equilibrium levels of gene flow between semi isolated populations, as quantified by Wright's fixation index, F_{ST}. This would allow only a small number of reproductively capable escaped farmed salmon spawning in wild populations every generation.

In considerations of the Endangered Species Act listing of populations of westslope cutthroat trout (*Oncorhynchus clarki lewisi*) in danger of hybridization with rainbow trout (*O. mykiss*) and Yellowstone cutthroat trout (*O. c. bouvieri*), Allendorf *et al.* (2004) suggested that listing only non hybridized populations was the only alternative that could be defended from the perspective of possessing local adaptations important for long term persistence of this sub species. An alternative criterion, allowing 10% introgression from the other taxa, was discarded because it could lead to hybridized populations acting as a source for further introgression. These considerations deal with sub species and species differences, and may be too conservative for our Atlantic salmon study that deals with farmed and wild population differentiation and introgression. The developmental and evolutionary

forces acting on farmed Atlantic salmon are so unlike those in the wild that two distinct biologies are being created within the Atlantic salmon species (Gross, 1998; see also Roberge *et al.*, 2006; Christie *et al.*, 2016). Gross (1998) even suggested that farmed and wild Atlantic Salmon be recognized as different "spe cies", and that farmed salmon be treated as "exotic" when they es caped to the wild, as a measure to prevent further impact from aquaculture. The calculation of *P*(*Wild*) at the level of individuals has an immediate use in practical management and conservation. In many rivers, hydropower companies have to compensate for the reduction in natural productivity of a river by releasing hatchery produced fish. In other rivers, releases of offspring from local brood stock is practiced on a voluntary basis. Regardless of purpose, a genetic test compulsory for all brood stock being used was introduced in 2014 by the Norwegian Environment Agency to limit the likelihood of spreading farmed salmon genotypes through stock enhancement. In autumn 2014, the calculation of individual *P*(*Wild*) led to 14% of potential broodstock in Norway being discarded for genetic reasons, and in 2015, 18% of potential brood stock was discarded (Karlsson *et al.*, 2015, 2016). In the highly impacted Hardangerfjord rivers (average introgression 13.2%), calculations of *P*(*Wild*) during autumn 2015 showed that only 83 of 141 fish (escaped farmed salmon excluded) qualified as wild origin brood stock to create a live gene bank for the most impacted populations.

The probability distribution of *P*(*Wild*) may help characterize the stage reached in an accumulation of farmed introgression. In some populations, the probability distribution for being wild shows distinct modes with fish at several stages of introgression, including "pure wild", "hybrid" and "farmed" (Tufto, 2000). At later stages of introgression, with a wide range of admixed groups in the population, we expect a smoother distribution of individ ual *P*(*Wild*) values without distinct modes. At this time, the pro portion of individiuals with pure wild origin is low, and management has to be cautious to preserve all ecotypes (e.g. late spawning fish; upper river spawners) in the remaining historically wild populations (Hansen *et al.*, 2006).

To protect the genetic integrity of wild Atlantic salmon popu lations, only low levels of introgression from escaped farmed sal mon can be allowed into wild populations. We found significant introgression in half of the populations studied, and levels of in trogression >10% in nearly one quarter of the populations. The rivers we studied represent three quarters of the entire Norwegian wild salmon spawning population. Further introgres sion is likely, unless substantial reduction of escaped farmed sal mon in the wild, or sterilization of farmed salmon, can be achieved.

Supplementary data

Supplementary material is available at the *ICESJMS* online ver sion of the manuscript.

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RAPID COMMUNICATION

Predicting the impacts of escaped farmed Atlantic salmon on wild salmon populations

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Abstract: The escape of Atlantic salmon (*Salmo salar*) from aquaculture facilities can result in both negative genetic and ecological interactions with wild populations, yet the ability to predict the associated risk to wild populations has remained elusive. Here we assess the potential of a spatiotemporal database of aquaculture facility locations, production estimates, and escape events to predict the distribution of escaped farmed salmon and genetic impacts on wild populations in the Northwest Atlantic. Industry production data, reported escape events, and in-river detections of escaped farmed salmon were collected from across the Northwest Atlantic. Genetic estimates of impact were obtained using single nucleotide polymorphisms (95 loci) representing aquaculture and wild salmon throughout the region (30 populations, 3048 individuals). Both the number of escaped farmed salmon detected at counting facilities and the magnitude of genetic impacts were positively correlated with a cumulative spatial measure of aquaculture production. Our results suggest that the risk of escapees and genetic introgression from wild–farmed salmon interactions can be assessed using information on farm production characteristics. This represents a first step in predicting the impact of existing cage-based farms on wild Atlantic salmon.

Résumé : Si l'échappement de saumons atlantiques (*Salmo salar*) d'établissements piscicoles peut se traduire par des interactions génétiques et écologiques négatives avec les populations sauvages, la capacité de prédire le risque associé pour ces populations sauvages demeure limitée. Nous évaluons le potentiel d'une base de données spatiotemporelles sur les emplacements d'établissements piscicoles, les estimations de la production et les cas d'échappement pour la prédiction de la répartition de saumons d'élevage échappés et de leurs impacts génétiques sur les populations sauvages dans le nord-ouest de l'océan Atlantique. Des données de production provenant de l'industrie, les cas d'échappement signalés et les détections en rivière de saumons d'élevage échappés ont été compilés pour toute cette région. Des estimations des impacts génétiques ont été obtenues en utilisant des polymorphismes mononucléotidiques (95 sites) représentant les saumons d'élevage et les saumons sauvages à la grandeur de la région (30 populations, 3048 individus). Le nombre de saumons d'élevage échappés détectés dans des lieux de comptage et l'ampleur des impacts génétiques sont tous deux positivement corrélés à une mesure spatiale cumulative de la production aquacole. Nos résultats donnent à penser que les risques d'échappement et d'introgression génétique découlant d'interactions de saumons sauvages avec des saumons d'élevage échappés peuvent être évalués en utilisant de l'information sur les caractéristiques de la production piscicole. Il s'agit d'une première étape dans la prédiction des impacts de pisciculture à base de cages sur le saumon atlantique sauvage. [Traduit par la Rédaction]

Introduction

The Atlantic salmon (*Salmo salar*) is one of the most valuable aquaculture fish species, and the salmon aquaculture industry is expanding worldwide (FAO 2016). Despite continued refinement of production methods and technology, the escape of aquaculture individuals into the wild environment occurs regularly due to weather events, predator attacks, or losses during regular operations (Bentsen and Thodesen 2005; Glover et al. 2017; Jensen et al. 2010). Estimates suggest two million farmed Atlantic salmon escape into the North Atlantic Ocean every year (Schiermeier 2003) with negative evolutionary and ecological impacts on wild salmon populations (Bolstad et al. 2017; Fleming et al. 2000; McGinnity et al. 2003; Skaala et al. 2012; Weir et al. 2005). Farmed salmon are both genetically distinct (reviewed by Glover et al. 2017) and phenotypically divergent from wild salmon, with differences such as accelerated growth rates, delayed maturity, behavioural differences, and reduced immunity (Fleming et al. 1996; Jonsson and Jonsson 2006), contributing to reduced survival in the wild (Fleming et al. 2000; McGinnity et al. 1997, 2003; Skaala et al. 2012). Interbreeding between escaped farmed salmon and wild salmon has been widely documented (Bourret et al. 2011; Clifford et al. 1998; Crozier 1993; Glover et al. 2012, 2013; Karlsson et al. 2016; Skaala et al. 2006) and can lead to phenotypic changes in native wild populations (Bolstad et al. 2017; Fraser et al. 2010), which in turn can reduce the fitness of wild populations (Fraser et al. 2008; McGinnity et al. 2003; Verspoor et al. 2015). Although interactions between wild

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and escaped domestic Atlantic salmon have repeatedly been identified as major threats to the persistence of wild populations (COSEWIC 2010; DFO 2008, 2013; Forseth et al. 2017; USASAC 2016), methods to predict and mitigate impacts remain lacking.

Wild Atlantic salmon populations in the Northwest Atlantic have declined in recent decades (Chaput 2012; ICES 2016) resulting in the closure of both commercial and recreational fisheries (COSEWIC 2010; DFO 2008, 2013; USASAC 2016). The factors responsible for these declines likely vary across regions (COSEWIC 2010; Parrish et al. 1998); however, genetic interaction with escaped farmed salmon has been identified as a major concern (DFO 2013; Ford and Myers 2008; Verspoor et al. 2015). Previous attempts to quantify the frequency of escape events and distribution of escaped farmed salmon in rivers have been limited, but available data suggest they are present in the majority of rivers near (<300 km) the aquaculture industry in Atlantic Canada (Morris et al. 2008). Moreover, recent genomic evidence indicates significant hybridization between wild and farmed salmon has occurred in southern Newfoundland (B. Wringe and I. Bradbury, unpublished data), which may further negatively affect threatened wild salmon populations (COSEWIC 2010; DFO 2013). Despite continued evidence of genetic impacts on wild salmon populations (Bolstad et al. 2017; Bourret et al. 2011; Clifford et al. 1998; Crozier 1993; Fraser et al. 2008, 2010; Glover et al. 2012, 2013; Karlsson et al. 2016; McGinnity et al. 2003; Skaala et al. 2006; Verspoor et al. 2015), the ability to predict population-level risks to inform management efforts remains limited. Predictive models linking aquaculture industry production characteristics, the distribution of escaped farmed salmon, and impacts on wild populations are ultimately needed to assess and manage risks to wild Atlantic salmon. A first step in developing these predictive models is to identify variables that are associated with the distribution of escaped farmed salmon or associated impacts on wild populations.

The main objective of this study is to identify factors associated with distribution of escaped farmed salmon in Atlantic Canada and the genetic risk from escaped farmed salmon to wild salmon populations. Results could be used to inform aquaculture management regarding the conservation of wild populations. Specifically, we explored the ability of a spatiotemporal database of aquaculture facility and production data to predict (i) the distribution of escaped farmed salmon and (ii) the genetic impacts on wild populations in Atlantic Canada. This work builds on the database and analysis published by Morris et al. (2008) of escaped farmed salmon detections from Maine and Atlantic Canada and extends previous analyses with the inclusion of industry production data and new genetic measures of impact on wild populations.

Methods

Data retrieval and sampling

Data compiled for this study were of three types: (i) aquaculture site production and location data for the Northwest Atlantic region; (ii) reports of escaped farmed salmon in Northwest Atlantic rivers (refer to online Supplementary material, Table S1[1]); and (iii) population genetic estimates of introgression between wild and farmed Atlantic salmon from southern Newfoundland. For the aquaculture site locations and production data, the resolution of available information differed by jurisdiction, so analyses were designed to address any associated assumptions and limitations due to these differences. The numbers listed below represent all licensed aquaculture sites in Newfoundland, New Brunswick, Nova Scotia, and Maine for the period of 2005–2015. Inventory data were obtained for a total of 78 aquaculture sites in Newfoundland for the period of 2005–2015 (provided by G. Perry, Aquaculture Management, Newfoundland Region, Fisheries and Oceans Canada (DFO); Fig. S1A[1]). Using the reported information on farm-stocked fish (e.g., introductions, transfers, harvest numbers, and mortalities), an annual estimate of the mean number of fish per site was calculated. For the province of New Brunswick, data were obtained during annual provincial monitoring activities (i.e., actual counts of fish in cages on the day of monitoring) for 89 sites during the years 2012 to 2015, while annual stocking license data (i.e., the maximum number a site is permitted to stock annually) were available for 2005–2012 (provided by T. Lyons, New Brunswick Department of Environment and Local Government, and G. Cline, Aquaculture Management, Maritimes Region, DFO). Detailed inventory data for Nova Scotia were not available at the time of analysis, so annual stocking license information for 18 sites during the years 2005–2015 were used (provided by E. Parker, Aquaculture Management, Maritimes Region, DFO). Annual stocking license data from 17 sites in Maine were also available for 2005–2015 (provided by D. Bean, National Marine Fisheries Service, National Oceanic and Atmospheric Administration).

Owing to discrepancies in aquaculture production monitoring between provinces and states, for all region-wide analyses, specific production numbers were not involved. Instead, each aquaculture site was assigned a value depending on whether it was fallow (0) or stocked (1) for each year. For analyses including genetic data (see below), the spatial extent was restricted to Newfoundland so that annual estimates of fish per site could be used. Further details are provided in analysis sections below.

Reports of escaped farmed salmon detected in rivers throughout the study region were obtained via opportunistic surveys and dedicated sampling and monitoring programs (Table S1 and Fig. S1B[1]), as in Morris et al. (2008). Academic and governmental Atlantic salmon research programs, aquaculture management authorities, and conservation organizations (e.g., Atlantic Salmon Federation) were asked to provide information on any escaped farmed salmon encountered. New reports of river-specific presence (with counts when available) or absence were added to the database compiled by Morris et al. (2008). Additional records were obtained from monitoring facilities (counting fences) in the Magaguadavic River in New Brunswick (since 2002) and Garnish River (since 2015) and Conne River in Newfoundland (since 1987). Scientific gillnetting and angling were also conducted in several Newfoundland rivers during the fall seasons of 2015 and 2016.

Genetic analysis

Genetic analysis was used to quantify the degree of introgression between escaped farmed salmon and wild populations in southern Newfoundland. Data examined included a combination of previously published sources, including (i) single nucleotide polymorphism (SNP) array data (Bradbury et al. 2015; Moore et al. 2014; B. Wringe and I. Bradbury, unpublished data) and (ii) targeted genotyping of a subset of this larger array using a 96 SNP assay (B. Wringe and I. Bradbury, unpublished data). The 5568 SNP array developed by the Centre for Integrative Genomics (Norway) (Bourret et al. 2013*a*, 2013*b*) was used for 611 individuals from the Maritimes and southern Newfoundland. For the targeted genotyping of 96 SNPs (B. Wringe and I. Bradbury, unpublished data), a further 2703 individuals from southern Newfoundland were genotyped. The total SNP data set was composed of a total of 3314 individuals, including 156 individuals (adults) of known aquaculture ancestry and from 47 wild Atlantic salmon populations from the Maritimes and southern Newfoundland, plus two aquaculture populations. 15 rivers from southern Newfoundland had data for 2 years (2014 and 2015), providing a total of 64 populations spanning the Maritimes and southern Newfound-

[1]Supplementary data are available with the article through the journal Web site at http://nrcresearchpress.com/doi/suppl/10.1139/cjfas-2017-0386.

land. Results presented here are limited to southern Newfoundland populations ($n = 26$).

The methodology for the 5568 SNP array is described in detail in Bradbury et al. (2015). In summary, only SNP genotypes with a call rate greater than 0.95 were retained, and SNPs were removed where minor allele frequencies were <0.05 or were missing in >0.15 of individuals. For the targeted genotyping of the subset of 95 SNPs, SNP type assays (Fluidigm) per the manufacturer's protocols were used (B. Wringe and I. Bradbury, unpublished data). This SNP genotyping was performed using 96.96 genotyping integrated fluidic circuits, read on an EP1 (Fluidigm), and analyzed using SNP genotyping analysis software (Fluidigm). Based on reanalysis of samples and following Pompanon et al. (2005), the genotype error rate was calculated to be 0.01% and consistent with other studies (0%–0.2%; see Hess et al. 2015; Jones et al. 2015; Larson et al. 2014; Petrou et al. 2014; Seeb et al. 2009).

We used the Bayesian clustering software STRUCTURE (Pritchard et al. 2000) to estimate population admixture values between domestic and wild populations (i.e., Q values ranging from 0 to 1, respectively). The R package ParallelStructure (Besnier and Glover 2013) was used to estimate K, the putative number of populations, from 1 to 30, replicated three times each. Each run had a burn-in of 100 000 Markov chain iterations, followed by 500 000 iterations. STRUCTURE was run with the admixture model and without population location priors. The degree of introgression with escaped farmed salmon was then calculated based on the mean proportion of each population's genome that could be attributed to the domestic baseline population.

Spatial analysis: propagule pressure

The river-specific risk from escaped farmed salmon was spatially quantified using propagule pressure, adapted from invasive species research where it represents the intensity of anthropogenic introductions of non-native species (Colautti 2005; Consuegra et al. 2011; Copp et al. 2007). In this study, propagule pressure was calculated for each river as either the presence of fish at an aquaculture site each year (from 2005 to 2015) or the number of fish stocked each year, divided by the distance to that site, and summed across all sites and years. For fine-scale analyses involving only Newfoundland, annual estimates of the number of fish per aquaculture site were divided by their respective distances to each river as follows:

$$\text{Propagule pressure for a given river } (R) = \sum_{i,y=1}^{s} \frac{F_{i,y}}{\text{LCD}(S_{i,y} \text{ to } R)}$$

where $S_{i,y}$ represents an aquaculture site in a given year (y), R is each river, $F_{i,y}$ is the number of fish at site S_i each year or presence of fish in aquaculture site S_i each year (0 or 1), and LCD represents the least-cost distance function.

For region-wide analyses, as numbers of stocked fish were not available for all jurisdictions, a value of one was assigned to aquaculture sites that were stocked in a given year, and unstocked sites were excluded (0/LCD = 0). The propagule pressure variable allows a river-specific measurement of aquaculture intensity that relates to both the proximity of aquaculture sites and the production levels of the sites. As such, rivers that were close to many aquaculture sites or close to a few very high-production sites would have stronger propagule pressures than rivers that were far from aquaculture sites or only near smaller-scale production sites. Propagule pressures were calculated for all rivers in the compiled escaped farmed salmon database and any rivers with genetic data, including Maritimes rivers reported in Moore et al. (2014) that were not included in this study's analyses (Fig. S3[1]).

To explore the utility of propagule pressure in predicting aquaculture impacts, three measures of impact were derived from the collated data: the presence of escaped farmed salmon in rivers, the number of escaped farmed salmon in rivers, and the amount of introgression observed in populations as reflected by the population mean Q values resulting from the Newfoundland STRUCTURE analysis (see above). Log-log regression models and GLMs (using Poisson and binomial distributions) were applied to examine the relationship between propagule pressure and these measures of impact. t tests and (or) χ^2 tests were used to assess the significant effects of explanatory variables on the measures of impact. AIC (Akaike information criterion) or corrected Akaike information criterion (AIC_c) where appropriate were used to compare and weight models according to their fit to the data and complexity. River size (measured as axial distance in kilometres) was also used as an explanatory variable to address the influence of population size. The R statistical environment (version 3.3.2; R Core Team 2016) and RStudio (version 1.0.136; RStudio Team 2015) were used for all visual and statistical analyses. Maps were produced using shapefiles downloaded from GADM.org via package raster. A package containing a function to calculate propagule pressure was written to facilitate this analysis and is available for download from GitHub via GNU General Public License (Package AQpress: https://github.com/freyakeyser/AQpress).

Results

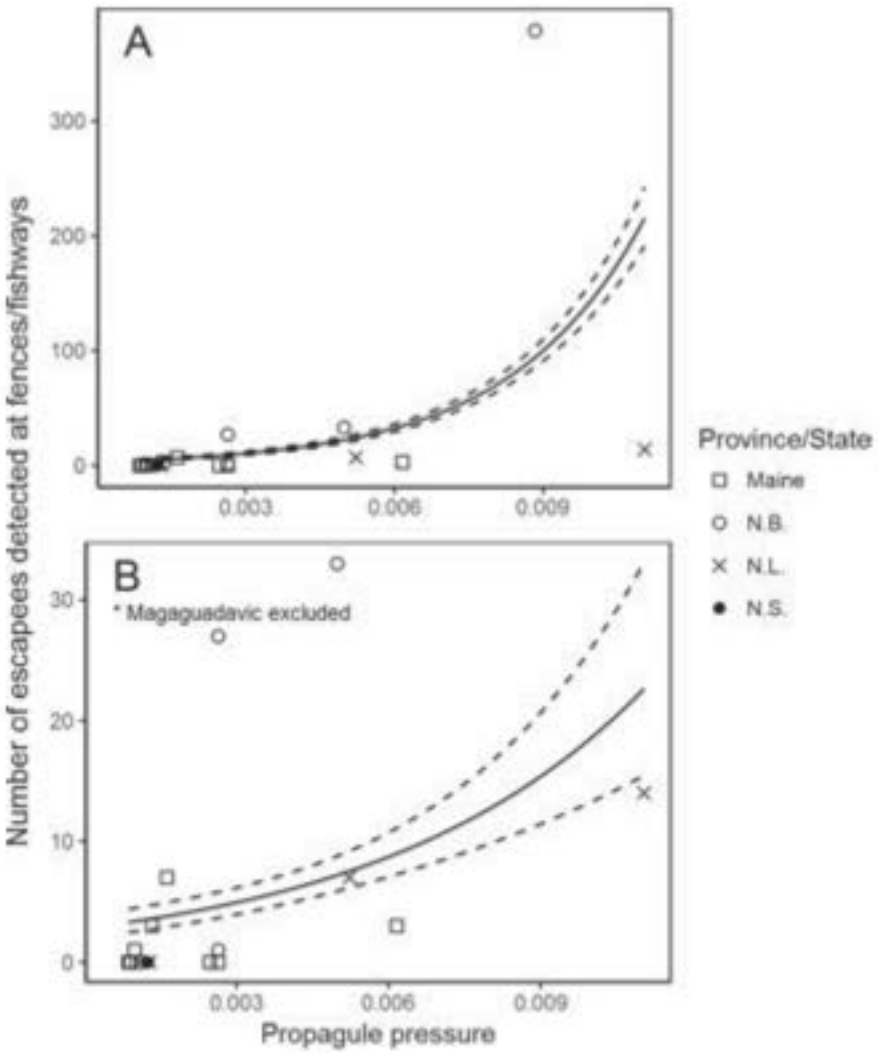
In total, an additional 217 records were added (annual river reports of escaped farmed salmon presence or absence) to the Morris et al. (2008) adult farmed escapee database, for a grand total of 467 records from the 1980s to 2016 (Table S1[1]). New records of presence or absence of escaped farmed salmon for 17 rivers for which no information previously existed were collected (mostly in Newfoundland; Morris et al. (2008) only presented records for two rivers in Newfoundland), as well as new records of presence or absence for a separate 17 rivers already in the Morris et al. (2008) database. These additions bring the total number of rivers in the database to 112 (Table S1[1]). A total of 1091 escaped farmed salmon have been detected since the publication of the (Morris et al. 2008) database, and our updated database contains detections of 9236 escaped farmed salmon in Northwest Atlantic rivers. Rivers in the Passamaquoddy Bay (outer Bay of Fundy) area had higher numbers of escaped farmed salmon reported than other areas (Fig. S1B[1]). In addition to the database of escaped farmed salmon reports, this study also incorporated genetic information for 29 Newfoundland rivers (Figs. S1A and S3 and Table S2[1]).

The presence of escaped farmed salmon in each river based on all data including opportunistic, standardized sampling data, and reports of escaped farmed salmon in each river (p value = 0.717, AIC = 35.7, null deviance = 31.8) were not significantly correlated with region-wide propagule pressures (Tables S3 and S4; Figs. S4 and S5[1]).

The total number of escaped farmed salmon detected using all data was significantly related to propagule pressure (p value < 0.05; Table S4[1]), although very few data points fell within the model's 95% confidence interval (Fig. S5[1]; AIC = 3671.024, null deviance = 3687.0). However, when only counting fence or fishway counts were used in the GLM (Poisson distribution), there was a significant positive exponential effect of propagule pressure (p value < 0.001) on the number of escaped farmed salmon in rivers, and the model fit was improved with AIC of 1064.5 and null deviance of 1941.7 (Fig. 1A; Table S5[1]). The removal of the reports from Magaguadavic River (an outlier value with 379 escaped farmed salmon detected) did not change this effect, though the AIC decreased to 213.5 (Fig. 1B; Table S5[1]).

Individual Q values for wild-collected Newfoundland samples ranged from 0.006 to 0.990 (Fig. S6[1]), representing both pure wild individuals (0) and pure aquaculture individuals (1). Population mean Q values ranged from 0.028 to 0.737, and rivers in Bay d'Espoir and Fortune Bay generally had higher Q values than other rivers. Most (70%) of the wild Newfoundland individuals had

Fig. 1. The numbers of escaped farmed salmon detected at counting fences and fishways and propagule pressures in Atlantic rivers. Solid lines show predicted relationships using generalized linear models with Poisson distribution including and excluding the Magaguadavic River (panels A and B, respectively). Dashed lines represent 95% confidence intervals of models. Both models are statistically significant ($p < 0.001$). Different symbols correspond to the province or state of detection. Propagule pressures were calculated region-wide using active (stocked) aquaculture sites from 2005 to 2015.



Q values < 0.5 (Fig. S6[1]). There was a significant positive log-log relationship (p value < 0.01, R^2 = 0.279) between population mean Q value and localized propagule pressures in Newfoundland (Fig. 2A; Table S6[1]) and a weak negative log-log relationship (p value > 0.05, R^2 = 0.071) between population mean Q value and river size, measured as axial distance in kilometres (Fig. 2B; Table S6[1]). A GLM using both propagule pressure and river size (AIC = 73.1, null deviance = 26.8) identified a significant log-log effect of propagule pressure (p value = 0.018) on population mean Q value, but no significant effect of river size (p value = 0.274; Table S7[1]), and model comparisons using AIC_c determined that the strongest model for population mean Q value did not include river size as an explanatory variable (Table S6[1]).

Discussion

Escaped farmed Atlantic salmon have been associated with both negative genetic and ecological interactions with wild populations (Bolstad et al. 2017; Glover et al. 2017; Verspoor et al. 2015). Accordingly, the ability to predict risk to wild populations is central to wild salmon conservation and aquaculture management. We evaluated the utility of industry production data and reported escape events to predict the distribution of escaped farmed salmon and the genetic impacts on wild populations in the Northwest Atlantic. Results indicated that increased numbers of escaped farmed salmon detected at counting facilities and an increased magnitude of genetic impacts in wild populations were both positively correlated with increased propagule pressure. These relationships suggest that the location and size of aquaculture facilities directly affects the magnitude of risk to wild salmon populations from escaped farmed salmon. Characterizing this risk represents an important step toward predicting the impact of cage-based Atlantic salmon aquaculture on wild Northwest Atlantic salmon populations. Relationships between the magnitude of aquaculture production and escaped farmed salmon occurrence and impacts have been previously observed in the Northeast (Fiske et al. 2006; Hansen et al. 1999), and Northwest Atlantic (Carr et al. 1997), but spatial variables have not been considered. This work extends previous studies that have documented the distribution of escaped farmed salmon in Atlantic Canada (Morris et al. 2008) and provided evidence of hybridization and introgression among wild and farmed salmon following escape events (B. Wringe

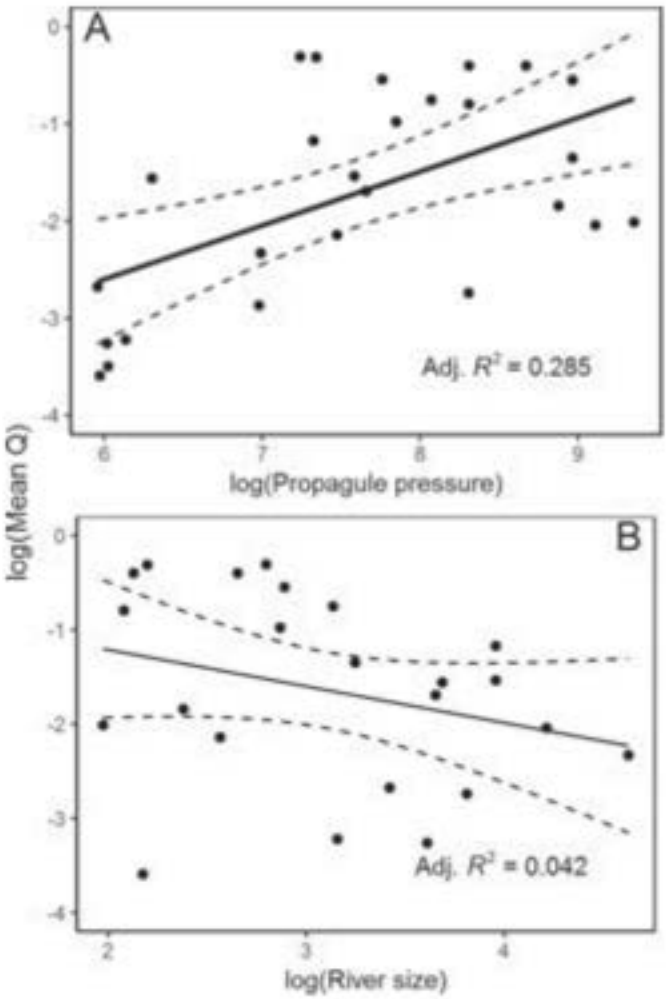


Fig. 2. Propagule pressures, river sizes, and mean population Q values for Newfoundland rivers (circles). Panel A shows log-log regression between propagule pressure and mean Q value ($p < 0.01$). Panel B shows log-log regression between river size and mean Q value ($p > 0.05$). Propagule pressures were calculated using Newfoundland inventory data (2005–2015), and river sizes are axial lengths (km). Solid lines show predicted effects on response; dashed lines show 95% confidence intervals.

and I. Bradbury, unpublished data) by using these data sources to test a novel approach to predict future impacts.

Escaped farmed salmon have been detected everywhere that net-pen salmon aquaculture occurs, with reports of millions of individuals escaping net-pens each year (Glover et al. 2017). In eastern North America, our analysis includes a total of 9236 escaped farmed salmon that have been detected since the 1980s, primarily in rivers proximate to the aquaculture industry. This likely represents a minimum number of detections, since identifying and quantifying escapes will depend on the location, timing, frequency, and extent of surveys carried out for this purpose. Information on escaped farmed salmon in eastern North America indicated that rivers in Passamaquoddy Bay, New Brunswick, and southern Newfoundland generally had the highest numbers of escaped farmed salmon. Both areas have high aquaculture production, and high numbers of escaped farmed salmon were previously reported by Morris et al. (2008). The distribution of escaped farmed salmon detected in fresh water has been correlated with the distribution of the industry in both Norway and Scotland (Glover et al. 2017). However, escaped European farmed salmon have also been reported as far away as the Arctic Ocean (Jensen et al. 2013), the Faroe Islands area (Hansen and Jacobsen 2003), and waters of Greenland (Hansen et al. 1997). The distribution of rivers in which escaped farmed salmon were detected here is well within the reported dispersal distance of escaped farmed salmon (Hansen and Youngson 2010) and is consistent with previous analyses (i.e., Morris et al. 2008).

Admittedly, reports of the presence of escaped farmed salmon are often not an unbiased sample, with opportunistic or targeted nonrandom sampling the norm. Research and monitoring has also focused primarily on Passamaquoddy Bay (Carr and Whoriskey 2006; O'Reilly et al. 2006; Whoriskey and Carr 2001) and southern Newfoundland due to the concentration of the aquaculture industry and consequent prevalence of escaped farmed salmon in these areas (DFO 2014; Verspoor et al. 2015). For this reason, the current data set for eastern North America likely lacks sufficient power to detect escaped farmed salmon at low prevalence outside these core areas. Furthermore, differences in data types and how they were collected means directly comparing counts obtained at the targeted monitoring facilities with intermittent or anecdotal counts (such as those obtained by angling, gillnetting, snorkel counts, etc.) may be difficult. As such, this underscores the need for standardized reporting and sampling methods, such as occurs in Norway (Anonymous 2017), to better quantify the distribution of escaped farmed salmon across the Northwest Atlantic region. This explains why we reported associations between propagule pressure and reports of escaped farmed salmon at fixed monitoring locations and excluded other data sources. This observation also supports the continued use of genetic monitoring (Bourret et al. 2011; Glover et al. 2017; Mjolnerod et al. 1997; B. Wringe and I. Bradbury, unpublished data) for impacts to gain a complete understanding of escaped famed salmon distribution and the consequences for wild populations.

Accordingly, evidence of genetic impacts due to interbreeding with escaped farmed salmon continues to accumulate on both sides of the North Atlantic (Bourret et al. 2011; Glover et al. 2017; Mjolnerod et al. 1997; Muhlfeld et al. 2017; B. Wringe and I. Bradbury, unpublished data). Our observation of a significant association between propagule pressure and the amount of introgression between farmed and wild salmon present supports the use of propagule pressure as a predictor of genetic impacts in wild populations. Similar links between propagule pressure and genetic introgression have been detected elsewhere (Bennett et al. 2010; Consuegra et al. 2011; Lamaze et al. 2012; Marie et al. 2012), supporting the use of propagule pressure as a management tool (Pritchard et al. 2007). Moreover, associations between the incidence of escaped farmed salmon in rivers and river-specific estimates of temporal genetic change (Glover et al. 2012) and admixture (Glover et al. 2013; Heino et al. 2015; Karlsson et al. 2016) have been reported. Our relationship between the number of escapees detected at counting fences and propagule pressure supports these findings.

Interestingly, although evidence of a relationship between the magnitude of genetic introgression and river size has been reported elsewhere (Glover et al. 2012, 2017; Heino et al. 2015; B. Wringe and I. Bradbury, unpublished data), it was not significant in this study. Glover et al. (2012, 2013) suggested that this relationship may be explained by larger (more robust) wild populations resisting introgression via increased competition on the spawning grounds and at juvenile stages. In contrast, our analysis suggests propagule pressure was the dominant factor influencing the amount of introgression detected. We restricted this analysis to the southern Newfoundland region because (*i*) the availability of industry production data allowed direct inclusion in our calculation of propagule pressure (i.e., rather than presence–absence of stocked sites) and (*ii*) increased divergence between wild and aquaculture salmon associated with a New Brunswick origin of the current domestic line increased the power to quantify levels of introgression with the marker panel used. Extension of the anal-

ysis to other regions may be possible in the future with improved industry data and targeted genomic panels (B. Wringe and I. Bradbury, unpublished data).

We suggest that propagule pressure could be a useful step toward the prediction of escaped farmed salmon impacts in Canada. The relationships with propagule pressure would likely benefit from the inclusion of more data and (or) data collected specifically to measure range-wide impacts, as has occurred with success in Norway through a standardized monitoring program and formal risk assessment (Taranger et al. 2015). Error in our study may also be attributed to the geographic distances measured or better resolution of the distances involved, and the incorporation of behavioural data on the movements of escaped farmed salmon may improve these relationships. The lack of consistency among available provincial or state inventory and (or) stocking license records prevented an in-depth, region-wide analysis of aquaculture production in this study. Furthermore, we identified significant differences in escape event reporting requirements across the region. Improved data collection on inventory and reporting requirements for escape events should improve understanding of escaped farmed salmon distribution, behaviour, and impacts (Jensen et al. 2010). Despite these challenges, we were able to calculate the propagule pressure variable successfully across the study region. Accurate estimates of propagule pressure could potentially be directly incorporated into spatial planning as a way to mitigate impacts of escaped farmed salmon on wild salmon populations.

This study has updated the current knowledge on the distribution and prevalence of escaped farmed salmon in rivers of the Northwest Atlantic and represents the initial steps in testing the use of a cumulative spatial measure of aquaculture production to predict the impacts of escaped farmed salmon on wild populations. The propagule pressure associations with impact identified here provide a useful metric that could be integrated into a risk management framework to inform aquaculture management activities and identify mitigation strategies.

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Current Opinion in Virology

Emerging viruses in aquaculture

Frederick SB Kibenge

Aquaculture remains the world's fastest-growing sector producing food of animal origin. Unlike in terrestrial animal agriculture, in aquaculture both farmed and wild aquatic animals in the same water column experience the same virus challenges. Additionally, the burgeoning international aquaculture expansion and expanding global trade in live aquatic animals and their products have been accompanied by long distance geographical redistribution of aquatic animal species and their viruses. The outcome is a continuous emergence of viral diseases in aquaculture, which may be driven by virus factors, animal host factors, environmental factors, and/or anthropogenic factors. Examples of emerging viruses in aquaculture include viral haemorrhagic septicaemia virus, infectious haematopoietic necrosis virus, infectious salmon anaemia virus, piscine orthoreovirus, Tilapia lake virus, Covert mortality nodavirus, Shrimp hemocyte iridescent virus, and Abalone herpesvirus.

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Introduction

Aquaculture, the intensive water rearing of fish, mollusks, and crustaceans, remains the world's fastest-growing sector producing food of animal origin. As in the case of terrestrial animal agriculture, bringing together large numbers of animals than occur naturally involves substantial animal stress which facilitates virus multiplication and clinical disease. However, aquaculture presents unique challenges in contrast to all other intensive animal production systems, in that the aquatic farmed and wild animals occur in the same water column, and the aquatic environmental parameters cannot be very closely controlled as for captive livestock agriculture (for example as in the poultry and swine industries). Viruses, carried by wild aquatic animals where they are often not sufficient to sustain the natural transmission cycle density, are readily facilitated by the high density of hosts in aquaculture, which with the associated chronic stress provide opportunities for the emergence of viral diseases [1••]. Additionally, the burgeoning international aquaculture expansion and expanding global trade in live aquatic animals and their products have been accompanied by rapid long-distance geographical redistribution of aquatic animal species and their viruses with emergence in the same or different aquatic animal species. The outcome of these events is a continuous emergence of viral diseases in aquaculture, which may be driven by virus factors, animal host factors, environmental factors, and/or anthropogenic factors [1••]. For example, the wide use of 'cleaner fish' in marine farmed salmonids as a biological control for sea lice *Lepeophtheirus salmonis* in Europe and Canada is now considered a new route of emergence of viruses (such as viral haemorrhagic septicaemia virus (VHSV) and Cyclopterus lumpus virus) in fish aquaculture [2]. This practice is not only akin to mixing of species in fish farms, but has routinely involved use of wild-caught cleaner fish directly in the salmon farms or as broodstock for hatchery-raised cleaner fish [2]. Moreover, progressive farming practices now enable discovery of emerging viruses through surveillance and laboratory diagnosis. Indeed, several new viruses infecting aquatic organisms have been discovered through Next-generation sequencing (NGS) methods [3].

Several emerging, and re-emerging viruses in aquaculture will be highlighted in this overview. Many are listed by the World Organization for Animal Health (OIE), which means that countries free of these viruses can refuse imports of live aquatic animals and their products from areas that have not been declared virus-free, regardless of existing free trade agreements [4].

Carrier status in global movement of live aquatic animals and their products

The potential for dissemination of aquatic viruses because of aquaculture and movements of live cultured aquatic animals or their eggs is extremely high where persistent viral infections occur in the absence of clinical disease (i.e. 'healthy carrier' aquatic animals/subclinical infections in aquatic animals). Although life-long infections are known to occur among herpesvirus infections and retrovirus infections, there are several other virus groups where infection is not cleared by the host and the virus persists in a carrier state including species susceptible to infection without displaying clinical signs, age-related resistance to virus infection (e.g. adult fish), and

infection with virus strains of low pathogenicity. There could also be situations of persistent infections where the virus level falls below detectable levels but not completely cleared from the host. All such infected animals are considered 'healthy' and may pass regulatory inspections for movement and/or export. This would be expected not only for new emerging viruses like piscine orthoreovirus (PRV) and tilapia lake virus (TiLV), which have been in existence but unknown until they were discovered [1••], and diagnostic tools developed not only for their detection, but also for re-emerging viruses such as VHSV, infectious haematopoetic necrosis virus (IHNV), infectious pancreatic necrosis virus (IPNV), and infectious salmon anaemia virus (ISAV) that cause persistent viral infections associated with lower virus levels in affected fish that may be difficult to detect through routine surveillance programs [5]. Most recently, 8000 juvenile Atlantic salmon at a commercial hatchery in Washington State-USA had to be destroyed because they tested positive for a strain of PRV found in Iceland. The virus is considered to have originated from fish eggs imported from Iceland. The source company for the eggs reported that they have an optional service of screening against PRV customers may choose as an extra risk measure to avoid vertical transmission (Owen E, 2018. https://salmonbusiness.com/egg-supplier-responds-to-washington-prv-salmon-cull/). In both examples above of new emerging viruses and re-emerging viruses where broodstock would have been persistently infected, the viruses would be disseminated via broodstock, fry or smolt movements, or egg transport into disease free farms, zones or countries. Where apparently 'healthy' aquatic animals are delivered to processing plants, the viruses would be disseminated via global trade in aquaculture products. In areas where these viruses are enzootic, clinical disease may manifest with the introduction of virus in imported aquatic material as for example with IPNV in Ireland where all reported clinical outbreaks were associated with imported IPNV isolates. In case of IHNV, in European countries where the main mode of virus transfer is by trade in infected fish, IHNV may remain undetected once introduced on a farm site [5].

The situation is even more concerning where international regulatory methods of control (e.g. for OIE listed diseases) dictate depopulation of affected farms upon virus detection in a few animals with few or none with clinical disease. In such situations, the affected animals may be allowed for human consumption and through international trade serve to introduce virus to new geographical areas. For example, White spot syndrome virus (WSSV), a highly infectious virus with a very wide crustacean host range has spread to all prawn-producing countries in the world with global movement of live shrimp. Until 2016, the Australian prawn industry was considered free of WSSV. Australia's biosecurity arrangements were breached by WSSV from Asia resulting in an outbreak in commercial *Penaeus monodon* prawn farms in Queensland in November December 2016. The most likely route of infection appears to be via imported infected retail prawns used for human consumption and as bait by fishers (Loynes K. 2017.https://www.aph.gov.au/About Parliament/Parliamentary Departments/Parliamentary Library/pubs/rp/rp1718/Chronology/WhiteSpotDiseaseAustralia). It is generally accepted that freezing seafood results in reduced infectivity of associated aquatic viruses.

Selected emerging viruses in fish aquaculture

Viral haemorrhagic septicaemia (VHS) virus (VHSV)

VHSV belongs to the species *Oncorhynchus 2 novirhabdo virus*, genus *Novirhabdovirus* within the family *Rhabdo viridae* [6]. Genotyping in accordance with VHSV G-gene and N-gene reveals four major genotypes (I IV) that correspond with the broad geographical origins and host specificity of isolates. VHS is a notifiable disease to the OIE [7]. VHSV has been isolated from more than 82 marine and freshwater fish species, with at least 44 of these species shown to be susceptible [7] although its economic importance is primarily to the rainbow trout and turbot aquaculture in Europe and Japanese flounder (*Paralichthys olivaceus*) in Japan and olive flounder (*Para lichthys olivaceus*) in Korea.

VHSV is assumed to be endemic among a wide range of marine and anadromous fish species in the northern hemisphere [7], occasionally emerging in aquaculture as shown by transmission events reported for rainbow trout reared in marine and brackish waters in Finland, Norway, and Sweden, and the recent detections of VHSV III in wrasse species (*Labridae*) used as cleaner fish in Atlantic salmon farms in Scotland and VHSV IVd in wild lumpfish (*Cyclopterus lumpus*) brought to a land-based farm in Iceland, to serve as broodfish [8••].

Infectious haematopoietic necrosis virus (IHNV)

IHNV belongs to the species *Oncorhynchus 1 novirhabdo virus*, genus *Novirhabdovirus* within the family *Rhabdo viridae* [6]. In contrast to VHSV in the same genus, IHNV has a relatively narrow host range restricted to salmonids, fish families *Oncorhynchus* and *Salmo*. Genotyping according to the glycoprotein gene reveals five major genogroups. Three of the genotypes, on the basis of a 303-nucleotide variable region ('mid-G'), are designated as U (upper), M (middle), and L (lower), respectively, to correlate with the geographic areas in the Pacific Northwest of North America; the fourth and fifth genogroups based on the full-length glycoprotein gene, are 'E' and 'JRt' or 'J', consisting of European and Japanese rainbow trout isolates, respectively. IHNV is endemic to western North America where it was first described in Sockeye salmon (*Oncorhynchus nerka*) fry hatcheries in the early 1950s, and is considered to have spread to Europe and Japan via shipments of IHNV-contaminated rainbow

trout eggs or fry. IHNV appears to travel through Europe without significant restrictions, termed viral 'tourism' as a consequence of frequent fish trade between private farms [9••]. IHN is a notifiable disease to the OIE [10]. Phylogenetic analysis of recent IHNV isolates in China indicate existence of a recently introduced virus via transfer of eggs or fish from North America where endemic virus continues to circulate undetected [11].

Infectious salmon anaemia virus (ISAV)

ISAV belongs to the species *Salmon isavirus*, genus *Isavirus* within the family *Orthomyxoviridae*. Genotyping based on the haemagglutinin-esterase (HE) gene reveals two basic genotypes, North American and European. ISAV strain designation is mostly based on sequence deletions/insertions in a 35-amino acid highly polymorphic region (HPR) of the HE protein [12]. Viruses without any deletion/insertion in HPR are designated ISAV-HPR0 to indicate 'full-length HPR' and are resistant to growth in cell culture, nonpathogenic, replicate only in epithelial cells of Atlantic salmon gills, and cause transient infection [12]. All ISAV isolated in fish cell lines to date from clinical disease have deletions in HPR relative to HPR0 and are referred to as ISAV-HPR-deleted (ISAV-HPRΔ). Virulent ISAV-HPRΔ targets endothelial cells resulting in systemic haemorrhagic disease. ISA is one of the most important salmonid viruses and is notifiable to the OIE [13]. Since 2012, ISAV outbreaks have been reported mostly in Norway, Canada and Chile. ISAV-HPRΔ was detected by RT-PCR but could not be isolated in cell culture, at a Chinese entry-exit port in 1 of 79 batches of eviscerated fresh salmon imported from Norway in 2015; the shipment was disposed of without entering Chinese aquaculture [14]. China currently has one of the world's biggest fully submerged net cage farming Atlantic salmon in the Yellow Sea (Owen E, 2018. https://salmonbusiness.com/chinas-gets-ready-to-harvest-first-batch-of-farmed-salmon-from-huge-deep-sea-fully-submersible-fish-cage/). The level of risk of introducing ISAV into a disease free country via importation of frozen whole salmon or fillets may be lower than with non-frozen salmon products as ISAV is sensitive to freezing and thawing [15].

Another fish orthomyxovirus, rainbow trout orthomyxovirus (RbtOV) isolated from juvenile rainbow and spawning steelhead trout (both *Oncorhynchus mykiss*) has been suggested to belong to a new genus, proposed name *Mykiss virus*, in the family *Orthomyxoviridae* [16]. RbtOV appears to have a relatively low prevalence in trout populations, grows in cell culture but is nonpathogenic in fish [16].

Tilapia lake virus (TiLV)

TiLV is a new orthomyxovirus of fish. It has a genome of 10 segments of linear negative sense single stranded RNA. It belongs to the species *Tilapia tilapinevirus*, genus *Tilapinevirus* within the family *Orthomyxoviridae*. Since its discovery as the etiological cause of massive losses of tilapia in Israel and Ecuador in 2009 [17••], TiLV has emerged as a significant cause of fish disease with mortality rates of 10 90% in farmed tilapia and the wild population in 12 countries across 3 continents (Asia, Africa, South America) [18]. TiLV represents an important risk for the fast-growing worldwide tilapia production sector. Tilapia is the world's second-most-farmed fish after carp [19]. It is possible that international trade may have been circulating TiLV worldwide through movement of live fish for aquaculture in the absence of knowledge of the existence of an associated risk [19,20]. It was recently shown that TiLV is inactivated in tilapia fillets stored at −20°C for 90 120 days [21] demonstrating that frozen seafood (e.g. whole fish or fillets) imports may be associated with lower risk of virus dissemination than non-frozen products. TiLV has not yet been detected in North America tilapia stocks [22].

Salmonid alphavirus (SAV)

SAV belongs to the genus *Alphavirus* within the family *Togaviridae*. SAV is the cause of pancreas disease (PD) and sleeping disease (SD), viral diseases of serious concern for salmon aquaculture in Northern Europe [23,24]. Genomic, antigenic, and histopathological studies have shown that SPDV and SDV isolates are closely related strains of the same virus now referred to as SAV. Six different subtypes of SAV (SAV1-6) have been identified using phylogenetic analysis with partial glycoprotein E2 and nonstructural protein-3 (nsP3)-gene sequence data, providing evidence that some subtypes are dominant in certain geographical regions [25], and each subtype likely represents a single and separate introduction to aquaculture from a wild reservoir in or around the North Sea. SAV has been isolated from wild common dab *Limanda limanda* and plaice *Pleuronectes platessa* in Scotland and Ireland. The disease, which was first recorded in 1976 in Scotland, has continued as a significant threat to sustainable salmon production in Scotland, Ireland, Norway, France, Spain, Germany, Switzerland, and most recently Poland. SAV infections are on the OIE list of notifiable aquatic animal diseases [25]. To date there has been no confirmed reports of SAV in North America [26].

Piscine orthoreovirus (PRV)

PRV belongs to the family *Reoviridae*, subfamily *Spinareovirinae*. The PRV genome comprises of 10 segments of double-stranded RNA and all of them have been sequenced [27,28]. PRV is considered to be ubiquitous in farmed Atlantic salmon. It is an emerging virus of salmon aquaculture that is associated with an ever-increasing list of clinical syndromes including heart and skeletal muscle inflammation (HSMI) in farmed Atlantic salmon in Norway, Chile and BC-Canada [27,29 31]. The PRV genomic segment S1 sequence differentiates PRV isolates into two genotypes, I and II [28,29], and each of them into two major subgenotypes designated Ia and Ib,

and IIa and IIb (Kibenge *et al.*, unpublished). Figure 1 shows the PRV genotypes and subtypes, and their geographical locations and associated clinical conditions.

Selected emerging viruses in crustacean aquaculture

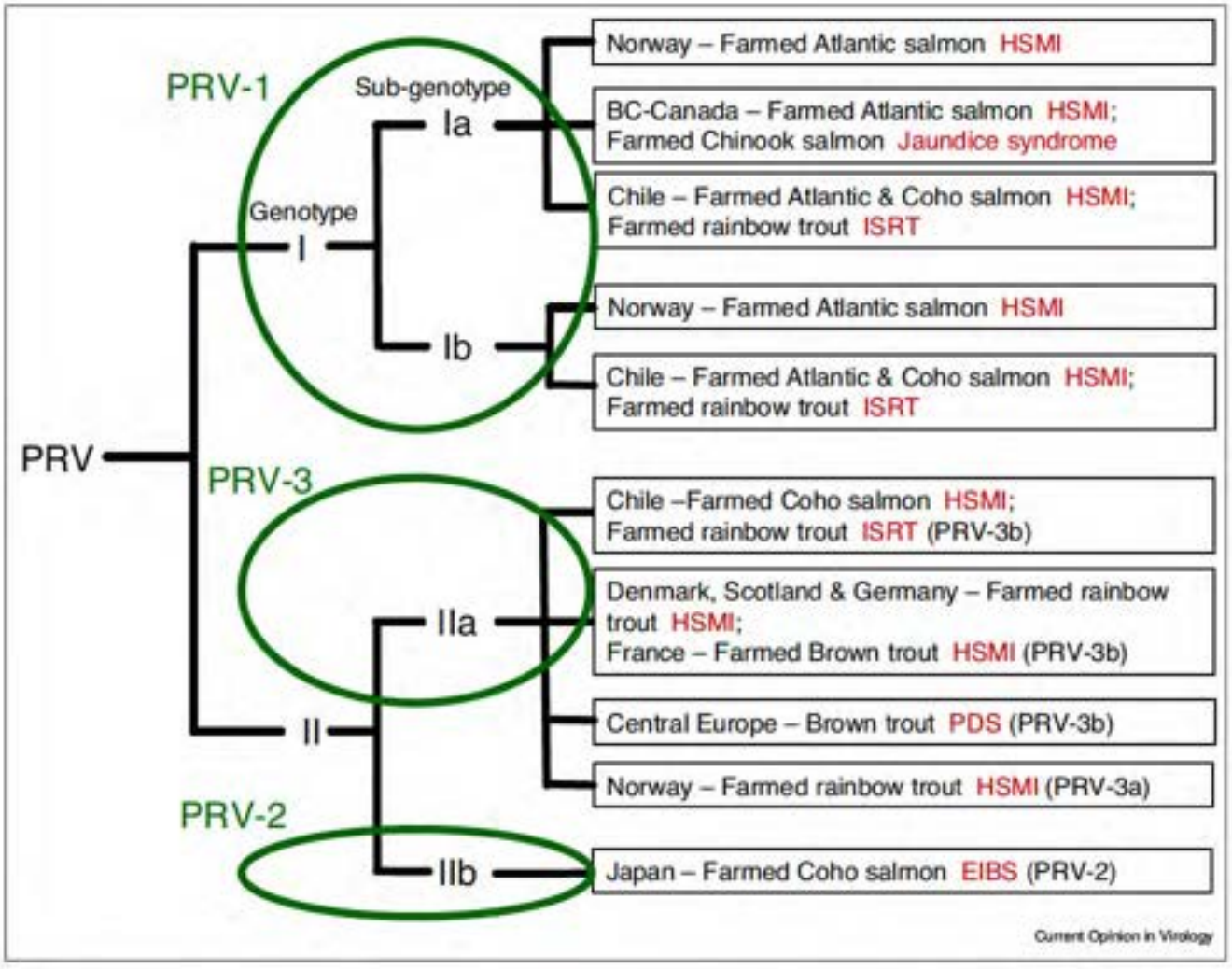
Shrimp hemocyte iridescent virus (SHIV)

A new virus of the family *Iridoviridae* isolated in China, results in a high mortality rate in white leg shrimp (*Litopenaeus vannamei*) [32]. The virus is proposed to be a member of the new genus *Xiairidovirus* [33] in family *Iridoviridae*. SHIV was detected in *L. vannamei*, *Fenner openaeus chinensis*, and *Macrobrachium rosenbergii* in samples collected during 2014 2016 from 5 provinces in China [32].

Covert mortality nodavirus (CMNV)

CMNV is a new virus of the family *Nodaviridae*, genus *Alphanodavirus*. It is the cause of viral covert mortality disease of shrimp [34] which has caused serious loss in China since its emergence in 2002 2003. Shrimp infected with CMNV are commonly found in deep water on the

Figure 1



Piscine orthoreovirus (PRV) genotypes, subtypes, geographical location and associated fish diseases. Phylogenetic analysis of genome segment S1 groups PRV into two genotypes (I and II) and four subgenotypes (Ia, Ib, IIa and IIb) [28,29, Kibenge *et al.*, unpublished]. Following order of discovery, Genotype I is also referred as PRV 1, subgenotype IIb as PRV 2 [29], and subgenotype IIa as PRV 3 [Kibenge *et al.*, unpublished]. All the PRV 3 isolates can be further subdivided into PRV 3a from rainbow trout from Norway [41,42], and PRV 3b from the rest of Europe [42 44] and Chile [45,46].

HSMI = Heart and skeletal muscle inflammation in farmed Atlantic salmon [29 31]. In other fish species (coho salmon, rainbow trout, brown trout), the disease is referred to as 'HSMI like' disease [29,41,42,44].

Jaundice and anemia (Jaundice syndrome) in farmed Chinook salmon in BC Canada [47,48].

ISRT = Idiopathic syndrome of rainbow trout in fared rainbow trout in Chile [45].

EIBS = Erythrocyte inclusion body syndrome in farmed juvenile coho salmon in Japan [29].

bottom of the shrimp pond rather than swimming on the surface or in shallow water like shrimp infected with White spot syndrome virus (WSSV) [35]. The disease causes economic losses in hatcheries and farms due to high mortality rates of up to 80% commonly found within 60 80 days post-stocking. CMNV should not be confused with other nodavirus infections such as infectious myonecrosis virus (IMNV), *Macrobrachium rosenbergii* nodavirus (MrNV) and *Penaeus vannamei* nodavirus (PvNV) [35]. These viruses do not cause hepatopancreatic atrophy and necrosis, unlike CMNV.

CMNV has a wide host range among cultured shrimp species, with a high prevalence and wide distribution in Southeast Asia, and Latin American countries [34]. CMNV was found in eleven species of invertebrates collected from shrimp ponds of cultured shrimp species affected with VCMD, which may be vectors and reservoirs of CMNV [36]. CMNV has also naturally crossed the species barrier (i.e. jumped species) and infected several species of fish such as *Mugilogobius abei*, a common marine fish in shrimp farming ponds and coastal water in China, another marine fish *Chaeturichthys hexanema* found in the Yellow sea [37], and farmed Japanese flounder (*Paralichthys olivaceus*) [38••].

Selected emerging viruses in molluscan aquaculture

Abalone herpesvirus (AbHV)

AbHV is the cause of abalone viral ganglioneuritis (AVG) in farmed and wild abalone primarily in Australia and Chinese Taipei [39] and is listed by the OIE [40]. The virus is a member of the family *Malacoherpesviridae* [39] which includes Ostreid Herpesvirus-1 and is tentatively placed in a new genus *Haliotivirus*. The disease first occurred in Australia in 2005 [40].

Future perspectives

Aquaculture is important now and will continue in the future as a principal source of animal protein for human consumption, as will the global trade in live aquatic animals and their products. Aquatic animal viral diseases are inherent in aquaculture, and they continue to negatively impact aquaculture significantly. Considering that seafood is the most traded commodity globally, it, therefore, virtually impossible to have 'aquatic virus-leakproof' international borders. The implementation of strict biosecurity measures on aquaculture farms on land, in lakes and the sea, and in processing plants or other natural source for aquaculture helps to limit but does not eliminate the risk of dissemination of aquatic viruses. Biosecurity management will remain an on-going effort for the foreseeable future. The best options for keeping abreast of the continuous emergence of viral diseases in aquaculture are ideally at the farm level where better knowledge about the viral diseases and their improved diagnosis, inspection and surveillance programs translate into higher profits for the farmer and, therefore, motivation for a sustainable industry.

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- • of special interest
- •• of outstanding interest

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Population-specific ranges of oceanic migration for adult Atlantic salmon (Salmo salar) documented using pop-up satellite archival tags

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ARTICLE

Population-specific ranges of oceanic migration for adult Atlantic salmon (*Salmo salar*) documented using pop-up satellite archival tags

Gilles L. Lacroix

Abstract: Pop-up satellite archival tags identified differences in oceanic migration of Atlantic salmon (*Salmo salar*) kelts from three distinct Canadian populations. Kelts from two endangered populations were restricted to coastal areas near home rivers, whereas kelts from a persisting nearby population migrated to the Labrador Sea and towards the Flemish Cap. Kelts spent most time near the surface (0–5 m), but coastal migrants undertook repeated daytime dives (10–40 m), associated with feeding, upon marine entry and progress was slow (8–23 km·day^{-1}). Distant migrants moved rapidly along the continental shelf (10–50 km·day^{-1}) against prevailing ocean currents, remaining near the surface, except for deep dives (100–1000 m) when crossing ocean channels and at the shelf edge. Home range water temperatures (0–15 °C) indicated that kelts avoided warmer adjacent areas in summer. Kelts did not avoid cold coastal habitat (0–5 °C) in winter, but avoided the surface layers. Kelt migration mimicked that of postsmolts of similar origins, with water temperature acting as a directive or controlling factor. Containment of kelts from endangered populations in coastal habitat was probably responsible for the disappearance of repeat spawners.

Résumé : Des étiquettes émettrices détachables ont permis de déceler des variations dans la migration océanique de charognards de saumon atlantique (*Salmo salar*) de trois populations d'origines canadiennes. Les charognards de deux populations en voie de disparition se limitaient aux zones côtières près de leurs cours d'eau d'origine, alors que les charognards issus d'une population qui persiste à proximité migraient vers la mer du Labrador et le Bonnet Flamand. Les charognards passaient la majeure partie de leur temps près de la surface (de 0 à 5 m), mais les individus dont la migration se limitait à la côte effectuaient de multiples plongées diurnes (de 10 à 40 m) associées à leur quête de nourriture immédiatement après leur entrée en mer, et se déplaçaient lentement (de 8 à 23 km·jour^{-1}). Les individus qui migraient sur de longues distances se déplaçaient rapidement le long du plateau continental (de 10 à 50 km·jour^{-1}) contre les courants océaniques dominants, demeurant près de la surface, sauf pour effectuer des plongées profondes durant la traversée de chenaux océaniques et en bordure du plateau. Les températures du domaine vital (de 0 à 15 °C) indiquaient que les charognards évitaient les zones attenantes plus chaudes durant l'été. Ils n'évitaient pas les habitats côtiers froids (de 0 à 5 °C) l'hiver, mais évitaient les couches de surface. La migration des charognards s'apparentait à celle de post-saumoneaux d'origines semblables, la température de l'eau jouant un rôle déterminant. Le confinement dans des habitats côtiers de charognards issus de populations en voie de disparition expliquerait vraisemblablement la disparition des saumons à pontes antérieurs. [Traduit par la Rédaction]

Introduction

Current knowledge of the oceanic migration and distribution of Atlantic salmon (*Salmo salar*) is generally based on the recovery and reporting of tags from extensive conventional tagging programs in Europe and North America in the second half of the 20th century. This revealed the vast ocean transits of North American salmon to areas of the Northwest Atlantic off western Greenland and in the Labrador Sea (e.g., Ritter 1989; Reddin and Friedland 1993; Miller et al. 2012). It supported the earlier assumptions of range that were based on the distribution of catches of untagged salmon in experimental fisheries (May 1973; Lear 1976; Jensen and Lear 1980). However, the information was both spatially and temporally patchy. As a result, the identification of oceanic range and migration routes for Atlantic salmon was potentially biased by the incidental nature and methods of tag recovery (e.g., Meister 1984; Reddin and Shearer 1987; Montevecchi et al. 1988). Additionally, these investigations assumed that migration timing and behaviour of tagged hatchery salmon were representative of naturally migrating populations and, often, that all individuals from a population or region migrated along similar routes and to similar destinations. As a result, inferences about possible migration routes and range for different salmon populations — coastal versus distant migration, western versus eastern North Atlantic range limits — have remained unresolved (Jessop 1976; Dadswell et al. 2010). The assumption of a shared migration range for all North American salmon populations could lead to unlikely expectations of marine survival for some populations that may have different migration strategies and destinations and a failure to recognize threats to their survival (Lacroix 2008, 2013).

In North America, Atlantic salmon populations listed as endangered are generally located at the southern end of the range (COSEWIC 2006; Department of Interior and Department of Commerce 2009). Their legal protection highlights the dire consequences of habitat loss and increased mortality of Atlantic salmon in those regions in the past 50 years. It also emphasizes the need to identify their spatial extent, properties of their habitat, and threats to their survival (Fisheries and Oceans Canada 2007). The coastal distribution and migration of Atlantic salmon postsmolts from several population units in the Gulf of Maine (GoM) watershed, listed as endangered, have been identified in surface trawling surveys (Lacroix and Knox 2005; Sheehan et al. 2011; Lacroix et al. 2012) and by using ultrasonic telemetry (Lacroix et al. 2005; Kocik et al. 2009; Lacroix 2013). However, these methods have had limited success when used over a wider range in the North Atlan-

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tic. Data storage tags (DST) have been used to monitor the thermal preferences of Atlantic salmon in coastal habitat (Reddin et al. 2004, 2006), and they could be used over the entire marine phase. However, the small DSTs used on salmon do not record geolocation, they must be recovered, and they only provide information for survivors. Large pop-up satellite archival tags (PSAT) that calculate light-based geolocation daily and archive detailed temperature and depth records have been used successfully since the late 1990s on large pelagic fish, such as Atlantic bluefin tuna (*Thunnus thynnus*) that spend time near the surface, to resolve ocean-wide migrations (Lutcavage et al. 1999; Block et al. 2005). These tags detach from fish and float to the sea surface at preset times or at death and transmit archived data via satellites, eliminating the need to recover tags. A small version of these large PSATs became available in 2007 that could potentially be used to monitor the ocean migration and fate of adult Atlantic salmon and resolve uncertainties about the habitat of endangered populations.

The Atlantic salmon is iteroparous and can survive to spawn multiple times, either in consecutive or alternate years, repeating the migration between home rivers and ocean feeding grounds (Jonsson et al. 1991; Fleming 1996). Those that survive after spawning in the fall usually overwinter in the rivers as kelts until spring, but kelts may also migrate downstream early in the winter to avoid severe ice conditions and find suitable habitat in lakes, large estuaries and, rarely, in coastal habitat (Huntsman 1938; Cunjak et al. 1998; Hubley et al. 2008). The range in return rates between spawning events reported among regions (0%–90%) indicates that large differences in kelt survival at sea occur among salmon populations (Ritter 1989; Jonsson and Jonsson 2004; Niemelä et al. 2006). Recent studies have shown that kelt survival was high (90%–95%) during the early stages of migration in estuarine and coastal habitat, indicating that this was not a critical phase for kelts despite their weakened condition after overwintering in rivers (Hubley et al. 2008; Halttunen et al. 2009). Thereafter, little is known of the kelt life stage despite the importance of repeat spawners in stock maintenance (Bardonnet and Baglinière 2000), especially during historical periods of low recruitment (Niemelä et al. 2006).

In North America, some of the highest return rates for kelts, indicative of high marine survival, were recorded for Atlantic salmon populations from the inner Bay of Fundy (BoF) in the GoM watershed (Jessop 1986; Ritter 1989; Cunjak et al. 1998). The majority of inner BoF salmon first spawn after 1 year at sea, and historically, they spawned multiple times in consecutive years thereafter (Ducharme 1969; Ritter 1989). Periods of high and low recruitment have alternated for these salmon populations, but there was no rebound after the last major decline in recruitment in the 1980s (Jessop 1986; Gibson et al. 2003). Repeat spawners all but disappeared, and the inner BoF salmon was almost extirpated and it was listed as endangered (COSEWIC 2006). An extended period of high mortality of salmon at sea would be catastrophic to a population that relied heavily on the repeated return of kelts from a cohort for recruitment and that had a high proportion of repeat spawners of higher fecundity (Jessop 1986). In contrast, some nearby salmon populations in the outer BoF have maintained a higher marine return rate during the same period (Jones et al. 2010). Historically, these outer BoF salmon tended to have a higher proportion of adults first returning after more than 1 year at sea and a lower proportion of repeat spawners than the inner BoF salmon. These differences in life cycle and survivorship among salmon populations suggest that kelts from different population units may have exploited different marine areas to recondition.

In this study, the feasibility of using PSATs on adult Atlantic salmon was first evaluated. A protocol was developed for programming and attaching tags to salmon kelts taking into account their size, anadromy, and duration of the marine phase. Then salmon kelts from inner BoF populations with different migration timing (i.e., fall–winter and spring) and from an outer BoF population that migrates in the spring were tagged with PSATs to identify and compare migratory behaviour, routes, and range in the ocean. This study is the first to provide daily locations for individual kelts during part of the marine phase, which made it possible to verify previously inferred migration routes. Additionally, the following hypotheses were evaluated: (i) kelts of different natal and genetic origin in the BoF have a different migration and exploit different marine feeding grounds; (ii) kelts that migrate at different times behave differently, which results in marine range differences; and (iii) kelt migration mimics that of postsmolts of the same natal origin and common factors control their migration at sea. Differences in migratory strategy and marine habitat exploited by kelts of different origins could be responsible for the differences in marine survival and adult returns reported for salmon populations in different regions of the BoF (Gibson et al. 2003; Jones et al. 2010). Additionally, by recording their swimming depth and ambient temperature throughout the migration in relation to location, it was possible to look at the effects of the environment on kelt activity and to evaluate factors that may direct or control outbound migration and result in different migratory strategies. Ultimately, the goal was to obtain much needed information on the marine habitat of populations of Atlantic salmon either listed or under consideration for listing as endangered because of increased mortality at sea (COSEWIC 2006, 2010).

Materials and methods

Kelt capture

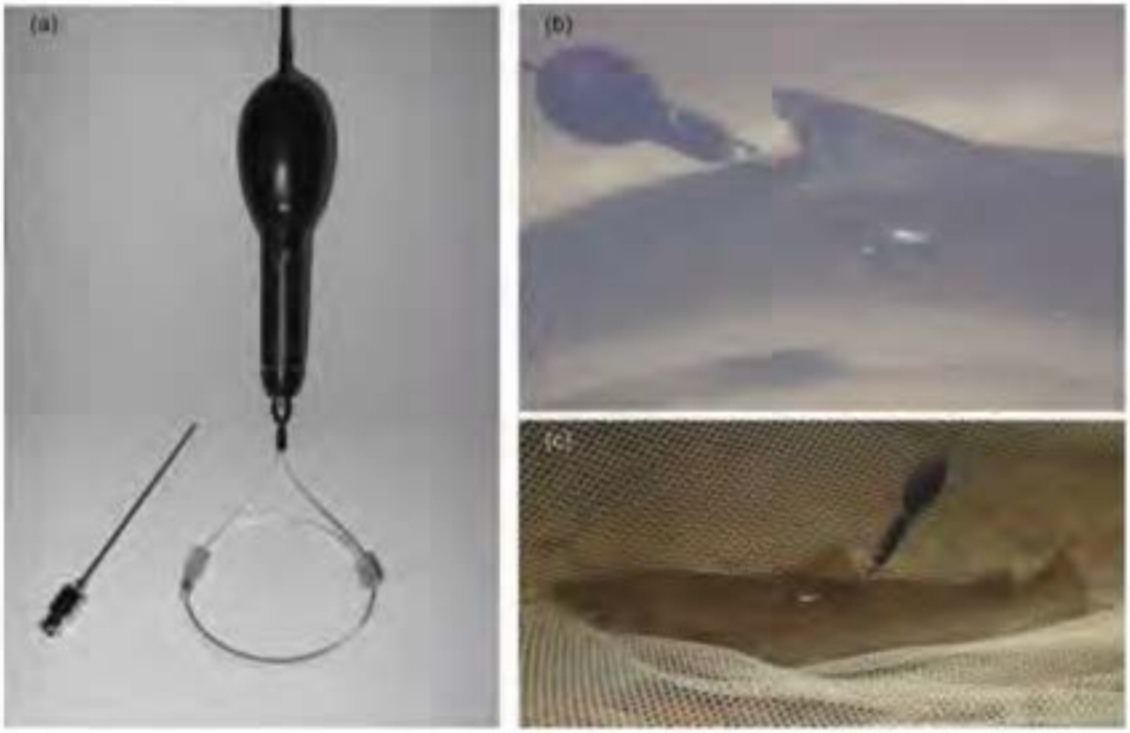
Atlantic salmon kelts were captured in three rivers in different regions of the BoF, Canada, and tagged with PSATs (n = 55). The salmon in each river were from genetically distinct population units (Verspoor et al. 2002), some of which had different seasons for kelt migration. The goal was to tag kelts as they were leaving the rivers to monitor and compare their ocean migration and habitat.

The Big Salmon River (BSR), located inside the BoF along the coast of New Brunswick, is an inner BoF river with a salmon population listed as endangered under Canada's Species at Risk Act (COSEWIC 2006). Many of the kelts in this river migrate back to sea soon after spawning in late fall and then in early winter, and few remain in the river until spring (G.L.L., unpublished data). Naturally migrating salmon kelts from BSR were captured by angling from shore in several pools near the river mouth in November of 2008 (n = 8), 2009, (n = 5), 2010 (n = 8), and 2011 (n = 8). They were tagged and released at the capture site, and they were allowed to recover in a floating net pen (3 m^3) in the river before and after tagging. Extensive angling efforts in this river during April–May of 2008 and 2009 yielded no spring kelts for tagging.

The Gaspereau River (GR), located deep inside the BoF in the Minas Basin, Nova Scotia, is another inner BoF river with an endangered salmon population (COSEWIC 2006). The river has a small hydropower dam 18 km from the river mouth where returning adults were captured in a trap in the summer and fall of 2008 and 2009. Those determined to be of GR origin were then held and spawned at a hatchery (Coldbrook Biodiversity Facility, Nova Scotia). Fish from the 2008 run were kept over winter, and they were tagged at the hatchery and released the same day near the river mouth in May 2009 (n = 6). Fish from the 2009 run were tagged at the hatchery and released together in December 2009 (n = 6). Kelts were released in both fall and spring because of uncertainty in the timing of the kelt migration and the unknown effects of the dam on overwintering in the lower reaches of the river.

The Hammond River (HR), located in the lower Saint John River system along the coast of New Brunswick, is an outer BoF river with a small, persisting salmon population (Jones et al. 2010). However, the Saint John River system has recently been included

Fig. 1. (*a*) X-tag with attachment loop completed and secured with crimps (covered with latex tubing) without threading the monofilament through the fish (7.6 cm tagging needle shown for reference) and (*b*, *c*) X-tag attached to Atlantic salmon recovering in net pen in the river before release.



in the New Brunswick outer BoF group of rivers with salmon populations proposed for listing as endangered (COSEWIC 2010). Based on past angling records, kelts from HR naturally migrate over several weeks in the spring after ice breakup, and they were captured by angling from a boat in several pools near the river mouth and tagged in April of 2009 (n = 5), 2010 (n = 5), and 2011 (n = 4). They were tagged and released at the capture site.

The physiological stress on salmon associated with catch-and-release angling and handling was reduced by tagging at times when the water temperature in the rivers was <4 °C and by avoiding exhaustive angling (Brobbel et al. 1996). Lures with single, barbless circle hooks (#2 or #4) were used to avoid internal damage. Kelts are more tolerant of angling and handling than bright salmon that have recently entered the river, and neither mortality in the short term (Brobbel et al. 1996) nor long-term effects or delayed mortality were expected (Thorstad et al. 2007; Halttunen et al. 2010).

Tag settings and tagging procedure

The PSATs were standard rate X-tags with antifouling coating from Microwave Telemetry Inc. (MWT, Columbia, Maryland; specifications available from http://www.microwavetelemetry.com/fish/Xtag/specifications.cfm). They were programmed for emergency release at 1250 m for crush-depth protection and for constant-depth release and reporting from dead fish or floating tags. Constant-depth settings were customized to avoid premature release. Activation of the constant-depth feature was delayed by 31 days postdeployment to allow kelts to leave rivers where they often held positions at constant depth. A maximum time-at-depth of 8 days and a constant-depth band of 2 m were used to account for the long, uninterrupted periods that salmon may spend near the surface during migration at sea. Most tags were set to detach after 4 months to ensure pop-up before salmon returned to fresh water to spawn again, because the tag release mechanism does not work in low conductivity water. Later in the study, after it was determined that tagged salmon usually did not return early to the rivers, some 6-month tags were deployed (six tags in each of 2010 and 2011 in BSR and four tags in 2011 in HR) in the hope of increasing monitoring range.

Tag attachments were prepared by threading a single 50 cm strand of monofilament (Jinkai 1.05 mm, 90 lb. fluorocarbon leader; 1 lb. = 0.453 kg) through a 2 cm length of vinyl chafe tube (1.3 mm internal diameter, ID) inserted through the tag attachment point (Fig. 1*a*). The strand was folded and cinched together up to the tag with overlapping pieces of chafe tube (1.6 mm ID covered by 2.2 mm ID, and 1–2 cm long depending on fish size), leaving two free strands leading to the tag.

Tags were activated 1–2 h before release. During tagging, in situ or at the hatchery, kelts were restrained and kept submerged in a padded measuring and handling trough that covered the head. The fish were measured and lateral scales were taken to determine age and spawning history (Shearer 1992). A 16-gauge by 7.6 mm stainless steel veterinary needle was pushed through the dorsal musculature under the anterior edge of the dorsal fin. Strand end #1, on which an aluminium alloy sleeve (#1) (Jinkai size I or J) and a 1 cm length of latex tubing had been threaded, was pushed through the needle, and the needle was removed leaving only the strand through the fish. The needle was then pushed through the musculature under the dorsal fin, about 2 cm from the posterior edge of the dorsal fin, but from the opposite direction. A second metal sleeve (#2) and latex tube were threaded on strand end #1, which was pushed through the needle and the fish as before. Strand #1 was used to pull the tag close to the posterior base of the dorsal fin, and the monofilament loop going through the fish was closed by threading strand end #1 through metal sleeve #1 and latex tube and crimping the metal sleeve. Strand end #1 was cut off at the crimp, and the latex tube was slipped over the crimp to prevent abrasion on the side of the fish. Strand end #2 from the tag was then threaded through metal sleeve #2 and latex tube already on the monofilament on the other side of the fish, the metal sleeve was crimped, and the latex tube was pushed over

it after trimming the strand end. As a result, the tag was effectively attached to the fish at four points, which helped to stabilize the tag, reduce lateral motion, and distribute pull from the tag through the dorsal musculature (Fig. 1b). Having separate strands leading from the tag to each side of the fish prevented the corkscrewing motion of tags observed when a single attachment tether and anchor point is used. The tag either floated upwards when the fish was stationary (Fig. 1c), or it was gradually pushed down depending on swimming speed. The length of the tag attachment varied with fish size to position the tag close to the fish but off its back when swimming and to avoid the antenna tip touching the tail.

Tag attachments were tested, and the behaviour of tagged salmon was observed in trials conducted before and during the study. Farmed adult Atlantic salmon (60–70 cm, fork length) were tagged with dummy X-tags ($n = 2$) and a prototype tag of similar shape ($n = 10$) in June 2008, and they were held at sea for 2 months in the large net pens used for salmon farming. During the study, hatchery-reared salmon of similar size to those caught in the rivers were tagged with dummy X-tags at the same time as the wild fish each year. These were held in an exterior concrete Swede pond (11 m) at a hatchery (Mactaquac Biodiversity Facility, New Brunswick) with several hundred salmon of similar size for periods of 4 to 8 months ($n = 16$). Periodic observations found tagged salmon to be feeding and as active as the untagged fish. There were no attachment failures and no tagging mortality in any of the trials. In addition, recovery of a tagged kelt in the GR trap, when it returned to the river after 2 months at sea, showed that the fish was in excellent condition and had been feeding and that scale loss or abrasions from the tag were minimal. The puncture points through the dorsal musculature had healed, and the tag attachment was strong and secure.

Transmitted and recovered tag data

While deployed, X-tags recorded and archived ambient light ($<4 \times 10^{-5}$ lux at 555 nm), temperature (resolution 0.16–0.23 °C), and pressure (depth resolution 0.34–5.4 m, 0.34 m archived) at 2 min intervals. After tag detachment and pop-up, a subset of the archived data was transmitted through Argos receivers on polar-orbiting satellites (National Oceanic and Atmospheric Administration, US Department of Commerce). Transmitted data included sunrise and sunset times each day based on light thresholding and temperature and pressure records at 15 min intervals for 4-month tags and at 30 min intervals for 6-month tags. The percentage of transmitted data successfully received varied with pop-up locations and time of year, but it was usually sufficient to produce daily geolocation estimates with few gaps. The accuracy reported by MWT for these light-based geolocation estimates is ±1° for latitude and ±0.5° for longitude.

Transmitted data associated with geolocation at sea was obtained from 39 of the 55 tags (71%), and only data for those kelts were used. The fate of the other 16 tags was as follows: eight tags transmitted from land along the rivers, most likely because of predation by observed bald eagles (*Haliaeetus leucocephalus*) and possibly by small mammals; three tags from the fall group released in GR were later found and returned, and the recovered data confirmed that these kelts survived and remained in the river over winter beyond the programmed end date; and five tags did not transmit, possibly because the kelts never left fresh water. A high proportion of the tags on fish that were at sea (26%) were eventually found after they washed up on shore and were returned for a reward. The archived data (2 min intervals) for these tags were recovered and used instead of the transmitted data.

Location estimates and trajectories

The majority of raw geolocation estimates (>95%) were made using transmitted sunrise and sunset times obtained when the fish was near the surface (0–2 m), and they provided near-continuous series of daily positions to reconstruct trajectories. In the case of recovered tags, geolocation estimates were improved by processing the raw light data (revised estimates provided by MWT). The raw geolocation estimates, starting at the time of departure and latitude and longitude at the river mouth, were processed with a state-space model (UKFSST) that used the unscented Kalman filter (UKF) and sea surface temperature (SST) to improve geolocation estimates and generate a "most probable" track with geolocation errors for individual kelts while at sea (Lam et al. 2008). Departure from the river was based on detectable changes in environment according to transmitted data. The UKFSST model was used with 1° Reynolds satellite-derived SST and tag-measured SST (depth <10 m) to geolocate where possible. In some cases ($n = 7$) where UKFSST was unstable or tended to over-smooth the SST and increase the search radius, the simpler KFTrack model was used (Sibert et al. 2003). In those cases, comparisons with SST satellite images were in good agreement, and KFTrack provided a more probable track that stayed away from land. For a few extreme cases where groups of successive locations were shifted on land, a secondary bathymetric correction that constrained estimated locations based on daily maximum depth was applied (Galuardi et al. 2010).

The greatest day-to-day variability in raw geolocation estimates usually occurred while kelts were near shore and in coastal habitat with high turbidity and low transparency. Raw geolocation estimates were most consistent when the fish were migrating offshore, and occasional anomalous location records resulting from dive-related shifts in the estimated times of sunrise and sunset were removed. Large errors in latitude estimates associated with proximity of the equinoxes (Royer and Lutcavage 2009; Sibert et al. 2009) occurred in four cases where kelts were at large during the spring equinox period. In all of the above cases, the models used were effective at filtering and reducing errors in geolocation estimates (Lam et al. 2008).

For kelts that survived to the preset end date, the migration end point was the first Argos Doppler measurement of location (class 2 or 3) after pop-up. The end point of tags reporting before the end date was determined as follows. For tags ($n = 11$) retained inside a predator for some time (1–8 days constant darkness), the location used was on the date of predation along a reconstructed track that included the postpredation period. For tags ($n = 2$) that remained attached to a kelt carcass on the bottom after a predator attack (8 days to constant-depth release), the end point was considered to be close to the first Argos reporting location because the depth and temperature records indicated that there was no movement or drift between death and pop-up. In all other cases of mortality, the end point was taken along the track on the date that kelt activity ceased and the tag surfaced. Some tags detached prematurely from live kelts ($n = 5$) when constant depth release was activated because the kelt remained too long within a narrow depth band, and the Argos location at pop-up provided an accurate end point.

Estimates of model parameters relevant to kelt movement varied within reasonable limits. The model diffusion coefficient range of 95–430 nautical miles^2·day^{-1} (1 nautical mile = 1.852 km) was well within limits for adult salmon migrating at a sustained swimming speed of 2 body lengths·second^{-1}. The mean variance for model estimates of latitude was 0.263° (95% confidence limit = 0.008) and for longitude it was 0.373° (95% confidence limit = 0.011). Maps of filtered daily location estimates and tracks for all kelts at sea and of their estimated end locations were produced using ArcGIS (Environmental Systems Research Institute, Inc., Redlands, California).

Classification of migratory behaviour and habitat

The start and end times defined earlier for marine migration were used to delimit the temperature and depth time series, reported as Greenwich Mean Time (GMT), of each kelt to identify

behaviour modes and classify habitat use. Kelts were grouped for analyses by origin and migration timing: outer BoF spring (OBS), inner BoF spring (IBS), and inner BoF fall (IBF). Analyses of frequency distributions for temperature and depth were used to assess and compare diurnal and seasonal patterns. The daily times of sunrise and sunset reported by PSATs and, when available, the more accurate time of change in light intensity from the 2 min light records of recovered tags were used to define light and dark periods. In addition, light data from recovered tags were used to improve sunrise and sunset times for tags with high day-to-day variability if the kelts were located in the same area (<0.5° longitude) on the same date. The early and late parts of each migration season were defined as follows: April–June and July–August for OBS and IBS groups and November–February and March–April for the IBF group. Time-weighted mean and weighted sample variance were calculated for each interval class in a frequency distribution for a kelt group, because the number of days of observations at sea was different for each kelt in a group, and the number of records in a day varied according to tag duration and extent of transmitted or recovered data. Comparisons of mean values in an interval (light versus dark period or early versus late migration) were made using the χ^2 test of independence between proportions. The nonparametric Mann–Whitney U test was used for comparisons of group means.

The behaviour of individual kelts was inferred by interpreting the temperature–depth time series and distance travelled, and they were classified according to migration route and range established by the track and end location. Daily travel distance and speed between estimated locations were measured for each kelt using Home Range Tools 1.1 (HRT) for ArcGIS 9.3 (Rodgers et al. 2007). Track length was the cumulative daily travel distance between home river and end points, and migration rate was the average of daily travel speed estimates. End point distance from the home river was also determined for each kelt by measuring a straight-line, over-water route that excluded meandering and nondirected movements. Both end point distance and track length were measured to provide an insight into mechanisms of migrations (e.g., straight-line orientation versus transport by currents) and to highlight differences in behaviour among groups (e.g., migration versus feeding).

The amount of time spent by kelts in different regions at sea was determined independently of estimated geolocations and tracks using SST records for individual kelts. This provided an alternate method of establishing marine habitat use and for validating the estimated tracks. It relied on the rapid, marked changes in the temperature time series that occurred when a kelt migrated from one region with a specific thermal profile to another with a different profile. Sharp changes in SST and the temperature profile (i.e., isothermal and well-mixed versus thermally stratified) were used to identify times of transit between regions and calculate the amount of time spent in each region. At a given time, the BoF was usually isothermal and had little spatial variation in SST, the northern GoM was mostly isothermal and SST was warmer and more variable than in the BoF, the Scotian Shelf was thermally stratified and SST was usually warmer than in the BoF–GoM, the Laurentian Channel was thermally stratified and had the warmest SSTs, and the North Atlantic off Newfoundland and the Labrador Sea were thermally stratified and had the coldest SSTs of any region. Additionally, daily maximum depth recorded was compared with bathymetry to validate region allocation made using temperature records. The weighted mean proportion of time spent in the BoF, GoM, and North Atlantic were calculated for each group of kelts. The different thermal regions in the North Atlantic were combined because of the low sample sizes in each region.

Habitat analysis

The marine distribution of all kelts in each of the three groups was mapped together by time period, and the utilization distribution (UD) of each group-by-time was calculated using HRT (Rodgers et al. 2007). The kernel-density estimation method was used, and all locations for kelts in a group were included in the analysis (Worton 1989). The kernel probability density estimator was calculated using both the fixed and adaptive methods. Smoothing parameters or bandwidth (h) in the model were estimated by least-squares cross-validation (LSCV) and biased cross-validation (BCV) and compared with the reference bandwidth (h_{ref}). The grid or raster cell size used to calculate probability density estimates was 3000 UTM units and was always smaller then the bandwidth. The method and smoothing parameter that minimized bandwidth and resulted in the "best fit" relative to the observed distributions were selected to construct 50%, 75%, and 99% UDs and display home range and core marine habitat. Best fit to the data was obtained using the BCV fixed kernel estimate of bandwidth for the IBF group and the LSCV adaptive kernel estimate for the IBS group. In both cases, these provided output similar to that generated using 0.30–0.40 h_{ref} kernels. For the OBS group, where dispersal was extensive and the migration range was up to 5000 km, both the LSCV and BCV methods failed to minimize bandwidth and resulted in extensive over-smoothing and unrealistic UDs, and the "ad hoc" method of scaling h_{ref} was used as described by Rodgers et al. (2007). For OBS kelt locations, the best fit was obtained using a 0.30 h_{ref} kernel.

Results

The PSATs for 39 kelts that migrated back to sea provided 2326 days of archived data, 70% of which was in marine habitat (Table 1). All kelts from the IB groups had only spawned once, whereas two of the OBS kelts had spawned twice and were therefore going back to sea for the third time. After tagging, kelts spent some time in rivers and estuaries, entering the BoF per se on different dates: 19 April – 13 May for the OBS group, 8–19 May for the IBS group (exit dates from the Minas Basin), and 11 November – 23 February for the IBF group. The mean PSAT monitoring period at sea did not differ among groups, but the range in time at sea was large (Table 1). The latest date that data were obtained for a kelt in each group was 13 August (OBS), 16 July (IBF), and 17 May (IBF).

Kelt end points (i.e., last location regardless of cause) were more widespread for the OBS group than for the two IB groups (Fig. 2). Most kelts with <30 days at sea ended up in the BoF–GoM area, regardless of origin. Thereafter, end points for the OBS group extended beyond this area (Fig. 2*a*), whereas most of the kelts from the IBS and IBF groups with >30 days at sea ended up in BoF–GoM (Fig. 2*b*). Both end point distance from home river and track length increased exponentially with time at sea for the OBS group (Fig. 3*a*), and end points for kelts from this group were significantly farther away than for the two IB groups (Table 2). There was no correlation between end point distance and time at sea for the two IB groups (Fig. 3*a*), but track length was correlated with time at sea for the IBF group (Fig. 3*b*). Mean track length for the OBS kelts was two or more times greater than for kelts from the two IB groups, but the differences were not significant because of the wide range in track length in each group (Table 2). Four kelts from the OBS group had track lengths >2000 km, and the longest track was 5000 km. Only two of the kelts from the IB groups had tracks >2000 km, but six kelts had tracks of at least 900 km. This did not necessarily increase their end point distance, and most kelts from IB groups ended up within 300 km of their home river. Track length for the sole GR kelt migrating in the fall was 279 km, and its migration rate was similar to that of BSR kelts in the IBF group.

Table 1. Characteristics of Atlantic salmon kelts from different groups in the Bay of Fundy (BoF) tagged with pop-up satellite archival tags (PSATs) in 2008–2011 and summary of monitoring periods according to habitat and reporting status.

	Kelt group				
Kelt and PSAT metrics	OBS	IBS	IBF	*F*	*P*
Kelt characteristics					
River origin (*n*)	HR (13)	GR (4)	BSR (21), GR (1)		
Mean fork length (cm)	71.0±10.7	63.6±7.9	62.1±8.0	4.140	0.024
	61.0–90.0	56.0–71.0	52.0–80.5		
Sex ratio (M:F)	4:9	2:2	10:12		
Sea age ratio (1:2)	4:9	2:2	16:6		
Total PSAT monitoring period (days)					
River–estuary	263.2 (31.7%)	23.0 (11.2%)	418.6 (32.4%)		
Marine	566.7 (68.3%)	182.2 (88.8%)	872.0 (67.6%)		
All habitats	829.9 (100%)	205.2 (100%)	1290.6 (100%)		
Mean PSAT monitoring period (days)					
River–estuary	20.2±5.8	5.7±4.9	19.0±25.7	0.863	0.430
	10.2–26.9	2.1–12.4	0.3–96.7		
Marine	43.6±32.1	51.3±21.4	39.6±38.1	0.204	0.816
	1.4–101.0	21.3–70.9	1.6–134.0		
All habitats	63.8±34.4	51.3±21.4	58.7±47.1	0.153	0.859
	20.3–123.1	21.3–60.6	4.9–169.5		
Marine period					
End report (*n*)	97.7±4.6 (2)	—	126.1±11.2 (2)	2.757	0.392
	94.5–101.0	—	118.2–134.0		
Early report (*n*)	33.8±23.3 (11)	51.3±21.4 (4)	31.0±27.0 (20)	1.067	0.356
	1.4–74.2	21.3–70.9	1.6–113.1		

Note: Kelt groups are outer BoF spring (OBS), inner BoF spring (IBS), and inner BoF fall (IBF). Rivers are Hammond River (HR), Gaspereau River (GR), and Big Salmon River (BSR). Sample sizes (*n*) and all values are for kelts with tags that reported from marine habitat. Mean values are reported ± standard deviation, the range is given under the mean, and results of the ANOVA (*F* statistic and *P* value) are shown. Data for tags that reported on preset date (End report) and those that reported prematurely (Early report) are presented separately.

The migration rate of OBS kelts was as high as 49.5 km·day^{-1}, and the mean migration rate for this group was significantly greater than that of kelts from the IB groups (Table 2). There was a threshold at 20–25 km·day^{-1}, with OBS kelts generally migrating at rates above and those from IB groups below this threshold (Fig. 3*c*). Despite OBS kelts being significantly larger than IB kelts (Table 1), the migration rate of kelts was not significantly correlated with fork length within any of the three groups (Fig. 3*c*). End location was not related to size or previous spawning history.

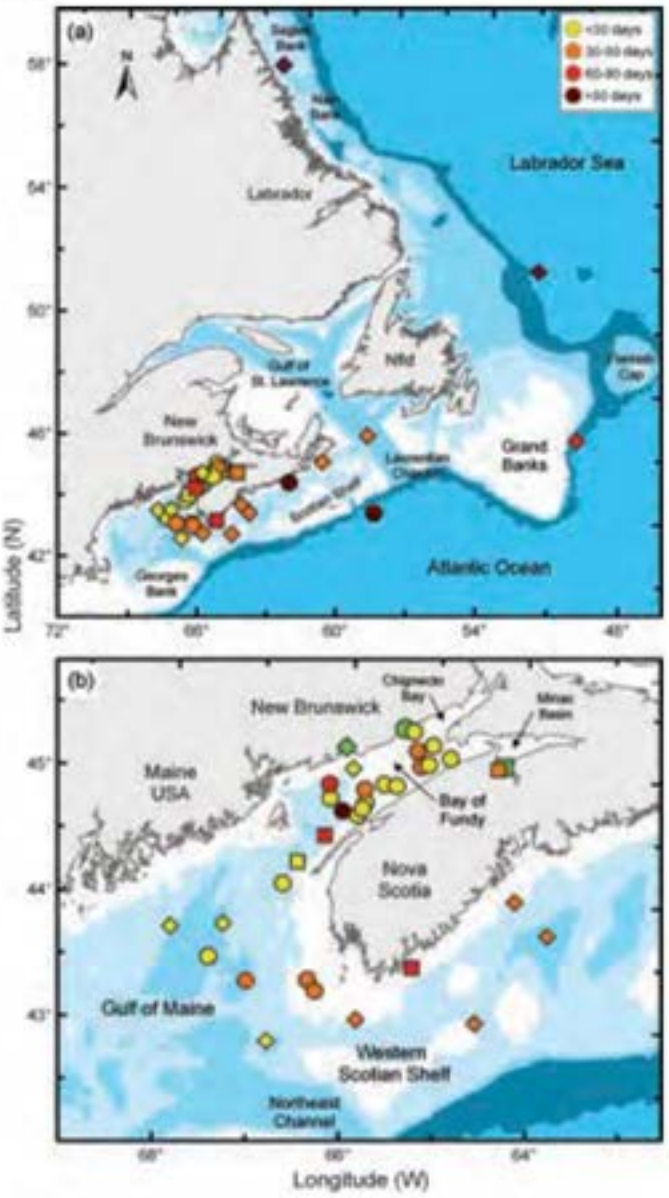
Migratory behaviour

Kelts from the OBS and IB groups with tracks >60 days at sea generally provided excellent examples of the differences in migratory behaviour of inner and outer BoF salmon (Fig. 4). The OBS kelt with the longest time at sea (101 days) initially migrated rapidly from the BoF and GoM during May (Fig. 4*a*), remaining near the surface (depth 0–5 m) with infrequent, shallow dives (10–20 m; Fig. 4*b*). Water temperature fluctuations were small as this kelt migrated through isothermal waters in the BoF and northern GoM. After leaving the GoM, periods of diving and swimming near the surface alternated as it migrated across the Scotian Shelf and Laurentian Channel in June, then around the Grand Banks of Newfoundland and up along the coast of Labrador in July. Migration then slowed in August as the kelt reached northern Labrador and then remained in coastal habitat on the Nain and Saglek banks. The entire migration was on the continental shelf, but on several occasions, this kelt moved to the shelf edge where it made some deep dives (300–500 m) and then turned back towards the coast. Water temperature increased as it migrated along the Scotian Shelf and across the outflow from the Gulf of St. Lawrence (GoSL), and then temperature declined gradually as it migrated around Newfoundland and north into the Labrador Sea. The overall temperature ranged from <0 °C during deep dives off Newfoundland and Labrador to 16 °C in the surface outflow of the GoSL, but temperatures were generally in the 4–10 °C range. This kelt sustained an average migration rate of 49.5 km·day^{-1} over a 4998 km track length, and the end point distance of 2972 km from the home river indicated that migration was relatively direct.

In contrast, during a period of 118 days at sea, the IBF kelt migrated at an average rate only 17.6 km·day^{-1}, and although the track length was 2091 km, the end point distance was only about 914 km from the river. This IBF kelt initially spent several months — December to mid-February — in the BoF and northern GoM (Fig. 4*c*). This period was characterized by frequent daily dives of 20–50 m (Fig. 4*d*). Water temperature did not fluctuate during these dives in isothermal waters of the BoF and northern GoM, but there was a seasonal decline from 10 °C in November to <4 °C by late January. Temperature increased as this kelt then moved into warmer waters on the western Scotian Shelf for several weeks, but it decreased rapidly to <2 °C in early February as it moved back near the coast of Nova Scotia. Thereafter, this kelt undertook a period of migration near the surface (depth 0–5 m) with few dives as it moved along the edge of the eastern Scotian Shelf. During this period, the temperature fluctuated more widely (2–12 °C), and it increased gradually as the kelt moved in and out of different water masses along the edge of the Scotian Shelf. This period of surface migration was interrupted by several deep dives (>400 m) when the kelt migrated over The Gully area near Sable Island. This kelt also remained on the continental shelf, following the shelf edge for more than a month without migrating into the adjacent warm waters of the Gulf Stream.

In both of these examples, the thermal profiles — change over time and extent of thermal stratification during daily dives — provided excellent validations of the general accuracy of geolocation estimates and tracks (Figs. 4*b*, 4*d*). The depth profiles, when examined in combination with the track and daily migration rate, clearly outlined periods of different behaviour. These included

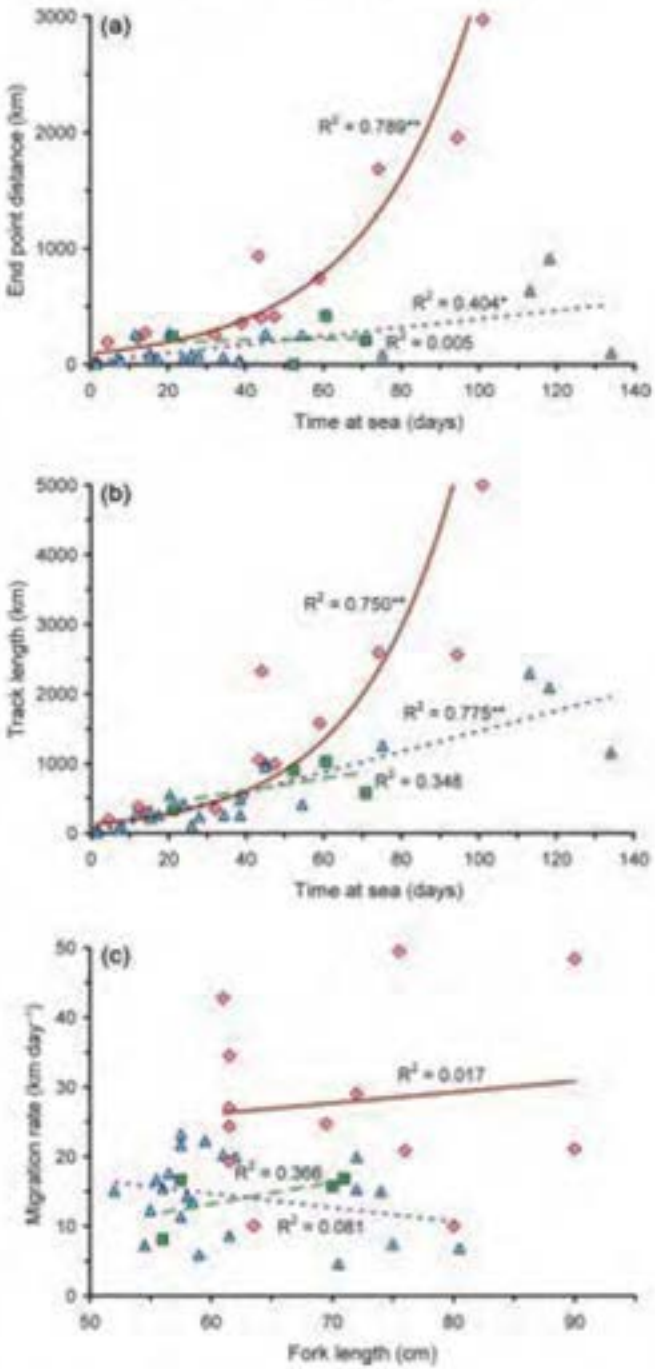
Fig. 2. (*a*) Full extent map of end locations for Atlantic salmon kelts from outer Bay of Fundy (BoF) spring (OBS, diamonds), inner BoF spring (IBS, squares), and inner BoF fall (IBF, circles) groups by reporting period (colour-coded by number of days at sea) and (*b*) expanded map of end locations near the BoF for these groups (same legend as in panel (*a*)) showing the mouth of the Hammond–Saint John River (green diamond), Gaspereau River (green square), and Big Salmon River (green circle).



(i) periods of rapid, extensive migration near the surface, (ii) periods of slow, localized migration with frequent dives, and (iii) brief interruptions of migration associated with localized deep dives. These deep dives occurred when kelts crossed channels along the migration route or as they moved to the edge of the continental shelf.

The deep diving behaviour was typical of OBS kelts that migrated to the shelf edge in the Labrador Sea and beyond the Grand Banks of Newfoundland (Fig. 5). These kelts migrated rapidly more than 2500 km in 74–95 days (Figs. 5a, 5d), remaining mostly near the surface, except for brief episodes of shallow dives. An initial period of relatively stable temperatures in the BoF–GoM was followed by periods where the daily water temperature range

Fig. 3. (*a*) End point distance from the home river and (*b*) track length in relation to time at sea and (*c*) migration rate in relation to fork length for Atlantic salmon kelts from outer Bay of Fundy (BoF) spring (OBS, diamonds), inner BoF spring (IBS, squares), and inner BoF fall (IBF, triangles) groups. The calculated regression lines (OBS, solid; IBS, dashed; IBF, dotted) and R^2 values with level of significance (*, $P < 0.01$; **, $P < 0.001$; ANOVA) are shown.



could at times vary widely (0–14 °C), especially during dives (Figs. 5b, 5e). Migration slowed for both kelts when they reached the shelf edge and made repeated deep dives. One kelt made repeated dives of 600–1000 m over 1 week in the Labrador Sea north of the northeast Newfoundland Shelf and the Funk Island Bank (Figs. 5a, 5b). The other kelt remained off the eastern and southeastern shoals of the Grand Banks over the period of 3–23 July, making repeated dives of 500–700 m (Figs. 5d, 5e).

Table 2. Marine migration characteristics of Atlantic salmon kelts from different groups in the Bay of Fundy (BoF) tagged with pop-up satellite archival tags in 2008–2011 summarized according to reporting status.

	Kelt group				
Metric and reporting status	OBS	IBS	IBF	*F*	*P*
End point distance (km)					
End report	2463.3±719.7	—	503.5±579.9	2.248	0.427
	1954.4–2972.2	—	93.4–913.6		
Early report	501.0±468.3	216.0±171.5	136.3±150.4	5.625	0.008
	20.5–1683.6	0.3–418.7	8.2–636.8		
Combined	802.9±876.9	216.9±171.5	169.6±219.5	5.935	0.006
	20.5–2972.2	0.3–418.7	8.2–913.6		
Track length (km)					
End report	3781.4±1721.0	—	1625.0±659.0	0.685	0.650
	2564.5–4998.3	—	1159.0–2090.9		
Early report	937.5±878.5	707.6±314.2	475.4±542.6	1.801	0.181
	20.1–2591.4	330.5–2830.5	9.1–2292.5		
Combined	1375.0±1425.0	707.6±314.2	579.9±633.6	2.865	0.070
	20.1–4998.3	330.5–1024.2	9.1–2292.5		
Migration rate (km·day^{-1})					
End report	38.2±15.9	—	13.1±6.3	1.079	0.563
	27.0–49.5	—	8.6–17.6		
Early report	25.9±12.2	14.3±4.2	14.4±5.8	7.423	0.002
	10.0–48.4	8.1–16.8	4.5–23.2		
Combined	27.8±12.9	14.3±4.2	14.3±5.7	10.512	0.0003
	10.0–49.5	8.1–16.8	4.5–23.2		

Note: Kelt groups are outer BoF spring (OBS), inner BoF spring (IBS), and inner BoF fall (IBF). Values are mean ± standard deviation, the range is reported under the mean, and results of the ANOVA (*F* statistic and *P* value) are shown. Data for tags that reported on preset date (End report) and those that reported prematurely (Early report) are presented separately.

There was no diurnal pattern associated with daylight during deep dives, and they often extended over more than 10–28 h (Figs. 5*c*, 5*f*). The descent was usually rapid (2–4 h), kelts remained in deep water for an extended period (6–12 h), and they then made a gradual, stepwise ascent back to the surface where they remained until the next group of dives. Abrupt changes of 6–8 °C in water temperature occurred as the kelts moved rapidly through the thermocline.

In sharp contrast with OBS kelts, most kelts from the IB groups remained in the BoF and northern GoM area during the first several months at sea, regardless of migration season (Figs. 6, 7). Their migration rate was slower, track length was shorter, and end points were comparatively closer to the rivers than for the OBS group (Table 2). One of the IBS kelts even returned to the Minas Basin within 2 months and re-entered the home river in July (Fig. 6*a*). Upon leaving the river, the IBS kelts soon started to make repeated dives in the 10–60 m range instead of migrating near the surface (Figs. 6*b*, 6*e*). There was a very clear diurnal pattern of repeated diving during daylight hours and remaining near the surface (<1 m) at night that persisted throughout the marine period, and the change in behaviour was very abrupt at dawn and dusk (Figs. 6*c*, 6*f*). This behaviour, typical of IBS kelts during May–July, was similar for IBF kelts during the December–May period (Figs. 7*c*, 7*f*). Regardless of whether they left the river in the fall or winter, soon after leaving the river, the IBF kelts started repeated diurnal, shallow dives that persisted throughout the winter, with few exceptions. At times, this diurnal behaviour within the confines of the BoF–GoM was interrupted by brief periods — usually only a few days — of near continuous migration near the surface, both day and night, as kelts changed locations (Figs. 6, 7). However, the diurnal diving behaviour resumed soon afterwards. While the kelts were in the BoF and northern GoM, water temperature was relatively constant, except for seasonal trends, and it did not fluctuate much during dives, confirming that they were in the isothermal waters of the BoF–GoM and not in thermally stratified habitat further offshore.

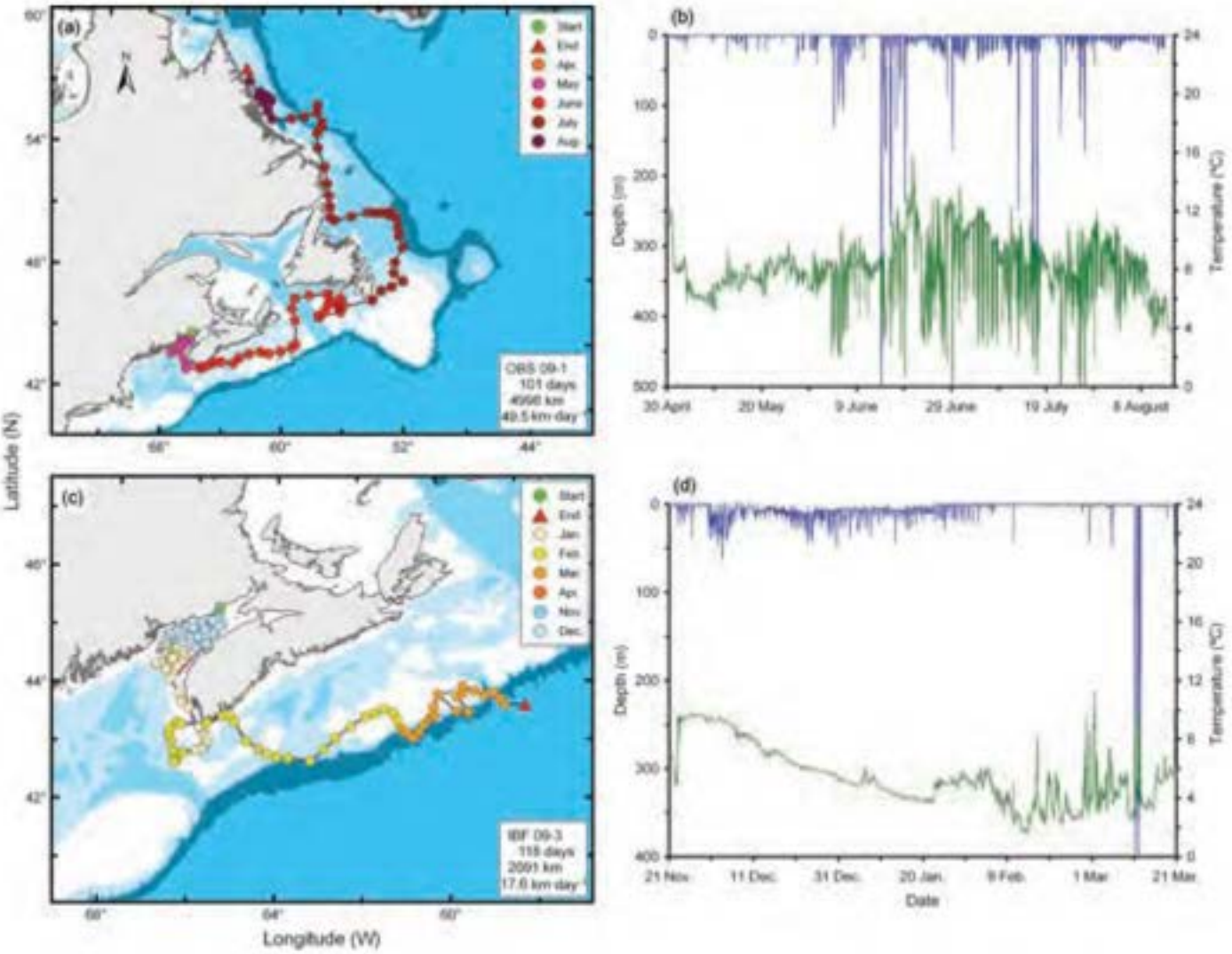
Regardless of differences in behaviour, the mean vertical distribution of kelts from all groups indicated that they spent most of their time 0–5 m below the surface (Fig. 8). During spring and summer, kelts spent 80%–90% of their time in this surface layer and spent about 10% more time near the surface at night than during daylight. However, the mean proportion of time spent at depths of 5–30 m was always significantly greater during daylight than at night (χ^2 test of independence, $P < 0.01$; Figs. 8*a*, 8*b*). During fall and winter, kelts were not as close to the surface as in the spring and summer (Mann–Whitney *U* test, $P < 0.01$), and the mean proportion of time spent in the 0–5 m band decreased to 60%–70% and that at 5–20 m increased accordingly (Fig. 8*c*). The time spent in the 5–10 m band in winter was, like that near the surface, predominantly at night, but this pattern reversed below the 10 m depth, where kelts spent significantly more time in daylight than at night (χ^2 tests of independence for each interval, $P < 0.01$). Individual depth profiles showed that during winter, kelts were usually at least 0.5–1 m below the surface at night (Figs. 7*c*, 7*f*), whereas in the summer they were <0.5 m from the surface at night (Figs. 6*c*, 6*f*). As mentioned earlier, there was no diurnal pattern associated with the deeper dives (>500 m) of OBS kelts (Figs. 5*c*, 5*f*).

Distribution and marine habitat characteristics

Estimated daily geolocations for all kelts in each group were used to identify the extent of migration and their marine distribution on a monthly and seasonal basis (Fig. 9). Kelts from the OBS group moved rapidly from the BoF–GoM in April and May to the Scotian Shelf and beyond as far as Newfoundland and the Grand Banks in May and June (Fig. 9*a*). By July, no OBS kelts remained in the BoF–GoM or on the western Scotian Shelf. During July and August, OBS kelts occurred along the south and east coast of Newfoundland and along the east coast of Labrador (Fig. 9*b*). They were generally on the continental shelf, but at times migrated to the shelf edge, either turning back onto the shelf to continue migrating or slowing down to exploit the slope habitat.

In contrast, there was no rapid departure of IBS kelts from the BoF. They remained in the BoF and northern GoM during May and June, and they were found close to the coast of Nova Scotia in July (Figs. 9*c*, 9*d*). Kelts from the IBF group, migrating back to sea in the

Fig. 4. Estimated tracks with daily locations (circles, colour-coded by month) and associated water temperature (green lines) and depth (blue lines) profiles for individual Atlantic salmon kelts from outer and inner Bay of Fundy (BoF) groups with the greatest end point distances from the home river: (*a*, *b*) outer BoF spring (OBS) kelt and (*c*, *d*) inner BoF fall (IBF) kelt.

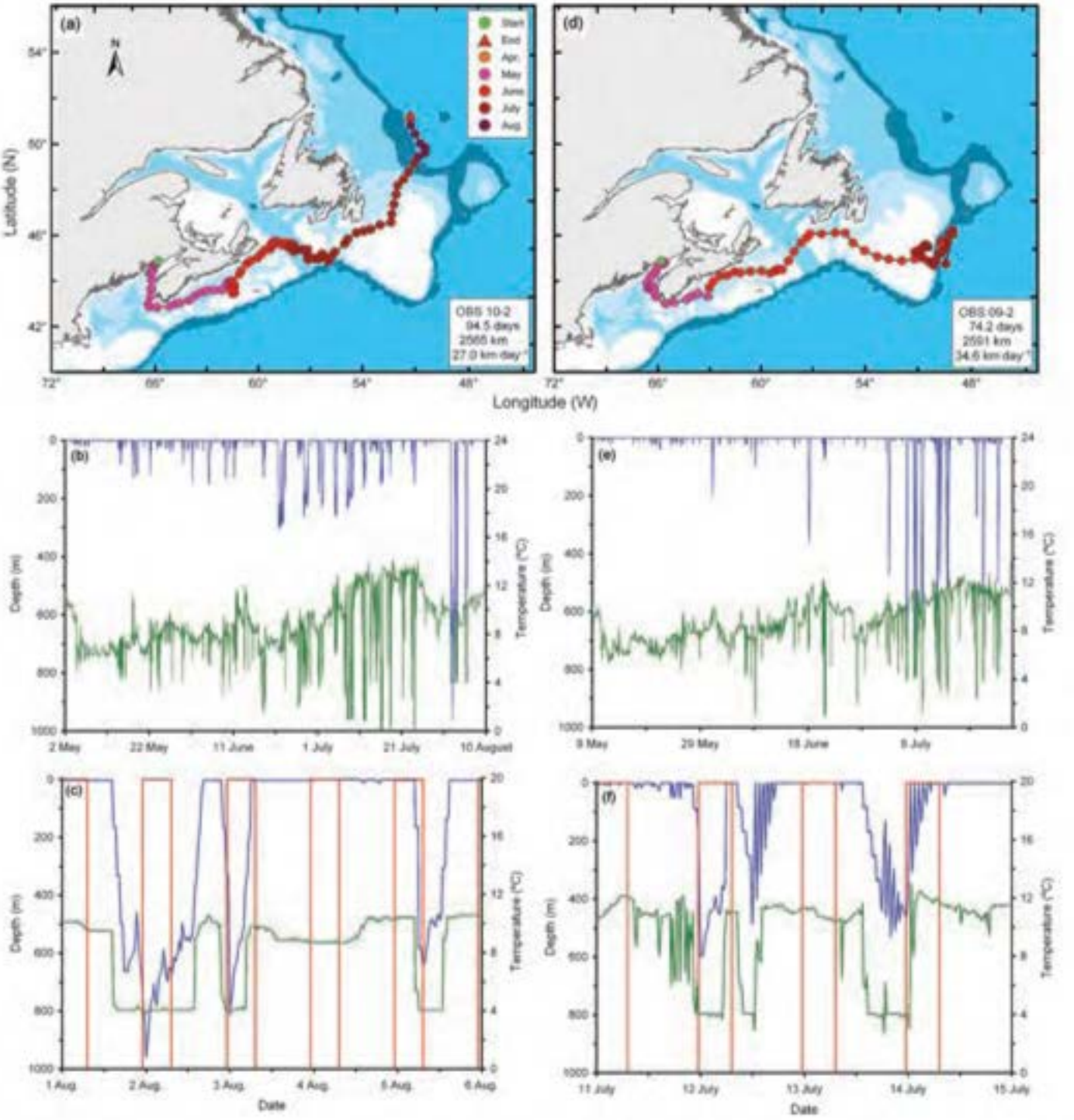


fall and during winter, also spent their first 2–3 months at sea in the BoF and northern GoM (Fig. 9*e*). The majority of these kelts remained in the same coastal area from February to May, but two kelts did leave the region and migrated along the Scotian Shelf, one during February and March and the other during May (Fig. 9*f*). Before that, these two kelts spent most of the winter in the BoF–GoM area. As winter progressed, kelts from the IBF group tended to disperse from the BoF to coastal habitat along the coast of Maine and at the southern tip of Nova Scotia, but they did not move into the southern GoM or Georges Bank area.

Kelts from both spring migration groups spent about 80% of their time near the surface, with little difference between the April–June and July–August periods (Figs. 10*a*, 10*b*). The rest of the time, they were mostly in the 5–30 m depth band, and seasonal differences were small and not significant (χ^2 tests of independence for each interval, $P > 0.05$). During the winter, IBF kelts spent significantly less time near the surface and more time in the 5–30 m depth band than IBS and OBS kelts did during the summer (Mann–Whitney *U* tests, $P < 0.01$; Fig. 10). The proportion of time in the 0–5 m depth band was significantly lower from February to May (44%) than earlier in the winter (70%) for this group, and time at depths of 10–30 m increased later in the winter (χ^2 tests of independence for each interval, $P < 0.01$; Fig. 10*c*). This avoidance of the surface layers was obvious for individual IBF kelts that spent the entire winter in the BoF, especially at times of minimum water temperature (Fig. 11*a*). There was some breakdown in the diurnal diving pattern at those times and avoidance of the surface layers at night in February and March (Figs. 11*b*, 11*c*). Kelts from both IB groups rarely dove deeper than 100 m (Figs. 10*b*, 10*c*), which was representative of the coastal habitat they occupied. In contrast, OBS kelts spent some time at depths of 100–1000 m, mostly in the later part of their migration when, at times, they moved to the edge of the continental shelf (Fig. 10*a*).

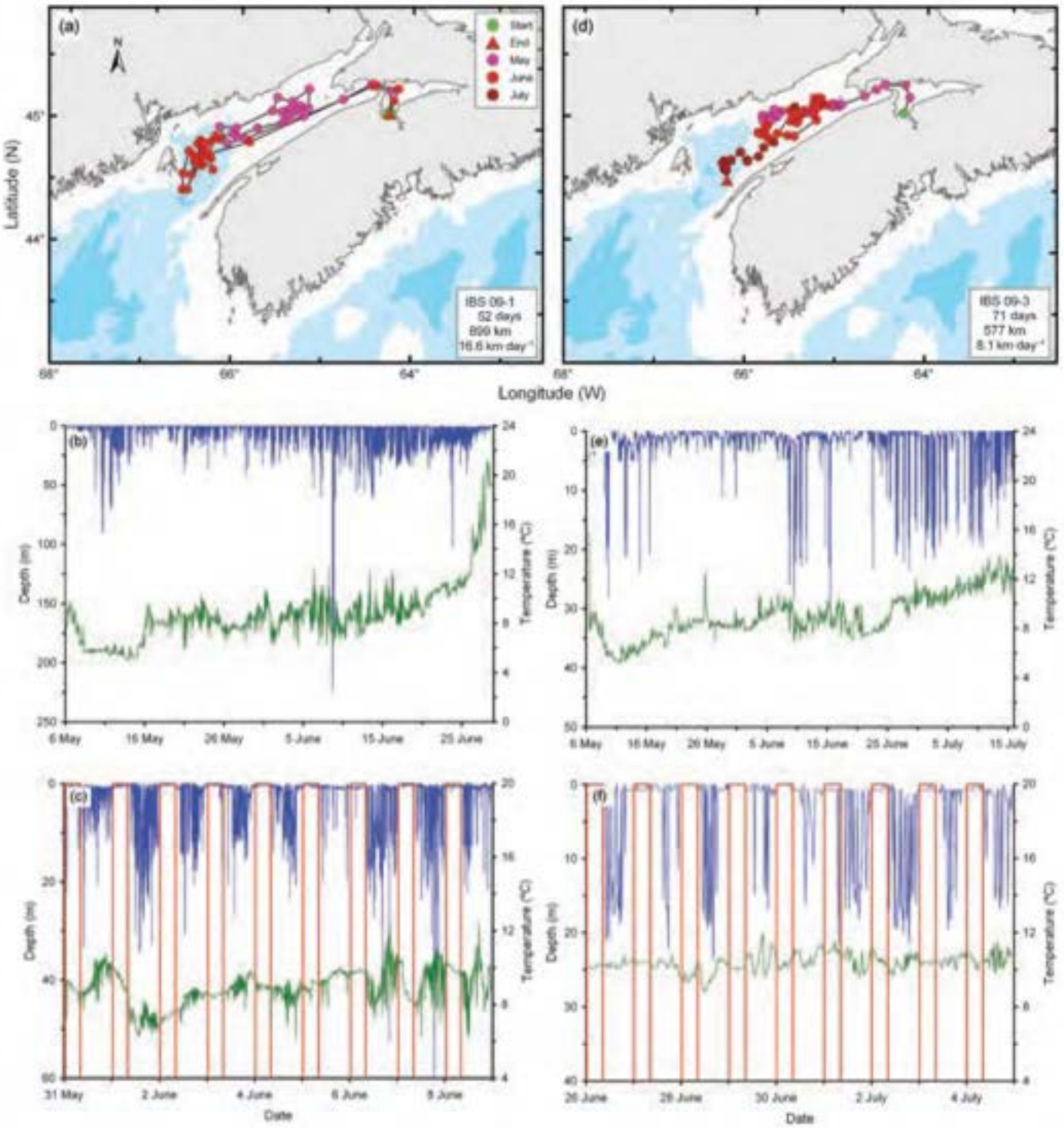
Water temperature was even more representative than depth of the seasonal habitat differences among kelts of different groups (Fig. 12). The range in temperature was widest (<0 to 16 °C) for OBS kelts that migrated through many different habitat areas, especially in July and August (Fig. 12*a*). The highest water temperatures were recorded by an OBS kelt when crossing the outflow from the GoSL and the lowest temperatures during deep dives along the coast of northern Labrador (Figs. 4*a*, 4*b*). The water temperature range was narrower for kelts from both IB groups that remained mostly in the isothermal waters of the BoF and northern GoM, and they were representative of seasonal temperature trends in those areas (Figs. 12*b*, 12*c*).

Fig. 5. (*a*, *d*) Estimated tracks with daily locations (circles, colour-coded by month), (*b*, *e*) associated water temperature (green lines) and depth (blue lines) profiles, and (*c*, *f*) selected water temperature (green lines) and depth (blue lines) profiles with times (GMT) of sunrise and sunset shown (red lines) during several deep dives for two Atlantic salmon kelts from the outer Bay of Fundy spring (OBS) group.



For spring migrating kelts, ambient temperature was significantly lower early in the migration than later, more so for the OBS group that left the BoF rapidly (Mann–Whitney *U* test, $P < 0.01$; Figs. 12*a*, 12*b*). The IBS kelts remained mostly inside the BoF during the summer, and they spent the most time of any group in water of 10–15 °C. In contrast, the OBS kelts, having left the BoF before summer, spent more time in cooler water, as they migrated to higher latitudes. For fall migrating kelts, water temperature fluctuated widely (1–11 °C) early in the winter as kelts first occupied cold habitat near the mouth of frozen rivers and then moved to the warmer but rapidly cooling waters of the BoF and northern GoM (Fig. 12*c*). Later in the winter, the range was much narrower for IBF kelts as minimum temperatures were reached in coastal habitat. These temperatures persisted into April, and as a result, kelts spent significantly more time in water of 3–5 °C in the late winter period than earlier (χ^2 tests of independence for each interval, $P < 0.01$). This was clearly seen for individual IBF kelts that remained in the BoF through the winter where they experienced water temperatures as low as 2 °C in February (Fig. 11*a*). At

Fig. 6. (*a*, *d*) Estimated tracks with daily locations (circles, colour-coded by month), (*b*, *e*) associated water temperature (green lines) and depth (blue lines) profiles, and (*c*, *f*) selected water temperature (green lines) and depth (blue lines) profiles with times (GMT) of sunrise and sunset shown (red lines) during several periods with frequent dives for two Atlantic salmon kelts from the inner Bay of Fundy spring (IBS) group.



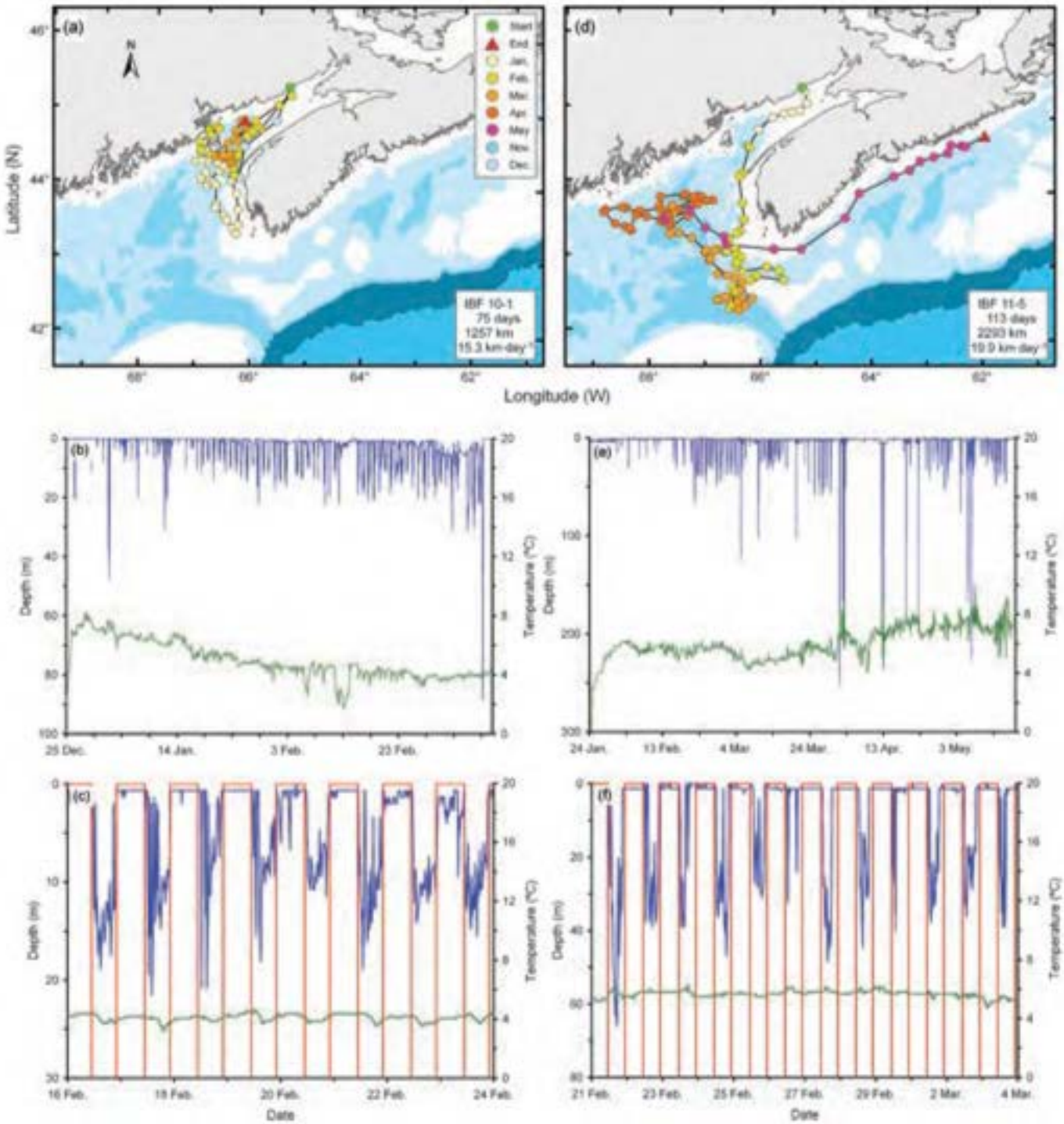
these times, the kelts avoided the surface water layers (Fig. 11*b*). Overall, kelts from all three groups experienced a wide range of temperatures, but they rarely ventured beyond the cool waters (<15 °C) of the continental shelf, avoiding adjacent warmer waters in the southern GoM and Georges Bank, GoSL, and Gulf Stream.

Home range and marine habitat utilization distributions

The kernel density analysis of daily geolocation estimates at sea clearly outlined the differences in home range (99% UD) among the three groups of kelts (Fig. 13). The home range of OBS kelts extended from the BoF, to the northern GoM, along the length of the Scotian Shelf, to the south and east coasts and banks of Newfoundland and north along the coast of Labrador. The range extended from 41°N to 59°N latitude and from 46°W to 70°W longitude, and it remained mostly on the continental shelf (Fig. 13*a*). The southern half of the GoM and the GoSL were excluded.

The home ranges of IBS and IBF kelts were contained within that of OBS kelts, but their extent was very different from that of

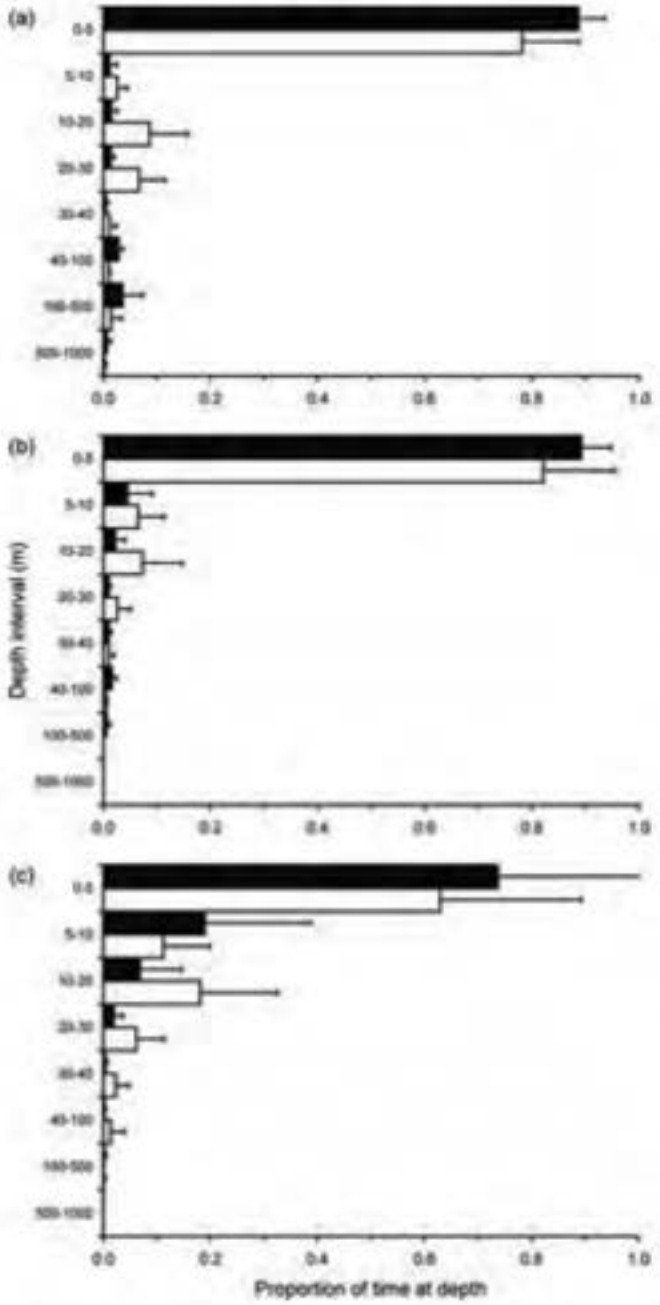
Fig. 7. (*a*, *d*) Estimated tracks with daily locations (circles, colour-coded by month), (*b*, *e*) associated water temperature (green lines) and depth (blue lines) profiles, and (*c*, *f*) selected water temperature (green lines) and depth (blue lines) profiles with times (GMT) of sunrise and sunset shown (red lines) during several periods with frequent dives for two Atlantic salmon kelts from the inner Bay of Fundy fall (IBF) group.



OBS kelts. The IB kelts were mostly in the BoF, northern GoM, and around the southern tip of Nova Scotia, regardless of season of migration (Figs. 13*b*, 13*c*). Kelts from the IBF group ranged further into the GoM than the IBS group, and their range extended from the coast of northern Maine to the coast of Nova Scotia and around Nova Scotia to coastal and offshore areas of the Scotian Shelf. The range of IB kelts was within 42°N to 46°N latitude and 58°W to 69°W longitude.

The 50% and 75% UDs indicated where the majority of kelt locations were concentrated and the areas of highest potential impact on each population. For OBS kelts, the 50% UD extended from the river mouth, through the outer BoF and northern GoM, around the southern tip of Nova Scotia on the western Scotian Shelf and to some extent onto the eastern Scotian Shelf (Fig. 13*a*). The distribution extended to nearshore habitat around the southern and Atlantic coasts of Nova Scotia as far as the LaHave Basin. The 75%

Fig. 8. Weighted mean (+ weighted standard deviation) proportion of time spent at different depths during night (dark bars) and day (open bars) by Atlantic salmon kelts from (*a*) outer Bay of Fundy (BoF) spring (OBS), (*b*) inner BoF spring (IBS), and (*c*) inner BoF fall (IBF) groups.



UD extended along the length of the Scotian Shelf to the south coast of Newfoundland and onto the Grand Banks of Newfoundland towards the Flemish Cap.

For IBS kelts, the 50% and 75% UDs were limited to several areas in the BoF that included the Minas Basin, the inner and outer BoF, and the Grand Manan Basin at the mouth of the BoF (Fig. 13*b*). The 75% UD extended to several small patches in the northern GoM. For IBF kelts, the 50% UD was broader and less patchy than for IBS kelts, mostly because of the larger number of location points used, and it extended from shore to shore from the inner BoF out to the Grand Manan Basin, with several patches in the northern GoM (Fig. 13*c*). The 75% UD fanned out into the northern GoM, extending down the coast of Maine, along southwest shore of Nova Scotia, and onto the western Scotian Shelf.

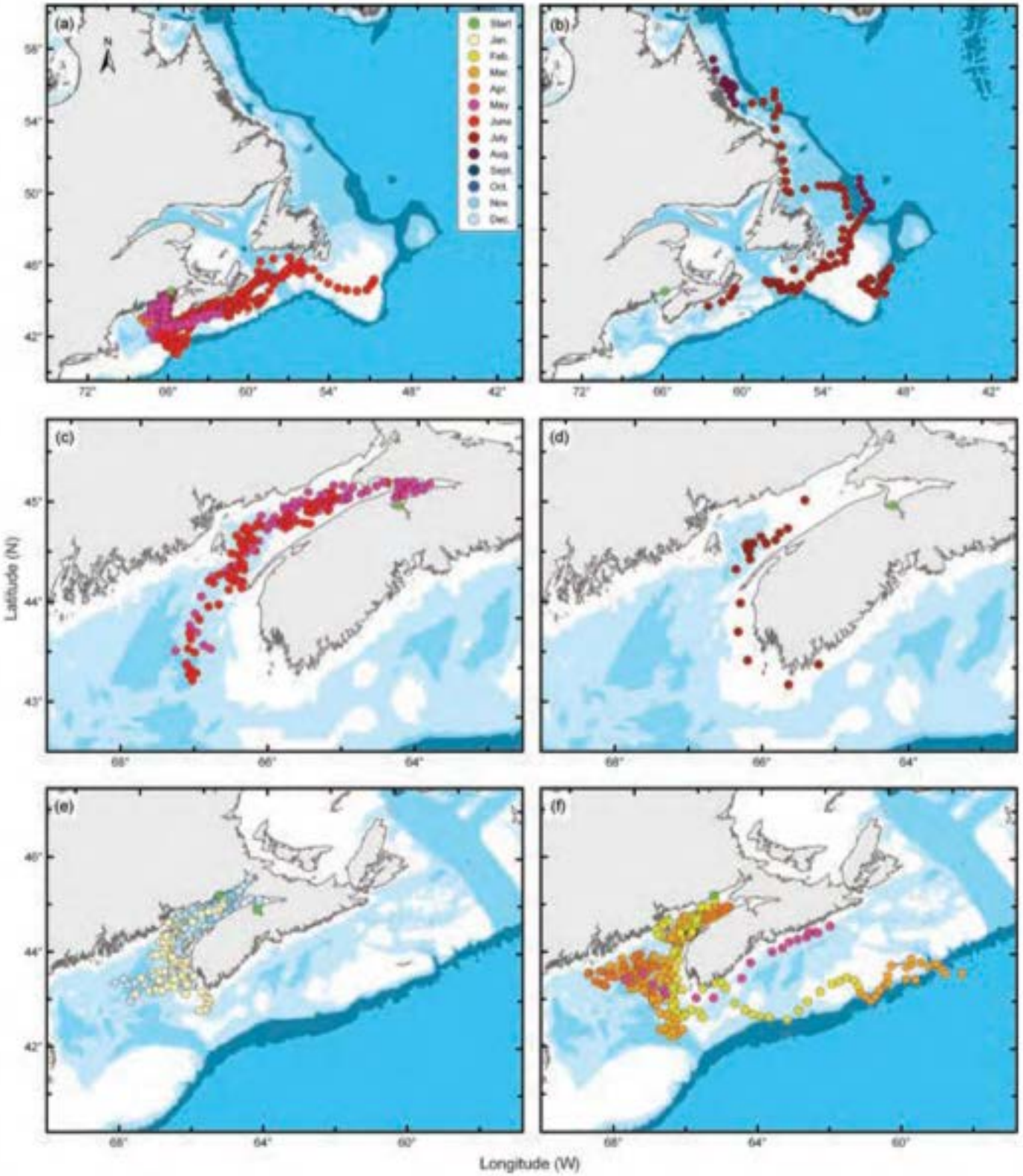
The estimates of time at sea spent in different marine regions based on individual temperature profiles, independent of estimated geolocations, were used to provide an alternate measure of home range or habitat use (Fig. 14). The change from isothermal profile to one with thermal stratification provided an accurate separation between the BoF–GoM and other regions of the North Atlantic used by kelts, and the temperature difference between the BoF and GoM usually provided a good indicator of transition between these two regions (*e.g.*, Figs. 4*b*, 4*d*). The OBS kelt group spent significantly less of the time at sea in either the BoF or GoM (<15% in each area) than in the North Atlantic where they occurred >70% of the time (Mann–Whitney *U* test, $P < 0.01$). In contrast, both the IBS and IBF kelt groups spent significantly more time in the BoF (46%–60%) and GoM (28%–43%) than in the North Atlantic, where they occurred <12% of the time (Mann–Whitney *U* tests, $P < 0.01$). Kelts migrating in the winter (IBF group) spent more time in the BoF than the GoM, whereas those from the IBS group spent similar amounts of time in both of these regions. Differences in habitat use between kelts from OB and IB groups were large and independent of the time of year that IB kelts were at sea.

Discussion

Atlantic salmon kelts from three genetically distinct populations originating from different regions of the BoF in the GoM watershed and with different migration timing were tracked and monitored during their migration back to marine feeding grounds after spawning. All metrics measured — estimated track and end location, end point distance from the home river, track length and migration rate, home range and habitat utilization distributions — indicated that the range in oceanic migration of kelts from inner BoF groups (IBS, IBF) was restricted compared with kelts from the outer BoF group (OBS). Differences in migratory behaviour between inner and outer BoF kelts, especially at the start of the marine period, influenced the time spent in different marine habitat areas and destination. Behaviour was apparently influenced by season of migration, time spent in rivers and estuaries to recondition before migration, and, as a result, differences in environmental conditions encountered during migration. Although home range was similar for kelts from the two inner BoF groups, there were some differences in behaviour and habitat between the inner BoF spring and fall groups that resulted from migration at different times of the year when energy reserves, thermal habitat, and prey availability differed.

The study demonstrated the potential value of deploying small PSATs on pelagic fish such as Atlantic salmon as a fishery-independent method to monitor behaviour and marine migration. The salmon kelts were some of the smallest (fork length, 52–90 cm) pelagic fish successfully tagged with PSATs to date, and their migration near the surface made them an excellent platform for light-based geolocation in contrast with other species where lengthy periods at depth severely limited geolocation (Aarestrup et al. 2009; Neilson et al. 2009). As a result, accurate daily locations produced tracks for kelts at sea that were verified by horizontal and vertical thermal gradients along the migration route. The tags greatly increased the spatial, vertical, and temporal ranges in which salmon were previously monitored at sea. Post-tagging mortality and tag loss due to attachment failure were considered unimportant because tag retention was 100% and mortality due to tagging was nil in trials associated with the study. However, many tags reported prematurely because of kelt mortality associated with predation by large pelagic fish identified from the archived data and other predators along the migration route (G.L.L., unpublished data). Nevertheless, the time at sea for a high proportion of tags on kelts was sufficient to describe and compare their behav-

Fig. 9. Estimated daily locations (circles, colour-coded by month) for all Atlantic salmon kelts from outer Bay of Fundy (BoF) spring (OBS), inner BoF spring (IBS), and inner BoF fall (IBF) groups, early and late in the migration: (*a*) OBS April–June, (*b*) OBS July–August, (*c*) IBS May–June, (*d*) IBS July, (*e*) IBF November–January, and (*f*) IBF February–May.



iour, migration, and habitat because migration patterns were evident early in the marine period.

Large gaps were filled in the migration route of North American Atlantic salmon previously inferred for salmon stocks from the southern end of the range from patchy recoveries of conventional tags (Ritter 1989; Miller et al 2012). Kelts of outer BoF origin tended to follow a similar coastal route along the continental shelf until they reached the south coast of Newfoundland. However,

Fig. 10. Weighted mean (+ weighted standard deviation) proportion of time spent at different depths early (hatched bars) and late (dark bars) in the migration by Atlantic salmon kelts from (*a*) outer Bay of Fundy (BoF) spring (OBS April–June and July–August), (*b*) inner BoF spring (IBS May–June and July), and (*c*) inner BoF fall (IBF November–January and February–May) groups.

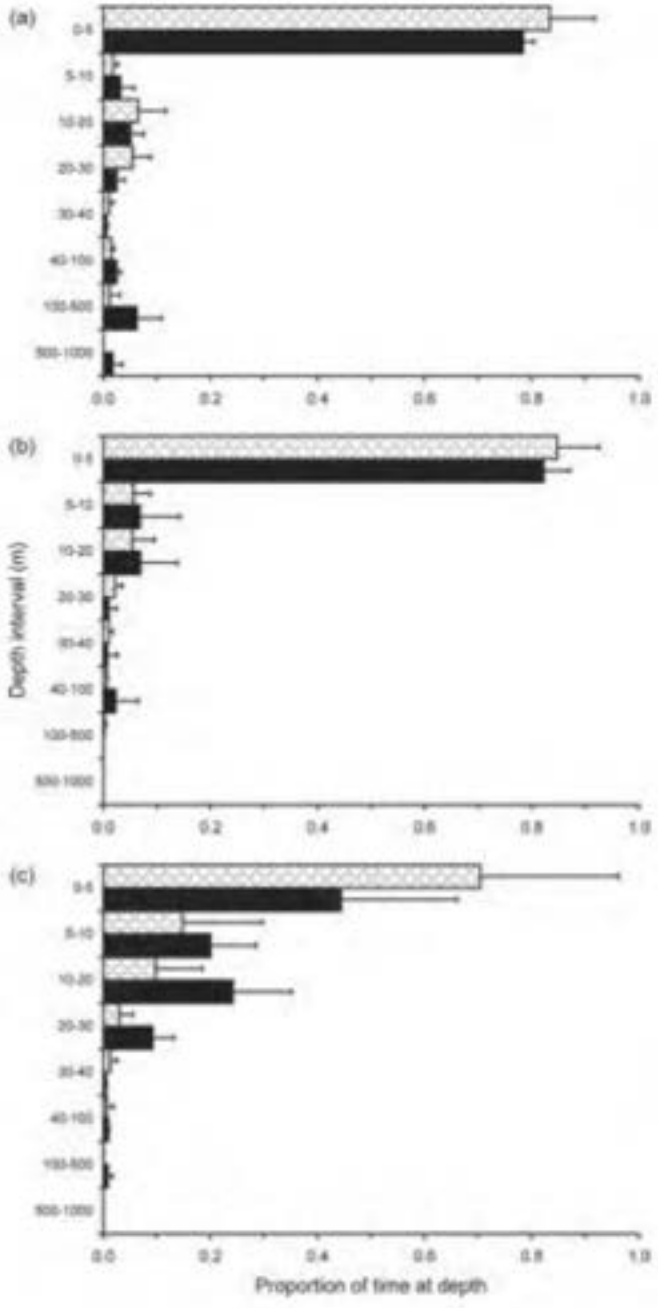
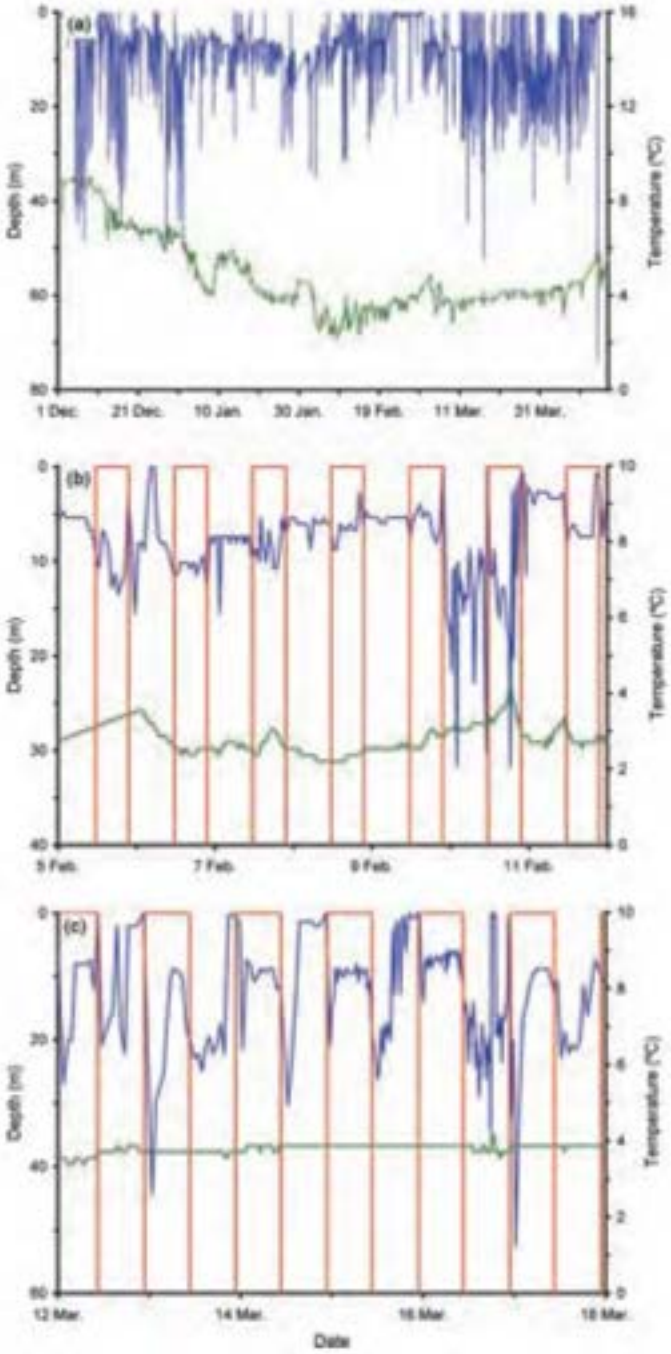


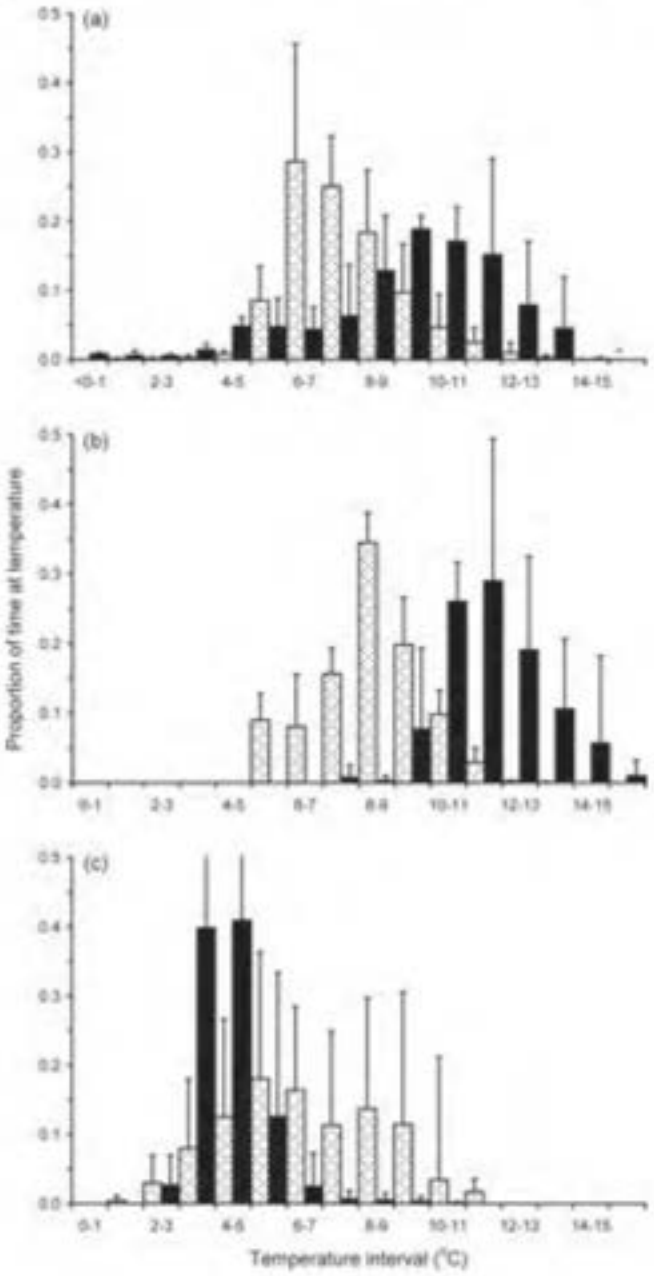
Fig. 11. (*a*) Water temperature (green line) and depth (blue line) profiles for an Atlantic salmon kelt from the inner Bay of Fundy (BoF) fall (IBF) group during the period spent in the BoF in 2009–2010 and (*b*, *c*) water temperature (green lines) and depth (blue lines) profiles with times (GMT) of sunrise and sunset shown (red lines) at times of minimum ambient temperature in the winter.



thereafter the kelts that survived moved in separate directions: (*i*) along the coast to northern Labrador, (*ii*) out towards the Labrador Sea, and (*iii*) out towards the Flemish Cap. Upon reaching these areas, the migration rate of kelts slowed and their behaviour changed from migrating near the surface to that of repeated diving, probably associated with feeding. This indicated that they may have reached their general destination or, at least, suitable temporary feeding areas. This dispersal in the later phase of migration to different feeding areas indicates flexibility in the migration routes and range of Atlantic salmon. They may not all follow the same route or aggregate in the Labrador Sea off western Greenland as previously inferred (Reddin and Friedland 1993).

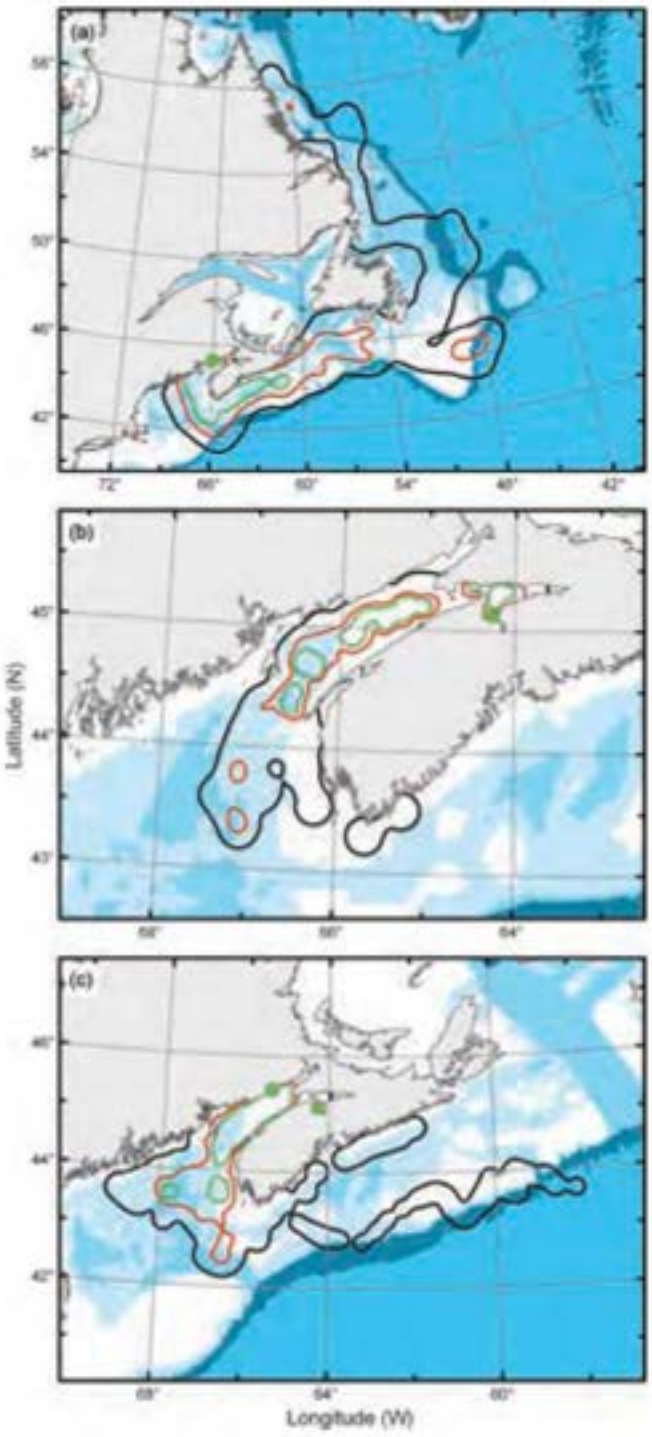
Migration tracks for kelts of outer BoF origin reaching distant feeding areas in the North Atlantic did not support the open ocean model for marine migration of adult Atlantic salmon. This model proposes that adult salmon are transported counterclockwise along the North Atlantic Current and from the western to eastern North Atlantic and then transported in northerly currents of the North Atlantic Subpolar Gyre (Dadswell et al. 2010). Instead, kelts

Fig. 12. Weighted mean (+ weighted standard deviation) proportion of time spent at different water temperatures early (hatched bars) and late (dark bars) in the migration by Atlantic salmon kelts from (*a*) outer Bay of Fundy (BoF) spring (OBS April–June and July–August), (*b*) inner BoF spring (IBS May–June and July), and (*c*) inner BoF fall (IBF November–January and February–May) groups. Mean proportion <0.001 in intervals >15 °C in early period (+) is shown in panel (*a*).



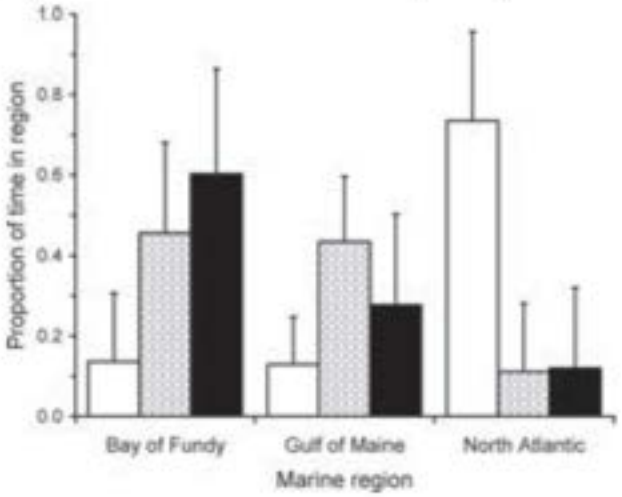
migrated rapidly against the prevailing coastal Labrador Current that flows southward along Labrador, then around the eastern and southern banks of Newfoundland and Flemish Cap and along the Scotian Shelf (Lazier and Wright 1993; Lavender et al. 2000). Kelts of both inner and outer BoF origin avoided moving far off the Northwest Atlantic Continental Shelf, and as a result, they were not entrained in the Gulf Stream that flows northeast along the outside of the Scotian Shelf and then into the North Atlantic Current (Krauss 1986; Dadswell et al. 2010).

Fig. 13. Marine habitat utilization distributions (UDs) for Atlantic salmon kelts from different groups: (*a*) outer Bay of Fundy (BoF) spring (OBS), (*b*) inner BoF spring (IBS), and (*c*) inner BoF fall (IBF). Isopleths for 50% UD (green outlines), 75% UD (red outlines), and 99% UD or home range (black outlines) and rivers of origin (green circles) are shown.



Migrating kelts usually remained near the surface (depth <2 m) for extended periods and rapidly covered large distances (>2000 km). Kelts from the OBS group displayed this behaviour as they left the river and estuary and, as a result, were out of the BoF within days. The extended period (10–27 days) spent feeding in the extensive estuary of the Saint John River in the spring, with its

Fig. 14. Weighted mean (+ weighted standard deviation) proportion of time at sea spent in different marine regions by Atlantic salmon kelts from outer Bay of Fundy (BoF) spring (OBS, open bars), inner BoF spring (IBS, hatched bars), and inner BoF fall (IBF, dark bars) groups estimated from individual water temperature profiles.



abundance of forage fish, probably helped these kelts recondition enough to undertake this rapid migration instead of starting to feed immediately upon marine entry. Atlantic salmon that overwinter in rivers and estuaries often feed for a period in the spring before undertaking the marine migration (Cunjak et al. 1998; Hubley et al. 2008). Migration near the surface continued for >1 month at a high rate (25–50 km·day^{-1}), and it was only infrequently interrupted by brief periods of deep dives. These kelts made extended (>24 h) deep dives (100–1000 m) when they crossed deep areas such as the Laurentian Channel (200–500 m) between the Scotian Shelf and south banks of Newfoundland or when they migrated to the edge of the continental shelf and depth increased rapidly to >2000 m on the slope. Migration rate slowed during these brief episodes of repeated deep diving, but there was none of the diurnal periodicity normally associated with feeding or predator avoidance in coastal habitat. When crossing the Laurentian Channel with warm surface waters flowing out of the GoSL, the dives may have helped kelts avoid high SSTs and seek a temporary thermal refuge or may have provided cues for orientation (Døving et al. 1985). The nutrient-rich, highly productive boundary areas with upwelling slope water at the shelf edge probably presented a foraging opportunity for salmon (Hansen and Quinn 1998). Later in the migration, the frequency of episodes of repeated shallower dives increased in these areas and migration slowed. These dives were probably associated with prey seeking and feeding as kelts replenished energy reserves after 1–2 months of near-continuous, rapid migration against prevailing currents.

The behaviour of kelts from both inner BoF groups differed markedly from that of outer BoF kelts. Shallow diving activity (mostly <50 m) started immediately upon entering the BoF and continued as long as kelts remained in coastal habitat in the BoF and GoM, both in summer and winter. There was a diurnal periodicity in vertical movements, apparently triggered by sunset and sunrise, with kelts remaining near the surface at night and making repeated dives through the day. It was suggested that the swimming depth of postsmolts during early marine migration (i.e., closer to the surface at night than in the day) depended on changes in light intensity, but that during daytime, irregular dives were initiated in response to other unidentified factors (Davidsen et al. 2008). Feeding, thermoregulation, and predator avoidance have been suggested as reasons for diurnal vertical movements and daytime dives by migrating salmon (Hansen and Quinn 1998; Tanaka et al. 2000; Reddin et al. 2004). Juvenile salmon typically seek shelter in rivers during daytime to avoid predators, and this behaviour may persist during marine migration, especially in coastal habitat. Pacific salmon apparently seek the coolest thermal refuge that they can exploit by vertical movement, especially in areas with large thermal differences in the water column (Tanaka et al. 2000). This behaviour was unlikely to be important in the vertically isothermal waters of the BoF and northern GoM. However, feeding activity at a time when energy reserves needed to be restored may be a more likely explanation. Daytime diving activity involved repeated dives with a return to the surface rather than a constant time at depth, indicative of prey-seeking behaviour instead of predator avoidance or negative phototaxis. The frequency and changing depth of these dives in different habitats were probably in response to the diurnal distribution and availability of prey in the water column (Lacroix and Knox 2005). Kelts from the inner BoF groups effectively used the BoF and northern GoM to feed and recondition early in the migration in the same way as spring kelts in large rivers use estuaries with an abundance of forage fish. This behaviour reduced their migration rate and was conducive to extended residency in coastal habitat, which would have fostered the annual return of repeat spawners that was characteristic of inner BoF salmon (Ducharme 1969; Ritter 1989).

The diurnal diving of inner BoF kelts, associated with feeding, was usually only interrupted by brief periods when kelts remained near the surface, both day and night, for several days. During these periods, there was a sharp change in ambient temperature indicative of a transition between habitat areas with different SSTs (e.g., BoF, GoM, and Scotian Shelf). These changes from vertical to horizontal movements could be searches for more abundant prey. In summer, orientation related to thermal preferences may have been involved in these transitions. Atlantic salmon postsmolts generally seek cool thermal refuges and avoid SSTs >15 °C in summer (Lacroix 2013). Longer interruptions in diurnal diving were only seen in winter when, after an extended period in the BoF–GoM, two inner BoF kelts migrated near the surface to different areas of the Scotian Shelf. This migration phase was similar to that of outer BoF kelts in the spring, and it was followed by some deep dives. However, these IBF kelts did not go as far as OBS kelts did, and they could have returned to the home river that year, as expected for salmon from BSR (Jessop 1986).

There were seasonal differences in vertical habitat used by spring and fall kelts. Spring kelts spent >80% of their time in the 5 m band below the surface in summer, whereas fall kelts spent more time at depths of 5–30 m and less time in the surface layer during winter. This difference in vertical habitat could be a response to seasonal differences in prey distribution in the water column. However, the increased use of deeper layers for fall kelts, especially during the second half of the winter, could be an avoidance reaction related to changes in thermal habitat. The ambient temperature for kelts decreased markedly over winter, and >80% of the time was spent in a narrow range of 3–4 °C from February to May, but habitat with SSTs <3 °C was rarely used. At this time, kelts were often 5–10 m deeper between dives than in summer, and they were rarely in the top 2 m of the surface layer even at night. A supercooling of the surface layer that can kill Atlantic salmon held in sea cages occurs in coastal areas of the BoF during the coldest period in winter (Saunders 1987). Frequent winter storms with strong winds promote supercooling without ice formation at the surface, resulting in SSTs below the lethal freezing temperature of Atlantic salmon in seawater (–0.76 °C; Fletcher et al. 1988). It is likely that kelts, unlike salmon held in captivity, avoided supercooled water (<0 °C) at the surface by going deeper during cold episodes in the winter.

Kelts used diverse habitat over wide latitudinal (41°N–59°N) and vertical (0–1000 m) ranges, but they always remained within an ambient temperature range of 0–15 °C, often going through the

full range in a single dive. In summer, they did not use extensive adjacent habitat with warmer SSTs, suggesting that thermotaxis may have been involved in finding suitable habitat. The OBS kelts migrated rapidly from the BoF and through a corridor along the Scotian Shelf bounded by the warm water of the Gulf Stream flowing northwards along the edge of the shelf on one side and by the outflow of warm water from the GoSL on the other side, always avoiding these warm water masses. This migration corridor was effectively closed to IBS kelts when, because of their delayed migration while feeding in the BoF, they became surrounded by warm water masses (SSTs >20 °C) in the southern GoM and on the Scotian Shelf from mid-June onwards (School of Marine Sciences, University of Maine, satellite images available at http://wavy.umeoce.maine.edu/index.htm). The IBS kelts avoided these warm waters and remained in coastal areas of the BoF and northern GoM with localized upwelling of cold water entering the GoM through the Northeast Channel. Habitat in the Eastern Maine Coastal Current and along the Fundy coast and southern tip of Nova Scotia provided a summer thermal refuge (10–15 °C) for kelts. These areas were within highly productive coastal currents and gyres that would have provided excellent foraging habitat for kelts through the summer (Lacroix and Knox 2005).

When in the North Atlantic, OBS kelts often experienced the full temperature range (0–15 °C) during a single deep dive into cold Labrador–Subarctic Slope water along the edge of the Newfoundland and Labrador shelves. Atlantic salmon are capable of withstanding brief exposure to 0 °C water because of their low blood freezing temperature (Fletcher et al. 1988). The summer temperature range recorded by DSTs on Atlantic salmon kelts in coastal waters of Newfoundland was 0–25 °C, but most of the time in marine habitat was spent in water <15 °C (Reddin et al. 2004). Although their tags did not measure depth, the authors inferred that the lowest temperatures probably occurred during deep dives. The temperature–depth profiles obtained for kelts in the present study confirmed their assumption that kelts make deep dives possibly associated with feeding.

Kelts residing in the BoF and northern GoM over winter used habitat with temperatures of 2–10 °C. A preferred temperature range of 4–10 °C for Atlantic salmon, based on their distribution in the Northwest Atlantic (Reddin and Friedland 1993), has been used to define critical habitat for the endangered inner BoF salmon population by inferring that both lower and higher temperatures limited range at sea (Amiro et al. 2003). However, the detailed records from PSATs in this study and from DSTs (Reddin et al. 2004) do not support the assumption that the BoF was mostly unsuitable for Atlantic salmon in the winter. They indicated that marine habitat with a wider temperature range can be tolerated and successfully exploited during long periods. Kelts from the IBF group spent most of their time at 3–4 °C during the coldest winter months, and they remained in the BoF when SSTs were <0–4 °C in winter by avoiding the surface layer. Low SSTs in winter may have reduced their vertical range, but they did not limit their range inside the BoF. These results demonstrated how the BoF provided important winter habitat to the endangered inner BoF salmon. In addition, low SSTs (<4 °C) on the Scotian Shelf in late winter did not prevent migration of inner BoF kelts to that area.

The migration rates, routes, and destinations of inner and outer BoF kelts tended to mimic those of postsmolts from their region (Lacroix et al. 2005; Lacroix 2013). As a result, kelts and postsmolts of similar origin probably exploit the same marine habitat either in coastal areas or in the North Atlantic (Jessop 1976; Ritter 1989; Reddin and Short 1991). Philopatry (i.e., site fidelity) is normally associated with the return migration of Atlantic salmon to home rivers for spawning. Experience gained while migrating as smolts and olfaction appear to be required for Atlantic salmon to navigate precisely along the coast when returning to home rivers or coastal marine sites (Sutterlin et al. 1982; Døving et al. 1985; Hansen et al. 1993). The mechanisms involved in the oceanic migration of salmon are not as clear, but some form orientation probably occurs (Harden-Jones 1968; Hasler 1971; Hansen and Quinn 1998).

Simulated trajectories for migrating Atlantic salmon were most accurate if the model included both thermotaxis and rheotaxis into the swimming behaviour (Booker et al. 2008). A thermotactic response was probably involved in controlling the range of both inner and outer BoF kelts and postsmolts. Kelts showed an avoidance of adjacent habitat with SSTs >15 °C in spring and summer similar to that observed for postsmolts (Ritter 1989; Lacroix 2013). The range of kelts increased in winter when SSTs were <10 °C, but a thermotactic response was possibly involved in the avoidance of the supercooled surface layer. The temperature-related habitat constraints for kelts also suggest that a physiological response related to optimal feeding and growth or swimming performance may have been involved, with temperature acting as a controlling factor (i.e., affecting metabolism and activity) rather than as a directive factor (sensu Fry 1947). Although the distant migration of outer BoF kelts against prevailing currents seems to imply a possible rheotactic response, it is unclear how salmon could maintain this freshwater response to currents when offshore without the needed visual clues (Fraenkel and Gunn 1961). The tendency of Atlantic salmon to follow coastlines for long distances may have helped (Reddin and Lear 1990; Hansen et al. 1993). The exploratory dives made by kelts when they crossed deep channels and at the shelf edge, transitioned between water masses with different thermal profiles, migrated across large-volume outflows of lower salinity, or encountered landmasses may also have provided orientation cues — thermohaline, bathymetric, and olfactory — that helped them maintain a relatively direct trajectory in the ocean.

Differences in adult return rates among salmon populations of inner and outer BoF origin were reported starting with the decline of inner BoF salmon populations in the 1980s and reconfirmed in the decade since 2000 (Gibson et al. 2003; Jones et al. 2010). During this 30-year period, marked changes occurred in coastal areas of the BoF, and as a result, the potential causes of salmon mortality increased (Lacroix et al. 2004; Lacroix and Knox 2005; Lacroix 2008). The relative success of kelts of inner and outer BoF origins was most likely influenced by their migration strategy and home range. The habitat used by kelts to recondition at the start of migration (i.e., estuary or BoF) affected their behaviour and migration rate. Predator avoidance would have been difficult for the inner BoF kelts that migrated slowly compared with outer BoF kelts that left the BoF rapidly within several days. Thereafter, exposure to potential causes of mortality in the BoF and northern GoM would have increased for inner BoF kelts that spent the majority of their time at sea in this coastal habitat. In comparison, the rapid migration of outer BoF kelts along the Scotian Shelf and in the North Atlantic should have helped them survive in habitat areas where potential causes of mortality have remained vague. A prolonged increase in mortality of inner BoF kelts in coastal habitat would have resulted in the disappearance of the multiple repeat spawners that returned annually to inner BoF rivers and maintained recruitment (Jessop 1986; Ritter 1989).

The shortened migration range and extended residency of kelts of inner BoF origin throughout the year in coastal habitat in the BoF and northern GoM and near shore along the Fundy and Atlantic coasts of Nova Scotia emphasize the need for a cautious management approach in habitat defined by the 50%–75% UDs to protect the endangered inner BoF salmon. Similarly, the seasonal migration corridor used by salmon of outer BoF origin along the Atlantic coast of Nova Scotia and southern coast of Newfoundland (50%–75% UDs), often close to shore, should probably be protected since New Brunswick outer BoF salmon are under consideration for listing as endangered (COSEWIC 2010). In both situations — residency and migration — salmon occurred mostly at depths of <5 m and exploited upper trophic levels (0–30 m), indicating

the importance of surface habitat. Coastal developments and activities near the surface in these habitat areas (e.g., salmon aquaculture and pelagic trawl or other surface fisheries) that could impact endangered, wild Atlantic salmon populations from the BoF should be scrutinized and, if necessary, restricted when implementing a recovery strategy for endangered populations (Fisheries and Oceans Canada 2010).

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REVIEW

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A global synthesis of peer-reviewed research on the effects of hatchery salmonids on wild salmonids

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Abstract

Hatcheries have long produced salmonids for fisheries and mitigation, though their widespread use is increasingly controversial because of potential impacts to wild salmonids. We conducted a global literature search of peer-reviewed publications (1970–2021) evaluating how hatchery salmonids affected wild salmonids, developed a publicly available database, and synthesized results. Two hundred six publications met our search criteria, with 83% reporting adverse/minimally adverse effects on wild salmonids. Adverse genetic effects on diversity were most common, followed by effects on productivity and abundance via ecological and genetic processes. Few publications (3%) reported beneficial hatchery effects on wild salmonids, nearly all from intensive recovery programs used to bolster highly depleted wild populations. Our review suggests hatcheries commonly have adverse impacts on wild salmonids in freshwater and marine environments. Future research on less studied effects—such as epigenetics—could improve knowledge and management of the full extent of hatchery impacts.

KEYWORDS
artificial propagation, hatchery salmonids, hatchery supplementation, salmonid captive-breeding, salmonid enhancement, salmonid stocking

1 | INTRODUCTION

For over one hundred years, hatcheries have been used to propagate and release salmonids across the globe (Jonsson, 1997; Waples, 1991; Zaporozhets & Zaporozhets, 2004), largely to subsidize fisheries, attempt to mitigate for habitat loss and overexploitation (Araki & Schmid, 2010; Hilborn, 1992; Maynard & Trial, 2014) and, more recently, to try to rebuild depleted populations of wild salmonids (Berejikian & Van Doornik, 2018; Hagen et al., 2021; Hess et al., 2012). Hatchery salmonids currently underpin many recreational, commercial, and (in the lower-48 of the United States in particular) legally obligated mitigation and tribal treaty fisheries, but the pervasive reliance on hatcheries remains contentious (Claussen & Philipp, 2022; Harrison et al., 2019; Kleiss, 2004). Although there is substantial evidence that hatchery salmonids generally have lower relative fitness than wild salmonids (Bouchard et al., 2022; Christie et al., 2014; Milot et al., 2013), continuing debate centers on the broad potential effects of releasing hatchery salmonids into nature and their potential impacts on sympatric wild salmonids (see Section 2 and Figure 1 for the definition of effect and impact), particularly when it comes to recovery of threatened and endangered populations (Araki & Schmid, 2010; Paquet et al., 2011; Young, 2013).

Evaluating and synthesizing the breadth of potential hatchery effects is complicated, however, because results may depend on

several factors. For instance, while adverse effects on wild salmonids have been commonly reported, others have found beneficial effects (Maynard & Trial, 2014; Miller et al., 1990; Naish et al., 2007), and publications cover a range of potential effects on different "Viable Salmonid Population parameters" (VSP: McElhany et al., 2000)—distribution (Laffaille, 2011), diversity (Bernaš et al., 2014), abundance (Willmes et al., 2018), and productivity of wild salmonids (Nickelson, 2003)—that may occur through different pathways such as ecological or genetic processes (Allendorf, 1991; Flagg et al., 2000; Neff et al., 2011), disease (Lamaze et al., 2014), or fishing (Hilborn & Eggers, 2000; Naish et al., 2007). Further, responses can differ among species (Araki & Schmid, 2010); the existing body of literature encompasses numerous salmonid species, and within species, there can be very different life histories such as individuals that migrate to the ocean and back (anadromous) or remain and mature in freshwater (resident) (Gossieaux et al., 2019; Maynard & Trial, 2014; Naish et al., 2007).

The source broodstock and intent of the hatchery program could also influence the type and magnitude of effects on wild fish. Traditional "production" type hatchery programs generally breed only hatchery individuals, often from a non-local source, and stock them to provide fisheries, and consequently, their effects could differ from modern "supplementation" programs that integrate some wild fish into their broodstock (to reduce genetic impacts) and release fish to enhance fisheries and the number of naturally spawning adults (Araki & Schmid, 2010; HSRG, 2015; Naish et al., 2007, Table 1). Moreover, smaller-scale "recovery" programs, including some captive breeding efforts, that rely solely on wild fish as broodstock to provide a short-term, conservation boost to highly depleted wild populations (Berejikian & Van Doornik, 2018; Janowitz-Koch et al., 2019) may offer more conservation benefits to wild salmonids than longer running supplementation programs that try to achieve multiple goals (Bowlby & Gibson, 2011; Naish et al., 2007).

Finally, large releases of hatchery salmonids also raise the potential for ecological effects in the North Pacific Ocean (Ruggerone & Irvine, 2018). An emerging body of research suggests hatchery salmon have triggered density-dependent responses in several co-mingling populations of wild salmonids, including but not limited to, reduced survival (Fukuwaka & Suzuki, 2000; Cunningham et al., 2018), growth (Kaerlyama et al., 2011), fecundity (Shaul & Geiger, 2016), and body size and abundance (Ruggerone et al., 2012).

The immense body of literature makes it difficult to interpret the information and results succinctly (Araki & Schmid, 2010). Research on the potential effects of hatchery salmonids on wild salmonids dates to the early-1900s and spans numerous species and three continents (Jonsson, 1997; Lichatowich, 2001; Maynard & Trial, 2014; Zaporozhets & Zaporozhets, 2004). In practice, scientists, managers, and policymakers may be familiar with studies in their region and on species they are tasked with managing and conserving but may be unaware of research outside their immediate scope of focus. For example, there have been numerous hatchery studies on Atlantic salmon (*Salmo salar*) and brown trout (*Salmo trutta*) that commonly reference one another (Horreo et al., 2014; Nilsson et al., 2008) and there are several publications on brook charr (*Salvelinus fontinalis*) (Bruce et al., 2020; Létourneau et al., 2018; Marie et al., 2010), yet those results are rarely cited or utilized in research on Pacific Salmon and vice-versa (e.g., Tatara & Berejikian, 2012; Wang et al., 2002). Accordingly, while several studies have reviewed hatchery effects on wild salmonids (Fraser, 2008; Naish et al., 2007), few have covered both *Oncorhynchus* and *Salmo* spp. (e.g., Araki & Schmid, 2010; Maynard & Trial, 2014), and to our knowledge, none have attempted to account for the entire breadth of publications for all species across the globe from freshwater to the ocean.

An evaluation of the overall body of peer-reviewed literature seems particularly valuable given the ongoing debate over hatchery practices in the western United States and other regions where salmonid recovery efforts are underway. A synthesis of publications from across the globe, covering various species and spanning freshwater and saltwater ecosystems would consolidate a broad array of literature and findings, and offer comprehensive insight into the patterns and processes of how hatchery salmonids potentially affect wild salmonids (Figure 1). For example, a synthesis could help determine: (1) How many studies have been published and how is the research distributed by year, country, species, and life history? (2) What proportion of publications reported adverse or beneficial hatchery effects on wild fish and how did those results vary by year, country, species, and life history? (3) Do potential effects differ based on the type of hatchery program? (4) Which VSP parameters (abundance, productivity, diversity, spatial distribution: McElhany et al., 2000) are most affected and what are the most common pathways of hatchery influence, such as genetic or ecological processes? and, (5) How many publications have evaluated potential hatchery effects in the open ocean and what are the general results so far? In turn, such an effort would help illuminate gaps in knowledge and areas for future research, increase the breadth of information available to decision-makers, and improve opportunities for collaborative research among scientists across different regions and countries.

2 | METHODS AND SYNTHESIS

2.1 | Objective and focus

Our objective was to collate all relevant peer-reviewed publications from across the globe and synthesize the main results—as presented by the authors—to answer broad-scale questions that are important to those tasked with researching, managing, and conserving salmonids (Figure 1). We also sought to incorporate the publications into an easily accessible database that can serve as a standing resource and be updated by scientists as new information comes to light (Appendix S1). In this effort, we reviewed only publications that explicitly and quantitatively evaluated whether stocking of hatchery salmonids affected the diversity, abundance, productivity (including effects on growth and survival as components of productivity), and distribution of wild salmonids via genetics, ecology, fishing, or disease (e.g., Berejikian & Van

FIGURE 1 Infographic displaying the rationale for the synthesis of research on how hatchery salmonids affect wild salmonids, how we define the terms effect(s) and impact(s), the literature search process, and the factors we considered when evaluating results from each publication. Although we identified 206 total publications, there are 207 total entries because Levin and Williams (2002) was counted twice, once for an adverse effect and once for no effect.

Doornik, 2018; Reisenbichler & Rubin, 1999). We did not seek to review publications that only compared *differences* between hatchery and wild salmonids, such as studies on the relative fitness of hatchery and wild individuals (e.g., Christie et al., 2014) unless the research also directly evaluated whether those effects influenced the recipient wild population of salmonids (e.g., Araki et al., 2009). Similarly, though epigenetic influences (i.e., effects arising through altered gene expression rather than changes to the genetic code) are increasingly recognized as important mechanisms for domestication (Le Luyer et al., 2017), we did not include epigenetic studies here because so far they have not directly addressed impacts to VSP characteristics in wild populations (but see Section 4 for emphasis that this topic deserves greater attention, and future iterations of our database will incorporate relevant studies as they become available). Ours was not a formal meta-analysis of quantitative effects, nor an assessment of fisheries that hatcheries can provide unless the study also examined whether fisheries potentially affected wild salmonids. Last, we use the terms effect(s) and impact(s) interchangeably, acknowledging they do not necessarily imply causation and can encompass statistical associations and/or model weights.

2.2 | Literature search

We conducted a literature search of peer-reviewed global publications focused only on research that directly evaluated how releases of hatchery salmonids potentially affected VSP characteristics of wild salmonids (*Oncorhynchus*, *Salmo*, *Salvelinus*, *Thymallus*) living in nature. We did not find any relevant literature on *Hucho* or Coregoninae. We used a modified search strategy based on guidelines from the Collaboration for Environmental Evidence for conducting a literature synthesis (Haddaway et al., 2018; Pullin et al., 2022: Figure 2). We started our search date with 1970 because preliminary searches found few publications prior to 1970 that matched our criteria (Table 2). Primary publications from 1970 (capturing a ramping up of searchable, relevant research) through May 29, 2021, were discovered via two English language searches in Web of Science (WOS) (Figure 2). We then reviewed a broad suite of publications to identify appropriate search terms that were relevant to our topic of interest and covered the array of descriptors used to characterize potential effects of hatchery salmonids on wild salmonids. Based on this foundation, we conducted a topic search (TS) using the descriptors: TS=(((hatcher* OR supplement* OR stock* OR enhance* OR artificial production* OR captive born OR introduced) AND (salmon* OR salmoni* OR steelhead OR char OR trout OR Oncorhynchus OR Salvelinus OR Salmo OR Grayling)) AND (effect* OR affect* OR outcome* OR respon* OR result* OR reestablish* OR restor* OR recover* OR collaps* OR influence* OR impact* OR chang* OR alter* OR increas* OR decrease* OR strength* OR weak* OR prevent* OR eliminat* OR assist* OR improv* OR reduc* OR replace* OR benefit* OR differ* OR consequenc* OR implicat* OR contribut* OR compensat* OR imped*

TABLE 1 Definition, description, and alternative terms used to classify different types of hatchery programs found in the literature review.

Hatchery type	Source of broodstock	Intent	Also referred to as
Production	Uses all or nearly all hatchery fish for broodstock, often but not always founded on non-local or non-native stock	Produce fish to support fisheries; rarely have conservation intent	Traditional, stocking, planting, releasing, supplementation, ocean ranching
Supplementation	Uses a proportion of wild fish as broodstock to help integrate hatchery and wild gene pool	Enhance fishery and supplement wild/natural populations, often run indefinitely	Supplementation, enhancement, conservation, supportive breeding
Recovery	Uses all or almost all wild fish for broodstock to fully integrate hatchery and wild gene pool	Rebuild wild populations by providing boost in abundance, sometimes no fishery focus, and temporary	Supplementation, enhancement, supportive breeding, captive breeding, conservation

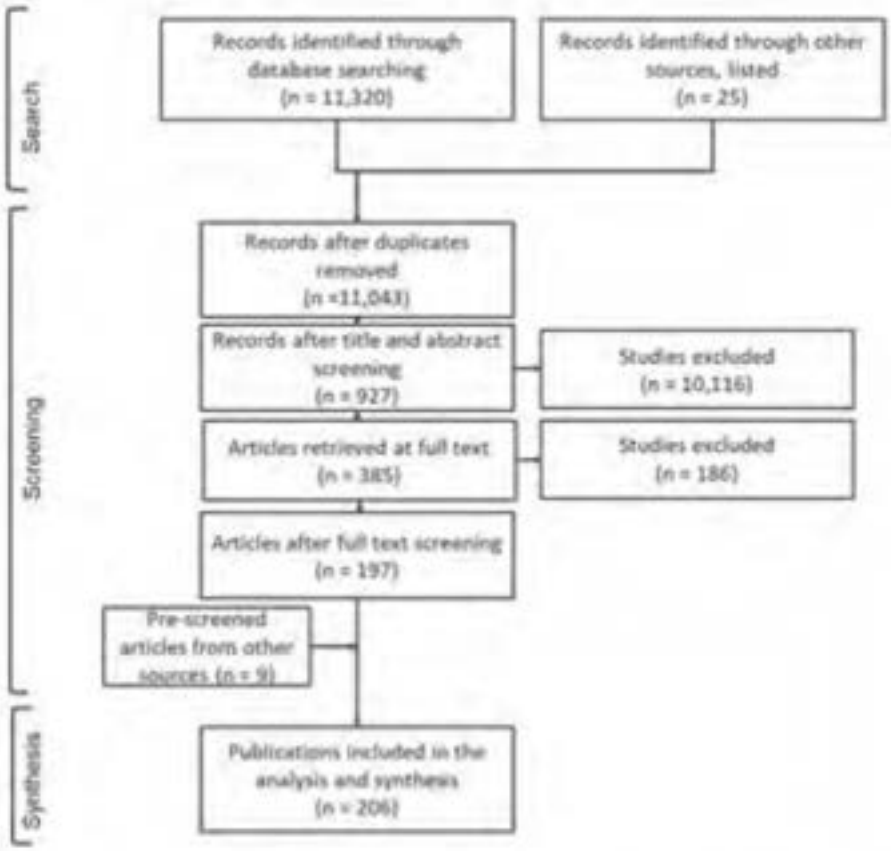

FIGURE 2 Flow diagram of the literature review process based on ROSES (RepOrting standards for Systematic Evidence Syntheses) flow diagram for systematic reviews (Collaboration for Environmental Evidence, 2018).

OR threat* OR caus* OR mask*) AND (gene* OR competition OR divers* OR producti* OR distribut* OR abundan* OR fitness OR demograph* OR evolution* OR ecolog* OR diverge* OR introgress* OR integrity* OR structure* OR life histor* OR portfolio OR size OR tim* OR space* OR spatial* OR densit* OR density dependen* OR growth OR surviv* OR predat* OR composit* OR interbreed* OR status OR trend OR hybrid* OR biomass OR disease* OR rate OR duration OR resilien* OR habitat* OR interspecific OR intraspecific OR regime OR manage*)). Next, we conducted a title search (TI) in WOS using the same descriptors.

2.3 | Selection process and criteria for inclusion

The WOS search revealed 11,320 potential publications, including 10,867 in the topic search and 453 in the title search (Figure 1). Following the decision tree outlined in Figure 2, duplicates were removed, and titles and abstracts were screened manually to identify publications that met the criteria to be eligible for our review (Table 2). To be included, first, the publication had to have been peer-reviewed and provide empirical data or a model that evaluated whether hatchery salmonids, via genetics, ecology,

TABLE 2 Criteria for inclusion of publications found during the search, including the type and year of the publication, hatchery type, the study focus, and review articles.

Criteria	Include	Exclude
Publication and years	Peer-reviewed in primary literature; 1970–2021	Non-peer-reviewed; prior to 1970
Hatchery type	Any production, supplementation, or recovery hatchery where fish are purposely released into nature	Net-pens where fish are not purposely released into nature
Study focus	Examined genetic, ecological, fishing, and/or disease effects of hatchery salmonids on wild salmonid abundance, productivity, diversity, and distribution	Examined how hatchery rearing and production affected wild salmonid performance, behavior, and traits (e.g., fitness of wild fish vs. hatchery fish)
Review publications	Contain new analyses, previously unpublished data	Summarize existing publications, no new analyses and/or data

TABLE 3 The sub-set of information for each publication that we used in our synthesis and summaries.

Attribute	Definition and/or classification
Year	Year study was published
Location	State, province, country of research
Hatchery species	Species of salmonid(s) that were studied
Life history	Did study focus on anadromous or freshwater resident (including freshwater migratory) species, or both
Habitat	Denotes whether study was conducted in freshwater or ocean or both
Hatchery type and intent	Hatchery classified as production, supplementation, recovery, or a combination thereof based on criteria in Table 1
Hatchery effect pathway	Denotes whether study examines, (1) genetic, (2) ecological, (3) fishing, or (4) disease effects, or combination thereof, on wild fish due to the presence of hatchery fish
Viable Salmonid Population parameter	Denotes whether study evaluates productivity, abundance, diversity, spatial distribution, or combination thereof
Genetic effect	Denotes which genetic attribute was analyzed, including diversity, population structure, effective population size, or a combination thereof
Effect on wild fish	Denotes whether hatchery effect on wild fish is adverse, minimally adverse, indeterminate, beneficial, or no effect if authors did not find any statistically significant effect

Note: See Table S1 in Appendix S1 for full description of all information included in the entire database.

fishing, or disease (i.e., hatchery effect process: Table 3), influenced VSP parameters that are fundamental to the viability of wild salmonids (McElhany et al., 2000). This also included publications that examined intra- and inter-species impacts of large releases of hatchery salmonids into the North Pacific Ocean (e.g., Frost et al., 2020; Ruggerone et al., 2012). Second, publications had to focus on hatchery programs that purposefully released fish into nature for fishing or conservation or both; we excluded publications on the effects of farmed salmon raised in net pens for direct consumption. Third, the search revealed numerous review articles. To minimize potential duplication, we only included reviews that contained new data or new analysis of previously collected data. Fourth, we excluded studies on inter-species impacts of introduced non-native resident salmonids, such as effects of non-native hatchery rainbow trout (*O. mykiss*) on native cutthroat trout (*O. clarkii*) in the United States' Intermountain West, because those results are clearly understood to be negative (Dunham et al., 2004; Hansen et al., 2019; Seiler & Keeley, 2009). Last, after reviewing papers on potential effects of hatchery salmonids in the open ocean, we identified and included an additional nine publications that were not found in the formal literature review (Figure 2).

2.4 | Classification and database of publications

We reviewed the full text of every publication that met our criteria with a strong focus on information that was most relevant to our synthesis, such as the study questions, the location and description of the hatchery programs, and the results of potential impacts on wild salmonids. Next, each publication was entered into a database created in R Core Team (2022), provided in Appendix S1,

and classified according to several relevant basic attributes so that each article entry includes associated columns with the authors, year, journal, DOI, the abstract, country, hatchery species, species interaction (e.g., intra- or inter-species hatchery effect), habitat (freshwater or ocean), life history (anadromous or freshwater resident or both), and study approach, which denoted whether it was an observation, model, experiment, or combination thereof (Table S1), but we only used a subset of these attributes in our analysis (Table 3).

We then classified the hatchery type and intent as production, supplementation, or recovery because previous studies (e.g., Berejikian & Van Doornik, 2018; Bingham et al., 2014; Bowlby & Gibson, 2011) and reviews (Araki & Schmid, 2010; Maynard & Trial, 2014; Naish et al., 2007) suggest potential effects on wild salmonids may vary in relation to the goal and broodstock sources of the hatchery program. We used criteria in Table 1 to define: (a) production hatcheries as those that solely or mostly use hatchery fish for broodstock, often but not always consisting of non-local or non-native strains, to produce fish for fisheries; (b) supplementation hatcheries as those that use a mixture of wild and hatchery fish for broodstock to improve genetic integration of the two populations and produce fish both to enhance fisheries and supplement natural spawners (e.g., Naish et al., 2007); (c) recovery hatcheries as those that use all or almost all wild fish for broodstock, including some captive brood programs, and produce fish solely to rebuild depleted stocks of wild salmonids (e.g., Berejikian & Van Doornik, 2018). Less commonly, we classified studies as including a combination of the different types of hatchery programs, such as Chilcote et al. (2011) which evaluated multiple stocks with a mixture of supplementation and production hatcheries.

Classifying the hatchery types was not always clear-cut, however. For instance, some publications used the term supplementation to describe the intent of hatchery programs that used non-local strains to "supplement" fisheries (e.g., Baer & Brinker, 2010; Baillie et al., 2016). Because they used non-local stocks and the hatchery releases were focused on production for fisheries, we classified them as production programs to be consistent with our criteria. In others, it was not clear from where the hatchery brood originated, but it was clear the focus was on fisheries (e.g., Hilborn & Eggers, 2000). Accordingly, we were cautious when classifying publications as supplementation programs unless there was sufficient information on the source of broodstock and intent (e.g., Fernández-Cebrián et al., 2014).

Next, we recorded the pathway of hatchery effect (i.e., genetic, ecological, fishing, disease) and VSP parameter(s) studied. Given the number of genetic publications on diversity, we further classified those studies according to the attribute that was analyzed, including diversity (e.g., Williamson & May, 2005), genetic population structure (e.g., Bruce et al., 2020), effective population size (e.g., Berejikian & Van Doornik, 2018), or a combination thereof such as both population structure and effective population size (e.g., Almodóvar et al., 2020).

We classified the hatchery effect on wild salmonids as adverse, minimally adverse, indeterminate, no effect, or beneficial (Table 3). To avoid any interpretative bias, we recorded the effect(s) directly as declared by the author(s). *Adverse and beneficial* refer to publications where the hatchery effect was determined by the authors to be harmful or helpful to the wild population, respectively. Adverse effects could include but are not limited to evidence of reduced productivity or abundance (e.g., Chilcote et al., 2011), or reduced diversity (e.g., Williamson & May, 2005) via unintended genetic introgression with hatchery fish (e.g., Cordes et al., 2006) or reduced effective population size (e.g., Gossieaux et al., 2019). Beneficial could denote effects such as evidence of increased effective population size (e.g., Hedrick et al., 1995), a demographic boost (e.g., Janowitz-Koch et al., 2019), or increased diversity and abundance from a critical level (e.g., Berejikian & Van Doornik, 2018). *Minimally adverse* refers to publications that found some negative effects on wild fish, but where those negative effects were inconsistent or explicitly reported by the authors as being minimal or slight (e.g., Finnegan & Stevens, 2008), while *indeterminate* refers to publications where both negative and positive effects were found (e.g., Small et al., 2009). *No effect* means the authors did not find a statistically significant effect for their measurement of choice (e.g., Wishard et al., 1984).

Last, we included an *effect summary*, a single sentence that encapsulated how the hatchery effects impacted the wild fish in relation to the VSP parameter(s) of interest. For instance, an effect summary could conclude that hatchery salmonids had a beneficial effect on the wild populations via increased genetic diversity (Berejikian & Van Doornik, 2018) or an adverse effect due to decreased genetic diversity (Bernaś et al., 2014).

2.5 | Questions and synthesis of information

After consolidating the research into a database, we synthesized the distribution of publications from 1970 to 2021 to summarize existing knowledge about how hatchery salmonids affect wild salmonids in freshwater and marine environments across the globe. Although the database contains a range of information which we provide in Appendix S1, hereafter we focus our analysis and results on five specific objectives:

1. To understand how the research effort was distributed, we first summed the total number of publications by year, country, species, habitat type, and life history.
2. Second, to synthesize the overall body of literature on hatchery effects on wild salmonids we summed the number of publications that reported adverse, minimally adverse, indeterminate, no effect, or beneficial effects on wild salmonids, and then calculated the proportion of different potential hatchery effects by year, country, species, and life history.
3. Third, we calculated the proportion of studies for each hatchery effect in relation to the hatchery's source of broodstock and

intent, which was classified as production, supplementation, recovery, or a combination thereof.

4. Fourth, to understand the potential ways hatchery fish impacted wild salmonids, for each hatchery effect we summed the number of publications in relation to the processes that contributed to the hatchery effect (genetic, ecological, fishing, disease, or a combination thereof), the affected VSP parameters (productivity, diversity, spatial distribution, and abundance, or a combination thereof), and if relevant, the type of genetic effect (diversity, population structure, effective population size, or a combination thereof).
5. Fifth, we tallied the number of publications that evaluated hatchery effects in the ocean and summarized the general results.

After evaluating those results, we identified potential data gaps and highlighted areas for future research in the Section 4.

3 | RESULTS

3.1 | Number of publications and database

After eliminating duplicates and reviewing titles, abstracts, and then full papers, we identified 206 relevant articles published between 1970 and 2021 (Figure 2). The literature search accounted for 197 of the publications, while nine studies in the ocean were identified through citations in other publications. One publication, Levin and Williams (2002), was counted twice in each component of the synthesis because the authors found adverse effects on one species and no effects on another; hence, hereafter we refer to 207 as the number of publications. The articles cover a wide range of observational studies, models, and experiments focused on *Oncorhynchus*, *Salmo*, *Salvelinus*, *and Thymallus* species in North America, Europe, and Asia. We also identified 50 review publications on the effects of hatchery fish on wild fish that could provide useful context and discussion points for this synthesis, though only four (Hilborn & Eggers, 2000; Naman & Sharpe, 2011; Ruggerone & Nielsen, 2004; Zaporozhets & Zaporozhets, 2004) provided new data and were therefore included in our synthesis (Appendix S1).

3.2 | Distribution of research by year, country, species, habitat, and life history

Our summary of publications revealed several results about how research was distributed in relation to several factors ranging from time to VSP parameters. First, the number of publications on the effects of hatchery salmonids on wild salmonids was unequal over time (Figure 3a). Publications per year steadily increased from 1973 and peaked at 15 publications in 2012, after which the number of publications per year slightly declined until the end of May 2021, when our search was concluded.

Second, we found publications from 22 different countries (Figure 3b). Among those, over half ($n = 113$) of the results focused on salmonid populations in the USA, followed by 20 in Canada, 11 in France, and 10 apiece in Spain and Norway (Figure 3b). Three to five publications each were found for the UK, Switzerland, Sweden, Poland, Russia, and Denmark.

Third, publications covered 15 species; among those, brown trout were the most researched with 39 publications, followed by steelhead ($n = 33$), Chinook salmon ($n = 28$), and Atlantic salmon ($n = 19$), compared to 14 publications on chum salmon, 11 on brook charr, and nine apiece on pink and coho salmon (Figure 3c). We also classified 11 studies as *Oncorhynchus* species, either because the analyses were not species-specific (e.g., Goodman, 2005) or they covered three or more species (e.g., Chilcote et al., 2011). One study was classified as Pacific salmon because they focused on multiple species of salmon in the ocean (Bigler et al., 1996), and we found two studies on grayling and one apiece for Amago salmon (*O. masou*), Arctic charr (*S. alpinus*), cutthroat trout, and golden trout.

Fourth, 181 studies evaluated hatchery effects occurring in freshwater, 23 in the ocean, and three were classified as both because they considered impacts in freshwater and the estuary (Levin & Williams, 2002; Nickelson, 2003). And, twice as many publications focused on anadromous life histories ($n = 132$) compared to resident life histories ($n = 64$), while only 12 publications included data on both life histories (Figure 3d).

3.3 | Synthesis and distribution of hatchery effects on wild salmonids

3.3.1 | All publications combined

Reported hatchery effects on wild salmonids ranged from adverse to beneficial, but the majority were adverse: 144 (70%) studies reported an adverse effect on wild salmonids and another 26 articles (13%) reported a minimally adverse effect (Figure 4). Thus, 83% of studies reported some degree of adverse effects from hatcheries on wild salmonids. Only seven publications (3%) reported beneficial effects of hatchery salmonids on wild salmonids, while 17 studies (8%) reported no hatchery effects on wild salmonids, and 13 (6%) were classified as indeterminate.

3.3.2 | Hatchery effects by year, country, species, and life history

Adverse or minimally adverse effects predominated the distribution of research across time, space, species, and life history. From 1970 through 2021, most publications each year reported adverse or minimally adverse effects on wild salmonids, except for 1994–1995 (Figure 3a). The first publication to report a beneficial hatchery effect occurred in 1995 followed by another publication in 2006,

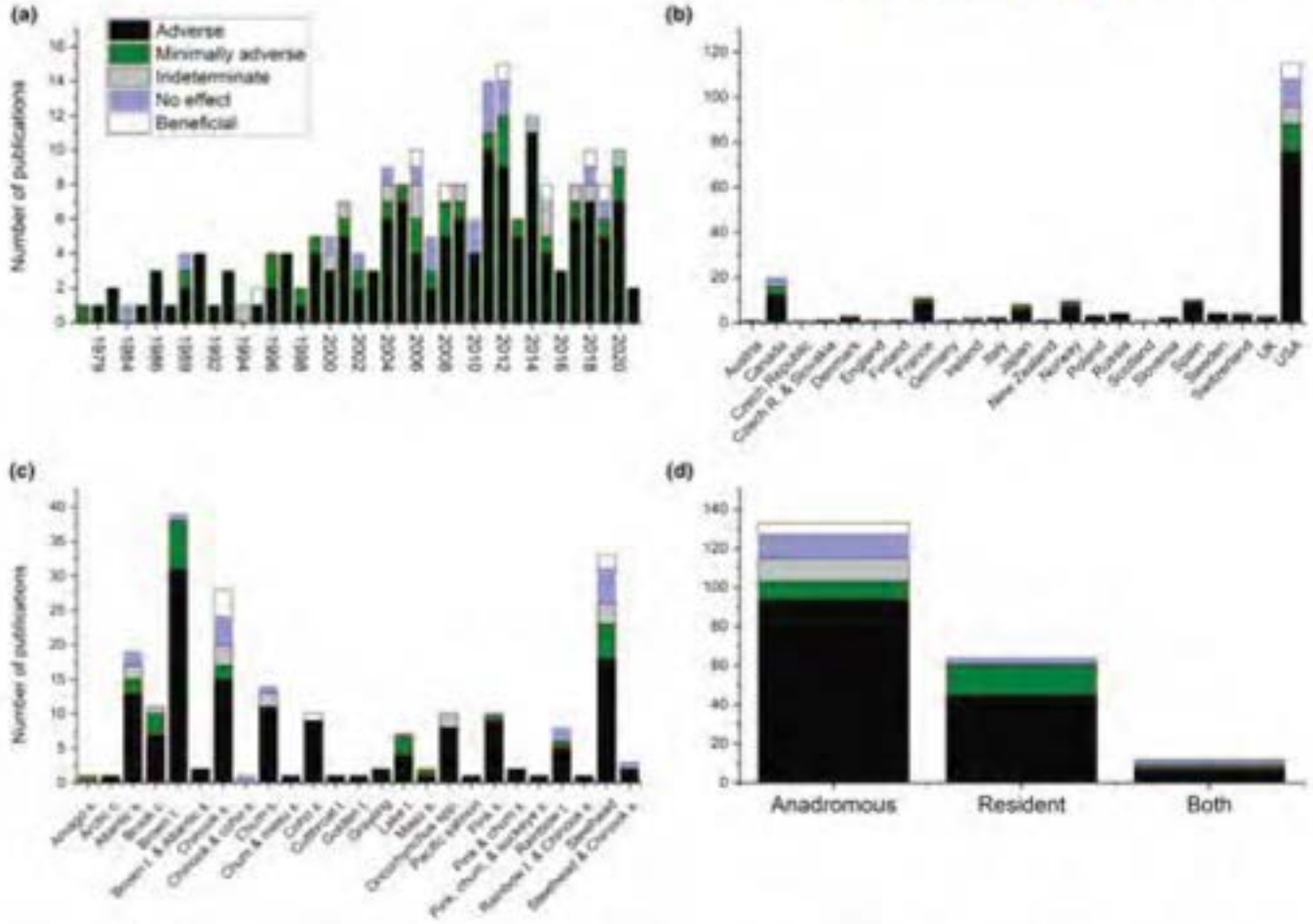

FIGURE 3 Distribution of publications by (a) year, (b) country, (c) species, and (d) life history in relation to the hatchery effect on wild fish, denoted as adverse, minimally adverse, indeterminate, no effect, or beneficial. Adverse and beneficial refer to publications where authors describe the hatchery effect as being negative or positive on the wild population, respectively. A minimally adverse effect refers to publications that found some negative effects on wild fish, but they were inconsistent, while indeterminate refers to publications where hatchery effects included aspects that had both negative and positive effects on the wild population or hatchery effects were almost immeasurable. No effect means that the authors did not find a significant hatchery effect on wild fish for the parameters they measured. In panel c., *Oncorhynchus* spp. refers to studies that focused on *Oncorhynchus* in general or included information on several species. There are 207 total entries because Levin and Williams (2002) was counted twice in each panel, once for an adverse effect on Chinook salmon and once for no effect on steelhead.

with the remaining five reports of beneficial effects being published thereafter as the number of publications increased.

Across the globe, 86 of 113 publications from the USA reported some type of adverse effect (adverse = 74, minimally adverse = 12), but it was also the only country to report beneficial effects (Figure 3b). In Canada and France, 12 of 20 studies and nine of 11 studies reported adverse effects, respectively, compared to nine of 10 in Spain and seven of 10 in Norway (Figure 3b). The Czech Republic and Scotland, with one study apiece finding no effect, were the only countries where an adverse or minimally adverse effect was not found, but overall, reports of no hatchery effect were rare outside North American countries.

For the most studied species, 37 of 38 brown trout publications reported adverse ($n = 31$) or minimally adverse hatchery effects ($n = 7$), compared to 17 of 28 for Chinook salmon (adverse = 15, minimally adverse = 2) and 15 of 19 studies on Atlantic salmon (adverse = 13, minimally adverse = 2; Figure 3c). For steelhead, 23 of 35 found adverse ($n = 18$) or minimally adverse effects ($n = 5$), including one study on "steelhead" from the Great Lakes where they are introduced (Bartron & Scribner, 2004); five of eight studies on resident rainbow trout also found adverse effects. Otherwise, 10 of 11 publications on brook charr and eight of nine each on pink and coho salmon reported adverse or minimally adverse effects, while beneficial hatchery effects were only reported for Chinook salmon, steelhead, and coho salmon.

Adverse and minimally adverse effects accounted for 102 of 132 publications on anadromous life histories and 60 of 64 publications on resident life histories (Figure 3d). Of the few publications that found a beneficial effect, six of seven were documented for the anadromous life history.

3.3.3 | Hatchery effect by hatchery type and intent

Most publications focused on production hatchery programs ($n = 143$) and more studies focused on supplementation programs ($n = 28$) than recovery programs ($n = 17$), while 19 studies accounted for a combination of production and supplementation hatcheries (Table 5). The proportion of studies reporting adverse effects on wild salmonids was 74% for production programs and 64% for supplementation programs. However, another 17% of the studies on production programs found minimally adverse impacts, while no minimally adverse effects were reported for supplementation programs (Table 5). On the contrary, 7% of the publications on supplementation programs found beneficial results and 17% indicated no effect, while 74% of the studies focused on both production and supplementation programs found adverse effects and 16% reported no effect.

For supplementation programs specifically, one publication reported a beneficial hatchery effect on abundance and productivity of natural-origin Chinook salmon (Fast et al., 2015) and another found releases of hatchery coho salmon increased abundance of naturally spawning fish without appearing to adversely affect wild productivity (Sharma et al., 2006). Nonetheless, adverse results from supplementation hatcheries were multiple and ranged from reduced diversity (Christie et al., 2012), productivity (Buhle et al., 2009), and abundance (Willmes et al., 2018) to altered run timing and spatial distribution (Hoffnagle et al., 2008).

The distribution of effects was more balanced for recovery programs, though the sample size was smaller (Table 5). Of the 17 studies on recovery hatcheries, the proportion of beneficial results (29%) was similar to the combined 30% of studies that found adverse (24%) and minimally adverse results (6%), respectively, while another 12% reported no effect and 29% were indeterminate. Of the five studies that reported beneficial effects from recovery hatcheries, four used all wild fish for broodstock, including two publications on the same long-term experiment on highly depleted populations of steelhead (Berejikian et al., 2008; Berejikian & Van Doornik, 2018) and the two on the same population of Chinook salmon (Hess et al., 2012; Janowitz-Koch et al., 2019). Adverse effects from recovery programs included decreased productivity in steelhead (Araki et al., 2009), reduced genetic structure (Lynch & O'Hely, 2001), and reduced diversity and productivity in Atlantic salmon (Bowlby & Gibson, 2011) and coho salmon (Willoughby & Christie, 2019).

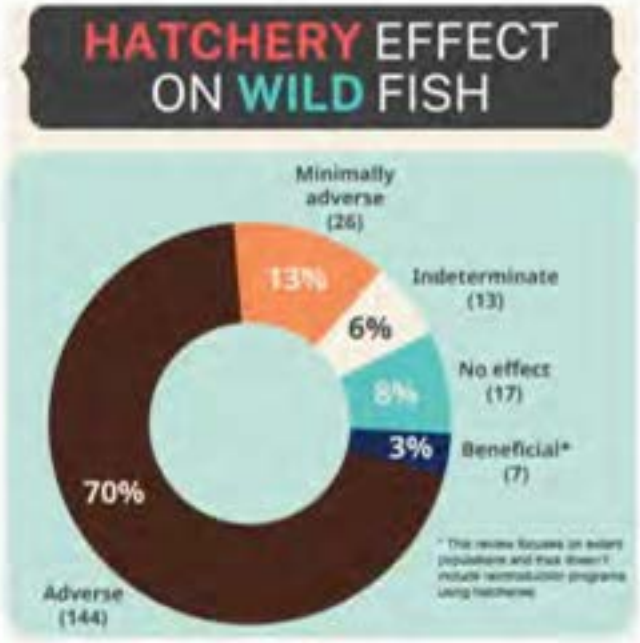

FIGURE 4 Donut plot displaying proportion (and number, in parentheses) of publications by the effect of hatchery salmonids on wild salmonids, including adverse, minimally adverse, indeterminate, no effect, and beneficial. There are 207 total entries because Levin and Williams (2002) was counted twice, once for an adverse effect and once for no effect.

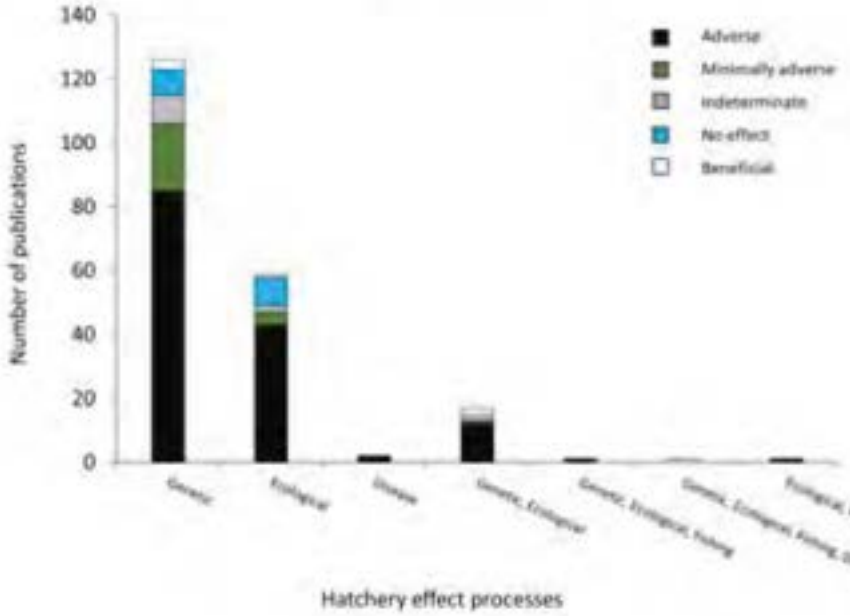

FIGURE 5 Distribution of publications in relation to the different processes through which hatchery fish affected wild salmonids, including ecological, genetic, fishing, disease, or some combination thereof in relation to the hatchery effect on wild population, denoted as adverse, minimally adverse, indeterminate, no effect, or beneficial. There are 207 total entries because Levin and Williams (2002) was counted twice in the ecological category, once for an adverse effect and once for no effect.

TABLE 4 Number of publications by their respective hatchery effect (adverse, minimally adverse, indeterminate, no effect, beneficial) on the Viable Salmon Population (VSP) parameter(s) of wild fish, including abundance (Abun.), distribution (Distr.), diversity (Diver.), productivity (Prod.), and various combinations thereof.

Effect on wild fish	Abun.	Distr.	Diver.	Prod.	Abun. & Distr.	Abun. & Diver.	Abun. & Prod.	Abun., Diver., and Prod.	Diver. & Distr.	Diver. & Prod.	Diver., Prod., & Distr.
Adverse	9 (1)	2	66	38 (14, 2)	1	3	14 (4)	4	1	4	1
Minimally adverse	2	1	20	3 (1)							
Indeterminate	1		8	2			2				
No effect		2	7	6 (4)	1		1				
Beneficial	1		1	2		2	1				
Total	13	5	102	51	2	5	18	5	1	3	1

Note: Adverse and beneficial refer to publications where authors describe the hatchery effect as being negative or positive on the wild population, respectively. A minimally adverse effect refers to publications that found some negative effects on wild fish, but they were inconsistent, while indeterminate refers to publications where hatchery effects included aspects that had both negative and positive effects on the wild population or hatchery effects were almost immeasurable. No effect means that the authors did not find a significant hatchery effect on wild fish for the parameters they measured. In cells with parentheses, the number refers to the number of studies in ocean, and if present, the second number after the comma refers to the number of studies in both the ocean and freshwater. Bolded and underlined values represent the VSP parameter with the greatest number of studies for each respective effect on wild fish. There are 207 total entries because Levin and Williams (2002) was counted twice in the productivity category, once for an adverse effect and once for no effect.

3.3.4 | Hatchery effect pathways and genetic effects

More publications ($n = 126$) tested or evaluated how hatchery salmonids affected wild salmonids via genetics than other pathways, and most reported adverse ($n = 85$) or minimally adverse effects ($n = 21$), while fewer were indeterminate ($n = 9$), found no effect ($n = 8$), or reported a benefit ($n = 3$) (Figure 5). Adverse effects also predominated ($n = 44$) among the 60 ecological studies, and 12 of the 17 articles focused on a combination of genetic and ecological processes found adverse results. Potential disease and fishery effects were far less studied. Outside of a review by Naish et al. (2007), we found only two publications that evaluated potential effects of disease and parasites (Lamaze et al., 2014; Robinson et al., 2020) and three that included fishery effects as a component of their research (Baer & Brinker, 2010; Fast et al., 2015; Hilborn & Eggers, 2000).

The strong genetic focus is why one VSP parameter, diversity, was also commonly represented in 102 publications, 86 of which reported adverse ($n = 66$) or minimally adverse effects ($n = 20$) (Table 4). This was particularly true for brown trout, where 35 of 39 publications focused on diversity. An additional 13 studies included genetic diversity as a component and 12 found adverse effects. Of the 115 genetic-centric studies, most focused on potential effects on population structure ($n = 59$), followed by various measures of genotypic/allelic diversity ($n = 25$) and effective population size ($n = 7$). The remaining 10 genetic articles were combinations of population structure, diversity, and effective population size.

Examples of adverse genetic effects included, but were not limited to, changes in population structure (Ayllon et al., 2006; Thaulow et al., 2012) stemming from an increased frequency of hatchery-origin alleles in wild populations (Caudron et al., 2009; Létourneau et al., 2018), reduced effective population size in wild populations with hatchery releases (Almodóvar et al., 2020; Hagen et al., 2021), replacement of wild salmonids by hatchery salmonids (e.g., Quiñones et al., 2013; Reisenbichler & Rubin, 1999), and reduced resistance to parasitic infections (Lamaze et al., 2014). In the single beneficial publication on diversity, a recovery hatchery program increased the effective population size in an endangered population of salmon (Hedrick et al., 1995), although as mentioned below, benefits to diversity were found in other publications that measured multiple VSP parameters.

After diversity, most publications focused on productivity, abundance, and a combination of productivity and abundance (Table 4). Of the publications on productivity, 30 were conducted in freshwater, 18 in the ocean, and three in both freshwater and an estuary. In freshwater, 22 of 30 studies found adverse effects on the productivity of wild salmonid populations (e.g., Chilcote et al., 2011; Jonsson et al., 2019; Skaala et al., 1996), while two apiece found no effect (e.g., Courter et al., 2019) or were indeterminate (e.g., Riley et al., 2005). In addition, nine of 13 studies on abundance and 14 of 18 studies on productivity and abundance in freshwater reported adverse effects, such as reduced productivity and abundance of wild salmonid populations (e.g., Byrne et al., 1992; Young, 2013) and reduced abundance and individual

TABLE 5 Number of publications (proportion in parentheses) and hatchery effect on wild fish by hatchery type.

Hatchery type	Adverse	Minimally adverse	Indeterminate	No effect	Beneficial
Production	108 (75%)	24 (17%)	4 (3%)	7 (5%)	0 (0%)
Supplementation	15 (64%)	0 (0%)	3 (11%)	5 (18%)	2 (7%)
Recovery	4 (24%)	1 (6%)	5 (29%)	2 (12%)	5 (29%)
Production, supplementation	14 (74%)	1 (5%)	1 (5%)	3 (16%)	0 (0%)

Note: Hatchery types include: production, supplementation, recovery, or a combination of production and supplementation or supplementation and recovery. Production refers to hatcheries that use all or nearly all hatchery fish for broodstock, which are often from a non-local source, and focus on producing fish for fisheries; supplementation refers to programs that integrate local wild and hatchery fish for broodstock to enhance fisheries and supplement wild populations; and a recovery program focuses strongly on conservation and uses mostly or all wild fish (fully integrated) to try and rebuild wild populations by providing a boost in abundance (often temporary). There are 207 total entries because Levin and Williams (2002) was counted twice in the production and supplementation category, once for an adverse effect and once for no effect.

condition of wild juveniles (Noble, 1991). The six remaining publications that reported benefits to abundance and productivity or a combination thereof all occurred in freshwater (e.g., Berejikian & Van Doornik, 2018; Janowitz-Koch et al., 2019). Effects on distribution and combinations of parameters including distribution were less represented than the other three VSP parameters (e.g., Hoffnagle et al., 2008; Love Stowell et al., 2015; Table 4).

3.3.5 | Hatchery effects in ocean

Hatchery effects on salmonids in the ocean involve competition for prey, potentially leading to reduced growth, body size and fecundity, delayed maturation, lower productivity, and fewer wild salmon. We found 23 studies on potential hatchery effects. Thirteen of those examined hatchery effects on local populations of wild salmon in the ocean, of which nine (69%) were adverse, one (8%) was minimally adverse, and three (23%) found no effect (Table S2). One of the three no-effect publications focused explicitly on potential juvenile competition in nearshore habitats during early marine residence (Sturdevant et al., 2011), while the other two focused on adult hatchery Chinook salmon production (Ohlberger et al., 2018; Nelson et al., 2019). Most other publications examined correlations between hatchery chum salmon and pink salmon and the productivity and growth wild adult salmon in the ocean (e.g., Cunningham et al., 2018; Frost et al., 2020; Ward et al., 2017).

We also included 10 studies that examined total salmon density effects on wild salmon in which hatchery salmon were an important component (additional studies involving density dependence at sea are available); nine (90%) of these studies reported adverse effects of density dependence on wild salmon while inferring an adverse effect of abundant hatchery salmon stemming from production hatcheries in Asia and North America (Table S2). Declines in the growth of all salmon species across most of their range are the most commonly observed effect of density dependence, including hatchery production (Bigler et al., 1996; Oke et al., 2020). Though not included in our analyses because it did not explicitly evaluate hatchery fish and in contrast to most results, Shuntov et al. (2019, 2020) argued that competition for prey at sea is minimal because prey biomass is exceptional and because salmon consume a small fraction of the available prey. However, this assessment cannot explain the density-dependent biennial patterns observed in Pacific salmon metrics (growth, abundance, productivity, maturation) in response to the biennial abundances of highly abundant pink salmon (Ruggerone et al., in press; Ruggerone & Connors, 2015; Ruggerone & Nielsen, 2004), of which many are hatchery fish (Ruggerone & Irvine, 2018).

4 | DISCUSSION

Hatcheries are used worldwide to produce salmonids for purposes ranging from providing fish for harvest to rebuilding endangered stocks and meeting Treaty responsibilities (Araki & Schmid, 2010; Maynard & Trial, 2014; Nalsh et al., 2007), but a strong dependence on hatcheries has also generated controversy and debate (Brannon et al., 2004; Claussen & Philipp, 2022; Harrison et al., 2019; Holt et al., 2008). Clarity in this discourse is partly obscured, however, by the sheer volume of complex research that dates back several decades, covers numerous species, and spans three continents, which makes it difficult to interpret succinctly the existing weight of evidence. We sought to provide a transparent, reproducible, and updatable synthesis and database of the current global research evaluating the impacts of hatcheries on wild populations, while purposefully not delving into the complex social and political desires or tribal Treaty and mitigation legal obligations surrounding hatcheries. Our review of over 50 years of peer-reviewed publications on how hatchery salmonids affect wild salmonids found most research reported adverse or minimally adverse hatchery effects across time, species, and countries, even for supplementation-type hatcheries, while reports of beneficial effects on wild salmonids were scarce except for a few very specific situations (e.g., Berejikian & Van Doornik, 2018; Hess et al., 2012). We hope this database serves as a useful standing resource that can be used and built upon to improve the breadth of science incorporated into decision-making.

Prior reviews have summarized overarching hatchery practices and processes, identified potential adverse impacts, and evaluated the role of hatcheries in salmonid fisheries and recovery (Fraser, 2008; Jonsson, 1997; Maynard & Trial, 2014; Naish et al., 2007). More similar to Miller et al. (1990) and Araki and Schmid (2010), we attempted to census the balance of existing peer-reviewed literature and provide summaries of each publication (Appendix S1). Miller et al. (1990) reviewed 316 hatchery projects, including numerous supplementation programs, across the western USA and Canada and in New England states working with Atlantic salmon. Of those, only 25 projects, or 8%, successfully supplemented existing runs of wild salmonids, and while adverse impacts to wild stocks were reported or postulated for almost every type of hatchery situation where the intent was to rebuild wild runs. The authors also suggested a bias toward not reporting negative or unsuccessful results. Two decades later, Araki and Schmid (2010) synthesized 266 hatchery case studies covering several species of fish, including 70 on salmonids, 51 of which (72%) reported adverse impacts ranging from deleterious effects of hatchery rearing on fitness in nature to reduced genetic variation in populations of hatchery fish. Our review of 208 publications found 70% reported adverse hatchery effects and another 13% found minimally adverse effects, while just 3% reported beneficial effects. Although we likely missed some relevant publications despite a transparent search process and did not include research on reintroductions using hatchery salmon (e.g., Liermann et al., 2017) or domestication effects on wild fish reared in hatcheries (e.g., Christie et al., 2016), the overall balance of results across three reviews and hundreds of studies appear relatively similar.

One possible reason for the preponderance of adverse effects across time, space, and species is most publications in our review assessed traditional, production hatcheries that focused on producing fish for fisheries, often but not always from non-local broodstock. Adverse effects on wild salmonids from such programs are well documented (Almodóvar et al., 2020; García-Marín et al., 1999; Marie et al., 2010). This was particularly true for brown trout, the most studied species, where many publications evaluated possible genetic effects of non-local hatchery stocks across Europe, often finding adverse genetic impacts (Araguas et al., 2017; Hansen et al., 2009; Thaulow et al., 2012). However, adverse effects also accounted for 63% of the publications that evaluated potential impacts from supplementation programs that use some or mostly wild fish and frequently employ breeding protocols to try to reduce deleterious genetic effects (Hutchings, 2014; Neff et al., 2011; Pinter et al., 2019).

Adverse effects from supplementation programs could be related to a suite of factors that are not dissimilar from production programs. For example, supplementation broodstock is generally derived from local populations to reduce potential genetic impacts; however, a review of 51 estimates of annual productivity from six studies on four salmon species found the relative fitness of early-generation hatchery individuals was about half that of wild fish (Christie et al., 2014), while another found hatchery salmonids displayed lower genetic variation than wild populations (Araki & Schmid, 2010). Interbreeding with individuals that have lower fitness and less diversity, among other differences, can reduce the diversity (Hagen et al., 2021), effective population size (Christie et al., 2012; Hagen et al., 2021), and productivity of wild populations (Goodman, 2005; Jonsson et al., 2019; Reisenbichler & Rubin, 1999). Depending on the intensity and duration of stocking, the gene pool of the wild population may eventually be compromised by high levels of hatchery influence, as evidenced by studies on brown trout in Europe (Fernández-Cebrián et al., 2014; Hauser et al., 1991; Pustovrh et al., 2012) and brook charr in North American (Létourneau et al., 2018); in the extreme, hatchery salmonids may replace wild fish (Largiadèr & Scholl, 1996; Quiñones et al., 2013).

In addition, although a key goal of supplementation hatcheries is to enhance opportunities for harvest, in some populations and years large numbers of returning hatchery salmon escape fisheries or are allowed intentionally to spawn, leading to many more total salmon than can be supported by the habitat and heighten density-dependent effects (HSRG, 2020; ISAB, 2015). We found studies where hatchery juveniles reduced the abundance and productivity of wild juveniles (Nickelson et al., 1986; Warren et al., 2014). Competition for habitat likely contributed to declines in wild coho salmon on the Oregon coast, USA, where density-dependent effects were five times greater for hatchery salmon than wild salmon and the productivity of several wild populations decreased as hatchery releases increased (Buhle et al., 2009; Nickelson, 2003). Adverse effects may thus depend on genetic and ecological pathways and the intensity of stocking, and such effects may be more common than anticipated if supplementation programs do not meet their own goals for reducing risk (e.g., targeted levels of wild integration into broodstock) and limitations of habitat capacity are not considered (Anderson et al., 2020). Regardless, interbreeding with less fit individuals and increased competition for habitat may help explain why both production and supplementation programs negatively influenced productivity of several populations of wild steelhead (Chilcote et al., 2011), and why a long-term effort to increase natural-origin Chinook salmon did not find a positive effect on abundance after releases were ceased (Scheuerell et al., 2015; Venditti et al., 2018).

Hatcheries can also benefit wild salmonids, though the situations appear nuanced. For instance, hatcheries have helped re-establish extirpated populations of salmonids (Galbreath et al., 2014), prevent extinction (Kline & Flagg, 2014), and jump-start recolonization following dam removal (Liermann et al., 2017). While those efforts did not meet the criteria for inclusion in our synthesis (e.g., effects on wild fish could not, or were not, evaluated due to extirpation or near extinction levels of abundance), in the publications we reviewed nearly all benefits occurred when recovery-type programs were used to provide a demographic boost to endangered populations of salmonids. Examples include small releases of hatchery smolts from a short-term, temporary captive-broodstock program to increase abundance and diversity of steelhead populations that were almost extirpated (Berejikian et al., 2008; Berejikian & Van Doornik, 2018), and a carefully controlled hatchery program that bred only wild fish to boost abundance of a highly depleted population of Chinook salmon (Hess et al., 2012; Janowitz-Koch et al., 2019). However, two of the four beneficial studies reported on the same populations, which tilts

the proportion of results given the relatively small number of publications, and other publications warn that even improved hatchery practices can still pose significant ecological and genetic risks to wild fish over the long term (Oosterhout et al., 2005), such as competition for food and habitat (ISAB, 2015) and reduced genetic diversity and divergence from the wild population (Bingham et al., 2014). Consequently, beyond 4–6 generations a loss in fitness can outweigh any increase in abundance from hatchery production and cause the population to decline (Bowlby & Gibson, 2011). Nonetheless, our review, like others (Maynard & Trial, 2014; Naish et al., 2007), suggests the balance of effects for recovery hatcheries is less skewed, with as many studies reporting beneficial or no effects as adverse ones.

Within the array of publications we reviewed, most research focused on hatchery effects that occurred via genetic interactions and found adverse impacts on wild salmonids, such as reduced diversity (Garcia-Marin et al., 1999; Perrier et al., 2013; Willoughby & Christie, 2019) and altered genetic structure of wild populations (Valiquette et al., 2014; Weigel et al., 2019; Wenne et al., 2016), though adverse effects on growth (Hasegawa et al., 2014, 2018; McMichael et al., 1997), productivity (Buhle et al., 2009; Nickelson, 2003) and abundance (Nickelson et al., 1986; Quiñones et al., 2013; Willmes et al., 2018) via ecological or both ecological and genetic processes were also reported. The frequency of adverse genetic impacts may have consequences for the resilience of wild fish moving forward. As an example, research on brown trout found long-term supplementation significantly reduced genetic diversity among locations and compromised the conservation of local genetic variation (Fernández-Cebrián et al., 2014), which threatened biodiversity in their southern range (Cagigas et al., 1999; Horreo et al., 2014; Splendiani et al., 2019). A tremendous amount of money and effort has been invested in restoring habitat to improve population productivity and increase carrying capacity (ISAB, 2015), and help offset future effects from climate change (Beechie et al., 2013; Bilby et al., 2022), an action demonstrated to increase wild fish abundance more effectively than species-specific stocking efforts (Radinger et al., 2023). Because the resilience of salmonids also depends on their functional genetic capacity to survive and reproduce in a changing environment (Kardos et al., 2021), future research could help illuminate the extent to which, if any, alterations to genetic diversity may influence returns on habitat investments where both hatchery and wild fish co-exist.

Our literature review also revealed an extensive body of research focused on potential effects of annual releases of 4.5 billion hatchery Pacific salmon into the North Pacific Ocean, which represents 40% of the total mature and immature salmon biomass in the North Pacific Ocean (Ruggerone & Irvine, 2018). The combination of publications on the specific abundance of hatchery salmon and overall abundance of hatchery and wild salmon at sea suggest heightened abundances, particularly of hatchery chum salmon and pink salmon, have triggered density-dependent effects in wild populations resulting in reduced growth, body size, fecundity, productivity, and abundance, and delayed maturation (Table S2). For example, research has found adverse effects of hatchery or total chum salmon abundance on the growth, productivity, and abundance of wild chum salmon (Frost et al., 2020; Kaeriyama et al., 2011; Ruggerone et al., 2012), of total hatchery and wild pink salmon and chum salmon on body size, age, productivity, and abundance of Chinook salmon across their range (Cunningham et al., 2018; Oke et al., 2020; Ruggerone et al., in press), and of hatchery pink salmon on productivity of wild sockeye salmon populations in British Columbia and Alaska (Connors et al., 2020). While it is difficult to disentangle correlation and causation, the strong biennial patterns in abundant pink salmon cannot be explained by the environment alone (Batten et al., 2018; Ruggerone & Connors, 2015; Ruggerone et al., in press) and, consequently, concerns for wild salmon have led scientists to call for international discussions, limits on hatchery production, and hatchery taxes (Holt et al., 2008; Malick et al., 2017; Peterman et al., 2012).

Considering the balance of the research herein, we selected four topics that remain underrepresented and seem important to clarifying science and management opportunities moving forward. First, effects on genetic diversity of wild salmonids are well studied but investigation of epigenetic effects as a possible biological pathway for these (and other) effects has only begun (Koch et al., 2022). Christie et al. (2016) found a single generation in a hatchery environment altered the expression of over 700 genes in steelhead. Other research has found similar results (Leitwein et al., 2022), even in the absence of genetic differentiation between wild and hatchery populations (Le Luyer et al., 2017), and the potential for the epigenetic changes to be passed along to offspring (Leitwein et al., 2021; Venney et al., 2023). Though the duration of impacts remains unclear it is hypothesized that heritable epigenetic effects may alter the evolutionary trajectory of wild populations, which is a critical issue to evaluate where hatchery salmonids are allowed to or are able to breed with wild salmonids (Skinner & Nilsson, 2021). Second, future research could illuminate the adaptive consequences of genetic changes sustained by wild salmonids (Neff et al., 2011) and whether accumulated effects inhibit their capacity to keep pace with climate change (e.g., Munsch et al., 2022) or respond positively to habitat restoration efforts. Third, large-scale experiments that evaluate multiple VSP parameters before, during, and after supplementation, such as Berejikian and Van Doornik (2018), are scarce, but well-designed experiments could help parse out natural spatial and temporal variability in environmental capacity from hatchery effects and offer greater clarity regarding the risks and benefits of hatchery programs.

Last, few publications evaluated disease or fishery effects despite demonstrated mechanisms of influence, such as decreased resilience to parasites associated with hatchery genotypes (see, Lamaze et al., 2014) and mixed stock fisheries on abundant hatchery stocks that are unsustainable for wild stocks (Naish et al., 2007). It is possible our search string did not fully capture the breadth of literature on fishery effects, or such analyses are less frequently published in peer-reviewed journals. Naish et al. (2007) analyzed fishery data from management reports and described a long history of overharvesting weaker wild stocks in intensive hatchery fisheries, which ultimately led to changes in fishery policy in the United States, but direct references to studies that met our criteria were rare. Understanding how such impacts have and continue to affect wild stocks could provide further insight, though in some cases identifying potential changes to

wild populations may require a longer-term perspective using historical data (e.g., McMillan et al., 2022).

5 | CONCLUSION

We created an easily accessible database focused on publications that examined potential effects of hatchery salmonids on wild salmonids, and then synthesized the large body of research to better understand how studies and potential hatchery impacts were distributed in relation to time, space, species, habitat, hatchery type, and other factors. Except in a few specific situations when recovery hatcheries were used to boost the abundance of wild salmonids threatened with extinction, hatchery effects on wild salmon were predominantly adverse across time, species, and countries, even when using more modern supplementation hatchery programs and practices. In addition, evidence indicates large releases of hatchery chum and pink salmon in the North Pacific Ocean alter the growth, survival, and abundance of wild salmonids that rely on the same common pool prey resource. These results have implications for conserving and sustaining wild salmonids and for extensive investments in salmon recovery across the globe. In conclusion, while there is a long history of debate over the widespread use of hatcheries, our results were consistent with prior reviews by Miller et al. (1990) and Araki and Schmid (2010), the combination of which clearly indicate that, from a scientific standpoint, hatcheries typically pose numerous risks that commonly result in negative impacts to the diversity, productivity, and abundance of wild salmonid populations. These negative impacts likely limit the efficacy of habitat restoration efforts aimed at rebuilding wild salmonid populations and the adaptive capacity of wild salmonids to keep pace with a changing environment, especially climate warming.

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CONFLICT OF INTEREST STATEMENT

The authors declare there are no competing interests.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available in the supplementary material of this article in the database provided in Appendix S2.

ETHICS STATEMENT

The manuscript has not been submitted for publication or published in another journal and because the manuscript is a review of prior publications on fish, information on ethical treatment of humans and animals is not applicable.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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REVIEWS AND SYNTHESIS

Infectious disease, shifting climates, and opportunistic predators: cumulative factors potentially impacting wild salmon declines

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Abstract

Emerging diseases are impacting animals under high density culture, yet few studies assess their importance to wild populations. Microparasites selected for enhanced virulence in culture settings should be less successful maintaining infec tivity in wild populations, as once the host dies, there are limited opportunities to infect new individuals. Instead, moderately virulent microparasites persisting for long periods across multiple environments are of greatest concern. Evolved resistance to endemic microparasites may reduce susceptibilities, but as barriers to microparasite distributions are weakened, and environments become more stressful, unexposed populations may be impacted and pathogenicity enhanced. We provide an overview of the evolutionary and ecological impacts of infectious diseases in wild salmon and suggest ways in which modern technologies can elu cidate the microparasites of greatest potential import. We present four case stud ies that resolve microparasite impacts on adult salmon migration success, impact of river warming on microparasite replication, and infection status on suscepti bility to predation. Future health of wild salmon must be considered in a holistic context that includes the cumulative or synergistic impacts of multiple stressors. These approaches will identify populations at greatest risk, critically needed to manage and potentially ameliorate the shifts in current or future trajectories of wild populations.

Introduction

Pacific Salmon are iconic fish that not only provide great economic, cultural and social benefit to humans (Lich atowich 1999) but are considered keystone species due in part to the tremendous nutrients they provide to both terrestrial and aquatic ecosystems as both live prey and decomposing carcasses (Cederholm et al. 1999). As anad romous fish, Pacific salmon hatch in freshwater lakes and streams, typically migrating to the ocean after 3 24 months where they may travel thousands of kilometers to reach feeding grounds before returning as mature adults for a single spawning event in the same natal rear ing areas in which they were born (see Groot and Mar golis 1991 for summary of the immense variation in life history within this general framework). Their high fidelity to natal streams and lakes has created strong genetic seg regation among populations shaped by both demography and selection, especially for species that migrate long dis tances upstream to spawn (e.g., Sockeye [*Oncorhynchus nerka*] and Chinook [*O. tshawytscha*] Salmon; Beacham et al. 2006a,b).

Productivity (measured as adults produced per spawner) of southern US populations of Coho (*O. kisutch*) and Chinook Salmon has been declining for decades; almost half of the most southerly distributed populations of Coho Salmon have become extirpated, while many others are listed as threatened or endangered (Nehlsen et al. 1991; Brown et al. 1994). In southern British Columbia, populations of Coho Salmon began declining in the 1980s, followed by Chinook Salmon in the late 1980s and Sockeye Salmon in the early 1990s (Beamish et al. 1995; Peterman and Dorner 2012; Beamish et al. 2012). Alternately, during this same period, Pink (*O. gorbuscha*) and Chum (*O. keta*) Salmon, both species that have the shortest duration of freshwater residency, have been increasing in productivity (Irvine and Fukuwaka 2011).

In Canada, owing to the high profile Fraser River salmon populations, the changes in fish population abundances have garnered much public and political attention. Coincident with the general patterns of declining productivity have been greater annual fluctuations in numbers of fish returning to the fishery (Sharma et al. 2013) which are often not accurately predicted by current management models (Haeseker et al. 2008; Hinch et al. 2012; Grant et al 2010). Predicting returns of Sockeye Salmon have been the most problematic, with preseason forecasts (defined as the mid point of the distribution of probable returns) off by 10s of millions of fish in some years (Peterman and Dorner 2011; Grant and MacDonald 2012). In 2009, Fraser River Sockeye Salmon experienced the lowest returns in over 60 years, with only 14% of the predicted 10.5 million returns arriving to the river (Peterman and Dorner 2011). This event combined with recent declines spurred Canada's Prime Minister to call for a public inquiry into the Decline of Sockeye Salmon in the Fraser River ('Cohen Commission', www.cohencommission.ca/en/). The following year was just as anomalous, with >28 million fish returning to spawn (S. Grant, unpublished data), nearly three times the median predicted by the run size forecast models, but still within the forecast range (Grant et al. 2010).

The Cohen Commission of Inquiry was tasked with assessing the scientific evidence to determine the cause of the declines in Fraser River Sockeye Salmon productivity as well as reviewing management practices and how scientific information is utilized to inform management decisions (Cohen 2012a). Although no single 'silver bullet' cause for the declines was identified, climate change impacting early ocean rearing conditions, infectious disease, predators, and aquaculture were considered perhaps most important of proposed factors, with a strong recognition that multiple cumulative stressors, some which may interact, were likely involved. In his final report (Cohen 2012b), Cohen suggested that the supporting science needs to move from basic understanding of adaptive responses to single stressors to predictive tools that can integrate the effects of multiple stressors.

While the situation for Coho, Chinook and Sockeye Salmon in BC appears dire for many populations, the fact that some populations are still performing moderately well suggest that both plastic and evolutionary mechanisms are contributing to responses to stressors associated with declines in abundance. In this special issue, we were asked to provide new insight into the evolutionary and ecological role of infectious disease in wild populations. Herein, we provide an extensive review of the conceptual background and current state of knowledge surrounding infectious disease impacts on wild salmon populations, and the potential interplay between two additional stressors, temperature, and predators, which may associate with salmon declines and influence or be influenced by infectious disease. We restrict most of our focus to microparasites (viruses, bacteria, myxozoans, and some fungi), as their instability and ability to exponentially replicate over very short periods of time enhances their potential for associating with population level impacts (Bakke and Harris 1998). This assertion is backed by several reviews of wildlife disease outbreaks around the world, for which very few have been caused by macroparasites (Dobson and Foufopoulos 2001; Lafferty and Gerber 2002). We present evidence for phenotypic variation among populations that may result in different outcomes from each of these stressors and explore the evolutionary mechanistic responses that have been demonstrated to date. We note that there is a bias in our examples toward wild salmon in BC. We then present four case studies that each present novel approaches to address hypotheses on ecological and evolutionary consequences of single and cumulative stressors involving infectious agents. These studies take a population approach rather than a traditional veterinary focus on diagnosis and treatment, similar to that of Lyles and Dobson (1993) and the review by Lafferty and Gerber (2002). These case studies all incorporate a broad based molecular microparasite monitoring approach capable of assessing the presence and load of dozens of microparasites at once and were performed as a 'proof of concept' for a new multidisciplinary research program on BC salmon health intended to support Pacific salmon management and conservation.

Synoptic review

We conducted an extensive literature review to put this section together and have chosen to focus the text more on conceptual discussion rather than on specific details about each microparasite. Key references for the conditions under which each of dozens of microparasites have been shown to impact salmon can be found in Table 1 and studies showing genetic associations with and transcriptional host

Table 1. M croparas tes known or suspected to cause d sease or econom c mpact n sa mon throughout the wor d

Microbe	Agent	Abbrev.	Disease	Disease in salmon	Present in BC	Risk to Sockeye	Hatchery	Carrier State Detection	Epidemic / high loss Associations	High-Risk Europe	Introduced to Chile	FW juveniles	FW adults	SW	Temperature responsive	Swim performance	Feeding Growth	Osmoreg.
A																		
Aeromonas hydrophila	Bacteria	Ahyd	Hemorrhagic septicemia	Seshadri et al. (2006)	X		X	Markwardt et al. (1989)							McCullough (1999); Croz ier et al. (2008)			
Aeromonas salmonicida	Bacteria	Asal	Furunculosis	Reith et al. (2008)	X	H	C	Markwardt et al. (1989); Austin and Austin (1993)	Emmerich and Weibel (1894)		1995	Evelyn et al. (1998)	Kent (2011)	Kent (2011)	McCullough (1999); Crozier et al. (2008)	Evelyn et al. (1998)		
Flavobacterium psychrophilum	Bacteria	CWD	Cold-water disease	Duchaud et al. (2007)	X	M	X	Nylund et al. (2011); Stephen et al. (2011)	Duchaud et al. (2007)	Duesund et al. (2010)		Stephen et al. (2011)	Kent (2011)		Stephen et al. (2011)			
Flavobacterium columnare	Bacteria		Columnaris		X	M		Austin and Austin (1993)	Pacha and Ordal (1963)					Duesund et al. (2010)	Holt et al. (1975)			
Salmon (Gill) chlamydia	Bacteria	Sch		Duesund et al. (2010)				Duesund et al. (2010) (?)	Duesund et al. (2010)	Duesund et al. (2010)				Duesund et al. (2010)				
Piscichlamydia salmonis	Bacteria	Pch	Contributing cause of Proliferative gill disease (PGD)	Duesund et al. (2010)				Duesund et al. (2010) (?)		Duesund et al. (2010)				Duesund et al. (2010)				
Piscirickettsia salmonis	Bacteria	Psal	Salmonid rickettisal septicemia	Larenas et al. (2003)	X	L	NP	Larenas et al. (2003)	Larenas et al. (2003)		1988			Kent 2011;				
Renibacterium salmoninarum	Bacteria	Rs (BKD)	Bacterial kidney disease	Wiens et al. (2008)	X	H	X	Wood and Yasutake (1956); Bullock and Herman (1988)	Skall et al. (2005)		1987	Mesa et al. (1999)	Elliott et al. (2013)	Elliott et al. (2013)			Pirhonen et al. (2000)	Price and Schreck (2003)
Rickettsia-Like Organism	Bacteria	RLO	Strawberry disease	X	X						1994		Lloyd et al. (2011) (trout)		Lloyd et al. (2011) (trout)			
Vibrio anguillarum	Bacteria	Vang	Vibriosis	Kent (2011)	X	H	C	Frans et al. (2011) (n)	Actis et al.. (1999)	Miyamoto and Eguc hi (1996)	2005			Kent (2011)	Egidius et al (1986)			Miyamoto and Eguchi (1996)
Vibrio salmonicida	Bacteria	Vsa	Cold-water vibriosis	Kent (2011)	X	H			0ivind et al. (1989)		2005			Oivind et al. (1989)	Egidius et al. (1986); 0ivind et al. (1989)			
Yersinia ruckeri	Bacteria	ERM	Enteric redmouth	Glenn et al. (2011)	X		X	Glenn et al. (2011)	Glenn et al. (2011)			Stephen et al. (2011)	Glenn et al. (2011)	Glenn et al. (2011)				

Table 1. (cont nued)

Microbe	Agent	Abbrev.	Disease	Disease in salmon	Present in BC	Risk to Sockeye	Hatchery	Carrier State Detection	Epidemic / high loss Associations	High-Risk Europe	Introduced to Chile	FW juveniles	FW adults	SW	Temperature responsive	Swim performance	Feeding Growth	Osmoreg.
B																		
Atlantic salmon paramyxovirus	Virus	ASPV	Contributing cause of proliferative gill inflammation (PGI)	Kvellestad et al. (2005)					Kvellestad et al. (2005)	Kvellstad et al. (2005)				Kvellestad et al. (2005)	Kvellestad et al. (2005)		Kvellestad et al. (2005)	
Erythrocytic necrosis virus	Virus	ENV	Viral erythrocytic necrosis (VEN)	Evelyn and Traxler (1978)	X	L	X		Evelyn and Traxler (1978)					Kent (2011)			Evelyn and Traxler (1978)	Haney et al. (1992)
Infectious hematopoietic necrosis virus	Virus	IHNV	IHN	Wertheimer and Winton (1982)	X	H	X	St-Hilaire et al. (2001)	Rucker et al. (1953); Traxler et al. (1998)			Traxler et al. (1997)	Traxler et al. (1997)	Traxler et al. (1997)	Hetrick et al. (1979); LaPatra et al. (1989)	Meyers (2006)		
Infectious pancreatic necros is virus	Virus	IPNV	IPN	Wolf (1988); Rønneseth et al. (2007)	X	L	X	Johansen and Sommer (2001); Ronneseth et al. (2012)	Bahar et al. (2013)	Wolf (1988)	(1998)	W olf (1988); Rønneseth et al. (2007)		Kent (2011)	Dobos and Roberts (1983)	Meyers (2006)	Meyers (2006)	
Infectious salmon anemia virus	Virus	ISAV	ISA	Nylund et al. (1994)				Plarre et al. (2005); Nylund et al. (2011)	Nylund et al. (1994)	Plarre et al. (2005)	(2001)			Thorud and Djupvik (1998)	Falk et al. (1997)	Meyers (2006)		
Pacific salmon parvovirus	Virus	PSPvV		?	Kent (2011)	UK												
Piscine myocarditis virus	Virus	PMCV	Cardiomyopathy syndrome (CMS)	Haugland et al. (2011)	? Brocklebank and Raverty (2002)				Løvoll et al. (2010)	Ferguson et al. (1990)		W iik-Nielson et al. (2012)	Wiik-Nielsen et al. (2012)	Wiik-Nielsen et al. (2012)		Haugland et al. (2011)		
Piscine reovirus	Virus	PRV	Heart and Skeletal Muscle Inflammatory Syndrome (HSMI)	Markussen et al. (2013)	Kibenge et al. (2013)			Palacios et al. (2010)	Løvoll et al. (2010)	Kongtorp et al. (2004); Finstad et al. (2012)	(2010)	Wiik-Nielson et al. (2011)	Wiik-Nielsen et al. (2011); Garseth et al. (2013)	Wiik-Nielson et al. (2012)		Kongtorp et al. (2004, 2008)		
Salmon alphavirus 1, 2, and 3	Virus	SAV 1/2/3	Pancreas Disease (PD) and Sleeping Disease (SD)	Graham et al. (2012)				Anderson et al. (2007); Nylund et al. (2011)	Snow et al. (2010)	Graham et al. (2012); Karlsen et al. (2013)		Nylund et al. (2003)		Karlsen et al. (2006)			McLoughlin and Doherty (1998)	
Viral encephalopathy and retinopathy virus	Virus	VER/VNN	Piscine nodavirus disease	Korsnes et al. (2005)					Plumb and Hanson (2011) - marine fish				(White Sturgeon)	Plumb and Hanson (2011)	Korsnes et al. (2005)			
Viral hemorrhagic septicemia virus	Virus	VHSV	VHS	Olesen et al. (1991)	X	L	X	Duesund et al. (2010)	Skall et al. (2005); Bowser et al. (2009)	Wolf (1988)		de Kinkelin et al. (1980)	Winton et al. (1991)	Skall et al. (2005)		Meyers (2006)	Baulaurier et al. (2012)	

Table 1. (cont nued)

Microbe	Agent	Abbrev.	Disease	Disease in salmon	Present in BC	Risk to Sockeye	Hatchery	Carrier State Detection	Epidemic / high loss Associations	High-Risk Europe	Introduced to Chile	FW juveniles	FW adults	SW	Temperature responsive	Swim performance	Feeding Growth	Osmoreg.
C																		
Gyrodactylus salaris	Ectoparasitic worm	Gyro		Johnsen and Jensen (1991); Mo (1994)			X		Johnsen and Jensen (1991); Mo (1994)	Malmberg (1993)		Stephen et al. (2011)	Soleng et al. (1998)					
Ichthyophthirius multifilis	Ciliate	IMR (Ich)		Bradford et al. (2010)	X	H			Bradford et al. (2010)				Bradford et al. (2010), Kocan et al. (2004)		Kocan et al. (2009)	Tierney and Farrell (2004)	Erickson (1965), Kocan et al. (2009)	
Nanophyetus salmincola	Fluke			Ferguson et al. (2012)										Jacobson et al. (2008)		Ferguson et al. (2012)	Ferguson et al. (2012)	Ferguson et al. (2012)
Neoparamoeba perurans	Amoeba	AGD	Amoebic gill diseas e	Kent (2011)	X	L	X	Nylund et al. (2011)			2006	Stephen et al. (2011)	X					
Spironucleus salmonicida Desmozoon	Flagellate			X	X													
Lepeophtherii (syn Paranucleospora theridion)	Microsporidium	NUC		Nylund et al. (2011)	X			Nylund et al. (2011)						Duesund et al. (2010)				
Facilispora margolisi	Microsporidium			?	Jones et al. (2012)									Jones et al. (2012)				
Loma salmonae	Microsporidium	Loma	Microsporidial Gill Disease of Salm on (MGDS)	Magor (1987)	X	L	**X**					Shaw et al. (2000)	Shaw et al. (2000)	Kent et al. (1995)				Kent et al. (1995)
Nucleospora salmonis	Microsporidium	Nsp	Chronic severe lymphobla-stosis	Kent (2011)	X	L	NP	Foltz et al. (2009)			1992	Kent (2011)	Kent (2011)	Kent (2011)				
Ceratomyxa shasta	Myxozoan	Cs	Ceratomyxosis	Stocking et al. (2006); Ray et al. (2010)	X	L	X	Foott et al. (2007)	Hallett et al. (2012)			Kent (2011)	Bartholomew (2010)	Kent (2011)	Stocking et al. (2006); Bartholomew (2009)		Meyers (2006)	
Kudoa thyrsites	Myxozoan	Kud		No	X		X			Moran et al. (1999)				Moran et al. (1999)				
Myxobolus arcticus	Myxozoan		Brain myxobolosis	?	X	L		Quinn et al. (1987)				Quinn et al. (1987)	Quinn et al. (1987)	Quinn et al. (1987)		Molesand Heifetz (1998)	Moles and Heifetz (1998)	
Myxobolus cerebralis	Myxozoan	Myx-18	Whirling disease	Kent (2011)		L									El-Matbouli et al.			

(continued)

Table 1. (cont nued)

Microbe	Agent	Abbrev.	Disease	Disease in salmon	Present in BC	Risk to Sockeye	Hatchery	Carrier State Detection	Epidemic / high loss Associations	High-Risk Europe	Introduced to Chile	FW juveniles	FW adults	SW	Temperature responsive	Swim performance	Feeding Growth	Osmoreg.
								Cavender et al. (2004)							(1998), Baldwin et al. (2000)	Moles and Heifetz (1998)		
Myxobolus insidiosus	Myxozoan			X										Ferguson et al. (2012)		Ferguson et al. (2012)	Ferguson et al. (2012)	Ferguson et al. (2012)
Parvicapsula kabatai	Myxozoan			Jones et al. (2006)	X								Jones et al. (2006)					
Parvicapsula minibicornis	Myxozoan	Pm		Bradford et al. (2010)	X	H		Foott et al. (2007)	Bradford et al. (2010)			Kent (2011)	Bradford et al. (2010), Kocan et al. (2004)	Kent (2011)	Bradford et al. (2010)	Wagner et al. (2005)	Ferguson et al. (2012)	Bradford et al. (2010)
Parvicapsula pseudobranchicola	Myxozoan	Parvi2	Parvic-capsulosis	Nylund et al. (2005)				Nylund et al. (2011)		(emerging) Nylund et al. (2005)				Nylund et al. (2005)		Nylund et al.. (2005)		
Tetracapsuloides bryosalmonae	Myxozoan	PKD/PKX		Kent (2011)	X	M		Clifton-Hadley et al (1986)	Clifton-Hadley et al. (1984)	Clifton-Hadley et al. (1984)		Clifton-Hadley et al. (1984)		Clifton-Hadley et al. (1986)	Clifton-Hadley et al. (1986)	Clifton-Hadley et al. (1984)	Clifton-Hadley et al. (1984)	Kent et al. (1995)
Ichthyophonus hoferi	Protozoan			Rahimian (1998)	X	M	NP	Rahimian (1998)	Sindermann (1957); Sindermann and Chenoweth (1993); McVicar (1999); Johnsen and Jensen (1991); Mo (1994)	Rahimian and Thulin (1996)		Uno (1990)	Uno (1990)	Uno (1990)	Kocan et al. (2009)	Koc an et al. (2009)		Uno (1990); Stephen et al. (2011)
Sphaerothecum destruens	Protozoan			Kent (2011)	X	L	NP					Kent (2011)	Kent (2011)	Kent (2011)				

Tab e conta ns terature references for bacter a (A), v ruses (B), and other m croparas tes (C), prov d ng ev dence of the env ronments and cond t ons upon wh ch each m croparas te s assoc ated w th d sease and enhanced pathogen c ty, and the sub etha mpacts on the phys o ogy and behav or of the host M croparas tes known to be n Br t sh Co umb a are noted w th an x, wh e emerg ng or exo- t c m croparas tes have terature referenced R sk to Sockeye, as noted by Kent (2011), s represented as h gh (H), moderate (M), and ow (L) M croparas te confirmat on n fish from hatcher es (x), net pens (NP) or cu ture, are as per Kent (2011) and Stephen et a (2011); Carr er state detect on s noted w th references for asymptomat c (a), chron c/pass ve (c), nonpathogen c serotype (*n*) and carr - ers where the m croparas te may have contr buted to death, but on the cause of death (?) Dates assoc ated w th m croparas te ntroduct on to Ch e are from b eta et a 2011 Genus and spec es names and 'et a ' are not ta s zed for readab ty References c ted n the Tab e but not referred to n the text are presented n Reference S1

responses to specific microparasites can be found in Table S1. While the tables are used extensively to demonstrate conceptual ideas in the text, we ask readers to refer to the tables themselves for pertinent references on specific microbes, as many are not repeated in the text; references only cited in the tables are provided under supplemental references.

Challenges facing the assessment of infectious disease impacts in wild fish

Disease causing microparasites are an inherent and natural component of ecosystems, greatly outnumbering free living organisms (Windsor 1998), and likely infect every organism on the planet (Poulin 1996). As a consequence, microparasites are considered to be one of the major selective forces driving evolution (Maynard Smith 1976; Eizaguirre and Lenz 2010). Wildlife epidemics are of increasing concern, with all major ecosystems on earth affected (Harvell et al. 1999; Dobson and Foufopoulos 2001).

In wild populations, it is difficult to isolate and quantify the effects of any single factor, such as infectious disease or environmentally induced stress, because we rarely observe wild fish die; they simply disappear (La and Cooke 2011). Moreover, it is generally assumed that weakened fish are the first to fall prey to the numerous avian, mammalian, and piscine predators, although direct demonstrations of this hypothesis are rare. Stress is known to play a role in fish disease outbreaks (Wedemeyer 1970); stressors above which animals are able to maintain homeostasis have deleterious consequences for survival (Barton 2002). Many infectious agents (hereafter microparasites or microbes) are opportunistic and do not impact survival unless fish are also stressed by other factors impacting immune system function, such as poor water quality or toxicants, which exacerbate (Barton et al. 1985) or attenuate (Pickering and Pottinger 1987) the cortisol response to a second stressor (Barton 2002). For example, the ubiquitous oomycete *Saprolegnia* generally invades fish that have been stressed or otherwise have weakened immune systems (Bruno and Wood 1999). Other microparasites may be associated with chronic infections that can impact behavior, condition, and performance, which may render fish less capable of continued migration and/or more vulnerable to predation or starvation. Even small effects of infectious agents on physiological state or behavior can potentially be critical to the fitness of wild fish if they impact energy allocation or the timing of key life history events (Bakke and Harris 1998). For example, impacts on growth can affect smolting (Marschall et al. 1998), early marine survival (Beamish and Mahnken 2001; Beamish et al. 2004), and predation rates (Hostetter 2009) in salmon. Finally, microparasites that cause acute disease may only do so in certain life history stages or in specific habitats (e.g., fresh water or salt water). Infectious hematopoietic necrosis virus (IHNV), endemic to wild Sockeye Salmon populations (Rudakova et al. 2007), is a good example; it can cause significant losses of fry and smolts in freshwater but diminishes to nearly undetectable levels in saltwater, often increasing in load in adult fish returning to spawn in freshwater, but not causing measurable disease (Traxler et al. 1997). Interestingly, this same virus is associated with devastating losses of Atlantic Salmon (*Salmo salar*) in ocean net pens (St Hilaire et al. 2002; Saksida 2006).

Most of what is known about disease impacts on salmon comes from fish in culture, where mortality is evident and measurable (Kurath and Winton 2011). Salmon enhancement hatcheries are abundant in the northeastern Pacific, accounting for 15.3% of the production of Coho and 18.6% of Chinook Salmon in Canadian commercial and Georgia Strait sport fisheries (Cross et al. 1991). In the Atlantic, 88% of Atlantic Salmon returning to US waters originated from hatcheries (Naish et al. 2008). From mortality events in these and other hatcheries around the world, there is a reasonable understanding of freshwater diseases important in a high density hatchery rearing environment. Aquaculture salmon have been reared in open ocean net pens since the 1970s in Europe and the East Coast of Canada and the United States, and the 1990s on Canada's West Coast and have been the primary source of information on infectious diseases impacting salmon in the ocean. However, as aquaculture is largely restricted to Atlantic Salmon, with only small numbers of farms culturing Chinook and Coho Salmon, information on ocean diseases impacting Sockeye, Chum and Pink Salmon is almost completely lacking (Kent 2011).

Fish health research generally follows events that start with observable mortality. Using a traditional veterinary diagnostic approach, abnormal feeding and swimming behavior and clinical signs of disease may be noted, followed by attempts at laboratory culture of infectious agents, histopathology to identify damage at the cellular level, and enzyme linked immunosorbent assays and/or PCR of suspect microparasites. In the event that an infectious agent is suspected but not identified, degenerate PCR sequencing may be attempted if there are suspected microparasites. Challenge studies may also be pursued to demonstrate that the disease observed in association with mortality is, in fact, infectious. *In situ* hybridization can be used to identify whether suspected infectious agents are associated with regions of tissue damage. If an infectious agent is identified, challenge research will follow the guidelines set out by Koch's postulates (1891) to establish a cause and effect relationship between the microparasite and clinical signs of disease. However, negative effects of subclinical infections in research are rarely reported (Kent et al. 2012).

Koch's postulates were updated by Fredericks and Rel man (1996) to incorporate modern molecular technologies as a powerful means for identifying yet to be cultured mi croparasites and for studying the host parasite relation ships. Previous to the advent of next generation sequencing (NGS), microparasites that were difficult to cul ture could exist for decades with no identified agent. Two heart diseases, heart and skeletal muscle inflammatory syn drome (HSMI) and cardiomyopathy syndrome (CMS), impacted the European aquaculture industry for at least a decade before viral agents were discovered [piscine reovirus (PRV) Palacios et al. 2010; piscine myocarditis virus (PMCV) Haugland et al. 2011]. In the northeastern Paci fic, erythrocytic necrosis (EN) has been associated with mortality in Chum and Pink Salmon for over three decades (Evelyn and Traxler 1978), and while inclusion bodies visi ble with histology could be used to determine the presence/ absence of the disease (Arkoosh et al. 2004), the sequence of the virus causing the disease was obtained only this past year (ENV; J. Winton, USGS, personal communication).

Even with the revised postulates, establishing a direct cause and effect relationship between microparasites and disease may not be possible in wild populations if pathoge nicity of an infectious agent causes infected fish to die and disappear before they are detected (Bakke and Harris 1998). Hence, despite abundant research on infectious dis ease impacts on fish in culture, our understanding of the ecological and evolutionary role of diseases impacting wild salmon populations is minimal (Bakke and Harris 1998; Kent 2011). Modeling studies assessing factors that may influence population fluctuations have implicated the potential role of disease (e.g., Levy and Wood 1992; Con nors et al. 2012; Fujiwara et al. 2014), but empirical research to identify specific infectious diseases that could shift population trajectories is limited.

The complex life history of anadromous salmon may blur the effects of disease epidemics and make them harder to detect (Bakke and Harris 1998). As there is limited pop ulation level monitoring for most salmon in BC, mortality that occurs during downstream river migration of smolts is often amalgamated with ocean mortality. Biotelemetry studies have recently shown that significant losses (up to 50%) can occur during downstream migration in two of the largest drainages in North America, the Fraser River in BC and the Columbia River in Washington/Oregon (Welch et al. 2009; Rechisky et al. 2013). Whereas in the Columbia River, downstream migration mortality is assessed regularly to address impacts of dams and alternate smolt transport systems (Schaller and Petrosky 2007), in BC, these oppor tunities are completely missed.

Over their highly migratory lifecycle, salmon may not only serve as vectors that can move microparasites from one environment to another (Walker and Winton 2010), but during the physiologically demanding shifts between freshwater, estuarine and marine ecosystems (Clarke and Hirano 1995), migrating salmon are also exposed to a suite of new microparasites carried in diverse host reservoirs, some of which may subsequently impact their perfor mance. Importantly, it is during these transition periods when some studies speculate that mortalities can reach very high levels in a short period of time (Bradford 1995; Beam ish et al. 2010), potentially high enough to exert strong evolutionary pressure on a population. Moreover, during these transition periods salmon from disparate environ ments converge, densities are maximized, and hormonal changes can cause immunosuppression (smolts Maule et al. 1987; adults Pickering and Christie 1980, 1981), providing an ideal environment for enzootic outbreaks of disease (Uno 1990). In southern BC populations of Sock eye, Chinook and Coho Salmon, levels of mortality in the early marine environment can be major determinants of year class strength (Beamish and Mahnken 2001; LaCroix et al. 2009). It is during this critical early marine period that many believe the key to declining productivity lies (Beamish et al. 2010; Peterman and Dorner 2011). While climate driven ocean conditions are hypothesized to play a major role (Chittenden et al. 2009; Rogers and Schindler 2011; Sharma et al. 2013), if disease were to contribute substantially to these mortalities in some or all years, genetic variance in susceptibilities to important disease causing microparasites may underlie some of the population level variances in returns. Importantly, density dependence is also strongly correlated with ocean produc tivity shifts (Elliott 1989), consistent with patterns expected if disease were a factor. However, as dying fish are virtually never observed, direct linkages with disease can be difficult to demonstrate. At the other end of the salmon life cycle, adult Pacific salmon migrate from the marine environment back to the freshwater rivers to spawn in the streams and tributaries in which they were born. As semelparous spe cies, returning Pacific salmon are simultaneously maturing, senescing, and starving, and hence, their condition and ability to fight infection is deteriorating over the last stretches of their migration, making them especially vulner able to additional environmental stressors and disease. Immunosupression induced by maturation hormones (Pic kering and Christie 1980) may also contribute to enhanced susceptibility by even opportunistic microparasites or those previously at a carrier state. In recent decades, the level of premature mortality experienced by salmon in major drainages in BC and Washington has escalated coincident with the general 2 3°C rise in river temperatures (Patterson et al. 2007; Keefer et al. 2008; Martins et al. 2011). For example, premature mortality for Sockeye Salmon return ing to the Fraser River to spawn was historically close to 15 20% but has been upward of 95% in some years, often

showing a high degree of genetic variation among populations within the drainage (Hinch et al. 2012). It is somewhat easier to associate these mortality events with infectious diseases, as some of the mortalities are observable as carcasses full of eggs lining the riverbanks. However, complex infections with multiple microparasites can obscure assigning a single disease as a cause of death; case studies I–III, presented below, delve into the complexity of microparasites carried by salmon returning to spawn.

Infectious disease impacts in wild salmon – what is known

Population level effects of infectious disease have been observed in wild freshwater and marine fishes, but not commonly in salmon (Kent 2011) possibly due to the reasons stated previously. Classic cases of disease epidemics in fish include widespread outbreaks of viral hemorrhagic septicemia (VHS) in several fish species in the Great Lakes (Bowser et al. 2009) and herring (*Clupea pallasi*), hake (*Merluccius productus*), and walleye pollock (*Theragra chalcogramma*) in the northeastern Pacific (Skall et al. 2005), a herpes virus introduced to Australian pilchards (*Sardinops sagax*) in the 1990s by bait fish (Murray et al. 2003) and causing mass mortalities over thousands of kilometers (Jones et al. 1997), sturgeon (*Acipenser nudiventris*) population crashes in the Aral Sea after introduction of *Nitzschia sturionis* (Bauer 1961) and chronic *Ichthyophonus hoferi* infections causing high mortalities in herring worldwide (Sindermann and Chenoweth 1993; Rahimian and Thulin 1996). The first record of epidemic disease in wild salmon was from a paper dating to the late nineteenth century documenting furunculosis outbreaks (caused by bacterium *Aeromonas salmonicida*) in Atlantic Salmon (Emmerich and Weibel 1894). Subsequently, outbreaks of furunculosis (Inglis et al. 1993), ulcerative dermal necrosis (UDN; Roberts 1993), and *Gyrodactylus salaris* (Johnsen and Jensen 1991; Mo 1994) have caused widespread conspicuous epidemics in wild populations of Atlantic Salmon in Europe. As well, the bacterium *Renibacterium salmoninarum* caused a major epidemic of bacterial kidney disease in Scotland in the 1930's (Smith 1964). In Pacific, salmon *Ichthyophonus* (Traxler et al. 1998) is suspected of associating with population level impacts in the marine environment, while in freshwater, population level mortality events have also been associated with *Ceratomyxa shasta* (Hallet et al. 2012), *Parvicapsula minibicornis* (Bradford et al. 2010) and *Ichthyophthirius multifiliis* (Kocan et al. 2004). Pacha and Ordal (1963) identified high *Flexibacter columnaris* infection rates as a potential cause for the decline of Columbia River Chinook, Sockeye, and Steelhead Trout (*Oncorhynchus mykiss*) in the early 1960s.

While macroparasites (defined as fish lice, tapeworms, nematodes, and some protozoan and fungal pathogens) can cause conspicuous harm to heavily infected individuals, they generally remain relatively stable over time and have limited impacts at the population level (sea lice may be an exception; Johnson et al. 1996; Krkošek et al. 2006) (Bakke and Harris 1998). Moreover, the complex life cycles of many macroparasites that require intermediate hosts to complete development further limits the range of environments where they can persist (Dobson and Foufopoulos 2001). Alternately, microparasites (e.g., viruses, bacteria, some protozoan, and some fungi) are very unstable, exponentially increasing over very short periods of time, and have a much greater potential as regulators of host population size and as selective agents (Bakke and Harris 1998). Given their volatile nature, microparasites are also associated with stronger immune responses that result in lasting immunity (Anderson and May 1979). For wild Norwegian Atlantic Salmon, a review by Bakke and Harris (1998) concluded that myxozoans, furunculosis, *G. salaris*, and sea lice are the pathogens of greatest threat. While viral diseases are common in cultured European salmon, they argued that there was no evidence of viral disease impacts on wild salmon, or of transfer of viruses from farmed to wild fish. In Pacific salmon off North America, a similar assessment of risk for population level impacts of disease in Sockeye Salmon was conducted by Kent (2011). Microparasites identified as 'high risk' included the IHN virus, well known to cause significant disease in juvenile Sockeye Salmon (Traxler et al. 1997), bacterial species *A. salmonicida* and *R. salmoninarum* that have been associated with highly observable hatchery losses of Coho and Chinook Salmon (Evelyn et al. 1998), *Vibrio* (*Listonella*) *anguillarum*, a bacterium associated with high losses of Pacific salmon in net pens (Actis et al. 1999), and two microparasites, *P. minibicornis* and *I. multifiliis* that have been associated with premature mortality of returning adult salmon (Kocan et al. 2004; Bradford et al. 2010; Table 1). Importantly, most microparasites that had never been assessed in Sockeye Salmon (of which there were many) were classified as 'low risk', and the review only included known endemics. Kent (2011) suggested that there was no evidence of exotic or uncharacterized salmon pathogens in BC. He also argued that because salmon would have evolved natural resistance to endemic microparasites, any associations of endemic microbes with declines would require enhanced susceptibility due to additional environmental stressors.

Some microparasites can transcend freshwater, estuarine and saltwater ecosystems, while others cannot (see Table 1 for full list and references). For some, pathogenicity may be diminished by the osmoregulatory demands associated with shifts between salinity environments, limiting their impacts to a single ecosystem. In other cases, like that for IHNV described previously, genetic variance in susceptibility of the host appears to drive patterns of differential

virulence between ecosystems. Alternately, there are numerous microparasites that can be transmitted in one environment but become more virulent in another. Some of the most devastating emerging viruses in European sal mon can be transmitted in freshwater ecosystems with no apparent ill effects on juveniles, but become virulent patho gens after entering the ocean [e.g., PRV (Løvoll et al. 2012) and PMCV (Wiik Nielsen et al. 2012)]. Infectious salmon anemia virus (ISAV) is an exception, as it is hypothesized that the avirulent wild type strain of the virus, HPR0, may be transmitted in freshwater but can readily mutate under conditions that are not well understood to become a viru lent pathogen in the marine environment (Plarre et al. 2012). A third pattern of differential virulence among eco systems is microparasites that are merely carried in the marine environment but become pathogenic during the energetically and physiologically challenging return migra tion of adult salmon to spawning grounds.

Sublethal effects of microparasites may be more detri mental to wild than cultured populations, as they may impact the ability to compete effectively for resources, to migrate to optimal environments for feeding and overwin tering, and to put enough energy into maturation to suc ceed in their once in a lifetime opportunity to spawn. Behavioral shifts are often the first line of defense when animals are stressed and are designed to lessen the proba bility of death or metabolic costs incurred by maintaining physiological homeostasis (Olla et al. 1980). Swimming performance is the behavioral trait perhaps most univer sally affected when animals are stressed and condition of fish is compromised (Webb and Brett 1973; Wedemeyer et al. 1990), with impairments in performance a good pre dictor of survival (Thomas et al. 1964). Given recent find ings that show enhanced robustness and disease resistance in fit fish (those that have undergone aerobic training exer cises), one might surmise that the relatively fitter wild fish would have an advantage over sedentary cultured fish (Cas tro et al. 2011). However, when swim performance is com promised, the impacts on survival of wild fish will be greater. Effects on swim performance have been associated with a wide array of parasitic and viral infectious agents in salmon (see Table 1). Appropriate food resources may improve favorable disease outcomes, such as reduced impacts of HSMI where functional feeds (high lipid/DHL content) reduced the viral load and lessened the pathology in heart tissues (Martinez Rubio et al. 2012). However, microbes that impact swim performance may also decrease feeding and growth in wild fish (Table 1). While impacts will be felt at most stages of development, there is mount ing evidence that impacts of reduced feeding and growth on survival of wild salmon in the early marine environment may be quite substantial (Beamish and Mahnken 2001; Beamish et al. 2004; Farley et al. 2007).

Infectious agents that cause disease in gill and/or kidney tissue are often associated with impaired osmoregulation and may indirectly impact salmon survival during salinity transitions. Osmotic stress during saltwater acclimation is metabolically challenging and can affect multiple energy intensive behavioral traits, including schooling, foraging activity, predator avoidance, and swimming performance, potentially increasing risk of predation (Järvi 1989; Hande land et al. 1996; Dieperink et al. 2002). Prolonged osmotic stress may reduce growth and increase susceptibility to opportunistic pathogens and additional stressors or at the extreme, result in complete osmotic failure and death. Osmoregulatory indices have been associated with reduced survival of adult salmon returning to spawn (Cooke et al. 2006; Crossin et al. 2009; Donaldson et al. 2010; Miller et al. 2011), and disease is one of the suspected drivers of this variation (Miller et al. 2011; Jeffries et al. 2012). Numerous microparasites have been associated with impaired osmoregulation, while others increase pathoge nicity during smoltification (Table 1).

Evolutionary drivers of disease resistance in salmon

It is expected that genetic diversity within host populations, especially associated with immune system processes, can buffer them against widespread epidemics (Altizer et al. 2003). Organisms with low disease response capability should be rapidly wiped from a population (Kronenberg et al. 1994), and hence, in the face of novel microparasite exposures, if populations are to remain viable they need to evolve resistance quickly. The cycle of adaptation and counter adaptation between microparasites and hosts cre ates an oscillatory dynamic of host and parasite genotypic frequencies and has been depicted as an 'evolutionary arms race' described under the 'Red Queen Hypothesis' (Van Valen 1973; Altizer et al. 2003).

Antagonistic coevolution between endemic micropara sites and their host populations has created a geographic mosaic in patterns of susceptibility of salmon to infectious diseases and is a potential driving force maintaining genetic variation in immune system processes (Bakke et al. 1990; Gjedrem et al. 1991). Salmon populations with historical exposure to particular diseases generally carry greater resis tance to those diseases (Zinn et al. 1977; Bower et al. 1995; Bartholomew 1998; Miller and Vincent 2008). Moreover, populations that have coevolved with specific infectious microparasites may show lower heritabilities than newly exposed populations, limiting the pace of future adaptation (Crozier et al. 2008). Genetic associations with resistance measured as survival under challenge testing have been demonstrated for a wide range of salmon microparasites (reviewed in Ødegård et al. 2011) of viral, bacterial, and parasitic origin (references in Table S1). Heritabilities range

between 0.14 (sea louse) to 0.62 (furunculosis) and are generally higher than those observed in livestock (Ødegård et al. 2011). Several studies have explored the genetic correlations between resistance against a variety of diseases; while most are positively correlated (Gjøen et al. 1997; Henryon et al. 2005), indicative of common immune related resistance genes, others may be negatively correlated or show no correlation at all (Ødegård et al. 2007; Kjøglum et al. 2008).

Disease resistance and the major histocompatibility complex

The complexity and polymorphism of the immune system suggests that it is indispensable for survival and argues for the importance of infectious agents as a selective force in natural populations (Bakke and Harris 1998). As such, we expect that host species exposed to a variety of microparasites should harbor a diverse array of resistance alleles or a range of inducible defences (Altizer et al. 2003). However, while most association studies in salmon have calculated heritabilities via familial associations with resistance, few have identified the underlying genetic mechanisms conferring resistance. There have been a fair number of targeted studies assessing associations between disease resistance and major histocompatibility complex (MHC) genes. MHC molecules play a crucial role in T cell mediated adaptive immune responses by binding self and parasite derived peptides for presentation to T cells (Potts and Wakeland 1990; Hedrick 1994). MHC class I molecules bind peptides produced within cells (e.g., derived from viruses, some microparasites) and generally elicit a cytotoxic response, while class II molecules bind peptides of exogenous infectious agents (e.g. most bacteria and macroparasites) generally resulting in a humoral (antibody) response.

Given the critical role in immune recognition of infectious agents and unprecedented levels of diversity displayed by MHC molecules, the evolutionary dynamics of the MHC has become a paradigm for adaptively important genetic diversity that is of relevance in ecology, population biology, and conservation (Sommer 2005; Piertney and Oliver 2006). Pathogen driven balancing selection derived through overdominance, negative frequency dependence or temporal/spatial heterogeneity in pathogen pressure is hypothesized to be the dominant force driving MHC evolution (Klein and O'huigin 1994; Parham and Ohta 1996; Hedrick and Kim 2000). It is expected that the maintenance of MHC diversity in wild populations assures resistance to a diverse array of microparasites, hence enhanced population viability (reviewed in Bernatchez and Landry 2003; Sommer 2005; Piertney and Oliver 2006; but see Radwan et al. 2010). We expect that in natural communities, adaptation to newly encountered microparasites or changes in microparasite virulence occurs on ecological rather than evolutionary timescales, necessitating selection based on pre existing genetic variation, referred to as 'standing genetic variation' (Barrett and Schluter 2008). MHC alleles associated with resistance or susceptibility to specific infectious agents of salmon have been identified in numerous laboratory challenge studies (ISAV Grimholt et al. 2003 and IHNV Palti et al. 2001; Miller et al. 2004; *A. salmonicida* Langefors et al. 2001; Lohm et al. 2002; *Piscirickettsia salmonis* Gomez et al. 2011) most consistent with the action of directional selection imposed by a single pathogen. Only a single study by Arkush et al. (2002), in which a series of bacterial (*V. anguillarum*), viral (IHNV), and parasite (*Myxobolus cerebralis*) challenges were conducted on inbred and outbred Chinook Salmon, demonstrated stronger single pathogen selection for heterozygosity than for a specific resistance allele (IHNV only). Hence, if pathogen driven selection is the dominant mechanism maintaining diversity of MHC molecules, the action of multiple pathogens is likely required.

The role of MHC genes in the evolution of local adaptation of anadromous salmon to differing microparasite communities among natal streams and lakes is supported by their higher level of population divergence than derived from demographics alone (Miller et al. 2001; Eizaguirre and Lenz 2010; McClelland et al. 2013). MHC allelic distribution patterns within salmon populations vary considerably, with some populations showing distributions more even than expected under neutrality (evidence of balancing selection), some less even (evidence of directional selection), and others showing no deviations from neutral expectations (Landry and Bernatchez 2001; Miller et al. 2001; Aquilar and Garza 2006; Campos et al. 2006; Dionne et al. 2007; Consuegra et al. 2011; McClelland et al. 2013). In Sockeye Salmon, the dominant class I (UBA) and II (DAB) loci show fluctuating patterns of allelic distribution across the species range that are not correlated between loci, suggesting that different selective forces are at play (McClelland et al. 2013). Most populations showing evidence of directional selection contain a single dominant allele that may be a resistance allele to a virulent infectious agent (McClelland et al. 2013). Over the entire range of Sockeye Salmon, there are only two alleles at the DAB locus observed at frequencies >90%, and one allele for UBA, and these are distributed across demographically distant populations (McClelland et al. 2013). Whether the same selective agents are responsible for maintaining each of these dominant alleles across distant populations is worth investigating in the future.

While numerous salmon population studies have contrasted allele frequency data for MHC and selectively neutral loci to demonstrate natural selection acting on the MHC over an ecological time scale (Miller and Withler 1997; Landry and Bernatchez 2001; Miller et al. 2001;

Aquilar and Garza 2006; Dionne et al. 2007; Peters and Turner 2008; McClelland et al. 2013), few have demonstrated in natural systems direct associations with pathogen resistance. A series of field studies based on wild Canadian Atlantic Salmon populations in Quebec offer some of the first direct correlations between microbes and shifting MHC allele frequencies in a single generation in salmon. Dionne et al. 2007 identified an association between bacterial community diversity and MHC class IIβ diversity along a latitudinal thermal cline, similar to patterns originally observed in humans (Prugnolle et al. 2005). A subsequent study identified an association between a dominant myxozoan parasite and two MHC class IIβ alleles, one statistically associated with susceptibility to infection, and the other with resistance (Dionne et al. 2009). Over time, the frequency of the susceptibility allele and infection with the myxozoan parasite decreased, consistent with rapid pathogen driven directional selection based on standing genetic variation. A similar study on juvenile European Atlantic Salmon documented shifts in MHC allele frequencies over a six month period in the river, possibly indicative of pathogen driven selection, although in this case, pathogens were not monitored (de Eyto et al. 2011).

Genome scans for QTL's associated with disease resistance

Genomic scans for genetic loci quantitatively associated with disease resistance (dQTL) have recently been conducted for a small number of salmon diseases (see below; Table S1). Unlike the MHC association studies, a dQTL approach is not targeted, but rather assesses associations across hundreds to thousands of single nucleotide polymorphisms [SNPs] or microsatellite loci mapped evenly across the genome. This approach can be used to identify the genetic architecture of disease resistance for a given disease, including the number of significantly associated loci across the genome, their level of contribution, and whether epistatic relationships exist between loci (Kover and Caicedo 2001). Synthesis of dQTL's across a range of diseases will reveal the species level genetic architecture of disease resistance, identifying clusters of dQTL's impacting resistance to multiple diseases. This approach has been used effectively to identify breeding schemes for agricultural species of interest (e.g., maize Wisser et al. 2006).

The largest focus of dQTL research in salmon has been on two important viral diseases significantly impacting global aquaculture of Atlantic Salmon, ISA, and infectious pancreatic necrosis (IPN). QTL discovery and validation studies have been undertaken for each (Table S1). These studies identified single major QTL's associated with resistance to each viral disease. For IPN, virtually all of the variation in resistance in both freshwater and seawater was associated with a single dQTL on linkage group 21 (Houston et al. 2010). For ISA, a powerful dQTL was identified in linkage group 8 (Moen et al. 2004, 2007). Lack of a fully curated salmon genome sequence hampers the precise identification of genes associated with resistance using a QTL approach (Davidson et al. 2010; NCBI ASM23337v1). However, a comparative genomics approach identified a candidate gene linked by synteny in tetraodon and medaka genomes to the major QTL for ISA resistance that codes for a major regulatory protein of several genes that have been implicated in the response to ISAV infection (Li et al. 2011). A dQTL study on VHS also identified a single dominant QTL conferring resistance in Rainbow Trout (Verrier et al. 2013a). A subsequent study found no genetic correlation of this QTL with resistance to another fish rhabdovirus, IHNV (Verrier et al. 2013b).

Phenotypic variation in disease response through gene expression profiling

Damage is a central feature of infectious disease; the degree of damage caused to host tissue will impact the level of host response and the pathological outcome of disease (Casadevall and Pirofski 1999). As such, microparasites can be ranked based on the likelihood that they cause damage, and hence disease, as a function of the magnitude of the host response (Casadevall and Pirofski 1999). Gene expression profiling can elucidate the molecular basis of variation in susceptibility and response to disease derived from both plastic and genetic mechanisms. CDNA microarrays and Agilent oligonucleotide arrays offer a high throughput method to assess the activity of thousands to 10s of thousands of genes at once and are the mainstay of functional genomics research. Numerous salmon arrays have been developed in the past decade, the most recent of which are Agilent oligonucleotide arrays with 44 000 gene features spotted onto four subarrays on each slide (Taggart et al. 2008; Jantzen et al. 2011). Array technology has been applied to assess salmon host response to a large number of infectious agents, including virtually all of the 'high impact' and emerging viral diseases (e.g. IHN, ISA, CMS, HSMI, and pancrease disease [PD]), a few of the important bacterial diseases (furunculosis, vibriosis, and rickettsia), but very few parasitic diseases (except amoebic gill disease, whirling disease, PKD, and sea louse) (Table 1 disease names; Table S1 references).

Most disease focussed microarray studies have identified genes and biological processes up and down regulated in response to a pathogen. More importantly, a small number of studies have contrasted responses in high and low susceptibility fish or pathogen strains of high and low virulence that can begin to unravel the mechanistic basis of resistance (Miller et al. 2007; Wynne et al. 2008; Purcell et al. 2009). Across virtually all viral challenge studies, a powerful and systemic induction of antiviral and interferon

(IFN) dependent genes has been correlated with viral load and degree of tissue damage (see Table S1), mirroring the important role of IFNs in orchestration of antiviral responses in mammals. However, the salmon IFN response was also stimulated in response to bacterium *P. salmonis* (Tacchi et al. 2011) and myxozoan *M. cerebralis* (Baerwald et al. 2008). As a general rule, resistant and susceptible hosts are responding using highly congruent profiles of genes, but the level of response increases with susceptibility and virulence. Hence, it appears that in many cases, increasing the power of the host response is not sufficient to resist disease. Instead, more subtle variations in the pathways stimulated may underlie the levels of susceptibility of the host. For IPNV, survivors generally elicited a stronger innate immune responses (Marjara et al. 2011), whereas for IHNV, the efficiency of viral entry and strength of host down regulation of cell transcription and translation appeared to be more important determinates of susceptibility (Miller et al. 2007 and K. M. Miller, unpublished data; Purcell et al. 2011). Alternately, the strength of complement activation appeared to be more predictive of resistance to bacteria *A. salmonicida* (Škugor et al. 2009) and *Flavobacterium psychrophilum* (Langevin et al. 2012). A single study on ISAV contradicted the pattern of enhanced response with higher microbe loads and more susceptible hosts; Workenhe et al. (2009) found that a low virulent strain of ISAV elicited a stronger host response than highly virulent strains.

In the second case study described below, we combine quantitative data on microparasites carried by wild migrating salmon with a measure of host response defined by the transcriptional activity of a subset of immune and stress related genes to gauge which microbes may be associated with the most 'damage' to the host, hence potentially impacting performance of wild fish.

Evolution of microparasites

Microparasites evolve responsive and adaptive molecular traits that enable efficient adherence, entry and replication within the host (Pulkkinen et al. 2010). Virulent microparasite strains have greater infectivity, higher tissue degrading capacity and higher growth rates but are not generally selectively favored in nature if death of the host limits the population cycle of the microparasite (Pulkkinen et al. 2010). However, infectious agents that can maintain infectivity for months in fresh or seawater or in the soil will endure a lower fitness cost of host death and are thus more likely to undergo selection for increased virulence in natural populations (Pulkkinen et al. 2010).

There is strong empirical evidence that evolution for enhanced microparasite virulence can proceed quickly in a culture environment because local extinction of infectious agents after spikes of disease does not occur if there is no limitation on host animals (Anderson and May 1982; Frank 1996; Ebert and Mangin 1997; Altizer et al. 2003; Murray and Peeler 2005). Continuous introduction of naïve fish to meet production demands, selection of recovered fish, and lack of control methods for novel microparasites all contribute to the evolution of enhanced virulence (Kurath and Winton 2011). Cooccurrence of multiple genetically distinct microparasite strains within the same population will also favor virulence if more virulent strains have a competitive advantage (Nowak and May 1994; Frank 1996; Gandon et al. 2001; Read and Taylor 2001). Moreover, the use of drugs to suppress and kill parasites in cultured fish not only selects for drug resistance, but may also exacerbate selection for faster growth and transmission (Mennerat et al. 2010). Use of vaccines that reduce pathogen growth may also reduce the cost of virulence, selecting for higher virulence due to reduced risks of host death (Mennerat et al. 2010).

RNA viruses are the best examples of rapid evolution of virulence of microparasites in cultured salmon. In salmon, eight RNA viruses are associated with emerging diseases in aquaculture (IHNV, ISAV, IPNV, PMCV, PRV, viral hemorrhagic septicemia virus [VHSV], salmon alphavirus [SAV], Atlantic Salmon paramyxovirus [ASPV]), many of which show evidence of rapid evolution on farms. For example, in farmed Rainbow Trout, genetic analyses of more than a thousand isolates of IHNV show higher levels of genetic diversity, faster rates of evolution, and independent evolutionary trajectories compared to ancestral wild isolates (Troyer et al. 2000). Similarly, VHSV genotype I has undergone rapid evolution in domesticated Rainbow Trout in Europe, producing a number of highly virulent strains (Kurath and Winton 2011). In Norway, only the avirulent ISAV HPR0 strain has been observed in wild fish, whereas both HPR0 and virulent strains of ISAV are common in salmon net pens (Plarre et al. 2005, 2012). While horizontal transmission has been considered a dominant route of exchange of virulent strains of the virus, a recent study by Plarre et al. (2012) proposed that virulent strains are repeatedly evolving on ocean farms from HPR0 strains common in wild populations.

Increased virulence under culture is not limited to viruses. Virulence of the bacterial pathogen *Flavobacterium columnare* in salmon fingerlings farmed in northern Finland is hypothesized to have evolved from fierce strain competition in high density rearing environments (Pulkkinen et al. 2010). The evolved virulent strains have higher infectivity and growth rates and are associated with increased severity of symptoms prior to death of the host. Moreover, they can transmit from dead fish and remain viable in sterilized water for months (Pulkkinen et al. 2010). Furunculosis has also increased in virulence in

cultured fish (Bakke and Harris 1998). In salmon aquaculture, there are attempts to minimize disease outbreaks and the evolution of enhanced virulence by limiting exposure between year classes and leaving sites fallow after harvest before new fish are introduced (Costelloe et al. 2001). For a more detailed description of parasite and pathogen evolution on salmon farms, see Mennerat et al. (2010).

Introductions of exotic microparasites

The introduction of novel microparasites may be associated with 'virgin ground' epidemics that progress quickly through previously unexposed populations and cause high mortality and striking reductions in host abundance (Altizer et al. 2003). However, to differentiate impacts associated with introduced diseases from those of climate or other factors that may influence population dynamics, abundance data before and after potential introductions are required (Hochachka and Dhondt 2000; Daszak et al. 2005; Lips et al. 2006; LaDeau et al. 2007). As a result, such outbreaks in species or populations that are not closely monitored would likely go undocumented; such is likely the case for wild salmon. The best examples of virgin ground epidemics come from terrestrial systems, with distemper outbreaks in European seals (Jensen et al. 2002), *Mycoplasma gallisepticum* and West Nile virus outbreaks in wild avian populations (Hochachka and Dhondt 2000; LaDeau et al. 2007), and outbreaks of a pathogenic chytrid fungus, *Batrachochytrium dendrobatidis*, threatening amphibian biodiversity in Panama (Lips et al. 2006). Whether new outbreaks are the results of 'host jumps' or introductions through natural shifts in carrier distributions due to climate or anthropogenic associated movements, we expect that if host populations have maintained sufficient diversity, emerging diseases will ultimately be both buffered by and change rapidly the genetic composition of host populations (Altizer et al. 2003). To date, there is more support for emergence from geographic proximity and opportunities for cross species transmission rather than genetic changes in the infectious agents themselves (Altizer et al. 2003). The best known example of species cross over caused by a mutation in the infectious agent is with the relatively benign feline parvovirus. In the 1970s, mutations in the capsid protein of the virus altered the recognition of the host transferrin receptor and caused the virus to be infective and highly virulent in canines, leading to epidemic outbreaks impacting wolves, coyotes and domesticated dogs (Parrish and Kawaoka 2005). Another example is the recent avian epizootics of high pathogenicity strains of H5N1 influenza A which jumped to mammals and caused small outbreaks and death in humans (Parrish et al. 2008).

In salmon, the homing response, which returns spawning salmon to their natal river, can serve to lessen natural exchange of microparasites between freshwater systems, and osmotic barriers associated with some microparasites would also reduce potential for exchange (Bakke and Harris 1998). These barriers to microparasite movements between freshwater systems would serve to enhance the variance in evolved resistance among populations, consistent with the patterns of MHC variation observed in anadromous salmon. Alternately, we expect that species or stocks that have lower site fidelity for spawning may be exposed to a larger array of microparasites and hence evolve a higher capacity for resistance. As conditions warm, successful colonization in more northerly latitudes may increase (Babaluk et al. 2000), enhancing the dispersal of microparasites among systems. For systems with no evolved resistance, new microbe introductions could result in localized disease outbreaks.

Translocation of microparasites through human activities is also a concern, and there are several documented cases where this has resulted in devastating effects. On a local level, translocation of fishes by anglers or enhancement facilities can introduce microparasites into systems where they were otherwise absent (Bakke and Harris 1998). Escapees from salmon farms are also a potential source of microparasite infections in wild fish, although examples of such occurrences are rare. In Europe, farmed escapees have been blamed for furuncolosis outbreaks in wild fish (Johnsen and Jensen 1994). However, it is the large scale transfers of fish and eggs that are considered the highest risk toward introduction of nonendemic pathogens. The accumulation of exotic microbes in the Chilean salmon aquaculture industry (6 bacterial, 3 viral and 2 parasitic salmon pathogens; Table 1), which was salmon disease free when the industry started in the early 1990s, is strong evidence of this risk (Ibieta et al. 2011). In Europe, Bakke and Harris (1998) suggest that the most devastating impacts of disease transfer through fish movements has been furunculosis outbreaks that occurred originally during the nineteenth century coincident with movements of juvenile salmonids across the Atlantic and within Europe (Lund 1967), with a second reintroduction occurring more recently across Europe (Egidius 1987). As well, there is some evidence that *G. salaris*, which is endemic and nonpathogenic in Finland, has been introduced through the movement of Rainbow Trout from Finland into Russia (Mo 1994), Germany, Spain, Denmark, and Portugal (Malmberg 1993). Similarly, outbreaks of *M. cerebralis*, the causative agent of whirling, in the United States followed translocations of live Rainbow Trout from Europe, most notably Germany, after WWII (Bartholomew and Reno 2002). While there has been speculation that PRV newly discovered in BC salmon is a result of recent egg imports (Kibenge et al. 2013), there is no compelling evidence to date of diseases impacting wild Pacific salmon in North America that resulted from

egg transfers associated with the aquaculture industry. However, there is evidence to support the very high impact that an endemic North American virus, IHNV, has had on the exotic Atlantic Salmon that are the mainstay of the aquaculture industry (Saksida 2006).

Potential for exchange between wild and cultured salmon

As wild salmon populations in North America and Nor way have been declining in both numbers and productiv ity, aquaculture production has been increasing (Ford and Myers 2008; Walker and Winton 2010). There is growing evidence that in some regions, aquaculture may be a primary cause of declines in wild populations (Ford and Myers 2008). Reductions in fitness due to genetic introgression of farmed escapees (where endemic species are cultured) and transfer of disease are the main issues of concern (Heggberget et al. 1993). Disease exchange from aquaculture to wild fish may occur through the introduction of novel microparasites by translocations of eggs or juvenile fish, or as a result of artificially high car rier states of endemic microparasites due to high density rearing environments (Krkošek et al. 2006). Additionally, net pen farming could increase concentrations of myxo zoan parasites by creating optimal environments for their intermediate invertebrate hosts (e.g., annelid worms) in the eutrophic environment under salmon pens (Johnsen et al. 1993), potentially increasing their impact on both farmed and wild migrating populations (Bakke and Har ris 1998).

In aquaculture, fish can be reared at densities more than a thousand times those in natural environments (Pulkkinen et al. 2010). A fundamental principle of epidemiology is that populations should be most subject to host specific infectious disease when they are at high densities (Lafferty and Gerber 2002). This is a key tenet of the premise that populations in a culture environment will be more affected by disease than wild populations; given what we know about disease outbreaks on farms, this does appear to be the case (Ibieta et al. 2011). In the section on microbial evolution above, we discussed the factors in addition to density present in a culture environment that facilitate rapid evolution of enhanced virulence. However, most evi dence to date suggests that it is not the highly virulent mi croparasites produced by high density salmon culture that are the greatest risk to wild populations (Anderson 1979; Bakke and Harris 1998; Biering et al. 2013). For example, molecular monitoring of wild Atlantic Salmon and sea trout (*S. trutta*) in Norway revealed that only one of the five emerging viruses (PRV but not IPNV, SAV, ISAV, or PCMV) impacting the salmon aquaculture industry was present in >1.5% of wild fish, nor were the two most path ogenic bacterial microbes, *R. salmoninarum* and *A. sal monicida* present at appreciable levels among the 500 fish surveyed (Biering et al. 2013). These prevalence rates dif fered dramatically from those associated with the Norwe gian aquaculture industry, which had been experiencing particularly high incidence of IPNV and SAV. The question is, did affeced wild fish simply die unsampled or is there really a much lower infection pressure on wild fish (McVic ar 1997)?

Studies from terrestrial systems indicate that cultured animals can be important carriers of disease, even if the cultured species suffers little pathology (Lafferty and Ger ber 2002). Terrestrial examples of domestic/wild impacts of disease exchange are abundant and have involved bacterial, fungal, viral, and protozoan infectious agents that have reduced wild populations of affected species by 80 90%, occasionally causing local extinction (reviewed in Lafferty and Gerber 2002). In the aquatic realm, a survey from ProMED mail in 2000 revealed that hatcheries and aqua culture facilities were associated with the North American spread of ISAV and salmon sarcoma virus in Atlantic Sal mon, and whirling disease (*M. cerebralis*) and furuncolosis in trout (Dobson and Foufopoulos 2001). In Norway, dis ease outbreaks of gyrodactyliasis (caused by *G. salaris*) and furunculosis leading to severe declines in wild populations are highly correlated with the expansion of the aquaculture industry in the northwestern Atlantic and the Baltic during the first half of the 1980s (Johnsen and Jensen 1994; Heggb erget et al. 1993). The scale of *G. salaris* losses was so great in Norwegian salmon rivers that entire systems were treated with rotenone in an attempt to eradicate the parasite (Windsor and Hutchinson 1990).

Disease transfer between aquaculture and wild popula tions is not unidirectional; there are several documented cases where disease outbreaks on farms have occurred after transmission of infectious agents from wild fish; in fact, there are more substantiated reports of wild to aqua culture disease transfer than aquaculture to wild (viral transfer reviewed in Kurath and Winton 2011). A case in point in the northeastern Pacific are the widespread out breaks of IHNV soon after the Atlantic Salmon farming industry was established in the early 1990s (St Hilaire et al. 2002). As Atlantic Salmon are an exotic species in the Pacific Ocean, they had no natural resistance to microbes endemic to BC salmon. IHNV is endemic to BC and is particularly prevalent in Sockeye Salmon popula tions in freshwater (Rucker et al. 1953; Traxler et al. 1998; see sections above for more discussion on IHN). From 1992 1996, cumulative mortality from the IHN outbreaks on BC farms was close to 50%, similar to levels experi enced during a second outbreak from 2001 to 2003 (Saks ida 2006) associated with losses of over 12 million fish. Sequence level analyses resolved that outbreaks resulted from three separate introductions from viral strains

common in wild Pacific salmon populations from Alaska, BC and Washington State (Saksida 2006).

The probability of disease transfer between aquaculture and wild fish in the marine environment will largely depend upon the hydrographic regime around the net pens, the migration routes of wild fish and length of time that wild and farmed fish are in close contact, prevalence of infection, shedding rates, and the longevity of micropara sites outside of their host. Models that include detailed field observations and oceanographic mapping to define poten tial dispersal routes within and between host metapopula tions are rare (Bakke and Harris 1998). Research on sea lice dispersal patterns in Europe (Costelloe et al. 1996, 1998) and circulation models around salmon farms in BC to bet ter understand potential dispersal patterns of IHNV and sea lice (Foreman et al. 2012) are the exception. Without this research, the epidemiological consequences of open net pen farms associated with aquaculture, and of movements of juvenile salmon between river systems, cannot be ade quately assessed.

The transfer of disease between farmed and wild fish does not necessarily require direct contact between the two populations. Microparasites can also be transported by predatory birds (McAllister and Owens 1992) and fish (Glover et al. 2013), and by escapees from farms (Munro et al. 1976). Avian scavengers may travel long distances, spreading diseases between freshwater and marine habitats (Murray and Peeler 2005); IPNV has been found in the feces of scavenging sea gulls (McAllister and Owens 1992). Predatory wild Atlantic cod (*Gadus morhua*) have also been shown to be carriers of PRV likely originating from nearby salmon farms (Glover et al. 2013).

Direct exchange of microparasites between cultured and wild fish is certainly not the only route of microbe exchange. Many microparasites have intermediate inverte brate hosts; hence environments that foster naturally high densities of intermediate hosts may enhance levels of natu ral populations. Marine fish, such as herring (*Clupea pall asi*), threespine sticklebacks (*Gasterosteus aculeatus*), Pacific hake (*Merluccius productus)*, and Pacific sandlance (*Ammo dytes hexapterus*) are routinely cocaptured in aggregations of salmon smolts or in areas around salmon farms, and are known to harbor microparasites that can infect salmon. Salmon microparasites known to be carried by marine fishes include: Viruses VHSV, ISAV, and IHNV; bacterial microbes *R. salmoninarum*, chlamydia like organisms; microparasites *Loma* sp. (Nylund et al. 2002; Kent et al. 1998). Sea louse are important salmon macroparasites, and may be important vectors for viruses (e.g. ISAV Nylund et al. 1993; IHNV Jakob et al. 2011), bacteria (*A. salmon icida* Nese and Enger 1993) and microparasites (*Para nucleospora theridion* Freeman and Sommerville 2009; Jones et al. 2012).

Potential for exchange between hatchery and wild fish

The Salmon Specialist Group of the International Union for the Conservation of Nature (IUCN) listed 'negative effects of hatcheries and construction of artificial spawning habitat,' including the spread of disease to wild salmon, as one the their three major threats to Sockeye Salmon (Rand 2008). While there is relatively strong evidence for genetic impacts on fitness (reviewed in Naish et al. 2008), direct evidence for the role of disease is lacking (Stephen et al. 2011). We restrict our brief discussion of hatchery impacts herein to the transmission of disease.

Hatchery fish reared for enhancement are exposed to the same environments as wild fish for the marine phase of their life cycle and freshwater return migration; hence, other than at natal rearing areas (or hatcheries), the same endemic microparasite reservoirs are the source of infec tious diseases for both (Naish et al. 2008). However, when infectious diseases occur, the prevalence and intensity of infection may grow faster in a high density hatchery envi ronment than in the wild (Naish et al. 2008). As with aqua culture, hatchery fish may facilitate microparasite transfer through the intentional movement of cultured fish carrying undetected exotic microbes (examples include the spread of whirling disease in the United States and *G. salaris* in Europe discussed previously) and amplification of endemic microparasites in high density rearing environments (released through untreated hatchery effluents), for which there is limited direct evidence (Naish et al. 2008). Inten tional release of infected fish can also occur, but the conse quences of these releases on wild fish have not, to date, been monitored (Stephen et al. 2011). Perhaps the best example of this potential is in enhanced disease in hatchery and wild fish barged together to facilitate transport around dams in the Columbia River (Elliott et al. 1997). Alter nately, hatcheries that aim for disease free environments through use of well water may release large numbers of sus ceptible naïve fish to the environment, which may cause localize outbreaks of disease (Naish et al. 2008).

Lack of regular microparasite monitoring in hatchery and wild stocks may largely explain the limited data avail able to assess disease interactions between hatchery and wild fish (Krueger and May 1991). Disease monitoring programs vary widely between individual hatcheries (Ste phen et al. 2011) but are largely limited to broodstock assessments for a small number of vertically transmitted microparasites [e.g. *R. salmoninarum* and IHNV in the Pacific Northwest (Stephen et al. 2011); *R. salmoninarum*, furunculosis, and IPNV in Norway (Biering et al. 2013)]. Veterinary diagnostics may be performed during mortality events. Case reports from BC enhancement hatcheries have identified a wide range of pathogens, including viral (3), bacterial (7), microsporidian (2), myxozoan (2), protozoan

(2), ameba (1), ciliate (1), and an ectoparasitic worm (1) infecting salmon in BC hatcheries (Table 1).

Climate change shifts the balance

There is an increasing concern about the potential ways in which global warming or climate change can alter the severity or distribution of diseases affecting aquatic animals (Harvell et al. 1999; 2002; Lafferty et al. 2004; Marcogliese 2008; Echaubard and Lesbarrères this issue). Most infec tious agents have short generation times and large popula tion sizes. Moreover, strong selection following ecological changes, like those associated with shifts in climate, might accelerate pathogen evolution (Altizer et al. 2003). Latitu dinal diversity gradients for pathogen richness track those of general species diversity increasing from the poles to the equator (Rohde and Heap 1998; Rohde 1999; Guernier et al. 2004), with temperature a contributing factor gener ating this variation (Clarke and Gaston 2006). Retreat of perennial sea ice has shown acceleration in recent decades (Comiso et al. 2008); subsequently increased Arctic passage has the potential to promote range expansion of various marine species and their associated pathogens across ocean basins (Post et al. 2013). A comprehensive understanding of host/pathogen relationships and their nuances among species, populations and life stages (e.g., salmonids) is criti cal to anticipating region specific impacts on disease potential within the context of spatially varying climate related changes in associated abiotic factors (e.g., tempera ture; Altizer et al. 2013).

It has been well established that temperature is a critical environmental factor that affects the progression of disease in fish (Wedemeyer 1996). High water temperature (HWT) can affect disease progression through direct effects on host physiology that compromise immune system function or direct effects on microparasites that alter their replication rate (Noe and Dickerson 1995; Marcogliese 2001), likely involving both plastic and evolutionary mechanisms. Tem perature increases can also impact development rate and timing of release of microparasites from intermediate hosts, potentially increasing densities and and extending exposure periods for migratory fish (Stocking et al. 2006; Ray et al. 2012; Chiaramonte 2013). Hence, migration timing, often associated with river temperature, is an important aspect impacting disease potential, especially via microparasites with intermediate hosts (e.g. *C. shasta*, *P. minibicornis*).

HWT has been identified as a source of stress especially during crucial life history stages such as adult spawning migration (Crossin et al. 2008; Eliason et al. 2011, 2013a; Clark et al. 2012) and is a primary factor affecting adult survival relating to overall fitness (Martins et al. 2011, 2012a,b). Thermal tolerance has been classified by several studies as species or population specific, consistent with historic temperatures (Lee et al. 2003; Farrell et al. 2008; Clark et al. 2011; Eliason et al. 2011, 2013b), and likely the result of selection (Crozier et al. 2008). Studies of handling stress at elevated temperature across a wide range of species have shown that deleterious effects occur within the bounds of a preferred temperature range rather than above (Gale et al. 2013); hence, additional stressors such as mi croparasite infection could have enhanced impacts at even slight temperature increases. Water temperatures above the thermal optimum could adversely affect swimming stamina of naturally migrating fish or fish evading predators and fishing nets in the river, regardless of infection status (Far rell et al. 2008). Sustained swim performance is substan tially inhibited between 18 to 21°C, above which fish can no longer maintain homeostasis and is immediately lethal (Farrell et al. 2009). Such inhibition is supported by observed migration failure of wild stocks when river tem peratures exceed 18°C (Crossin et al. 2008; Jeffries et al. 2012; Keefer et al. 2008; Martins et al. 2011, 2012a). Pro posed mechanisms contributing to decreased stamina and migration failure in the presence of HWTs include increased energy use (Rand et al. 2006), decreased dis solved oxygen (Eliason et al. 2011), as well as severe micro parasite infections resulting in lower critical swim speeds and longer recovery rates. (Tierney and Farrell 2004; Wag ner et al. 2005; Kocan et al. 2009). Decreased swim perfor mance arising from infection could increase exposure time to HWT and vulnerability to predation, further exacerbat ing the potential for cumulative impacts.

If differences in microparasite virulence under HWTs result from reduced condition of the host (i.e., a weaker immune response), then evolutionary variance in suscepti bility to temperature stress may play a larger role than plas tic responses in pathogen temperature outcomes, with predicted greater tolerance to microparasites in fish with greater resistance to HWT stress. Hence, direct effects of temperature on pathogen virulence may manifest differ ently among populations depending on evolved variances in temperature susceptibility; animals that are not stressed directly by high water temperatures may be more refractory to pathogens showing enhanced virulence with tempera ture. Moreover, the degree of energy allocation to the immune response may be pathogen and host dependent at HWT, as exemplified in the relatively stronger transcrip tional response of Atlantic cod to viral versus bacterial pathogens under HWT conditions (Hori et al. 2013).

Cumulative chronic and acute stressors impacting sal mon stocks are in need of quantitative evaluation using a multivariate approach (Johnson et al. 2012) and evolution ary perspective to anticipate population specific variability in HWT responses of host and pathogen. A large scale multispecies evaluation of disease potential in adult salmon during spawning migration and in response to both

thermal and fisheries stressors has produced preliminary findings presented in our third case study described below. Using a microparasite screening approach of wild fish col lected and held in a laboratory setting, we manipulated temperature during a simulated migration to monitor dif ferences in microbe load and associated mortality trends between temperature treatments.

Predators the ultimate cause of death of infected wild fish?

During their marine life, Pacific salmon experience variably heavy mortality rates that generally exceed 90% (Bradford 1995). Mortality arising from nonanthropomorphic preda tion is thought to be less common in homeward migrating fish upon river entry, but still can occur from marine mam mals and bears (Quinn and Kinnison 1999), and may be mediated by other stressors like fisheries interactions (i.e., postrelease predation of discards by seals; Donaldson et al. 2011). The losses in the marine environment are thought to be caused primarily by predation in the first few weeks to months following ocean entry, and by weakened condi tion due to food limitation during the first winter at sea (Beamish and Mahnken 2001). While mortality from both causes is thought to be size and condition dependent (Willette 2001; Hurst 2007), the supporting evidence tends to be indirect and inferential. For example, size selective mortality is typically inferred from reconstructions of fish lengths from recovered hard parts (scales and otoliths) (Healy 1982), but this method precludes an assessment of variation in body mass, condition or health, and rarely are characteristics of survivors and nonsurvivors compared simultaneously. Although size selective survival for salmon is commonly recognized (e.g., Saloniemi et al. 2004), at times it is not observed (Welch et al. 2011) or the effect is negative (Ewing and Ewing 2002). Nonetheless, conditions that lead to decreased growth and energy storage are expected to increase mortality rates and ultimately decrease returns of adult salmon (Beamish et al. 2004). For example, environmental conditions that lessen the quality and avail ability of food can decrease growth rates resulting in poor physical condition (Tocher 2010; Duffy and Beauchamp 2011; Tomaro et al. 2012). Poor physical condition can reduce salmon health and survival directly through immune suppression and susceptibility to pathogens (Peters et al. 1988; Arkoosh et al. 2006). Poor body condi tion has also been linked to a reduction in the capacity of fish to evade predators under controlled conditions (reviewed in Mesa et al. 1994), and in river environments (Hostetter 2009). However, the actual sources of mortality let alone the role of body condition and other indices of the health of juvenile salmon in determining their suscepti bility to predators while at sea remain a black box given the difficulty of studying fish in such a dynamic environment over enormous geographic scales ultimately facing an array of potential predators. This becomes even more confound ing given the likely potential for conflating interactions between environmental conditions, competition, disease, and predation.

Selection of prey in poor body condition is a widespread phenomenon in terrestrial systems (Murray 2002; Husse man et al. 2003). The tendency for terrestrial predators to take substandard prey is linked to hunting strategy where predators that pursue their prey are more likely to take indi viduals in poorer condition compared to those with ambush tactics given impeded escape ability and/or state dependent risk taking (Fitzgibbon and Fanshawe 1989). Similar pat terns of prey selection are often assumed to operate for sal mon in the ocean (Burke et al. 2013). A laboratory study by Mesa et al. (1998) demonstrated that Chinook Salmon chal lenged with *R. salmoninarum* were more susceptible to pre dation by Northern Squawfish (*Ptychocheilus oregonensis*) and Smallmouth Bass (*Micropterus dolomieu*) under experi mental conditions. We found a single field study that assessed the impact of condition and microparasites on pre dation in wild salmon (Hostetter 2009). They documented external condition characteristics (e.g., body injuries, descal ing, external signs of disease, fin damage, and ectoparasite infestations) of tagged out migrating Steelhead Trout smolts in the Columbia River and noted that recoveries of tags at downstream colonies of Caspian Terns (*Hydroprogne caspia*) and Double crested Cormorants (*Phalacrocorax aur itus*) not only indicated that smaller Steelhead were taken but that predation was highest on Steelhead displaying signs of poor condition. Moreover, external condition was corre lated with the presence of selected pathogens detected by histopathology and molecular analysis. While the indices of condition were somewhat qualitative and the suite of patho gens restricted, results are intriguing.

In general, condition based susceptibility and the role of disease in the marine environment remains untested given that predator/prey interactions are difficult, if not often impossible to observe. In case study IV, we identify one predator/prey system that is amenable to observation and direct testing of the condition based predation hypothesis. The Rhinoceros Auklet *Cerorhinca monocerata* is an abun dant, pursuit diving seabird that consumes copious quanti ties of salmon post smolts, delivering them whole and intact to nestlings (Thayer et al. 2008). During migration, the vast majority of juvenile salmon from southern and central BC stocks funnel past aggregations of hundreds of thousands of auklets that breed on colonies scattered along BC's Central and North coast (Tucker et al. 2009, 2012). We were able to collect freshly caught smolts from auklet nests and contrast their condition and infection status with that of smolts in the general population. Although the scale of the study was small, it is one of the few studies able to

make direct contrasts between predated and unpredated salmon in the field.

Perspective on moving forward

Establishing a direct cause and effect relationship between pathogens and disease may not be possible in wild populations if pathogenicity of an infectious agent causes infected fish to die and disappear before they are detected (Bakke and Harris 1998). Hence, to understand the role of infectious diseases on wild salmon, it is important that we merge both field studies that allow for the discovery of factors associated with survival in complex natural environments with controlled laboratory studies that can test hypotheses gained from field studies and provide a stronger mechanistic basis to findings. There is a strong foundation of research on distributions and impacts of salmon macroparasites in wild salmon, largely because these are readily observable either to the naked eye or using microscopy (Margolis and Arthur 1979; McDonald and Margolis 1995; Bennett et al. 1998; Kent et al. 1998; Arkoosh et al. 2004; Ferguson et al. 2012). Microparasites have received much less focus in wild fish, despite the fact that they have caused the most devastating impacts on cultured fish. Bacterial kidney disease (BKD), vibriosis, ceratomyxosis, and enteric redmouth have, however, been observed in wild migrating fish (Arkoosh et al. 2004; Kent et al. 1998; Fujiwara et al. 2011; Rhodes et al. 2011). A small number of studies that have conducted sequential sampling have used overdispersion (mean to variance ratios) of parasites as indirect evidence of mortality (Gordon and Rau 1982; Kalbe et al. 2002; Jacobson et al. 2008). Alternately, use of negative binomial distributions truncation technique described by Crofton (1971) has been a widely accepted model for macroparasites (see Scott and Smith 1994; Ferguson et al. 2011).

Traditional diagnostic approaches relying on observed mortality events are not sufficient to study disease in natural systems. The probability of finding near moribund infected fish in random samples of wild caught salmon is low, and damage at the cellular level that characterizes different types of diseases may be difficult to resolve with histology. Because successful cell culture generally requires a moderate load of viruses or bacteria (Templeton et al. 2004), culture may additionally miss detection of animals at an early stage of infection. Moreover, culture based methods may underestimate microparasite presence, as all microparasites are not cultivable (e.g., PRV, PMCV, ISAHPR0, others). ELISA's can be an effective diagnostic method to identify well characterized infectious agents but are not generally as sensitive as molecular approaches (Sandell and Jacobson 2011). Quantitative RT PCR is generally the most sensitive method to detect presence and load of microparasites (Purcell et al. 2011), but in some instances may not be as sensitive as culture based methods for diagnosing disease, as it is unable to determine whether a microbe present in a tissue is viable and actively replicating (Purcell et al. 2013).

The fact is that we have not adequately characterized the range of microparasites that wild salmon carry, especially in the marine environment. Most of the recently discovered microparasites associated with emerging diseases in Europe have not even been assessed for the presence in North America. Hence, we, along with numerous other scientists studying wildlife populations (Bakke and Harris 1998; Dobson and Foufopoulos 2001), argue that a broad characterization of the microparasites carried in the wild would provide a good foundation to research aimed at establishing the role of infectious disease in natural systems.

BC salmon health initiative

We have developed a multidisciplinary research program, the Strategic BC Salmon Health Initiative (SSHI; http://www.genomebc.ca/portfolio/projects/fisheries projects/strategic salmon health initiative/) that merges the fields of genomics, epidemiology, histopathology, virology, parasitology, fish health, veterinary diagnostics, and salmon ecology to assess the potential role of infectious disease as a cofactor in wild salmon declines. The core of this research is the evaluation and application of a high throughput microfluidics platform for the identification and quantification of important viral, bacterial, protozoan, and fungal microparasites that may influence the health and survival of native populations of BC salmon. Using this technology, the research will characterize broadly the range of microparasites carried by wild salmon, assess variance in diversity and loads of microparasites carried in populations of wild and cultured salmon during smolt out migration and adult return migration, assess genetic variance in host response to specific microparasites, conduct association studies between microparasites, host immune genes, and fate (using biotelemetry), and assess in experimental studies which microparasites are further stimulated to replicate under elevated temperatures and handling stress (catch/release fisheries). This program is also integrating histopathology to identify lesions associated with cellular damage that may be associated with high loads of specific microparasites, important to begin to link microparasite carrier states with potential for disease. Epidemiology studies will incorporate full viral genome sequencing to characterize the distribution and potential for exchange of viral microparasites of interest. NGS will also be used for viral discovery research. Laboratory challenges of understudied microparasites that carry the greatest potential for population level impacts (i.e., of sufficient prevalence and load, possibly with evidence of shifting prevalence/load over

migration, and associated with strong genomic responses and evidence of cellular damage) will follow to determine cause and effect relationships between microparasites and disease and to determine under what conditions disease occurs. Ultimately, if microparasites potentially associated with salmon productivity are identified, studies that pro vide the evolutionary framework upon which disease ensues based on genetic variation in microparasites and host will be pursued.

To effectively tackle cumulative impacts of multiple stressors, we are clearly going to need to employ modern, sophisticated tools, and approaches for studies conducted in natural systems. Ideally, these would merge molecular based monitoring tools, genetic markers to differentiate populations, gene expression profiling to assess condition and health, biotelemetry to relate biological and physiologi cal metrics of condition and health with shifts in behavior and fate, and oceanographic data to incorporate abiotic factors. Herein, we present a series of three 'proof of con cept' field studies and one laboratory study that utilize a combination of novel approaches to explore the range of microparasites potentially impacting wild salmon popula tions and the cumulative impacts of genetics, temperature stress and predators on the diversity and loads of micropar asites and ultimate disease outcomes. In future, the intent is to merge these approaches with full genome scans (i.e., QTL discovery and full parental genotyping of hatchery fish) that will provide a greater mechanistic understanding of the evolutionary impacts of cumulative stressors on wild salmon populations.

The highlighted studies were developed to test a number of null ecological and evolutionary hypotheses, including (i) there are no genetic differences in the diversity, range and load of microparasites carried by wild salmon popula tions that have reared in a common ocean environment (adult liver study), (ii) microparasite carrier states are not predictive of migratory fate of return migrating salmon, and if they were, there are no population specific differ ences in microparasite associations (2010 tracking study), (iii) temperature and handling stress do not impact micro parasite replication or virulence, or subsequent survival of salmon (Coho handling study), and (iv) there is no associa tion between salmon infection status and seabird predation (Auklet study).

Foundations of the novel and merging of technologies presented in our studies

Molecular technologies are rapidly changing the ways we approach ecological and evolutionary research and the depth of information that can be gained both quickly and relatively cheaply. Common and emerging applications, with examples in aquatic/salmon biology, include:

1) Genetic assessments, which when based on a small num ber of markers (e.g. microsatellites or SNPs) have been used to identify population compositions of mixed pop ulation samples of Pacific salmon routinely applied in salmon management and to identify population specific migration routes (Beacham et al. 2008; Tucker et al. 2009, 2012), and to identify population of origin of individuals used to assess the performance and condi tion of different stocks groups across diverse habitats (Cooke et al. 2008; Miller et al. 2011; Hinch et al. 2012). Genome scale genetic assessments (e.g., fully mapped microsatellites, RAD tag sequencing) have been used in QTL discovery and to identify adaptive genetic variation among populations (Houston et al. 2008; Miller et al. 2012). Herein, we apply genetic population identification based on microsatellite loci and SNPs on wild caught individuals to assess (i) the relative impact of genetic variation (at the population level) in micro parasite diversity and load, and (ii) to determine the importance of genetic variation (at the population level) in microparasite associations with migratory fate.
2) Gene expression profiling to elucidate response to stres sors, based on both targeted gene 'biomarker' approach (e.g. qRT PCR of biomarkers known to associate with disease, stress, environmental adaptation; Elder et al. 2008) and genome based approaches [e.g., microarrays assessing the activity of 10s of thousands of genes or NGS of RNA transcripts (RNA Seq) (Salem et al. 2012)]. Herein, we employ a targeted gene approach alongside the microparasite monitoring applied on a microfluidics platform.
3) Monitoring systems to determine the presence and rela tive abundance/load of species/strains of interest (e.g., microparasites, harmful algal bloom species, planktonic communities, gut contents, and invasive species). Research on microbial communities is perhaps the far thest along when it comes to large scale molecular based monitoring, with NGS approaches used to simul taneously identify species compositions and functional trajectories of common place microbial communities (MacLean et al. 2009). Molecular virology has also uti lized a NGS approach to discover viruses that control phytoplankton bloom cycles (Suttle 2007). However, research and monitoring of infectious agents is far behind, largely employing single assays as a diagnostic tool to assess potential associations of a small number of microparasites with disease and mortality. Herein, we expand on this approach and present studies that utilize a microfluidics qRT PCR platform that can simulta neously run 96 TaqMan assays on 96 samples (Fluidigm BioMark™, Fluidigm Corp., San Francisco, CA, USA). This system has similarly been used in microbial water monitoring (Ishii et al. 2013). We apply this system for

the first time to monitor the presence and load of up to 45 salmon microparasites and verified the key findings of a subset of microparasites on the commonly used ABI 7900 platform.

Biotelemetry

The field of biotelemetry has been used effectively to track migratory pathways of a large range of organisms (Ropert Coudert and Wilson 2005); in the marine realm, sharks, marine mammals, salmon (Rechisky et al. 2013), and tuna have commonly been studied. In 2003, we began merging biotelemetry with nondestructive biopsy sampling of blood and gill tissue from adult salmon (see Cooke et al. 2005 for details on the technique) to determine whether there were associations between indices of physiological condition and migratory behavior and fate (reviewed in Cooke et al. 2008 and Hinch et al. 2012). Over multiple years' study, timing of river entry and migratory fate were found to be associated with osmoregulation and stress in return migrating Sockeye Salmon (Cooke et al. 2006; Crossin et al. 2007, 2009; Donaldson et al. 2010). In 2006, we expanded the physiological component of this research to include functional genomics (Miller et al. 2011). The functional genomics study identified a single mortality related signature (MRS) associated with premature mortality in the river no matter if salmon were tagged in the marine environment, the lower river, or at spawning grounds, providing strong evidence that the condition of salmon in the marine environment impacted the success of migration in the river. Based on the biological processes stimulated within the MRS, we hypothesized that this signature was associated with a response to viral infection. Case study II was a further expansion on this approach, merging molecular monitoring of microparasites and host genes associated with immunity and stress with biotelemetry to explore the linkages between microparasite carrier states, salmon condition, and migratory fate of wild caught Sockeye Salmon returning to spawn in the Fraser River in 2010.

Case studies

Overview

In the following case studies, we assessed diversity and load of a suite of microparasites and conducted association analyses to determine both the factors that explain variations in microparasite distributions (case studies I III) and the impact of microparasite carrier states on the fate of wild migrating salmon (case studies II and IV). Note that we did not directly assess 'disease state' as defined by levels of cellular damage, and we did not attempt to culture microparasites to determine whether they were viable. We did, however, merge host gene expression analysis in case study II to assess which microbes are eliciting a strong response in the host. Given that the microarray studies reviewed above universally show that intensity of host transcriptional response is highly correlated with susceptibility and disease, we use these data to assess which microbes carry the greatest potential for disease at the time the fish were sampled. Future studies will merge histopathology and gene expression analysis with molecular monitoring to identify whether pathology at the molecular and cellular levels is associated with high load carrier states of microparasites.

Methods

Fluidigm BioMark

In all studies, we conducted qRT PCR of microparasites, and in some cases, host genes using TaqMan assays run on the Fluidigm BioMark™ platform. We focus largely on microparasites known or suspected to associate with diseases in salmon worldwide (Table 2). Some of the microparasites on our panel are known endemics to BC, others are known to be present in other species but not previously assessed in the species of focus, are recently identified in BC salmon but not extensively studied, or are associated with emerging diseases in Europe but not previously assessed in northeastern Pacific salmon populations (Table 1). Most microparasite assays were from the literature, although a small number were designed in house with Primer Express 3.0.1 software (Life technology, Burlington, ON). Herein, we present results for microbe assays that show strong correlations between the BioMark™ and ABI 7900 platforms, that have been sequence confirmed to verify that the assay is picking up the intended microbe, and that with few exceptions, carry efficiencies above 85%. A full evaluation of the platform performance and impacts on assay sensitivity and specificity for each assay is underway in phase 2a of the SSHI.

The Fluidigm BioMark™ microfluidics platform can run 96 assays against 96 samples at once (9216 reactions on a single dynamic array). As our microparasite TaqMan assays are run in duplicate, we ran up to 45 unique assays and 2 3 housekeeping gene controls per run. We followed manufacturer instructions on the temperature and cycle conditions. Technical details for RNA and cDNA preparation are in Miller et al. 2011 and for the Fluidigm BioMark™ are presented in Data S1.

In each study, tissues were collected in the field and preserved in RNAlater (Qiagen, MD, USA) for 24 hours at 4°C and then frozen in −80°C. In destructively sampled fish, gill, whole brain, liver, head kidney, white muscle, and heart tissues were sampled in the field, whereas in nondestructively sampled fish, only gill was taken. The tissues utilized for microbe monitoring varied by study, as outlined below. Note that in this broad screening, the tissue assessed

Table 2. Overview of the microparasites included in case studies

Microbe	Agent	Literature Citation	Efficiency	Prevalence over Case Studies			
				I	II	III	IV
Aeromonas hydrophila	Bacterium	Lee et al. (2006)	0.83	N/A			
Aeromonas salmonicida	Bacterium	Keeling et al. (2013)	0.93	N/A			
Flavobacterium psychrophilum	Bacterium	Duesund et al. (2010)	0.97	19% *	38%*		1%
Piscichlamydia salmonis	Bacterium	Nylund et al. (2008)	0.77				
Piscirickettsia salmonis	Bacterium	Corbeil et al. (2003)	0.97	N/A	<1%		
Renibacterium salmoninarum	Bacterium	Suzuki and Sakai (2007)	0.94	N/A			
Rickettsia Like Organism (Strawberry disease)	Bacterium	Lloyd et al. (2011)	0.94	N/A	4%*	71%	
Salmon (Gill) chlamydia	Bacterium	Duesund et al. (2010)	0.88		3%		4%
Vibrio anguillarum	Bacterium	MGL	N/A	N/A			
Vibrio salmonicida	Bacterium	MGL	N/A	N/A	N/A	N/A	
Yersinia ruckeri	Bacterium	Glenn et al. (2011)	0.98	N/A			
Atlantic salmon paramyxovirus	Virus	Nylund et al. (2008)	0.92	N/A			
Erythrocytic necrosis virus	Virus	J. Winton (pers. comm.)	N/A	N/A		N/A	
Infectious hematopoietic necrosis virus	Virus	Purcell et al. (2006)	0.97	<1%	1%		
Infectious pancreatic necrosis virus	Virus	S. Clouthier (pers. comm.)	0.97	N/A			
Pacific salmon parvovirus	Virus	MGL	0.96	27% *			23%
Piscine reovirus	Virus	Wiik Nielsen et al. (2011)	0.90	<1%	19%*		
Salmon alphavirus 1, 2, and 3 (PD/SD/HSS)	Virus	Andersen et al. (2007)	0.91	N/A			
Salmonid herpesvirus/Oncorhynchus Masou Herpes Virus	Virus	MGL	N/A	N/A	N/A	N/A	
Viral encephalopathy and retinopathy virus	Virus	Korsnes et al. (2005)	0.90	<1%	<1%		
Viral hemorrhagic septicemia virus	Virus	Jonstrup et al. (2013)	0.88	N/A			
Gyrodactylus salaris	Ectoparasitic worm	Collins et al. (2010)	0.89	N/A			
Ichthyophthirius multifiliis	Ciliate	MGL	0.91	N/A	14%*	98%*	1%
Nanophyetus salmincola	Fluke	MGL	0.80	N/A			
Spironucleus salmonicida	Flagellate	MGL	0.98	N/A			
Paranucleospora theridion (syn. Desmozoon lepeophtherii)	Microsporidium	Nylund et al. (2010)	0.78	<1%	19%		28%
Facilispora margolisi	Microsporidium	MGL	0.83	N/A	1%		
Loma salmonae	Microsporidium	MGL	N/A	N/A	32%*	N/A	1%
Nucleospora salmonis	Microsporidium	Foltz et al. (2009)	0.99	18%	30%	10%	
Ceratomyxa shasta	Myxozoan	Hallett and Bartholomew (2006)	0.97	N/A	20%*	100%*	1%
Kudoa thyrsites	Myxozoan	Funk et al. (2007)	0.90	<1%		54%	
Myxobolus arcticus	Myxozoan	MGL	0.96	N/A	<1%		2%
Myxobolus cerebralis	Myxozoan	Kelley et al. (2004)	0.89	N/A			
Parvicapsula kabatai	Myxozoan	MGL	0.96	N/A		N/A	12%*
Parvicapsula minibicornis	Myxozoan	Hallett and Bartholomew (2009)	0.98	N/A	34%*	100%*	35%*
Parvicapsula pseudobranchicola	Myxozoan	Jørgensen et al. (2011)	1.29	<1% *	3%		7%*
Tetracapsuloides bryosalmonae	Myxozoan	Bettge et al. (2009)	0.91	N/A	1%*	38%	N/A
Cryptobia salmositica	Protozoan	MGL	N/A	N/A	N/A	N/A	
Ichthyophonus hoferi	Protozoan	MGL	0.88	N/A	1%	2%	5%
Sphaerothecum destruens	Protozoan	MGL	0.82	N/A		2%	2%

Case studies I and II assessed ocean, river, and spawning ground adult Sockeye, using liver (I) or gill (II) tissue. Case study III surveyed mixed tissues from adult freshwater migrating Coho. In case study IV, liver and gill tissue from ocean migrating Sockeye post smolts was assessed. The null 0% prevalence noted as ( ), assays not assessed within the case study noted as N/A, significant microparasite marked as (*). Prevalence values presented from case study III include only held fish. Primers obtained from publication are noted with literature citation (MGL primers subject to request). References cited in the Table but not referred to in the text are presented in Reference S1.

may not be the primary infective tissue for all microbes monitored; hence, in studies only assessing single tissues (e.g., case studies I and II), we may be underestimating overall microbe carrier states. Individual genetic popula tion identification was performed for all Sockeye Salmon studies on all samples except those collected at spawning grounds (Beacham et al. 2005).

QRT PCR results were exported as a heatmap csv file and imported into GenEx (www.multid.se) for data prepa ration and statistical analysis. Data from multiple arrays

were combined within GenEx and the average of the duplicated samples calculated. Samples amplifying products from only one duplicate were treated as negative; negatives were all given a threshold cycle (CT) of 50. We used a conservative cut off of CT<27 to score individuals as 'positive' or 'detected' for the calculation of prevalence; this equates to a CT of 35 36 on the ABI 7900 and is near the upper limit of reliable repeatability on the ABI instrument. Pearson correlation tests and principal components analysis (PCA) were performed in Genex. Multivariate analyses of variance (MANOVAS) were applied with a randomization procedure (Efron and Tibshirani 1993) in R (R v. 2.15.3; R Development Core Team 2008) to generate test statistics for main effects and interactions in pairwise comparisons. For each analysis, factor labels were randomly permuted 10 000 times to build a permutation distribution rather than compare test statistics to normal distributions. Significance levels were then computed by determining the number of times the reference distribution gave a test statistic equal to or greater than the observed value. If the overall test was significant, pairwise *post hoc* tests were applied to determine which microbes were driving the differences observed. *Post hoc* univariate and multivariate t tests were also compared with the permutation distributions to determine where the significant differences occurred. Bonferroni corrections were conducted to minimize Type II errors when performing multiple tests; only results significant after correction are reported. The impact of microparasite diversity (count of all detected microbes per individual) and load (count of microbes with CT<20) were additionally explored in some studies using nonparametric Mann Whitney *U* test or Chi Square statistics, respectively.

Data preparation and analysis of host genes was also performed in GenEx Enterprise (www.multid.se), in which duplicates were averaged, missing values were filled with column mean, values were corrected for PCR efficiencies (from serial dilutions run), data were normalized (delta CT) with three reference genes (78d16.1, MrpL40 and CoilP84), and values were converted to relative quantities with pooled sample data (delta delta CT), and $\log_2$ transformed. To determine whether there was an association of host gene expression with specific microparasites, a nonparametric Mann Whitney *U* test was performed, with a threshold value of 0.00088 to keep the overall risk of type I error at 0.05 under multiple testing.

Results

Case study I

Are the prevalence, load, and diversity of microparasites correlated with host stock, environment, and/or year? Case study I was a preliminary assessment of the microparasites in liver tissue of return migrating Sockeye Salmon. A total of 758 Sockeye Salmon were collected from 2005 through 2010 (6 years) from test fishery ocean trawlers in the marine environment (Johnstone Strait, Juan de Fuca Strait), freshwater trawlers in the lower Fraser River, and by beach seine or netting at spawning grounds (as per Miller et al. 2009; experimental design in Table S2). Sockeye have a strong cyclic abundance pattern within populations, and hence, it was not possible to sample all populations in all years (population*year could not be evaluated).

Individual microparasite prevalence over all samples ranged from 0 27%, with six of the 11 microbes surveyed amplifying products with CT<27 in at least two samples (Table 2). Most detected microparasites were present in at least some fish before freshwater entry with the exception of *Kudoa thyrsites*, Gill chlamydia and *P. salmonis*. The three most prevalent microparasites were bacterium *F. psychrophilum*, parvovirus, and microsporidian *Nucleospora salmonis* (Fig. 1). Two way MANOVAs revealed that environment ($P < 1 \times 10^{-4}$ in both comparisons) and population ($P < 1 \times 10^{-4}$ in stock comparison) were the main contributors to the overall microparasite variation. However, an interaction term was also significant between environment and stock ($P < 1 \times 10^{-3}$). Individuals carried one to three microbes, and with the exception of three of four viruses surveyed (parvovirus, PRV, Viral encephalopathy and retinopathy [VER]), the environmental trend showed enhanced overall microbe prevalence, diversity, and load toward the spawning grounds (Fig. 1).

Flavobacterium psychrophilum prevalence increased toward the spawning grounds for Chilko, Quesnel and Shuswap populations (Fig. 1). This bacterium is the causative agent of bacterial coldwater disease and is a freshwater pathogen mostly known for its impact on Rainbow Trout fry (reviewed in Starliper 2011); it has not previously been assessed in Sockeye Salmon, and was considered to be of low risk by Kent (2011) due to lack of evidence that Sockeye Salmon were susceptible. An increase in load was observed at the spawning grounds (Fig. 2), suggesting that the bacterium was actively replicating and being transmitted among individuals during migration in the river.

The myxozoan parasite *Parvicapsula pseudobranchicola* was only observed in a small number of fish in 2005 almost exclusively in the Quesnel population (data not shown). While the myxozoan was present in two of three environments (ocean and spawning grounds), the lowest CT was 22, indicating only a moderate load (~10^2 copies). *P. pseudobranchicola* is considered a marine parasite primarily infecting gill tissue, and has been associated with mortalities on salmon farms in Norway (Karlsbakk et al. 2002). There are no data to indicate its pathogenicity in freshwater, and no previous studies in wild fish, or studies documenting its presence in BC (but see also case study

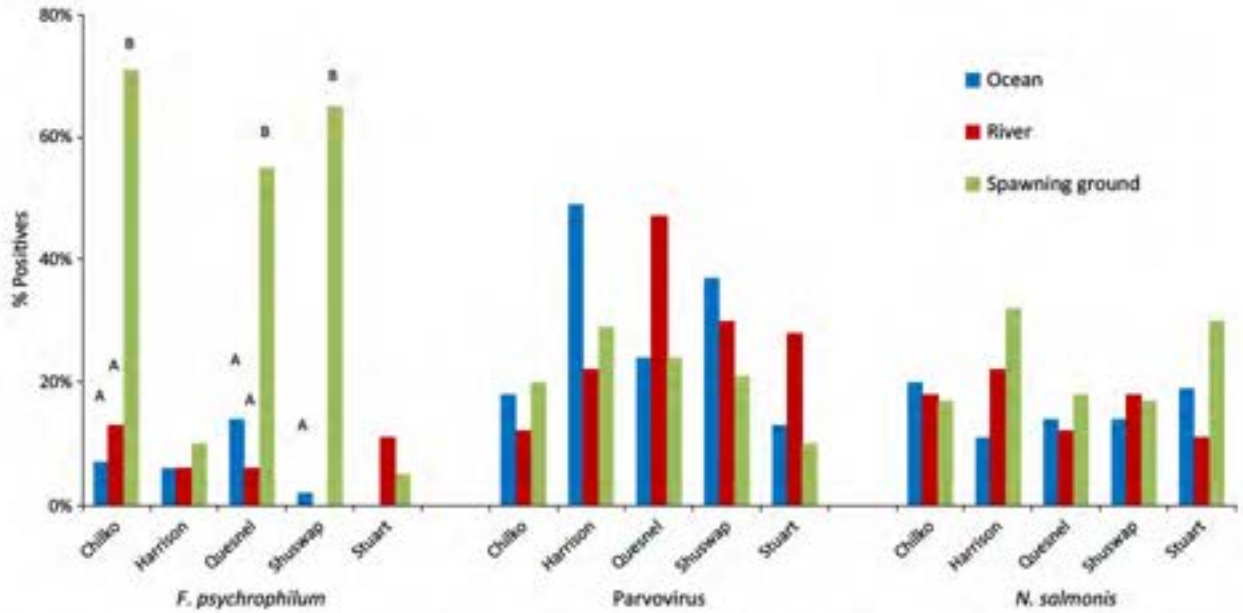

Figure 1 Distribution of *Flavobacterium psychrophilum*, Pacific salmon parvovirus and *Nucleospora salmonis* among stocks and environments in liver tissues of return migrating Sockeye Salmon (case study I). Environment explained the highest source of variation, with increasing prevalence at spawning grounds in three (Chilko, Quesnel, Shuswap) of the five stocks. Microbes that were significantly different between environments within each stock are indicated by differing letters (i.e., A and B; $P < 0.001$, 1 way MANOVA).

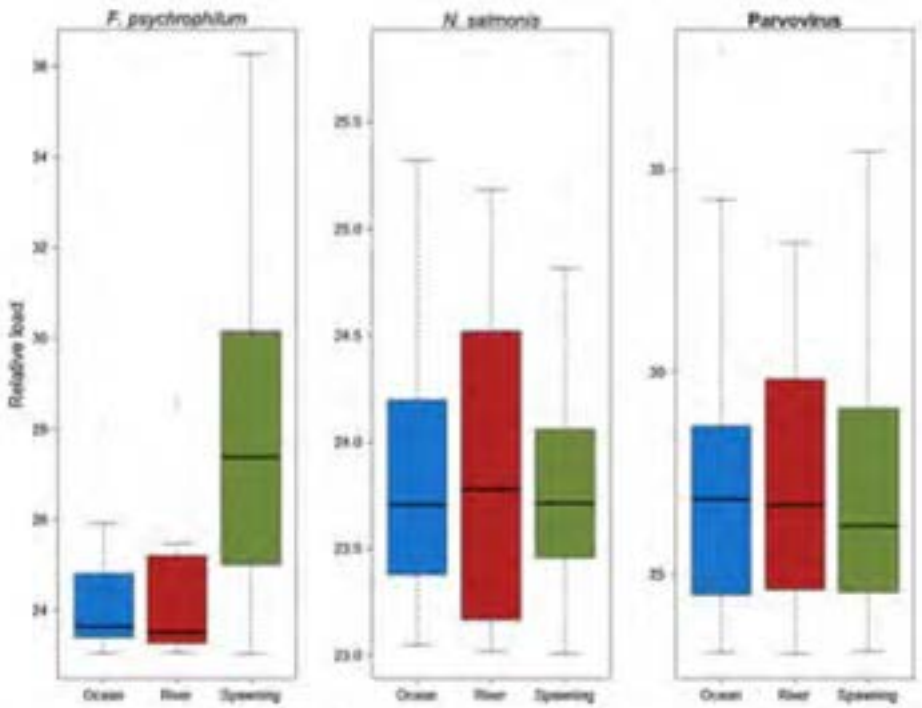

Figure 2 Box plots contrasting the distributions of relative loads (50 CT) of three microparasites (*Flavobacterium psychrophilum*, *Nucleospora salmonis*, and Parvovirus) among adult Sockeye Salmon over three environments (ocean, river, and spawning grounds) (case study I). Only samples with detections were used in the calculation of relative loads.

IV). The presence of this parasite in BC has been confirmed through sequencing (data not shown).

The Pacific salmon parvovirus, recently discovered in Sockeye Salmon through NGS (K. M. Miller, unpublished data), was highly prevalent in return migrating Sockeye Salmon, with its distribution more variable by population than environment. Parvovirus is a DNA virus, and these data were based on cDNA, hence we were monitoring the active production of viral transcripts rather than merely the presence of the virus. Parvovirus was present in all years and was in relatively lower prevalence in Chilko (overall prevalence of 16% vs >34% in Harrison; Fig. 1). There was a trend toward lower prevalence at the spawning grounds for four of the five stocks (all but Chilko; Fig. 1). Twenty one percent of fish amplifying parvovirus carried CT's <20 (>10^2 copies per well), with high load samples distributed across all environments and stocks (Fig. 2). It has not been determined yet whether parvovirus can cause disease in sal mon, but it is capable of transmission (K. M. Miller, unpublished data). However, due to enhanced immuno suppression of salmon during their spawning migration, we do not expect that they recover from infections; hence, it is possible that the consistent decreased prevalence toward the spawning grounds is associated with mortality, either directly or indirectly associated with parvovirus infection.

Microsporidian *N. salmonis* was the third most prevalent microparasite (18% over all samples), but did not show a differential distribution based on environment, year or population (Fig. 1). *N. salmonis* is considered by many to be the etiological agent of marine anemia, a disease that has been associated with mortality in Chinook Salmon and Rainbow Trout in the northeastern Pacific (Kent 2011). While this parasite appears to be fairly ubiquitous in adult Sockeye Salmon, it was not observed at high load (CT<20) in any samples (Fig. 2). The high prevalence, low variabil ity, and low load are indicative of a carrier state of this par asite in return migrating salmon.

Case study II

Are there microparasites associated with migration success of salmon returning to spawn in freshwater? In case study II, we assessed whether microparasites already carried by sal mon in the marine environment may be associated with premature migration mortality of return migrating adult Sockeye Salmon in the marine and freshwater environment. Analyses were performed on nondestructively sampled gill tissue collected in the summer of 2010 from fish tagged with acoustic or radio tags in the marine environment on the approach to the Fraser River [approximately 100 200 km from the river; see Crossin et al. (2009) and Miller et al. (2011) for tagging and sampling details]. Destructively sam pled gill tissue from the lower Fraser River and the Late Shuswap spawning grounds were additionally analyzed to identify microbes detected in freshwater only. Micropara site monitoring was conducted over 44 salmon micropara sites, and transcriptional variation in 58 host genes involved in stress, immunity, and associated with the MRS (described by Miller et al. 2011) was assessed (Table S3).

Genetic population identification determined the lake systems to which salmon were migrating. The study was performed on two populations, Chilko and Late Shuswap, with 57 and 125 fish tagged and tracked for each, respec tively (see Table S2 for experimental design). The two pop ulations chosen have similar migration distances to reach spawning grounds (629 km for Chilko, 484 km for Late Shuswap from the mouth of the river), and in recent years, have been migrating into the river during peak river tem peratures in August. This timing is normal for Chilko (a summer run population), which has been shown to be highly resistant to stress associated with HWTs (Eliason et al. 2011; Jeffries et al. 2012). The timing is about 6 weeks early compared with historic norms for Late Shuswap (a fall run population), which is highly susceptible to stress and mortality associated with HWTs (Jeffries et al. 2012). To minimize artefacts associated with tagging related mor tality (see Crossin et al. 2009 for details), we limited our analyses of acoustically tagged fish to those that were picked up at the first ocean receiver approximately 2 days travel time from the tagging location. The same could not be done for radiotracked fish, as radiotags cannot transmit in saltwater. Specific details on migration speeds, behavior, and mortality will not be presented herein.

Survival was assessed using days tracked and whether or not salmon arrived to spawning grounds. A PCA analysis was performed (as in Miller et al. 2011) to identify the major trajectories of the microbe data. A Pearson correla tion was performed between days tracked and each of the principal components (PCs) to explore potential associa tions with survival. Those that were significant were used in survivorship analysis performed as outlined in Miller et al. (2011).

Seven of the 44 microparasites assessed were detectable in at least 2% of the fish tagged in the marine environment. The most prevalent microparasites were *L. salmonae* (31%), PRV (29%), *N. salmonis* (32%), and *F. psychrophi lum* (21%). PC1 and PC2 together explained 96.9% of the total microbe variation and both were correlated with sur vival ($P < 0.05$). PC1 differentiated fish by the diversity of microparasites they carried, the extreme negative end com prised largely of survivors that were microbe free, and the extreme positive end containing fish carrying up to five microbes. For PC2, the positive end, which carried a dis proportionate number of unsuccessful fish, was heavily loaded with *L. salmonae* and PRV, while the negative end

contained more *P. theridion* positive fish. Survivorship analysis was performed separately for Chilko and Late Shu swap populations and was significant for PC2 in Chilko, for which there was a 20% differential in survival to spawn ing grounds (Fig. 3). Survivorship analysis was additionally performed based on *L. salmonae* and PRV positives and negatives, with both microparasites significantly associated with migration losses for the Chilko population only (Fig. 3; *P* values cited in figures). The strongest effect on survivorship was for *L. salmonae*, whereby fish positive in the marine environment carried a 9.6 times greater odds of dying before reaching spawning grounds ($P < 0.05$); the odds ratio for PRV was 2.3 but was not significant ($P > 0.05$).

The two microparasites associated with migration sur vival also elicited the strongest transcriptional response in the host. Twenty of the 58 host genes were differentially expressed (after multiple test correction) in association with PRV infection, and four for *L. salmonae* ($P < 0.0001$) (Fig. S1). For PRV, genes involved in immune regulation including complement formation (C7 and C3), T cell activation, signaling and cytolysis (ZAP7, CD4, PRF1), interferon response (IRF1), pro viral activity (HTATIP, EEF1AO, HNR1), viral pathogenesis (MMP25), and B cell activation (SAMSN), and genes associated with osmoregu lation (Na+K+ ATPase isoforms A1b and B1), osmotic stress (SHOP21), inflammatory response (RPL6), and feed ing (TMEM18) were differentially stimulated (Fig. S1). For *L. salmonae*, ZAP7, HTATIP, EEF1AO and one unknown (C486176) associated with the MRS (Miller et al. 2011) were similarly affected. PRV is associated with an emerging disease in Europe (HSMI; Palacios et al. 2010); this is the first study documenting this virus in Sockeye Salmon and indicating any associations between PRV and disease response and/or mortality in Pacific salmon. Microsporidi an salmon gill disease caused by *L. salmonae* can cause up to 30% mortality in farmed salmon (Kent and Speare 2005) and is associated with osmoregulatory dysfunction and disease in freshwater adult salmon (Table 1).

When marine and freshwater samples were analyzed together, 13 of the 44 microbes were detected, with a strong influence of environment ($P < 1 \times 10^{-5}$) on microbe communities. One microbe (*P. minibicornis*) increased in prevalence from the marine environment, with a slight

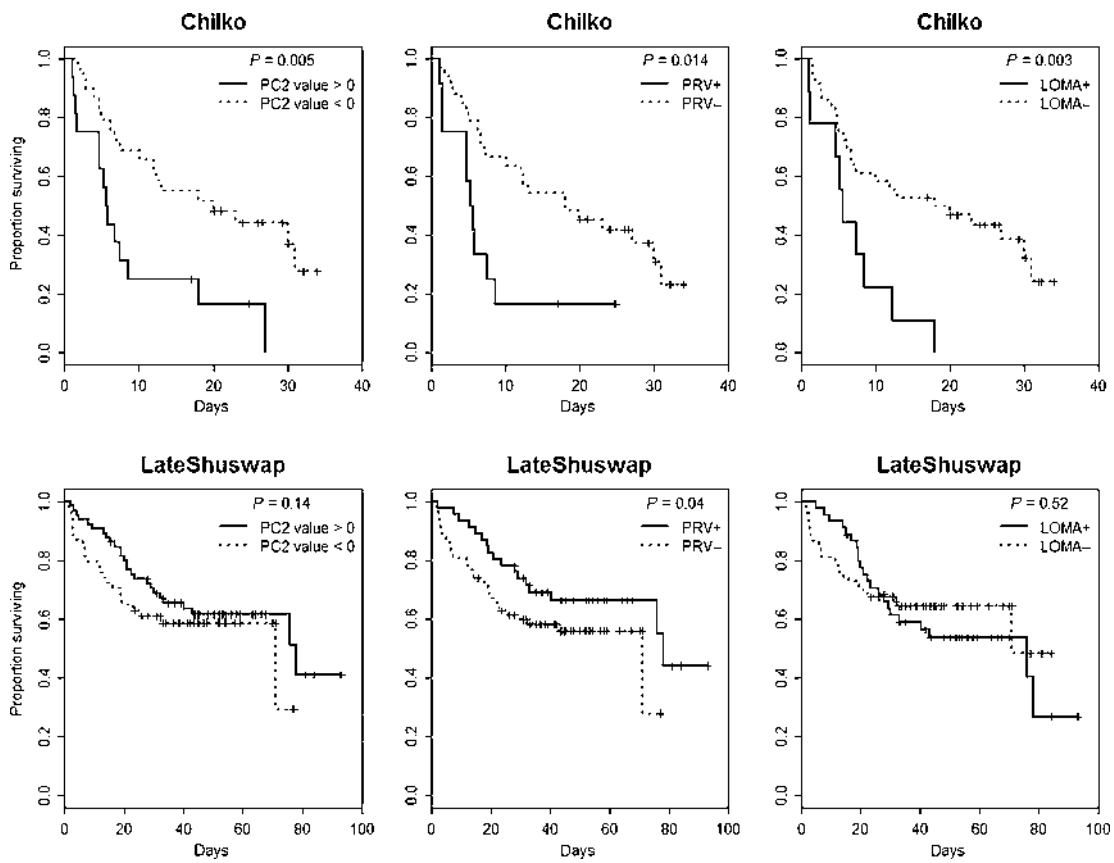

Figure 3 Survivorship analysis for Chilko (top) and Late Shuswap (bottom) stocks based on individual rankings for Principal Component 2 (PC2), and positive (CT<27)/negatives for Piscine Reovirus (PRV) and *Loma salmonae* (Loma) (case study II). *P* values are shown on top right.

decrease upon arrival at the spawning grounds (Fig. 4). Four microbes [*I. multifiliis*, *C. shasta*, *F. psychrophilum*, and Rickettsia like organism (RLO)] were largely picked up in freshwater and increased to spawning grounds (Fig. 4) and were highly correlated with each other ($P < 1 \times 10^{-6}$). Coincident with increased prevalence was a significant increase in microbe diversity from the marine to freshwater environment ($P < 1 \times 10^{-8}$). In freshwater, the four microbes picked up in freshwater were associated with the strongest host response, with 7 to 12 of the 58 host genes affected. *I. multifiliis* and *P. minibicornis* have been associated with prespawning mortality of Sockeye Salmon in previous studies (Table 1). RLO, predominantly observed at spawning grounds, is suspected to cause skin diseases red mark syndrome or strawberry disease in Rainbow Trout in the United Kingdom and USA; this disease is not known to cause mortality but decreases the commercial value of fish (Metselaar et al. 2010). There are no studies of this organism in Sockeye Salmon.

Case study III

What microbes undergo enhanced replication in elevated river temperatures and potentially impact survival of adult salmon during river spawning migration? Pacific salmon are ectotherms highly susceptible to changes in environmental temperature and individual susceptibility varies depending on species and stock specific thermal tolerance (Pörtner and Knust 2007; Farrell et al. 2008; Kent 2011; Eliason et al. 2013a). Successful completion of this once in a lifetime migration and spawning is imperative for continued propagation of Pacific salmonids and continued loss of spawners has drastic implications for future productivity. To increase our understanding of the disease potential of returning adult salmon during freshwater residence, we used a microparasite load assessment paired with measures of physiological impairment and mortality in an experimental setting. Adult Coho Salmon were collected from a tributary of the Fraser River at the Chilliwack River supplemental hatchery. Fish in 'silver' condition (i.e., fresh to the river) were transported to 8000 L experimental tanks at the DFO Cultus Lake Salmon Research Laboratory, Cultus Lake, BC and a subset of these fish ($n = 9$) were destructively sampled to assess microbe load upon collection. At arrival, tank temperatures were equal to that of the river (10°C); after a 24 h recovery period, the temperature in half of the tanks was increased to 15°C over 48 h, yielding two temperature treatments designated 'cool' and 'warm', with two tank replicates per temperature. After experimental day 14, a subset of survivors from each temperature group was destructively sampled (cool: $n = 17$; warm: $n = 18$); the remaining fish were sampled at experimental day 24 (cool: $n = 10$; warm: $n = 7$). Tissue samples from gill, liver, spleen, kidney, heart, muscle, and brain were homogenized separately, then 20 μL of aqueous phase from each tissue was pooled to capture the maximum breadth of microbe diversity within each individual. Molecular analysis was carried out following protocols described previously, and the experimental design is presented in Table S2.

To avoid any unknown effect of tissue degradation on microbe load, only surviving fish were used in this analysis. Total mortality was far greater among warm fish than cool

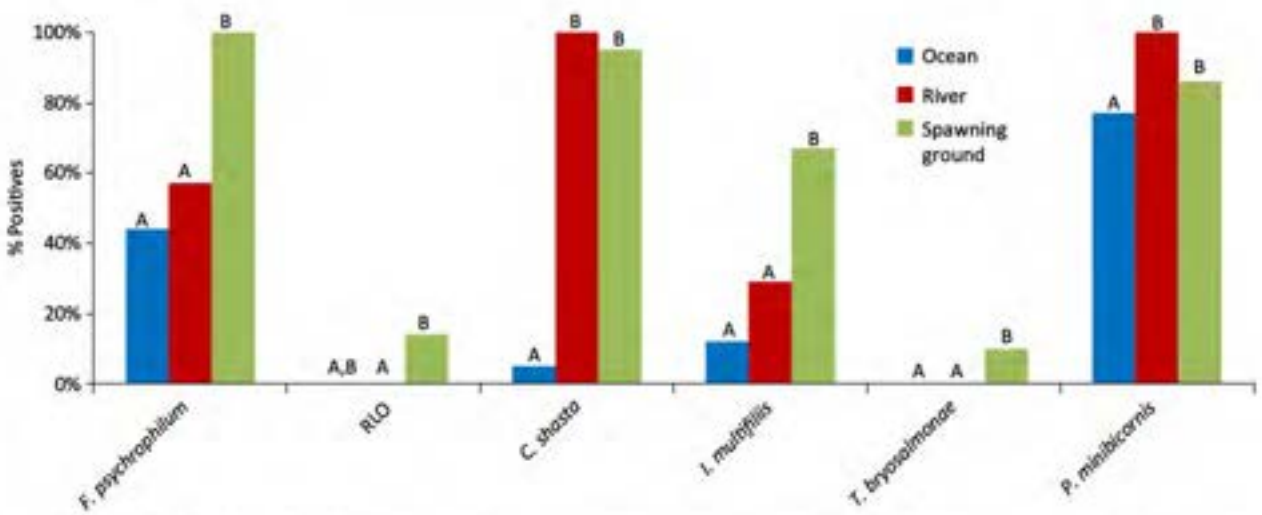

Figure 4 Distribution of key microbes among stocks and environments in gill tissue of return migrating Sockeye Salmon (case study II), including bacteria *Flavobacterium psychrophilum*, Rickettsia like organism (RLO), Myxozoa *Ceratomyxa shasta*, and *Parvicapsula minibicornis*, ciliate *Ichthyophthirius multifiliis*, and *Tetracapsuloides bryosalmonae*.

(39% and 3%, respectively; Chi squared test: $P < 0.001$). Given evidence of enhanced mortality of adult salmon at HWT (e.g. Jeffries et al. 2014; this study), our hypotheses were constructed within a framework of a microbe load threshold. In brief, we predicted microbe loads to increase across all individuals with time due to senescence and other natural factors contributing to decreased resistance of the host (see discussion above). Although microparasite repli cation may be restricted, senescing fish would not be expected to reduce microbe loads within their tissues (A. K. Teffer, unpublished data). Hence, a threshold load would ultimately be reached and either maintained (survivors) or advance to result in host morbidity. High load fish would thus drop out of the population and average loads of sur viving fish would remain unchanged over time. However, if HWT diminishes the resiliency of the host to infection, the load threshold for survival would be decreased at HWT, especially later in the migration and further along senes cence trajectory. Therefore, we hypothesized that while cool survivors would show no difference in microparasite loads between 14 and 24 days, warm survivors would show a decrease in average loads and truncation of load distribu tions at 24 days relative to 14 days, evident of decreased threshold load levels and a loss of high load individuals at HWT. Furthermore, this effect is only expected to be evi dent for pathogenic microbes (e.g., *P. minibicornis*), as nonpathogenic microparasites (e.g., *K. thyrsites*) could the oretically be present in high loads without causing mortal ity (Table 1).

Four myxozoan parasites (*C. shasta*, *P. minibicornis*, *K. thyrsites*, and *Tetracapsula bryosalmonae*), one ciliate (*I. multifiliis*) and one bacteria (Rickettsia like organism, RLO) were >50% prevalent in collected fish and incorpo rated into multivariate testing. Prevalence was largely unchanged in both cool and warm treatments (Fig. 5). However, results show significant differences in microbe loads between temperature groups (Fig. 6; MANOVA: $P = 0.001$) and a significant interaction between tempera ture and time ($P = 0.045$). This interaction was primarily attributable to temporal differences at HWT, though not significant after Bonferroni adjustment ($P > 0.0001$). Mar ginal differences in the relative loads of *C. shasta*, *I. multi filiis*, RLO and *P. minibicornis*, though not statistically significant ($P > 0.0001$), were apparent between 14 days and 24 days at HWT, each consistent with losses of high load individuals. There was no similar trend among cool temperature fish, and in fact, *P. minibicornis* loads were marginally higher, but not significantly so, at 24 days than 14 days. Our low sample sizes likely attributed to our inability to identify statistically significant differences, but trends in the data warrant further examination of how microbe load and composition respond to HWT stress within a survival context. We identify *C. shasta*, *I. multi*

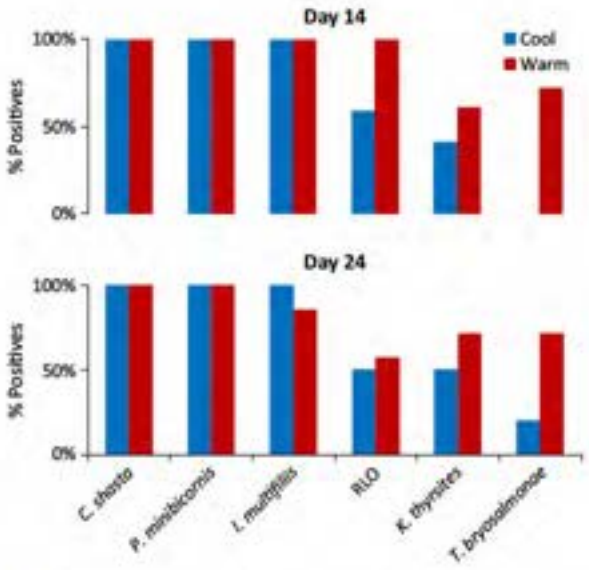

Figure 5 Percent prevalence of detections (CT <27) of four myxozoan parasites (*Ceratomyxa shasta*, *Parvicapsula minibicornis*, *Kudoa thyrsites*, and *Tetracapsuloides bryosalmonae*), one ciliate (*Ichthyophthirius multi filiis*) and one bacteria (Rickettsia like [RLO]) present at experimental days 14 and 24 in case study III.

filiis, and RLO as potentially pathogenic organisms affect ing adult coho salmon survival and support nonpathogenic designation despite enhanced replication at HWT of *K. thyrsites* and *T. bryosalmonae*. *Ichthyophthirius multifiliis* is known to have a thermally responsive life cycle (Ewing et al. 1986; Noe and Dickerson 1995; Matthews 2005; Ai hua and Buchmann 2001) and has been identified as patho genic in BC previously in the Skeena River, associated with high levels of prespawn mortality of returning adult sock eye salmon (Traxler et al. 1998). Detrimental effects of *P. minibicornis* infection on the health and survival of adult salmon in the Fraser River have been demonstrated (Wag ner et al. 2005; Crossin et al. 2008. Bradford et al. 2010) and our data complement previous findings by showing how cool temperature may enhance the ability of fish to maintain high microparasite loads. Clearly, interactions between microparasites within a temperature context as well as interactions with other stressors (e.g., fisheries cap ture) warrant further evaluation. Such an investigation is currently underway using repeated nonlethal sampling techniques to measure microbe load, reproductive status and physiological impairment of individuals throughout freshwater residence (A. K. Teffer, unpublished data).

Case study IV

Do salmon that fall prey to predators have higher micropara site diversity or load than those in the general popula tion? Studies on Red Grouse (*Lagopus lagopus scotica*)

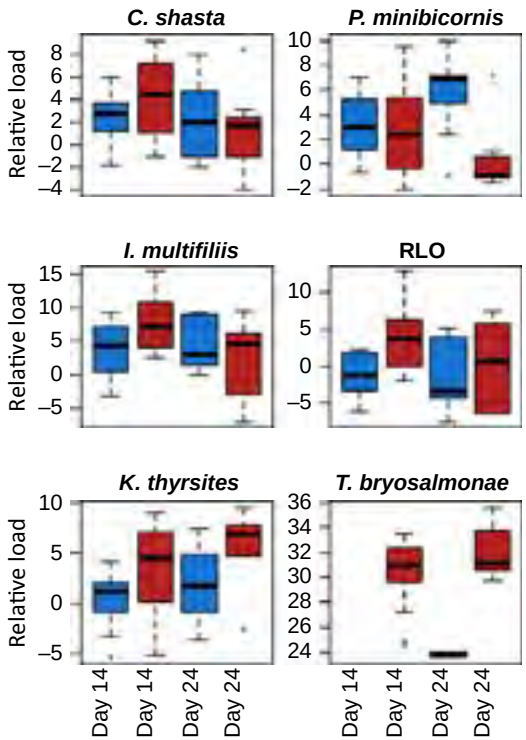

Figure 6 Box plots contrasting the distributions of relative loads (50 CT) of four myxozoan parasites (*Ceratomyxa shasta, Parvicapsula minibicornis, Kudoa thyrsites* and *Tetracapsuloides bryosalmonae*), one ciliate (*Ichthyophthirius multifiliis*) and one bacteria [Rickettsia like organism (RLO)] of adult Coho Salmon at collection (Day 1; *n* 9), after 14 days held at either cool (10°C; *n* 17) or warm (15°C; *n* 18), and after 24 days (cool: *n* 10; warm: *n* 7) (case study III).

and *Daphnia* support the hypothesis that infected animals are more prone to succumb to predation (Hudson et al. 1992; Johnson et al. 2006). To determine whether similar patterns exist in the ocean, we used predation by a colonial seabird as a model system to test the null hypothesis that the prevalence and/or load of microparasites in post smolts migrating in the ocean do not impact their susceptibility to predators. Additional factors associated with salmon condition, environmental factors (oceanographic conditions and prey quality), and genetic (population) factors that primarily influence body condition and health of salmon smolts will be pursued in subsequent studies.

In the eastern North Pacific, the timing of the seaward migration of Pink, Chum, and Sockeye Salmon smolts coincides with the chick provisioning period of Rhinoceros Auklets. Taking advantage of the fact that birds deliver whole fish to nestlings, we sampled auklet diets intensively at several large breeding colonies in BC. Sampling on colonies coincided with coast wide trawl surveys, enabling us to directly compare characteristics of auklet predated smolts against control, trawl caught smolts. Using this approach, we have established very clearly that the auklets disproportionately take smolts that are small and in poor condition (S. Tucker, unpublished data); herein, we examine the microparasite profiles of Sockeye Salmon to determine whether or not those that are predated have higher incidence and/or load of microparasites than those in the general marine environment where the auklets are feeding. We additionally assessed whether microbe profiles were influenced by size and condition (measured as the residuals of the length weight relationship over all fish sampled, and categorizing fish as above or below average mass to body length). Our null hypothesis is that microbes will not be associated with variance in size or condition factor.

Eighty six Sockeye Salmon post smolts collected from a trawl survey within Queen Charlotte Sound, BC were measured for fork length and weight. Seventy nine Sockeye Salmon post smolts were collected from auklet nesting colonies in Queen Charlotte Sound and treated similarly. Gill and liver tissues were combined for the monitoring of 40 microbes.

Thirteen of the 40 microparasites surveyed amplified products with CT<27 (Table 2; Fig. 7). MANOVA was applied to determine the relative roles of predation, size and condition factor, and their interaction terms, in the variances in microbe distributions among the 13 detected microparasites.

Microparasite distributions were differentiated between predated and trawl samples ($P < 0.001$), but no significant relationship with size, condition factor, or any interactive terms was observed ($P > 0.05$). Post hoc testing revealed significantly higher levels of three *Parvicapsula* parasites *P. pseudobranchicola* ($P < 0.001$), *P. kabata* ($P < 0.005$), and *P. minibicornis* ($P < 0.005$) in the predated Sockeye. *P. minibicornis* was highly prevalent with detection in 46% of predated fish and 24% in the general population (trawl) and a minimum CT of 7.7, indicative of a load of $>10^7$ (Fig. 7). *P. kabatai* was observed in 20% of predated fish and 4% of trawled, with a minimum CT of 15.3 load $>10^3$. *P. pseudobranchicola* was observed in 16% of predated and no trawled fish, with a minimum CT of 19.1 load $>10^2$ Predated fish also carried a higher diversity ($P < 0.001$) and load ($P < 0.001$) of microparasites than those in the general population (Fig. 8). Individual fish carried between 0 and 5 microparasites, with an average of 1.6 for predated and 0.9 for the general population. The vast majority of fish with >3 microparasites were predated, as were 11 of the 14 fish with 3 microparasites. Thirty nine percent of predated fish carried at least one microbe with a CT<20 (load $> 10^4$) versus only 16% in the general population. Moreover, whereas 6% of predated fish carried two microparasites at high load, none of the general population samples carried more than one high load microparasite.

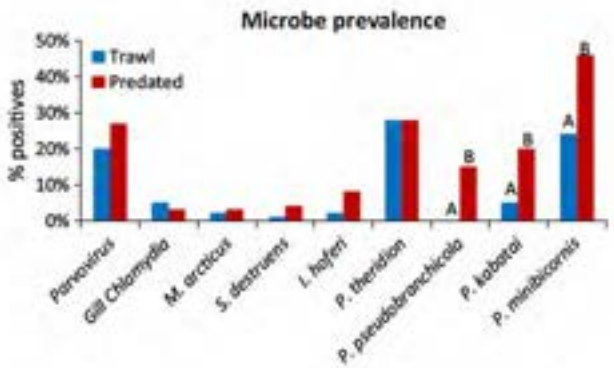

Figure 7 Percent prevalence of microbes (CT <27) identified in Sockeye Salmon from samples predated upon by Rhinoceros Auklet compared to those from a trawl survey in waters adjacent to sampled colonies (case study IV). A/B indicates a significant difference between groups, tested by microbe.

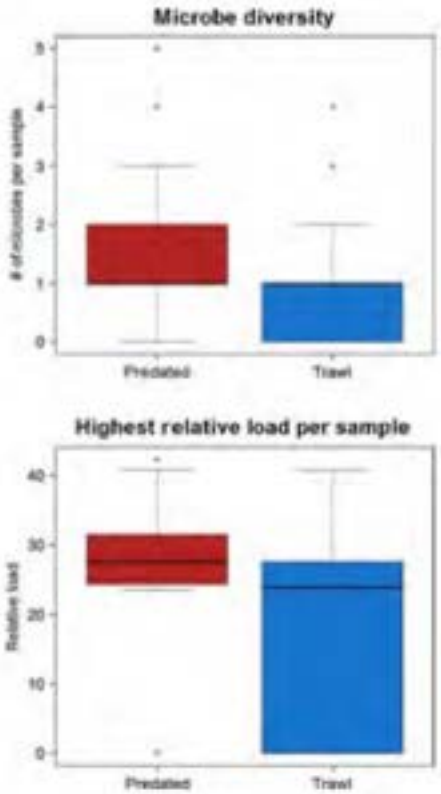

Figure 8 Box plots revealing differences identified in microbes found in Sockeye Salmon from samples predated upon by Rhinoceros Auklet compared with those from a trawl survey in waters adjacent to sampled colonies (case study IV). (A) Microbe diversity, indicating the number of distinct microbes carried per sample. (B) Highest relative load (50 CT) of any microbe within samples.

These data refute the null hypothesis that the condition of fish, as defined by the microparasites they carry, does not affect their probability of predation. While the majority (87%) of Sockeye Salmon post smolts that were preyed upon by auklets were in poor body condition (S. Tucker, unpublished data), there was no relationship between con dition and the microparasites they carry. However, how the presence of these parasites might contribute to the overall physical condition of fish, or what the manifestations might be with respect to health or to factors affecting predator avoidance remains unknown. Of the three myxozoan *Par vicapsula* parasites showing a significant association with predation, only *P. minibicornis* has been extensively studied in wild salmon, and then only in adults. *P. minibicornis* is picked up in freshwater where its' alternate host, the poly chaete *Manayunkia speciosa* (Bartholomew et al. 2006), resides. In return migrating salmon, *P. minibicornis* is asso ciated with severe infection and pre spawning mortality after 450°C accumulated thermal units in the river (Wag ner et al. 2005; Bradford et al. 2010); similar studies in smolts have not been published. *P. pseudobranchicola* is a marine parasite that has been associated with mortalities of farmed Atlantic Salmon in Northern Norway (Table 1; Karlsbakk et al. 2002), with impacts on swimming activity level and possibly vision (bleeding around the eyes) (Jørgensen et al. 2011), which could affect predator avoid ance. *P. pseudobranchicola* infection levels in Norway are higher in farmed than wild fish (Jørgensen et al. 2011). *P. kabatai* was first isolated from kidneys of Pink Salmon in Quinsam River, BC (Jones et al. 2006), and histologic lesions associated with the parasite from wild collected fish have been described (Saksida et al. 2012). Dual infections with myxozoan parasites can be a common occurrence and may have a synergistic effect increasing lethality of infection (Nichols and True 2007).

Discussion

Disease consequences not only depend on the spatial and temporal scale of a pathogen but also its impact in terms of mortality and morbidity (Peeler et al. 2007). Most studies of infectious agents impacting wild populations are associa tion based that is, they document parasite distributions (e.g., Margolis and Arthur 1979; McDonald and Margolis 1995; Bennett et al. 1998; Kent et al. 1998; Arkoosh et al. 2004; Sandell 2010; Ferguson et al. 2012), and occasionally assess shifts in prevalence and/or load to develop hypothe ses about impacts (e.g., Gordon and Rau 1982; Kalbe et al. 2002; Jacobson et al. 2008), but do not measure mortality directly. While our case studies also take an association based approach, the study merging acoustic tracking with microparasite monitoring was able to directly associate spe cific microbes with migration success, resolving two infec tious agents, a microsporidian parasite (*L. salmonae*) and a virus (PRV), that were correlated with premature migra tion mortality in one of two assessed stocks, and especially notable during marine migration. Moreover, we showed

that the microparasites most predictive of fate stimulated the strongest immune response in the host. In the predation study, we directly compared microparasite profiles of salmon being predated by auklets with those in the general population and showed that post smolts carrying any of three species of myxozoan *Parvicapsula* parasites were more likely to be predated. Moreover, we found that predated fish generally carried higher microparasite diversity and load. Climate shifts are expected to continue to impose further stress on already declining populations of salmon, creating an optimal environment for a range of infectious diseases to flourish. In a holding study, we showed that myxosporean parasites (*P. minibicornis* and *C. shasta*) and the etiological agent associated with strawberry disease (RLO) were responsive to temperature shifts in freshwater, rapidly increasing in load in Coho Salmon held at 10°C and 15°C, and then showing a truncated load distribution among 15°C survivors, suggesting a loss of high load individuals at high temperature. These data corroborate those of previous studies indicating temperature mediated responses of *C. shasta* and *P. minibicornis* infecting Chinook and Sockeye Salmon, respectively; salmon are exposed to both of these parasites in freshwater and both negatively impact survival (Table 1). As a whole, these studies highlight the potential importance of myxozoan and microsporidian parasites in wild salmon.

Microsporidia are related to fungi and infect a broad spectrum of taxa, with half of the known genera infecting aquatic hosts (Stentiford et al. 2013). In aquatic systems, their impacts range from cryptic to catastrophic, with the potential to drive host population cycles and ecological impact on species interactions within ecosystems (Stentiford et al. 2013). Microsporidian parasites have also been implicated in colony collapse disorder in bees (Higes et al. 2008) and are the most common infections among immuno compromised humans (Williams 2009). Myxozoans are highly diverse spore producing parasites that share a close phylogenetic relationship with cnidarians (Chang 2013). Myxozoans are largely aquatic, most with obligate development in teleost fish and annelid worm hosts (Kent et al. 2001). While their complex life cycle may reduce the likelihood of their sustaining persistent disease epidemics in the wild (Bakke and Harris 1998), three have been implicated in disease outbreaks in wild and cultured salmon (*M. cerebralis* causing whirling disease, *T. bryosalmonae* causing PKD, and *C. shasta* causing ceratomyxosis) and one has caused severe economic impacts on industry (*K. thyrsites* causes post harvest myoliquefaction of muscle tissue; Kent et al. 2001).

Coevolution between microparasites and their hosts will be most strongly felt in systems where population level impacts of pathogens occur. However, population level effects may be reduced in systems with strong density dependence, especially if infection related mortalities occur before density dependence is strongest (Fujiwara et al. 2014). In salmon, infection related mortality within populations that occurs just prior to smoltification may be less affected by density dependence, as competition for resources in the marine environment would not be limited to the size of one or a few populations, but the combined densities of hundreds of merging populations. However, if large scale mortalities were to occur during early marine residence, it is possible that reduced competition for resources could counter the negative impacts of infectious disease. However, on the west coast of Canada, competition for resources is hypothesized to be largely driven by the massive explosion of even year Pink Salmon populations (Ruggerone et al. 2003); hence, unless Pink Salmon were to also be affected, densities may not be reduced to sufficiently low levels to counter the impacts of disease. In the case of return migrating salmon, disease associated prespawn mortality may have a lower impact on population variance in years with high returns as density dependent competition for spawning resources may counter impacts of disease. However, in years with low density returns and high river temperatures excelerating the rate of disease development and reservoirs of microparasites with alternate hosts, strong population level effects of disease may be felt. Interestingly, in Sockeye Salmon, abundance of returns has been relatively stable in the dominant cycle year where millions of salmon return to spawn relative to other years that have experienced strongly declining abundance (Peterman and Dorner 2011), consistent with a relatively stronger impact of disease on populations when densities are low. The hypothesis that density dependence may reduce the impact of infectious disease at a population level is contrary to microbe host evolutionary theory, hence requires further study.

Multiple infections by various microparasites of salmonids present further complexity to the host/parasite/environment relationship (Thomas and Blanford 2003). The microbe monitoring data from all four case studies revealed a high percentage of BC salmon carrying coinfections of multiple microparasites. However, it was relatively rare for fish to carry high loads of multiple microbes at once (maximum observed in our studies was three microbes at CT<20 carried in a single tissue sampled from live fish). Given the range of infectious agents salmon are exposed to in their life time, and the potential that many microparasites may go into a carrier state upon recovery from disease (Table 1), coinfections are expected. The question is, are fish that carry higher microbe diversities in a generally poorer conditional state than those with lower diversity? The answer to this question probably depends upon the composition of the coinfection. In the predation study, we showed a direct correlation between microbe diversity and predation, suggesting a poorer conditional

state associated with microbe diversity. Similarly, Jacobson et al. (2008) and Sandell (2010) found that in surveys of post smolts in the ocean, higher parasite diversity, prevalence and loads within fish were observed in years of good relative to poor ocean productivity, and concluded that parasite infections were less tolerated when fish were otherwise stressed. There is also evidence from laboratory studies that particular coinfections may impact the pathogenicity of single microbes, essentially affecting their clinical and immunological evolution. For example, some viruses cannot replicate efficiently without coinfection; many parvoviruses in the dependovirus genus require coinfection with adenoviruses for replication (Anderson and Pattison 1984). A salmon parvovirus that was recently identified by NGS in Sockeye Salmon is phylogenetically close to the dependovirus genus, but whether it requires coinfection for replication is not yet known (KM Miller, unpublished data). Diseases stimulated by coinfections between viruses and microsporidian parasites are also common (Duncan et al. 2012; Toplak et al. 2013), but have not been studied extensively in fish. Given the very large number of microsporidian parasites observed in fish (Stentiford et al. 2013), this is an important area of future study. Alternately, some coinfections may be beneficial to the host. For example, in cell cultures, IPNV interferes with the IHNV replication (Alonso et al. 2003) and induces interferon activity which may act to suppress IHNV replication (de Kinkelin et al. 1992). As well, in trout infection with avirulent cutthroat reovirus induces an IHNV resistant state (Hedrick et al. 1994).

Predators may limit levels of infection within populations, thereby decreasing rates of host pathogen coevolution. In a culture setting, rapid removal of sick or dead fish is an effective way to keep disease under control (Jarp and Karlsen 1997; Murray and Peeler 2005). In natural environments, predation, natural and anthropomorphic (fishing) may reduce infectious disease by reducing host densities below certain thresholds (Dobson and May 1987). For microparasites primarily exchanged horizontally, if predators select infected fish at early stages of disease, they could decrease the threshold of infection related mortality, thereby decreasing exchange rates of microparasites and reducing the probability of epidemic levels of infection (Lafferty and Gerber 2002). In doing so, natural predation may increase the costs of high pathogen virulence if moribund fish are removed before transmission occurs, which would, in essence, decrease the rate of coevolution among microparasites and their hosts. In fisheries, if certain gear types were shown to selectively harvest fish infected with important disease causing pathogens, under some circumstances, evolutionary disease management strategies may actually warrant harvesting a portion of affected stocks to minimize disease impacts at spawning grounds. Alternately, for microparasites with alternate hosts, like the myxozoans in our predation study, predators could simply increase the probability of infection related mortality, thereby increasing the potential for coevolution, although this effect could be countered by reduced infection levels in the alternate host.

The ecological and evolutionary outcomes of cumulative impacts of climate, infection, and predation are hard to predict, as their direction will depend upon predator and host densities and how strongly temperature impacts microparasite replication rates and swim performance. Temperature can immunocompromise the host and increase the replication rate of numerous microparasites, increasing rates of infection and disease development; case study III corroborates this assertion. The cumulative impact of temperature and infection in the absence of predation should therefore increase rates of coevolution, especially in susceptible hosts. In a system with predators, impacts on swim performance may be felt at a lower level of infection when temperatures are elevated, increasing the vulnerability of fish to predation. If the density of predators is sufficiently high to reduce salmon densities while disease levels are still low, microparasite evolution may be reduced. However, if temperature impacts on microparasite replication rates are faster than predators can remove impacted fish, predators would have limited impact. In essence, the arms race of host microparasite evolution in wild populations could be enhanced with environmental stress and decreased under high levels of predation.

There is not likely a single stressor that can account for the massive declines in productivity and abundance of salmon in the northeast Pacific; rather the cumulative and interactive effects of multiple stressors are likely at play. The uncertainties in predicting evolutionary responses to cumulative stressors are considerably greater for organisms such as salmon that have complex, migratory life cycles (Crozier et al. 2008), as responses at one stage of development may impact subsequent states, and resistance may not impact all life history stages equally. It is imperative that we build a greater understanding of both plastic and evolutionary responses to individual stressors and determine whether cumulative effects are additive, antagonistic, or synergistic if we are to predict the outcomes of cumulative stressors and variation in population level responses.

Moving forward, there are many ways that modern technologies can improve the depth and breadth of ecological and evolutionary information required to assess the impacts of disease processes in natural systems. Merging of broad scale microbe surveillance with biotelemetry and assessments of cumulative stressors will provide greater insight into the microbes of most import to wild populations. Indeed, such multidisciplinary approaches are demanded by complex environmental problems (Cooke et al. 2008).

Evolutionary drivers of variation in microparasite susceptibility can additionally be incorporated into these 'natural' studies by linking data on MHC variation or by taking a dQTL approach. Gene expression profiling through microarrays or high throughput biomarker surveillance of host immune genes can be integrated to elucidate the microparasites that elicit strong responses in the host and for which MHC related defence mechanisms are important. These indirect correlative approaches on naturally migrating wild organisms will allow for the 'discovery' of potential linkages between microparasites, genetic susceptibilities, and probability of disease (via levels of immune stimulation) that can be followed up in laboratory studies to better understand mechanistic linkages with disease.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data S1. Fluidigm BioMark Methods.

Table S1. Overview of the microparasites for which genetic associa tions with disease resistance, identification of disease related quantitative trait loci (dQTL), and/or host response through microarray gene expres sion profiling studies have been determined.

Table S2. Design of case studies I (A), II (B and C), and III (D).

Table S3. Host gene Taqman assays assessed in Case Study II.

Figure S1. Gene expression of the 20 host genes significantly associ ated with PRV infection in case study II.

Council

CNL(06)48

Resolution by the Parties to the Convention for the Conservation of Salmon in the North Atlantic Ocean to Minimise Impacts from Aquaculture, Introductions and Transfers, and Transgenics on the Wild Salmon Stocks

The Williamsburg Resolution

(Adopted at the Twentieth Annual Meeting of NASCO in June 2003 and amended at the Twenty-First Annual Meeting of NASCO in June 2004 and at the Twenty-Third Annual Meeting of NASCO in June 2006)

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(Adopted at the Twentieth Annual Meeting of NASCO in June 2003 and amended at the Twenty-First Annual Meeting of NASCO in June 2004 and at the Twenty-Third Annual Meeting of NASCO in June 2006)

The Parties,

NOTING the provisions of the Convention for the Conservation of Salmon in the North Atlantic Ocean of 2 March 1982 (the "Convention"), which seeks to promote the conservation, restoration, enhancement and rational management of salmon stocks;

WELCOMING the achievements in salmon conservation by the Parties to the Convention, within the framework of the Convention, and the role of the North Atlantic Salmon Conservation Organization (the "Organization") therein;

NOTING that NASCO and its Contracting Parties have agreed to apply the Precautionary Approach to the conservation of salmon and acknowledging the need for measures taken in accordance with this Resolution to be consistent with the Precautionary Approach;

AWARE of the need for cooperation between the Parties in order to maintain and to restore the wild salmon stocks, and promote sustainable conservation and management of such stocks;

RECOGNISING the benefits, including the socio-economic benefits, which have resulted from the development of salmon aquaculture;

CONSCIOUS of the threats to the wild stocks of salmon from different human activities, including possible adverse effects from aquaculture, introductions and transfers and transgenics;

RECOGNISING that in order to protect wild salmon stocks from adverse impacts that can or might be caused by aquaculture, introductions and transfers, and transgenics, there is a need to take into account local conditions in determining appropriate management measures;

DESIRING to minimise the possible adverse impacts of aquaculture, introductions and transfers and transgenics on the wild stocks and noting the earlier initiatives taken by the Organization in this respect;

RESOLVE as follows:

ARTICLE 1

Cooperation between the Parties

The Parties shall cooperate in order to minimise adverse effects to the wild salmon stocks from aquaculture, introductions and transfers and transgenics.

ARTICLE 2

Definitions

For the purposes of this Resolution definitions are as given in Annex 1.

ARTICLE 3

Burden of Proof

Each Party, in accordance with the Precautionary Approach, should require the proponent of an activity covered by this Resolution to provide all information necessary to demonstrate that the proposed activity will not have a significant adverse impact on wild salmon stocks or lead to irreversible change.

ARTICLE 4

Risk Assessment

Risk assessment is integral to the implementation of the Precautionary Approach and serves to promote transparency in the decision-making process. Risk assessment should include identification of options and consideration of mitigation measures. The Parties should develop and apply appropriate risk assessment methodologies in considering the measures to be taken in accordance with this Resolution.

ARTICLE 5

Measures to Minimise Impacts of Aquaculture and Introductions and Transfers

Each Party shall take measures, in accordance with Annexes 2, 3 and 4 to this Resolution, to:

- minimise escapes of farmed salmon to a level that is as close as practicable to zero through the development and implementation of action plans as envisaged under the Guidelines on Containment of Farm Salmon (CNL(01)53);
- minimise impacts of ranched salmon by utilizing local stocks and developing and applying appropriate release and harvest strategies;
- minimise the adverse genetic and other biological interactions from salmon enhancement activities, including introductions and transfers;
- minimise the risk of disease and parasite transmission between all aquaculture activities, introductions and transfers, and wild salmon stocks.

Movements into a Commission area of reproductively viable Atlantic salmon or their gametes that have originated from outside that Commission area should not be permitted.

ARTICLE 6

Non-Indigenous Fish

No non-indigenous fish should be introduced into a river containing Atlantic salmon without a thorough evaluation of the potential adverse impacts on the Atlantic salmon population(s) which indicates that there is no unacceptable risk of adverse ecological interactions.

Introductions into any Commission area of reproductively viable non-indigenous anadromous salmonids or their gametes should not be permitted.

ARTICLE 7

Transgenic Salmonids

The Parties should apply the Guidelines for Action on Transgenic Salmon, CNL(97)48 (Annex 5), to protect against potential impacts from transgenic salmonids on wild salmon stocks. In view of the current lack of scientific knowledge on the impact of transgenic salmonids on wild salmon stocks, the use of transgenic salmonids should be considered a high-risk activity. There should be a strong presumption against any such use.

ARTICLE 8

River Classification and Zoning

For the purposes of developing management measures concerning aquaculture and introductions and transfers, Parties should, as appropriate, develop and apply river classification and zoning systems. Details of such systems should be established in accordance with the guidance in Annex 6.

ARTICLE 9

Mitigation and Corrective Measures

Where significant adverse impacts on wild salmon stocks are identified, the Parties should initiate corrective measures without delay and these should be designed to achieve their purpose promptly.

Mitigation measures can include activities to safeguard against potential future impacts (e.g. contingency planning, gene banks).

ARTICLE 10

Implementation

In order to have confidence that the wild stocks are protected from irreversible genetic change, from significant ecological impacts and from significant impacts of diseases and parasites, full implementation of the measures in this Resolution and its Annexes is essential. Local conditions may warrant consideration of stronger measures. All measures should be regarded as adaptable to improved salmon aquaculture technologies and methodologies (e.g. use of sterile fish, lice vaccines, etc.)

Where detailed agreements are developed by a regional Commission of NASCO in support of this Resolution, they will be appended. Appendix 1 indicates the current situation within the North American Commission. Appendix 2 contains a Memorandum of Understanding between Canada and the USA intended to reconcile the differences between the methods used to authorise introductions and transfers in the two countries. Any further guidelines to assist in implementing this Resolution will be annexed.

Each Party shall report annually to the Organization on the measures adopted and actions taken under Articles 5, 6, 7 and 9.

ARTICLE 11

Research and Development

Each Party should encourage research and data collection in support of this Resolution (as detailed in Annex 7) and should take steps to improve the effectiveness of the measures contained in this Resolution.

Each Party shall report annually to the Organization on the research and development carried out.

ARTICLE 12

Dissemination of Information

Educational materials should be developed and distributed to increase awareness of the risks that introductions and transfers of aquatic species may pose to wild salmon stocks and the need for the measures that control these activities.

Annex 1

Definitions relating to Salmon Aquaculture, Introductions and Transfers and Transgenics

Term	Definition
Containment	Physical containment: Prevention of escapes of farmed salmon into the freshwater and marine environments. Containment of diseases and parasites: Implementation of measures to prevent the transfer (spread) of diseases and parasites between aquaculture facilities and wild fish.
Epidemiological zones	Zones defined by lack or presence of specific pathogens.
Introduction	The intentional or accidental release of a species into an environment outside its native or natural range.
Mitigation stocking	Stocking conducted as a voluntary action or statutory requirement to mitigate lost production due to an activity that cannot be removed.
Non-indigenous	Not originating or occurring naturally in a particular environment; introduced outside its native or natural range.
Population	A group of organisms of a species occupying a specific geographical area.
Rehabilitation	The rebuilding of a diminished population of a finfish species, using a remnant-reproducing nucleus, toward the level that its environment is now capable of supporting.
Restoration	The re-establishment of a finfish species in waters occupied in historical times.
Risk assessment	The process of identifying and describing the risks of activities having an impact on fisheries resources, habitat or aquaculture before such activities take place; the process of identifying a hazard and estimating the risk presented by the hazard, in either qualitative or quantitative terms.
River classification	Designation of a river or watershed according to the degree of human impact.
Salmon aquaculture*	The culture or husbandry of Atlantic salmon, including salmon farming, salmon ranching and salmon enhancement activities.
Salmon enhancement	The augmentation of wild stocks in individual river systems by the release of Atlantic salmon at different stages in their life-cycles.
Salmon farming	Production system which involves the rearing of Atlantic salmon in captivity for the duration of their life-cycle until harvested.
Salmon ranching*	The release of reared Atlantic salmon smolts with the intention of harvesting all that return.
Salmonid*	All species and hybrids of the family Salmonidae.

Stock* (Management unit)	A management unit comprising one or more salmon populations.
Stock (local)	A stock from a river or tributary in close proximity to the river to be stocked. This may refer to rivers with a common bay of entry or closely related catchment areas.
Stocking	The deliberate release of Atlantic salmon into the wild at any stage of their life-cycle for enhancement, mitigation, restoration, rehabilitation or ranching purposes.
Transfer*	The deliberate or accidental transport of Atlantic salmon within their native or natural range.
Transgenic	An organism that has been modified by genetic engineering to contain DNA from an external source.
Wild salmon	Fish that have spent their entire life-cycle in the wild and originate from parents which were also spawned and continuously lived in the wild.
Zone	Geographic area reflective of the degree of degradation or manipulation of wild Atlantic salmon populations.

* for the purposes of the NAC Protocols, a different definition is used, see NAC(94)14

Annex 2

General Measures to Minimise Impacts

This Annex is designed to provide guidance to NASCO's Parties on minimising impacts of salmon aquaculture and introductions and transfers on wild salmon stocks. The guidelines will be regularly reviewed and updated as appropriate in the light of new scientific information and changing technologies and methodologies.

1. Siting and Operation of Aquaculture Activities

1.1 Salmon aquaculture facilities should only be located where hydrographical, epidemiological, biological and ecological standards can be met. Factors which may be taken into consideration include: availability of water supply and receiving waters for discharge; water quality and exchange; water depth; site protection; separation distances between aquaculture facilities; and distance from salmon rivers. Further guidance on containment is provided in Annex 3.

1.2 Consideration should be given to the establishment of "wild salmon protection areas" where salmon aquaculture is restricted or prohibited. Such protection areas may minimise genetic, disease, parasite and environmental impacts.

1.3 The designation of "aquaculture regions", where all the steps in the production process are carried out and which are separated from similar regions by areas without aquaculture, could also be considered. Such regions could provide a framework for management of the aquaculture industry and could assist in controlling the spread of fish diseases and parasites.

1.4 The separation distance between aquaculture facilities at marine sites should be based on a general assessment of local conditions. Wherever possible, different generations of salmon should be reared in separate locations. As local conditions permit, a fallowing regime should be practised as a means of minimising outbreaks of disease and parasites. Aquaculture production should be adapted to the holding capacity of an individual site and should not exceed density levels based on science and good husbandry practices.

1.5 Dead and dying fish should be removed immediately from aquaculture production facilities, taking into account worker safety, and weather and sea state conditions. Mortalities should be disposed of, along with waste materials, in an approved manner. Procedures should be established to address the effective removal and disposal of infectious material. Contingency plans should be established for the disposal of mortalities from emergency situations.

1.6 Depending on local regulations and protocols, tagging or marking or inventory tracking systems will be used in order to facilitate the identification of farmed salmon in the wild and their separation from wild fish, to determine the source of escapes and to assess the interactions of escaped farmed salmon with the wild stocks. These systems could be coupled with river monitoring and recapture systems that allow holding and close examination of returning fish in the rivers.

2. Diseases and Parasites

2.1 All steps in the aquaculture production process from hatchery to processing plant, including transportation of live fish materials, should be conducted in accordance with appropriate fish health protection practices. This includes attention to the application of appropriate husbandry techniques to minimise the risk of disease in the reared stock. These might include vaccination, use of optimal stocking densities, careful handling, frequent inspection of fish, proper diet and feeding regimes, avoidance of unnecessary disturbance of the fish, detailed health inspections, disinfection of transportation equipment and the use of foot baths at production facilities.

Specified diseases and parasites

2.2 Mapping of the presence of serious diseases and parasites should be used to establish epidemiological zones (either with or without specific pathogens). Management measures within these zones should include monitoring to confirm the disease status of a zone and eradication. These zones should be established for at least the following diseases: Viral Haemorrhagic Septicaemia (VHS), Infectious Haematopoietic Necrosis (IHN), Infectious Salmon Anaemia (ISA) and the parasite *Gyrodactylus salaris*.

2.3 Movements of live salmonids and their eggs from a zone where any of the specified diseases is present to a zone free of these diseases should not be permitted. However, movements of salmonid eggs may be permitted where there is minimal risk of transmission of the specified diseases or parasite.

2.4 A list of the prevailing infectious diseases and parasites, and the methods in practice for their control, should be maintained by the appropriate authorities.

Unknown diseases and parasites

2.5 Procedures should be established for the early identification and detection of, and rapid response to, an outbreak of any new disease or parasitic infection likely to affect Atlantic salmon. These procedures should include the establishment of official surveillance services responsible for the monitoring of the health of both wild and farmed fish. The procedures should also demand the rapid introduction of restrictions on the movement of salmonids in the case of an outbreak of a disease or parasitic infection until the status of the disease or parasitic infection is known.

2.6 Even with such procedures, it may not be possible to respond in time to prevent the spread of such a disease or parasitic infection. It is recommended that the Contracting Parties, when establishing or reviewing rules on transfers of fish, consider additional protective measures such as:

- **the establishment of zones:** the intention of such zones, between which the movement of live salmonid fish and their gametes should be restricted and which might be defined using geographical, climatic or biological criteria, is to limit the spread of parasites and diseases to wild stocks;

- **the movement of salmonids:** for disease prevention purposes, the trade in eggs is safer than the trade in live fish. It must, however, be recognised that

some serious diseases, such as IPN, BKD and IHN, may be transferred with eggs and ovarian fluid;

- **diseases of wild fish:** there is a need to strengthen and amend disease controls to minimise disease transfer between aquaculture activities and wild fish.

Health inspections of donor facilities

2.7 Movements of live salmonids and their eggs from hatcheries to areas containing Atlantic salmon stocks, or to facilities where there is a risk of transmission of infection to such areas, should only take place from facilities where regular inspections have not detected significant diseases and parasites.

Use of medicines and disinfectants

2.8 Medicines and disinfectants to control diseases and parasites must be used with care and in accordance with the manufacturer's instructions and any Codes of Practice, and in compliance with regulatory authorities.

3. Gene Banks

3.1 Various activities may result in serious adverse impacts on salmon stocks and strains such that the potential exists that a portion of the salmon genome is lost. In order to protect against this possibility, Parties should consider the establishment of gene banks for stocks that are in danger of extirpation. This could provide a source of genetic material for future restoration programmes.

Annex 3

Guidelines on Containment of Farm Salmon, CNL(01)53

Section 1: Introduction

1.1 The North Atlantic salmon farming industry and the North Atlantic Salmon Conservation Organization (NASCO) have established a Liaison Group. This Liaison Group recognised the importance of conserving and enhancing wild salmon stocks and of supporting a sustainable salmon farming industry and is seeking to establish mutually beneficial working arrangements in order to make recommendations on wild salmon conservation and sustainable farming practices. To this end the Liaison Group has developed guidelines on containment to apply throughout the NASCO Convention area.

1.2 Both Parties recognise that a number of guidelines and measures, outlined below, should apply to all salmon aquaculture activities. The Liaison Group should be updated annually on progress on the development of parallel measures in relation to these activities.

Section 2: Objectives

2.1 These guidelines are intended to result in the prevention of escapes of farmed salmon in the freshwater and marine environments.

Section 3: Site Selection

3.1 Sites shall be selected having regard to the capability of the equipment to withstand the weather and other environmental conditions likely to be experienced at that site;

3.2 In the interest of avoiding collision damage, equipment shall comply with the relevant national and international regulations regarding navigation and marking;

3.3 Careful consideration shall be given to the siting of land-based facilities, so as to minimise the risk of escapes from these facilities.

Section 4: Equipment and Structures

4.1 Nets, cages and mooring systems shall be designed, constructed and deployed to prevent escapes, having proper regard to the prevailing conditions at the site. Mooring systems should have a significant in-built safety margin;

4.2 Nets and cages should be marked with an identification number; adequate records of each net and cage in use should be maintained in order to assess its fitness for purpose;

4.3 Nets shall be: compatible with the cages with which they will be used; secured to the cage collar so that the collar alone bears the strain; and adequately UV-protected. Net weights shall be installed in such a way as to prevent damage to the nets;

4.4 Tank systems shall be designed to contain fish effectively and to minimise the chances of fish escaping. Where the outflow from tanks passes into a settling pond, the outflow from the settling pond should incorporate a screen of suitable size and construction to minimise the chances of fish escaping;

4.5 Effective predator deterrence methods shall be implemented as appropriate; these should be up-graded as improved, site-appropriate and cost-effective systems of proven efficacy become available; records of predator attacks that may have caused escapes should be maintained for audit;

4.6 Salmon farming systems should be upgraded as improved, site-appropriate and cost-effective systems of proven efficacy become available.

Section 5: Management System Operations

5.1 Farm management procedures shall ensure supervision by appropriately trained, qualified or experienced personnel. There is a need for constant vigilance during operations that could result in escapes;

5.2 Procedures shall be adopted to ensure that escapes are prevented during movement and handling of stocks (e.g. during stocking, counting, grading, transport, transfers, treatment and harvesting of fish), and during net changes and cleaning;

5.3 Regular preventative maintenance, inspection and repair procedures shall be adopted in order to prevent escapes;

5.4 Stress testing of all nets in use shall be conducted on a regular basis and testing protocols, minimum breaking strengths and thresholds for net replacement should be specified in action plans. Records of the results of the tests shall be retained throughout the period the net is in use;

5.5 When it is necessary to tow cages, great care shall be taken to avoid damage to the nets;

5.6 Storm preparation procedures shall be developed to minimise the risk of damage from storms detailing the actions to be taken to ensure that the site is made ready; after each storm all nets, cages and mooring systems shall be inspected for damage;

5.7 Vessels shall be operated so as to minimise the risk of accidental damage to the equipment;

5.8 Where practicable, security systems should be installed so as to deter acts of vandalism and malicious damage.

Section 6: Verification

6.1 Management systems should include as a minimum all details of introductions, grading, transfers, treatments, handling or any other incident or occurrence that may have led to an escape. These details shall be recorded and retained for audit. Detailed records should allow estimates of escapes to be made. It is recognised that not all discrepancies will be the result of escapes;

6.2 When an event occurs which leads to an escape defined as significant under the action plan, the operator shall advise the appropriate authorities immediately;

6.3 A site-specific contingency plan shall be developed for use when an event occurs which may have led to an escape defined as significant under the action plan. The contingency plan shall include details of the method of recapture to be used and the area and timeframe over which a recapture programme would apply. Efforts shall be made to recapture farmed salmon immediately provided that this is practicable and does not adversely affect wild Atlantic salmon populations;

6.4 Action plans should require appropriate authorities to take all reasonable efforts to issue permits for facilitating the contingency plans developed for each farm.

Section 7: Development of Action Plans

7.1 Each jurisdiction should draw up a national action plan, or regional plans, at the earliest opportunity, based on these guidelines. The action plan is the process through which internationally agreed guidelines on containment would be implemented at national or regional level through existing or new voluntary codes of practice, regulations, or a combination of both;

7.2 Each action plan should:

7.2.1 create a systematic basis for minimising escapes so as to achieve a level of escapes that is as close to zero as is practicable;

7.2.2 include a mechanism for reporting information on the level and causes of escapes;

7.2.3 include a mechanism for reporting and monitoring in order to assess compliance and to verify the plan's efficacy;

7.2.4 identify areas for research and development.

7.3 The action plan should be based on co-operation between industry and the relevant authorities and should include the allocation of responsibilities under the plan(s) and a timetable for implementation.

Section 8: Reporting to the Liaison Group

8.1 Each jurisdiction should advise the Liaison Group annually on progress in implementing its action plan(s).

Section 9: Revision

9.1 These guidelines shall be subject to revision, with the agreement of the Liaison Group, to take account of new scientific, technical and other relevant information.

Annex 4

Guidelines for Stocking Atlantic Salmon

I. Introduction

The term "stocking" is defined as "the deliberate release of Atlantic salmon into the wild at any stage of their life-cycle for enhancement, mitigation, restoration, rehabilitation or ranching purposes," as defined in Annex 1 of this Resolution.

Stocking is widely carried out by many government and private entities for the reasons listed above. While these programmes are sometimes successful, it is now known that stocking can also have negative impacts on wild salmon populations and other species and that poor hatchery practices may negatively impact the characteristics of the wild salmon population that we wish to conserve. Potential consequences include: depression of the survival and abundance of indigenous populations and straying of stocked fish into nearby rivers. There is thus a need to consider fully the risks as well as the benefits arising from stocking.

Codes of Practice for stocking are widely available as are very detailed stocking manuals. These codes and manuals are designed to address issues of local or national relevance.

The present document is designed to provide guidance to NASCO's Parties on applying the Precautionary Approach to the authorisation and conduct of any stocking of Atlantic salmon into the wild. The guidelines will be regularly reviewed and updated as appropriate in the light of new scientific information.

II. Rationale for Stocking

There are many possible causes for decline of Atlantic salmon populations and stocking may not be an appropriate solution. Where a river is at or close to carrying capacity there may be little or no benefit from stocking. In addition, stocking is carried out for ranching purposes.

NASCO's Guidelines on the Use of Stock Rebuilding Programmes, CNL(04)55, provide guidance on compliance assessment, evaluation of the problem, development of a management plan and monitoring and evaluation of progress. In addition, to assist its Parties in applying the Precautionary Approach, NASCO has developed a Decision Structure for Management of North Atlantic Salmon Fisheries, CNL31.332, and a Plan of Action for the Protection and Restoration of Atlantic Salmon Habitat, CNL(01)51. It is recommended that these documents be consulted in determining if stocking is an appropriate management response to a perceived problem.

In accordance with the Precautionary Approach appropriate risk assessment methodology should be developed and applied by the Parties to proposals for stocking. Proponents must provide all information necessary to demonstrate that a proposed stocking activity will not have a significant adverse impact on wild salmon populations or have an unacceptable impact on the ecosystem.

III. Guidelines for Conducting Stocking

A. Definition of river classes

For the purposes of these guidelines, three types of river are defined on the basis of the extent to which salmon and their habitats have been affected by human activities: Class I, Class II and Class III.

Rivers are classified as Class I when they are pristine. Class I rivers have no significant human-induced habitat alterations, and neither any history of introductions or transfers of fish into the watersheds nor any fish-rearing operations in the watersheds, and no aquaculture has been conducted in marine cage culture within a specified distance of the river.

Rivers are classified as Class II if one or more of the following conditions occur: the habitat has been altered; non-indigenous wild or hatchery-reared Atlantic salmon populations have been released; or aquaculture has been conducted in marine cage culture within a specified distance of the river. Non-indigenous species may be present in land-based facilities. Introduced species such as rainbow trout would be treated as indigenous if a population has been established for 10 or more years. Many rivers around the North Atlantic will belong to this class.

Rivers are classified as Class III if habitats have been altered or if fish communities are destabilised, such as the loss of component populations, or non-indigenous species are present.

B. Guidelines applicable to all rivers

1. Atlantic salmon of European origin, including Icelandic origin, should not be released in the North American Commission area and Atlantic salmon of North American origin should not be released in the North-East Atlantic Commission area.

2. Prior to any transfer of eggs, juveniles or broodstock, health inspections of the donor facility will be undertaken. No fish will be transferred from the facility to other facilities or released into waters within the NASCO Convention area if emergency diseases, as defined by national, state, or provincial authorities, are detected at the donor facility.

3. Fish with restricted diseases, as defined by national, state, or provincial authorities, may be transferred between facilities or released into waters within the NASCO Convention area, provided that this does not result in changing the disease status of the receiving facility or waters. These transfers must also comply with national, state or provincial regulations.

4. Where hatchery rearing programmes are used in support of stocking programmes specialist advice should be sought in order to minimise genetic impacts in resultant generations. Hatchery rearing programmes should comply with the following measures:

    (a) Wherever possible, use eggs or progeny of wild fish;

(b) Ensure that wild fish removal will not significantly adversely impact on donor population(s);

(c) Derive broodstock from all phenotype age groups and components of a donor population[1];

(d) Careful consideration must be given to the size of the effective breeding population and its management. Geneticists have generally recommended that a minimum of a random group of 50 pairs be used for each cohort. However, that advice may not always be appropriate. For rehabilitation projects, where wild populations may be severely limited (i.e. remnant populations and live gene bank situations), it is essential that specialist advice be sought in order to minimise genetic impacts in resultant generations;

(e) Ideally, for genetic reasons, each male should be mated separately with a female so that the contribution of all males is equal (i.e. do not mix milt of males prior to fertilization, which can promote sperm competition);

(f) Where a river, or tributary, has completely lost its salmon population(s), several populations might be used for stocking to provide wide genetic variability for natural selection. However, genetic advice should be sought;

(g) Where there are suitable areas of unoccupied habitat, stocking with eggs or fry is recommended as stocked populations will benefit from natural selection during the juvenile phase.

5. Stocking and management programmes should take account of the fact that most Atlantic salmon in rivers are structured into a number of populations.

C. Guidelines applicable to Class I rivers

1. *General*

(a) No Atlantic salmon reared in a fish culture facility are to be released into a Class I river, another river which has its estuary within an appropriate, specified distance of a Class I river, or a marine site that is within an appropriate, specified distance of a Class I river;

(b) In general, no non-indigenous[2] Atlantic salmon are to be released into a Class I river.

2. *Rehabilitation*

(a) Generally, rehabilitation is not necessary in Class I rivers. However, where human-induced or natural events impact on a Class I river the preferred methods are to improve degraded habitat and to ensure escapement of sufficient spawners through fisheries management.

[1] The term 'population' here is used to denote a genetic population, i.e. populations are groups of animals within which mating is more or less random and among which interbreeding is more or less constrained.
[2] Not belonging to the local genetic population.

3. ***Restoration (or establishment) of Atlantic salmon in a river or part of a watershed where there are no salmon***

(a) Expert advice should be sought to identify the best option, based on the genetic and ecological characteristics of the donor population or the habitat characteristics of the donor stream;

(b) Consideration should be given to the impacts on the existing fish community and fisheries.

4. ***Ranching***

(a) Atlantic salmon ranching should only take place at release sites located greater than an appropriate, specified distance from the estuary of a Class I river and if it is demonstrated that the activity will not significantly affect wild Atlantic salmon populations.

D. <u>Guidelines applicable to Class II rivers</u>

1. ***General***

(a) Atlantic salmon, with the exception noted in III-B-1 of these guidelines, may be considered for stocking, if fish health and genetic protocols are followed and risk assessments show, on the basis of careful ecological impact evaluation, that negative impacts on local populations of Atlantic salmon will be minimal. Use of non-indigenous fish should only be used as a last resort.

2. ***Rehabilitation***

(a) The preferred methods are to improve degraded habitat and to ensure escapement of sufficient spawners through fisheries management;

(b) If further measures are required, residual population(s) of wild fish should be used. If the residual populations are too small, thorough genetic and ecological assessments should be carried out to identify the best option for rehabilitation purposes.

3. ***Restoration (or establishment) of Atlantic salmon in a river or part of a watershed where there are no salmon***

(a) For restoration, use a population(s) from a tributary within the same watershed or from a nearby river(s) that has similar genetic and ecological characteristics to the original population(s);

(b) For establishment, use a population(s) from a tributary within the same watershed or from a nearby river(s) that has similar habitat characteristics;

(c) Consideration should be given to the impacts on the existing fish community and fisheries.

4. ***Ranching***

(a) Atlantic salmon ranching should only take place at release sites located greater than an appropriate, specified distance from the estuary of a Class II river and if it is demonstrated that the activity will not significantly affect wild Atlantic salmon populations.

E. Guidelines applicable to Class III rivers

1. ***General***

(a) Atlantic salmon, with the exception noted in item III-B-1 of these Guidelines, may be considered for stocking, if fish health and genetic protocols are followed and risk assessments show, on the basis of careful ecological impact evaluation, that negative impacts on local populations of Atlantic salmon will be minimal.

2. ***Rehabilitation***

(a) The preferred methods are to improve degraded habitat and to ensure escapement of sufficient spawners through fisheries management;

(b) Rehabilitation may be achieved by stocking cultured fish.

3. ***Establishment or restoration of Atlantic salmon in a river or part of a watershed where there are no salmon***

(a) For restoration, use a population(s) from a tributary within the same watershed or from a nearby river(s) that has similar genetic and ecological characteristics to the original population(s);

(b) For establishment, use a population(s) from a tributary within the same watershed or from a nearby river(s) that has similar habitat characteristics;

(c) Consideration should be given to the impacts on the existing fish community and fisheries.

4. ***Ranching***

(a) Ranching of Atlantic salmon should only be permitted if it is demonstrated that the activity will not significantly affect wild Atlantic salmon populations.

IV. Guidelines for Authorising Stocking

A. Introduction

Both proponents and agencies responsible for managing Atlantic salmon must ensure that the risk of adverse effects on wild Atlantic salmon populations from stocking is minimised.

B. Responsibility of proponent of stocking

1. Proponents must submit an application for stocking of Atlantic salmon to the permit-issuing agency (see Box 1).

2. The application should provide a full justification for stocking and sufficient documentary evidence to allow for an evaluation of the impacts of the proposed stocking activities on the wild Atlantic salmon and its habitats.

3. The lead-time required for notice and justification of stocking will be determined by the permit-issuing agency.

4. Proponents must report all stockings that are conducted.

C. Responsibility of those with the authority to issue permits

1. Enact laws to protect wild populations of Atlantic salmon and prevent the release of Atlantic salmon that will significantly affect the productivity of existing wild Atlantic salmon populations.

2. Draw the Guidelines to the attention of all proponents of stocking at the application stage.

3. Establish, maintain, and operate a permit system and inventory for all stockings of Atlantic salmon.

4. Enact regulations to control the stocking of Atlantic salmon.

5. Establish a formal scientific evaluation process to review all applications (private and government agencies) for the stocking of Atlantic salmon and recommend conditional acceptance or rejection of the proposed stocking(s) based on the potential impact on the ecosystem.

6. Establish an evaluation process to determine the effectiveness of stocking activities and their impacts on wild Atlantic salmon populations.

7. Within a class of rivers, each agency may be more restrictive in setting salmon stocking requirements.

8. Submit to NASCO, as requested, information of a scope to be determined by the Council in relation to the application of these Guidelines.

Box 1. Guidance for proponents in the preparation of stocking proposals

The following information should be provided to the permit-issuing agency with all applications to stock Atlantic salmon so as to enable the risk of adverse effects from the proposed activities on wild Atlantic salmon populations to be evaluated.

(1) Name the population and/or strain and, where available, its genetic characteristics, and include:

  (a) Time and quantity of stocking;

  (b) A list of anticipated future stockings;

  (c) A list of previous stockings.

(2) Area, place, river or hatchery from which the fish will be obtained.

(3) Proposed place of release and any interim rearing sites.

(4) Disease status of donor hatchery, river or other location from which fish are obtained.

(5) Disease status of recipient facility or stream (where available).

(6) Objectives of the stocking and the rationale for not using a local population (if such use is not proposed).

(7) Details of the available biological characteristics of the donor population. This would include such characteristics as run timing, time of spawning, age-at-maturity, size-at-age, etc. and potential for competition with local populations of Atlantic salmon in the recipient waters or nearby waters.

(8) Information on similar stockings.

(9) Proposed procedure for transportation from donor to recipient site.

(10) Measures to be taken to prevent transmission of disease agents and to reduce the risk of escape of fish.

(11) Species composition at proposed site of introduction and adjacent rivers.

(12) Climatic regime and water chemistry, including pH of waters at the site of proposed introduction and of adjacent rivers.

(13) Potential of stocked fish to disperse to nearby streams.

(14) A bibliography of pertinent literature.

(15) A plan for monitoring, in order to assess how successful stocking has been.

Annex 5

NASCO Guidelines for Action on Transgenic Salmonids, CNL(04)41

THE PARTIES to NASCO are aware of the development of transgenic salmonids. While there may be benefits from the introduction of such salmonids if, for example, they could not interbreed with wild stocks the Council recognises that there are also risks which may lead to irreversible genetic changes and ecological interactions.

The Council considers that there is an urgent need to take steps to ensure the protection of the wild stocks and has therefore agreed to cooperate to develop means such that transgenic salmonids cannot impact upon wild salmon stocks. The following specific steps are agreed.

The Parties will:

a) advise the NASCO Council of any proposal to permit the rearing of transgenic salmonids and provide details of the proposed method of containment and other measures to safeguard the wild salmon stocks;

b) take all possible actions to ensure that the use of transgenic salmonids, in any part of the NASCO Convention Area, is confined to secure, self-contained, land-based facilities;

c) inform their salmon producers of the potentially serious risks to wild stocks of this development and consult with the salmon farming industry on this matter through the Liaison Group established between NASCO and the international salmon farming industry;*

d) take steps, as appropriate, to improve knowledge on the potential impacts of transgenic salmonids on the wild salmon stocks and their habitat;

e) examine the trade implications associated with transgenic salmonids in accordance with World Trade Organization Agreements and other instruments of international law.

Furthermore, those Parties to NASCO that are also Parties to the Cartagena Protocol on Biosafety to the Convention on Biological Diversity should take into account the provisions of that Protocol.

*Note: At its Seventeenth General Meeting in Galway, Ireland, in September 1996, the International Salmon Farmers' Association (ISFA) adopted its Policy on Transgenic Salmon, which states that "In accordance with sound environmental practices, the ISFA firmly rejects transgenic salmon production".

Annex 6

River Classification and Zoning

For the purpose of developing management measures concerning aquaculture, introductions and transfers, Contracting Parties should classify their Atlantic salmon rivers. Where appropriate, consideration should be given to grouping neighbouring or biologically (or otherwise) similar river systems into complementary management zones. River classification and zonation systems are useful to identify specific rivers and/or areas that need special protection. For example, rivers and/or areas that have been subject to significant enhancement efforts may need to be differentiated from rivers and/or areas that have not. This could allow managers to easily identify the rivers and/or areas where future enhancement efforts may or may not be appropriate.

The NAC Protocols and the NASCO Salmon Rivers Database provide examples of river classification systems. Contracting Parties should consider these examples in developing classification systems that are appropriate to their needs. Parties are further encouraged to work co-operatively in developing such systems (e.g. NEAC Parties could develop a classification system that complements the Water Framework Directive).

In conducting a risk assessment for a proposed aquaculture, or introductions and transfers, activity, the classification of the river(s) and/or zone(s) should be taken into account and class/zone-specific factors should be considered. Furthermore, in developing measures appropriate to each class of river or management zone, it is recognised that local conditions are a very significant factor and should also be considered.

Annex 7

Research and Development and Data Collection

Research and data collection should be carried out, as appropriate, in support of this Resolution. Recognising that research requirements are continually developing, a list of current research areas is identified in this Annex. Where appropriate, successful research results should be taken forward to pilot testing

Areas for research and pilot testing include:

Sterile fish

Methodology and techniques for sterilization are now well developed; research should now focus on developing strains of sterile fish which could perform at a level similar to current strains of fish used in farm production. Trials should be encouraged to evaluate the performance of strains of sterile fish under production conditions.

Tagging and marking

Tagging and marking is being used on a small scale in order to facilitate the identification of farmed salmon in the wild and their separation from wild fish, to determine the source of escapes and to assess the interactions of escaped farmed salmon with the wild stocks. Full evaluation of those trials should be conducted in order to assess effectiveness, the feasibility of large-scale marking, and associated costs. Consideration should also be given to food safety, product quality and animal welfare.

Evaluation of production methods

There should be an ongoing evaluation of current and new production methods and technology (e.g. improved containment techniques, development of suitable strains of sterile fish, development of sea lice vaccines, etc.).

Aquaculture broodstock

Research is recommended on broodstock selection methodology to minimise impacts on wild salmon stocks.

Genetics

Great advances have been made in genetic research in the past decade. These methods should be applied in investigating, in greater detail, interactions between wild salmon and salmon of aquaculture origin, including the extent of hybridization, composition of stocks, and identification of disease strains and appropriate treatment.

Diseases and parasites

The transmission of diseases and parasites between salmon reared in aquaculture and the wild stocks is an area of considerable concern. Research on vectors for transmission, and methods

to prevent and control disease and parasite outbreaks in wild salmon and in aquaculture, should be encouraged.

Interactions

Information should be collected and analyzed on the extent of intermingling in rivers and at sea between wild salmon and salmon of aquaculture origin.

Risk assessment frameworks

There has been considerable activity in the development of risk assessment frameworks. There remains a need to identify the appropriate factors to be included in a risk assessment in order to evaluate the potential impacts of aquaculture, introductions and transfers, and transgenics on wild salmon stocks.

Biological impacts

Further work is recommended on biological interactions between wild salmon and salmon of aquaculture origin including competition and behavioural interactions that may affect the viability and success of the wild populations.

Escape prevention

Research into escape detection technologies and improved containment systems should be encouraged.

Appendix 1

North American Commission Protocols for the Introduction and Transfer of Salmonids Summary of Protocols by Zone, NAC(94)14

Note:

This document contains only summary Protocols and should be read in conjunction with document NAC(92)24.

1 ZONING OF RIVER SYSTEMS

The NAC has adopted the concept of Zoning for application of these protocols to the NAC Area. Three zones have been designated based on the degree of degradation or manipulation of the wild Atlantic salmon populations (Figure 1). The NAC recognizes that Atlantic salmon populations have been variously affected by human activities. These activities include over-harvesting, selective fishing, habitat degradation, mixing of stocks, introduction of non-indigenous fish species, and spreading fish diseases. Atlantic salmon stocks in northern areas (Zone I) have generally been least affected, and those stocks in the southern area (Zone III) have been most affected, by humans.

In order to allow operational flexibility within a Zone, river systems have been classified as Class I, II, or III rivers. Generally, rivers will have the same classification as the Zone in which they occur. For example, in Zone II, river systems will be mainly categorized as Class II. However, a river system may be assigned a higher classification than the Zone in which it is located (e.g. Class I river in Zone II) to allow additional protection for valuable Atlantic salmon stocks. In extenuating circumstances and if a river is sufficiently isolated from other rivers, it is acceptable to have a river with a lower classification than the Zone in which it is located (e.g. Class III rivers within Zone II or Class II rivers in Zone I).

All rivers are generally classified at the same level as the Zone designation. Member countries wishing to change the location of Zone boundaries or to have rivers of a lower classification within a Zone should submit their recommendations, with scientific justifications, to NAC.

2 DESCRIPTION OF ZONES

Zone I: Geographic Area: Northern Quebec, Labrador, Anticosti Island and the major salmon-producing rivers in Newfoundland north of Cape Ray and west of Cape Saint John; namely: all rivers from Cape Ray to Cape Anguille and in Bay of Islands, Lomond River, Portland Creek, River of Ponds, Torrent River, Castors River, St. Genevieve River, Western Arm Brook, Salmon River (Hare Bay), Northeast River (Canada Bay), and Main River (Sop's Arm).

Rivers are classified primarily as Class I. They are pristine rivers with no significant man-made habitat alterations, no history of transfers of fish into the watersheds, and no fish-rearing operations in the watersheds.

Zone II: Geographic Area: Quebec rivers flowing into Gulf of St. Lawrence south of Pte. des Monts, Gaspé region of Quebec, Magdalen Islands, Prince Edward Island, New Brunswick, Nova Scotia, Newfoundland (except rivers designated as Class I rivers, referenced above in description of Zone I) and State of Maine east of Rockland.

Rivers are classified primarily as Class II watersheds in which one or more of the following conditions occur: the habitat has been altered; non-indigenous wild or hatchery-reared Atlantic salmon stocks have been released; or aquaculture has been conducted in marine cage culture. Non-indigenous species may be present in land-based facilities. Introduced species such as rainbow trout would be treated as indigenous if a population has been established for ten or more years.

Zone III: Geographic Area: Lake Ontario, southern Quebec draining to St. Lawrence River, State of Maine west of Rockland, New Hampshire, New York, Connecticut, Massachusetts, New Jersey, Rhode Island, and Vermont.

Rivers are classified primarily as Class III watersheds in which habitats have been altered, or where fish communities are destabilized, or exotic species are present.

3 PROTOCOLS

3.1 Protocols applicable to all three Zones

(1) Reproductively viable strains of Atlantic salmon of European origin, including Icelandic origin, are not to be released or used in Aquaculture in the North American Commission Area. This ban on importation or use of European-origin Atlantic salmon will remain in place until scientific information confirms that the risk of adverse genetic effects on wild Atlantic salmon stocks is minimal.

(2) No live salmonid fishes, fertilized eggs, gametes, or fish products are to be imported from IHN enzootic areas, unless sources have an acceptable history of disease testing demonstrating the absence of IHN (e.g. Great Lakes Fish Health Disease Committee protocol requirements). IHN infected areas currently include State of Washington, Oregon, Idaho, California, Alaska, British Columbia, Japan, and parts of Taiwan and France.

(3) Prior to any transfer of eggs, juveniles or brood stock a minimum of three health inspections of the donor facility will be undertaken during the two-year period immediately preceding the transfer; and

- No fish will be transferred from the facility to other facilities or released in waters within the NAC Area if emergency diseases are detected at a rearing facility (see Annex III, Part II of NAC(92)24);

- Fish with restricted diseases may be transferred or released in the NAC Area provided that this does not result in changing the disease status of the receiving facility or waters. These transfers must also comply with

national, state or provincial regulations (see Annex III, Part II of NAC(92)24).

(4) Prior to any movement of non-native fishes into a river system or rearing site inhabited by Atlantic salmon the agency with jurisdiction shall review and evaluate fully the potential for interspecific competition which would adversely impact on the productivity of wild Atlantic salmon populations. Such evaluations should be undertaken, to the extent possible, with information on the river in which the introduction is to occur and from similar situations.

(5) Hatchery rearing programmes to support the introduction, re-establishment, rehabilitation and enhancement of Atlantic salmon should try to comply with the following measures:

(a) Use only F1 progeny from wild stocks;

(b) Derive broodstock from all phenotype age-groups and the entire run of a donor population;

(c) Avoid selection of the "best" fish during the hatchery rearing period; and

(d) During spawning, make only single paired matings from a broodstock population of no less than 100 parents. Should the number of one sex be fewer than 50, the number of spawners of the other sex should be increased to achieve a minimum effective population size (N_e) of 100.

$$N_e = \frac{4N♂N♀}{N♂+N♀}$$

3.2 Protocols applicable to Zone I

Zone I consists of Class I watersheds where every effort must be made to maintain the existing genetic integrity of Atlantic salmon stocks. The following summary protocols apply.

3.2.1 General within Zone I

No Atlantic salmon reared in a fish culture facility are to be released into a Class I river, another river which has its estuary less than 30 km from a Class I river, or a marine site less than 30 km from a Class I river (distances would be measured in a straight line(s) headland to headland).

No non-indigenous fish species, other than Arctic charr and brook trout, or non-indigenous Atlantic salmon stock is to be introduced into a Class I watershed.

3.2.2 Rehabilitation

Fisheries management techniques will be used to ensure sufficient spawners such that spawning escapement exceeds a minimum target level to maintain an effective breeding population.

Habitat that becomes degraded will be restored to the greatest extent possible.

3.2.3 Establishment or re-establishment of Atlantic salmon in a river or part of a watershed where there are no salmon

Use transfers of adults or juvenile salmon from the residual population in other parts of the watershed.

A nearby salmon stock which has similar phenotypic characteristics to the lost stock could be transferred if there is no residual stock in the recipient watershed and provided an effective breeding population is maintained in the donor watershed (See Section 3.1 (5)).

If the biological characteristics of the original stock are not known or there was no previous stock in the recipient watershed, then transfer broodstock or early life stages from a nearby river having similar habitat characteristics.

3.2.4 Aquaculture

(i) Rearing in marine or freshwater cages, or land-based facilities:

- Reproductively viable Arctic charr and brook trout may be reared in marine and freshwater cages and in land-based facilities;

- Rearing of other salmonids or non-indigenous fishes is not permitted in the marine environment within 30 km of a Class I river, in a Class I river, or in a watershed with its estuary less than 30 km from the estuary of a Class I river. (30 km is measured in a straight line(s) headland to headland);

- Rearing of reproductively viable indigenous species and reproductively sterile non-indigenous species is permitted in land-based facilities;

- Reproductively sterile salmonids may be reared in the marine environment, and/or in a watershed with its estuary greater than 30 km from a Class I river, provided that the risk of adverse effects on wild salmon stocks is minimal;

- Natural or man-made ponds which have adequate screening of the outlet and inlet streams, such that the risk of fish escaping is low, can also be treated as land-based facilities.

(ii) Commercial ranching:

- No commercial ranching of salmonids is permitted within 30 km of the estuary of a Class I river (measured in a straight line(s) headland to headland);

- At locations greater than 30 km from the estuary of a Class I river, reproductively sterile Atlantic salmon, reproductively viable brook trout or Arctic charr, and reproductively sterile non-indigenous species may be ranched provided that the risk of adverse effects on wild Atlantic salmon stocks are minimal.

3.3 Protocols applicable to Zone II

3.3.1 General within Zone II

Reproductively viable non-indigenous species, other than Arctic charr and brook trout, and reproductively viable Atlantic salmon stocks, non-indigenous to the NAC area, are not to be introduced into watersheds or into the marine environment of Zone II.

Restoration, enhancement and aquaculture activities are permitted in the freshwater and marine environments.

3.3.2 Rehabilitation

The preferred methods are to improve degraded habitat and ensure escapement of sufficient spawners through fisheries management.

If further measures are required, use residual stocks for rehabilitation and enhancement. If the residual stock is too small, select a donor stock having similar life-history and biochemical characteristics from a tributary or nearby river.

Stocking of hatchery-reared smolts is preferred, to reduce competition with juveniles of the natural stocks.

3.3.3 Establishment or re-establishment of Atlantic salmon in a river or part of a watershed where there are no salmon

To establish an Atlantic salmon stock, use a stock from a nearby river having similar stream habitat characteristics.

If re-establishing a stock, use a stock from a nearby river which has similar biological characteristics to the original stock.

It is preferable to stock rivers with broodstock or early life-history stages (eggs and fry); this would allow selection and imprinting by juveniles to occur.

If eggs are spawned artificially, use single pair matings and optimize the effective number of parents (See Section 3.1(5)).

3.3.4 Aquaculture

(i) Rearing in marine or freshwater cages, and land-based facilities:

- It is important to apply methods which minimize escapes;

- Reproductively viable Arctic charr and brook trout may be reared in marine and freshwater cages and in land-based facilities;

- Develop domesticated salmon broodstock using local stocks; or, if local stocks are limited, use nearby stocks;

- Reproductively viable non-indigenous species may only be introduced into land-based facilities where risk of escapement is minimal;

- Non-indigenous salmonid stocks may be introduced into the wild or used in cage rearing operations if the fish are reproductively sterile and the risk of adverse ecological interactions is minimal.

(ii) Commercial ranching:

- Commercial Atlantic salmon ranching will only be permitted at release sites located greater than 20 km from the estuary of a Class II river (measured in a straight line(s) headland to headland) and it is demonstrated that the activity will not negatively affect wild Atlantic salmon stocks;

- Non-indigenous species or distant national Atlantic salmon stocks may be used if the fish are reproductively sterile and the risk of adverse ecological interactions is minimal.

3.4 Protocols applicable to Zone III

3.4.1 General within Zone III

Indigenous and non-indigenous salmonid and non-salmonid [except reproductively viable Atlantic salmon stocks non-indigenous to the NAC Area] fishes may be considered for introduction or transfer if fish health and genetic protocols are followed and negative impacts on Atlantic salmon can be shown to be minimal using careful ecological impact evaluation.

3.4.2 Rehabilitation

Habitat quality should be upgraded wherever possible.

Rebuilding stocks can be achieved by controlling exploitation and by stocking cultured fish.

3.4.3 Establishment or re-establishment of Atlantic salmon in a river or part of a watershed where there are no salmon

Transfer source stocks from nearest rivers having similar habitat characteristics.

Stock with juvenile stages (eggs, fry and/or parr). If eggs are spawned artificially, use single pair matings and optimize the effective number of parents (Section 3.1(5)).

3.4.4 Aquaculture

(i) Rearing in marine or freshwater cages, or land-based facilities:

- Use of local stocks is preferred but non-indigenous stocks may be cultured;

- Marine cage culture can be widely practised; but preferred locations are at least 20 km from watersheds managed for salmon production (measurements are by straight lines from headland to headland);

- Culture of non-indigenous species in land-based facilities on Class III watersheds is permitted in adequately controlled facilities where risk of escapement is minimal.

(ii) Commercial ranching:

- Commercial ranching of salmonids is permitted if it is demonstrated that the activity will not negatively affect Atlantic salmon rehabilitation or enhancement programmes or the development of wild Atlantic salmon stocks.

4 GUIDELINES FOR APPROVAL OF INTRODUCTIONS AND TRANSFERS

Both proponents and agencies responsible for managing salmonids have a responsibility for ensuring that risk of adverse effects on Atlantic salmon stocks from introductions and transfers of salmonids and other fishes is low. Reasonable laws to protect wild stocks should be enacted by each agency, as necessary. Resource management agencies will determine protection for habitats with Atlantic salmon potential.

4.1 Responsibility of proponent

The proponent must submit an application for introduction or transfer of fishes to the permit-issuing agency. This request must provide a full justification for the introduction or transfer such that a complete evaluation will be possible prior to issuance of a permit. The list of information to be included in the justification for introductions and transfers is in Section 4.4 below. The lead time required for notice and justification of introductions and transfers will be determined by the permit-issuing agency. Proponents should be aware of the protocols established for introductions and transfers.

4.2 Responsibility of government agencies having the authority to issue permits

These agencies shall be those entities having the responsibility for fishery management within the receiving area. The responsibilities of the agencies shall include:

(1) Establish, maintain, and operate a permit system and inventory for all introductions and transfers of fishes;

(2) Enact regulations required to control the introductions and transfers of fishes as per established protocols;

(3) Establish a formal scientific evaluation process to review all applications (private and government agencies) for the introduction and transfer of all species and recommend conditional acceptance or rejection of the proposed

introductions and transfers based on the potential impact on the productivity of Atlantic salmon;

(4) Within the Zones each agency may be more restrictive in classifying individual watersheds. Rarely, a less restrictive classification may be applied to an individual watershed if its estuary is at least 30 km in Zone I, or 20 km in Zone II (measured in straight lines headland to headland) from a watershed with a higher classification;

(5) Annually, submit to the NAC Scientific Working Group the results of the permit submission/review process, and a list of introductions and/or international transfers proposed for their jurisdiction;

(6) Prevent the release of fishes which will adversely affect the productivity of wild Atlantic salmon stocks.

4.3 Responsibilities of the NAC Scientific Working Group on Salmonid Introductions and Transfers

(1) Maintain an inventory of all introductions of salmonids, transfers of salmonids from IHN-infected areas, and importation of salmonids across national boundaries into the Commission Area.

(2) Review and evaluate all introductions and transfers referenced in Section 4.3(1) above in relation to the NAC protocols and report the results to the North American Commission.

4.4 Preparation of proposals

The following information is required, by the permit-issuing agency, with applications involving introductions and transfers of salmonids, except for restocking into source river. This information will be used to evaluate the risk of adverse effects on Atlantic salmon stocks.

(1) Name the species, strain and quantity to be introduced or transferred, and include:

(a) Time of introduction or transfer;

(b) List anticipated future introductions or transfers;

(c) List previous introductions and/or transfers.

(2) Area, place, river or hatchery from which the fish will be obtained.

(3) Proposed place of release and any interim rearing sites.

(4) Disease status of donor hatchery, river or other location from which fish are obtained.

(5) Disease status of recipient facility or stream (where available).

(6) Objectives of the introduction or transfer and the rationale for not using local stock or species.

(7) For non-indigenous species, provide the available information on the proposed species' life-history, preferred habitat, potential parasites and disease agents, and potential for competition with Atlantic salmon in the recipient waters or nearby waters.

(8) Information on similar transfers or introductions.

(9) Proposed procedure for transportation from donor to recipient site.

(10) List measures to be taken to prevent transmission of disease agents and to reduce the risk of escape of fish.

(11) Species composition at proposed site of introduction and adjacent rivers.

(12) Climatic regime and water chemistry, including pH of waters at the site of proposed introduction and of adjacent rivers.

(13) For indigenous species determine the life-history and biological characteristics of donor stock. This would include such characteristics as run timing, time of spawning, age-at-maturity, size-at-age etc.

(14) Potential of introduced or transferred fish to disperse to nearby streams.

(15) A bibliography of pertinent literature should be appended to the proposal.

4.5 Evaluation of proposals

The evaluation of proposals will be the responsibility of the permitting agency and will focus on the risk to Atlantic salmon production and potential production associated with the proposed introductions and/or transfers. The evaluation will be based on the classification of the recipient watershed. All requests for introductions or transfers must provide sufficient detail (Section 4.4 above) such that the potential risk of adverse effects to Atlantic salmon stocks can be evaluated.

The evaluation of potential adverse effects on fish health will consider the disease history of the donor and recipient facility and/or watershed with specific reference to the potential for transferring emergency diseases. The risk of detrimental genetic effects of introducing a non-indigenous stock into a river will be evaluated taking into consideration the phenotypic and life-history characteristics of the donor stock, the biochemical information (mitochondrial/nuclear DNA and enzyme frequencies, if available), and geographic distance between donor and recipient locations. The evaluation of the risk of ecological effects on Atlantic salmon populations is more involved. Introduction of non-indigenous Atlantic salmon stocks and/or non-indigenous species will be evaluated by considering the life-history and habitat requirements of the transferred fish.

The introduction of non-indigenous species poses a significant risk to the productivity of the Atlantic salmon stocks. Evaluation will be by comparison of the habitat requirement and behaviour of both the proposed introduced species and the indigenous Atlantic salmon stock at all life stages. The habitat requirements and areas of possible interactions with Atlantic salmon have been described for 13 fish species (see Part IV, Ecological Subgroup report). These can be used to provide a cursory evaluation of the life-history stage at which interactions would occur. However, more detailed information on stocks and habitats in both donor and recipient locations would be required in the form of an envirogram (example is provided in Part IV). Where insufficient data are available, research will be required prior to permitting the introduction or transfer.

An outline example of the type of information which is available in the species summaries (Part IV) is presented below for rainbow trout:

(1) Conditions under which interactions may occur:

- spawning rainbow trout may overcut Atlantic salmon redds and displace developing eggs;
- competitive interaction of juveniles: (i) exploitative competition for food; and (ii) interference competition;
- rainbow trout juveniles are more aggressive than juvenile Atlantic salmon, and may displace salmon from pools; and
- large rainbow trout are piscivorous and could prey on all stages of young salmon including emigrating smolts.

(2) Low interaction:

- in streams which Atlantic salmon do not utilize;
- in streams in which salmon are well established; and
- aquaculture using sterile fish or land-based facility.

(3) Conditions under which no interaction would occur. It would be permissible to use reproductively viable rainbow trout:

- in habitats with pH less than 5.5;
- if rainbow trout are already present in recipient stream; and
- in disturbed ecosystems where Atlantic salmon are absent and sport fishing would be improved.

5 GLOSSARY

Applicant: See proponent.

Aquaculture: The culture or husbandry of aquatic fauna other than in research, in hobby aquaria, or in governmental enhancement activities.

Commercial ranching: The release of a fish species from a culture facility to range freely in the ocean for harvest and for profit.

Competition: Demand by two or more organisms or kinds of organism at the same time for some environmental resource in excess of the available supply.

Containment: Characteristic of a facility which has an approved design which minimizes operator error to cause escape of fish, or unauthorized persons to release contained fish.

Diversity: All of the variations in an individual population or species.

Enhancement: The enlargement or increase in number of individuals in a population by providing access to more or improved habitats or by using fish culture facility production capability.

Exotic: See introduced species.

Fish: A live finfish.

Fish culture facility: Any fish culture station, hatchery, rearing pond, net pen, or container holding, rearing, or releasing salmonids.

Gamete: Mature germ cell (sperm or egg) possessing a haploid chromosome set and capable of formation of a new individual by fusion with another gamete.

Genetics: A branch of biology that deals with the heredity and variation of organisms and with the mechanisms by which these are effected.

Indigenous: Existing and having originated naturally in a particular region or environment.

Introduced species: Any finfish species intentionally or accidentally transported or released by Man into an environment outside its native or natural range.

Introduction: The intentional or accidental release of a species into an environment outside its native or natural range.

Isolation: Means restricted movement of fish and fish pathogens within a facility by means of physical barriers, on-site sanitary procedures and separate water supply and drain systems and cultural equipment.

Mariculture: Aquaculture in sea water.

Native: See indigenous.

N_e: Effective population size $= \frac{4N♂N♀}{N♂+N♀}$

Niche: A site or habitat supplying the sum of the physical and biotic life-controlling factors necessary for the successful existence of a finfish in a given habitat.

Non-indigenous: Not originating or occurring naturally in a particular environment; introduced outside its native or natural range.

Population: A group of organisms of a species occupying a specific geographic area.

Predator: An individual that preys upon and eats live fish, usually of another species.

Proponent: A private or public group which requests permission to introduce or transfer any finfish within or between countries and lobbies for the proposal.

Quarantine: The holding or rearing of fish under conditions which prevent the escape or movement of fish and fish disease agents. (For a detailed description of a quarantine facility see Annex IX of Part II).

Rehabilitation: The rebuilding of a diminished population of a finfish species, using a remnant reproducing nucleus, toward the level that its environment is now capable of supporting.

Restoration: The re-establishment of a finfish species in waters occupied in historical times.

Salmonid: All species and hybrids of the Family Salmonidae covered by the AFS checklist special publication No. 12, "A list of Common and Scientific Names of Fishes from the United States and Canada (1980)".

Species: A group of interbreeding natural populations that are reproductively isolated from other groups.

Stock: Population of organisms sharing a common gene pool which is sufficiently discrete to warrant consideration as a self-perpetuating system which can be managed.

Strain: A group of individuals with a common ancestry that exhibits genetic, physiological, or morphological differences from other groups as a result of husbandry practices.

Transfer: The deliberate or accidental movement of a species between waters within its native or natural geographic range, usually with the result that a viable population results in the new locations.

Transferred species: Any finfish intentionally or accidentally transported and released within its native or natural geographic range.

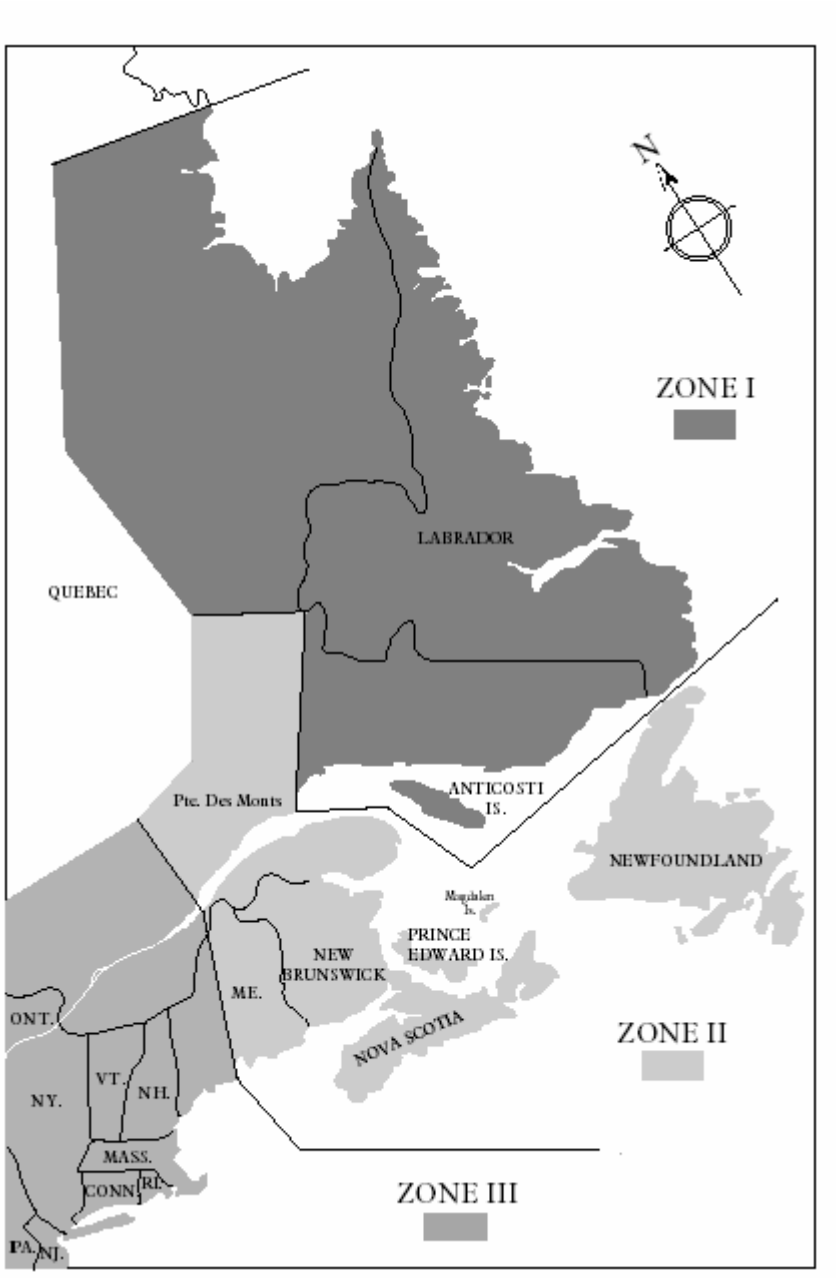

Figure 1.

Map of eastern Canada and northeastern USA showing the three zones designated for implementation of the Protocols. Certain rivers on the west coast of Newfoundland are designated as Zone I, even though Newfoundland is shown as being in Zone II.

Appendix 2

NAC(05)7

Memorandum of Understanding between Canada and USA

Preamble

The North American Commission (NAC) of NASCO recognizes the potential effects that introductions and transfers of aquatic species can have on fish health, genetics, and their ecology. In 2003, NASCO adopted the Williamsburg Resolution which referenced the NAC Protocols as contained in NAC(92)24 and ancillary document NAC(94)14. In Canada, the National Code on Introductions and Transfers of Aquatic Organisms was adopted in 2001. It is acknowledged that Canada and the United States utilize different methods within their countries for authorization of introductions and transfers. This Memorandum of Understanding is meant to reconcile the differences between the methods used but recognizes the common goal is the conservation and protection of wild Atlantic salmon.

Memorandum of Understanding

Canada and the United States have agreed to record the following in connection with the introductions and transfers of salmonids in the North American (NAC) area:

A. Authorizations of Introductions and Transfers

In Canada, the National Code on Introductions and Transfers of Aquatic Organisms is the mechanism for approval of introductions and transfers which is authorized by permits. In the United States, state and federal permits are the mechanisms for authorizing introductions and transfers.

B. Requirement to Report

The Parties agree to report to the NAC annually on any decision made under their respective jurisdiction that has an impact on the other jurisdiction. In particular, any decisions made that are not consistent with the NAC Protocols will be identified.

C. Requirement to Consult

The Parties agree to consult with each other if either jurisdiction receives a proposal for an introduction or transfer that may have an impact on the other, including any proposal that would be inconsistent with the NAC Protocols.

D. Need for Review

The Parties agree to convene the NAC Scientific Working Group, from time to time, to review the provisions of the Williamsburg Resolution with respect to developments that may have an application on introductions and transfers in the NAC area and provide recommendations to the Parties for their consideration and action, if required.

SLG(09)5

Guidance on Best Management Practices to address impacts of sea lice and escaped farmed salmon on wild salmon stocks (Adopted in June 2009 and Revised in June 2010)

1. Since 1990, NASCO has co-convened three major international symposia to ensure that it had the best available information on interactions between wild and farmed salmon to guide its decisions. In 1994, in response to the information presented at these symposia, NASCO adopted the 'Oslo Resolution' designed to minimise impacts of aquaculture on the wild salmon stocks. The Oslo Resolution had been developed in consultation with the salmon farming industry and, in order to strengthen this relationship, a Liaison Group was established in 2000. The objective of the Liaison Group is to establish mutually beneficial working arrangements in order to make recommendations on wild salmon conservation and sustainable salmon farming practices, to maximise potential benefits and to minimise potential risks to both. Through the Liaison Group Guidelines on Containment of Farm Salmon were developed and reports on progress with developing and implementing containment action plans are made to the Liaison Group. These guidelines, together with Guidelines on Stocking and elements to ensure consistency with the Precautionary Approach, were incorporated into a new Resolution, the Williamsburg Resolution, CNL(06)48, adopted in 2003 and amended in 2004 and 2006.

2. The most recent NASCO/ICES symposium held in Bergen in 2005 highlighted that while much progress had been made in addressing impacts of aquaculture and in better understanding the nature of these impacts, sea lice and escaped salmon were identified as continuing challenges both for the industry and the wild stocks and on which further progress was urgently needed. NASCO, therefore, decided that it would establish a Task Force comprising representatives of the Parties, the salmon farming industry and NASCO's accredited NGOs with the aim of: identifying a series of best practice guidelines and standards to address the impacts of aquaculture on wild salmon stocks; to identify knowledge gaps and research requirements to address them; and to consider if, and how, impact targets can be identified. In accordance with its Terms of Reference, the Task Force collated existing Codes of Practice as contained in document ATF(09)7 and developed this guidance on best management practices, framed around the elements of the Williamsburg Resolution, designed to achieve international goals to address the impacts of sea lice and escaped salmon on wild Atlantic salmon. The guidance provides a range of measures from which those most appropriate to the local conditions should be put into place to safeguard the wild salmon stocks.

3. This guidance is intended to supplement the Williamsburg resolution and to assist the Parties and jurisdictions: in managing salmon aquaculture, in cooperation with their industries; in developing future NASCO Implementation Plans; and in preparing their 2010 and subsequent Focus Area Reports on aquaculture and related activities. It is anticipated that the triennial reviews of the FARs will provide a mechanism for assessing progress towards achievement of the international goals. It is the intention that NASCO and its jurisdictions explore, in collaboration with industry, opportunities for cooperative scientific research in support of the goals.

	Sea lice	Containment
International Goals	**100% of farms to have effective sea lice management such that there is no increase in sea lice loads or lice-induced mortality of wild salmonids attributable to the farms.**	**100% farmed fish to be retained in all production facilities**
	Use Williamsburg Resolution as basic guidance, supplemented as below	
Best Management Practices (BMPs)	Area management, risk-based, integrated pest management (IPM) programmes that meet jurisdictional targets for lice loads at the most vulnerable life-history stage of wild salmonids.	Codes of Containment including operating protocols
	Single year-class stocking	Technical standards for equipment
	Fallowing	Verification of compliance
	Risk-based site selection	Risk-based site selection
	Trigger levels appropriate to effective sea lice control	Mandatory reporting of escape events and investigation of causes of loss
	Strategic timing, methods and levels of treatment to achieve the international goal and avoid lice resistance to treatment	Adaptive management in response to monitoring results to meet the goal
	A comprehensive and regulated fish health programme that includes routine sampling, monitoring and disease control	
	Lice control management programmes appropriate to the number of fish in the management area	
	Adaptive management in response to monitoring results to meet the goal	
Reporting & Tracking	Monitoring programme appropriate for the number of farmed salmon in the management area and sampling protocols effective in characterising the lice loads in the farms and wild salmonid populations.	Number of incidents of escape events and standardised descriptions of the factors giving rise to escape events
	Lice loads on wild salmonids compared to areas with no salmon farms	Number and life-stage of escaped salmon (overall number; % of farmed production)
	Lice-induced mortality of wild salmonids (e.g. as monitored using sentinel fish, fish-lift trawling, using batches of treated smolts)	Number of escaped salmon in both rivers and fisheries (overall number; % of farmed production) and relationship to reported incidents
	Monitoring to check the efficacy of lice treatments	

	Sea lice	Containment
Factors Facilitating Implementation	Development of a monitoring programme appropriate for the number of farmed salmon in the management area and sampling protocols effective in characterising the lice loads in the farms	Monitoring of rivers for escaped salmon
	Access to a broad suite of therapeutants, immunostimulants and management tools	Site appropriate technology
	Collation and assessment of site selection and relocation criteria	Advanced permitting to facilitate recapture and exchange of information on effectiveness of recapture efforts
	Regulatory regimes which facilitate availability of alternative sites, as necessary, to support achievement of the goal	Technology development (e.g. cage design, counting methods for farmed salmon, methods to track origin of escaped salmon and their progeny)
	Training at all levels in support of the goal and to increase awareness of the environmental consequences of sea lice	Training at all levels in support of the goal and to increase awareness of the environmental consequences of escaped salmon
	Monitoring of lice levels: in areas with and without farms; before, during and after a farm production cycle; and in plankton samples	Assessments of the relative risks to the wild stocks from escaped salmon from freshwater compared to marine facilities and from large but infrequent escape events compared to small but frequent escape events.

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AQUACULTURE ENVIRONMENT INTERACTIONS
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Quantifying the contribution of sea lice from aquaculture to declining annual returns in a wild Atlantic salmon population

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ABSTRACT: Atlantic salmon *Salmo salar* has shown declines in abundance associated with reduced survival during marine life stages. Key impacts on survival may include a changing ocean environment and salmon louse *Lepeophtheirus salmonis* infestation from aquaculture. A 26 yr record from the Erriff River (Western Ireland) was used to evaluate the contribution of sea lice from salmon aquaculture to declining returns of wild 1 sea-winter (1SW) salmon. Statistical models suggested that returns were >50% lower in years following high lice levels on nearby salmon farms during the smolt out-migration. The long-term impact of salmon lice was explored by applying predicted annual loss rates as a multiplier to observed 1SW salmon returns. This produced a 'lice-corrected' return time series, i.e. an estimate of how returns might have looked in the absence of a serious aquaculture lice impact. The corrected time series was adjusted to account for some reduction in recruitment due to lost spawners. Comparing observed and lice-corrected time series suggested that salmon lice have strongly reduced annual returns of 1SW Erriff salmon, but that the salmon lice impact does not explain a declining trend in this population.

KEY WORDS: *Lepeophtheirus salmonis* · *Salmo salar* · Salmon smolts · Salmon farming · Ricker stock recruitment

INTRODUCTION

Atlantic salmon *Salmo salar* is an iconic anadromous fish species that has shown marked declines in abundance in recent decades (Limburg & Waldman 2009). Decreased survival rate in the marine environment, rather than in natal rivers, seems to explain the current poor state of many salmon populations (ICES 2016). Marine survival can be partitioned into coastal (transitional and inshore waters) and oceanic (offshore and open ocean) components. The coastal component operates during the first migration of juvenile salmon (smolts) out of their natal river. Events during such early life stages can have an impact on subsequent marine survival of salmon (Holsman et al. 2012). The oceanic component refers to fish in summer nursery areas offshore and in winter feeding areas. In addition to natural mortality, each component of marine survival is influenced by anthropogenic pressures.

Coastal pressures frequently interact (Parrish et al. 1998) and include local pollution (Larsson et al. 1996, McCormick et al. 1998, Johnson et al. 2007) and increased rates of sea lice *Lepeophtheirus salmonis* infestation associated with salmon aquaculture (e.g. Krkošek et al. 2007, Costello 2009). Sea cage aquaculture causes sea lice on sympatric wild fish to increase (Frazer 2009). Marine survival of wild pink salmon has been related negatively to lice density on farmed salmon (Marty et al. 2010, Krkošek et al. 2011) and to observed lice infestation on out-migrating juvenile wild fish (Peacock et al. 2013). The negative impact of sea lice on salmonid survival appears to be exacerbated by warmer environmental conditions (Bateman et al. 2016, Shephard et al. 2016). In the ocean, salmon respond to large-scale climate

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forcing (ICES 2016) by the North Atlantic Oscillation (NAO) and the Atlantic Multi-decadal Oscillation (AMO) that drive sea surface temperature (SST) and thus salmon thermal habitat (Friedland et al. 1993, 2003, Jonsson & Jonsson 2004, Mills et al. 2013) and associated prey dynamics (Beaugrand & Reid 2012, Defriez et al. 2016). Recent studies suggest that ocean warming has had a negative impact on oceanic growth and survival (McCarthy et al. 2008, Todd et al. 2008, Friedland et al. 2009) and genetic diversity (Horreo et al. 2011) of Atlantic salmon.

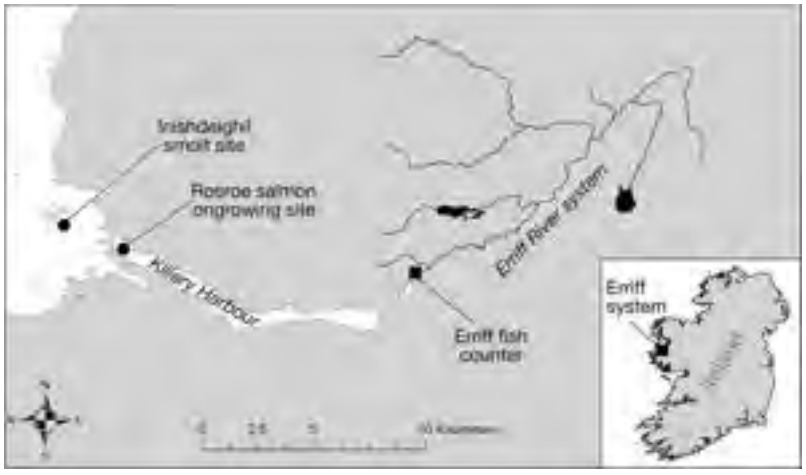

Fig. 1. Erriff River system and Killary Harbour, Ireland, showing the location of the salmon aquaculture sites and the fish counter

Strong environmental impacts on marine life stages of salmon have made it difficult to use observational data to separate the effects of sea lice from other effects on survival, and so much relevant work applies field trials using cultivated salmon smolts treated with anti-parasitic agents (Gargan et al. 2012, Krkošek et al. 2012). Some trials indicate that baseline survival of smolts has an important influence on the success of lice treatment, with poorer environmental conditions increasing vulnerability to sea lice impacts. Hence, population-level effects of sea lice on wild salmon cannot be estimated independently of the other factors that affect marine survival (Vollset et al. 2016). The contribution of sea lice to overall marine survival of wild Atlantic salmon remains an important knowledge gap, particularly in the context of changing oceanographic conditions and the long-term decline of many populations. Parsing out coastal sea lice effects might contribute to understanding of changing high-seas marine survival, and possibly guide management of lice on salmon farms to reduce impacts on wild populations (Peacock et al. 2013).

The Erriff River system in the west of Ireland is designated as a Special Area of Conservation for Atlantic salmon under the European Union Habitats Directive (92/43/EEC). This system has supported salmon angling since the late 19th century, with annual returns of fish to the river being recorded for several decades. Salmon aquaculture commenced in the Erriff estuary (Killary Harbour) in the mid-1980s, and licensed annual production increased from 450 t in 1986 to 2200 t by 2006. Levels of sea lice infestation on the Killary salmon farm have been recorded since 1991. The position of this salmon farm at the entrance to a narrow fjord (Fig. 1) makes the Erriff system an excellent 'natural experiment' on the possible effects of sea lice from aquaculture on marine survival of a wild Atlantic salmon population. We used a 26 yr record from the Erriff to investigate relationships between sea lice (salmon lice *Lepeophtheirus salmonis*; hereafter simply sea lice) infestation on the Killary salmon farm and annual returns of wild 1 sea-winter (1SW) Erriff salmon, while accounting for unexplained inter-annual variability in marine survival of this population.

MATERIALS AND METHODS

Study system

The Erriff River has a catchment area of 166 km^2 and discharges into Killary Harbour, a 15 km long fjord in the west of Ireland (Fig. 1). Data series used in the current study comprised:

(1) Annual wild Atlantic salmon *Salmo salar* returns: (a) count of 1SW Erriff fish returning to the river, and (b) estimated return (accounting for annual commercial fishing mortality at sea, *F*; see below) of Erriff salmon to the Irish coast (1987–2016).

(2) Annual aquaculture lice count estimate: average number of mobile (pre-adult and adult) sea lice (*Lepeophtheirus salmonis*) fish^{-1} on the Killary salmon farm (Fig. 2) in April (www.marine.ie/Home/site-area/areas-activity/aquaculture/sea-lice), multiplied by an estimate of the total number of fish on the farm (taken as 0 in 1986 prior to farming and recorded for 1991–2016. For years when the smolt on-growing site (Fig. 1) was active, estimated total lice from this site were added to the total for the salmon farm. The current analysis related the number of returning 1SW

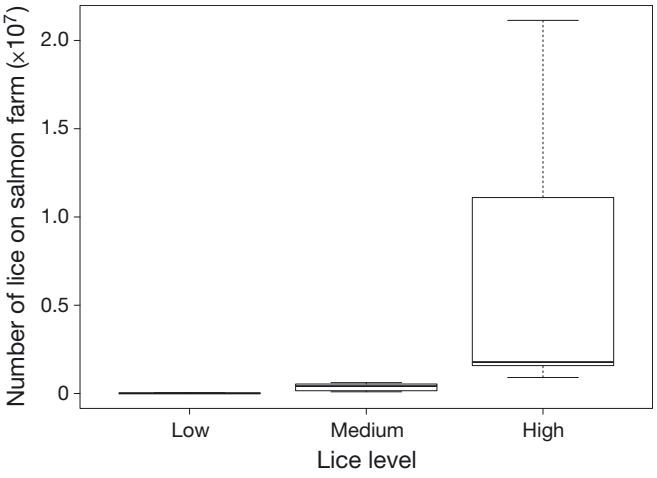

Fig. 2. Numbers of sea lice in each level of the categorical lice variable $Lcat2_i$. Summary statistics are the median of the data, the lower and upper quartiles (25% and 75%) and the minimum and maximum values. There are 8 data points (years) for the Low lice level, and 9 data points in each of Medium and High lice levels

salmon to the number of sea lice on aquaculture sites in the previous year, i.e. when those 1SW fish outmigrated as smolts. Aquaculture lice counts for April were used as an index of lice infestation pressure on wild migrating salmon smolts because records from 2002–2016 (N = 15 684 smolts, Inland Fisheries Ireland unpublished data) indicated that 88% of the wild salmon smolt run in the Erriff catchment occurs between 1 April and 10 May.

Estimation of annual wild salmon returns

Two salmon return series were used. Salmon entering the Erriff are recorded by a fish counter approximately 200 m upstream of the river mouth. The annual count of 1SW salmon S returning to the Erriff River in year i (S_{iRiver}) was calculated as the sum of 1SW salmon rod caught (killed) below the fish counter and the number recorded by the counter. S_{iRiver} represents exact known counts of fish entering the river, but does not account for variable levels of F prior to return. Estimated return to the Irish coast (S_{iCoast}) was estimated by using F time series to expand S_{iRiver}. Commercial drift and draft net fisheries for wild salmon both operated off the Irish coast during the early study period, viz. 1987–2006, but fishing was restricted to inshore draft netting from 2007–2016. F was calculated slightly differently for these 2 time periods:

(1) Combined (drift and draft net) mean annual exploitation rate F for 1SW salmon has been calculated for 2 west of Ireland salmon stocks: Corrib and Burrishoole (Ó Maoiléidigh et al. 2015). These averaged F estimates (Fig. 3) were used to raise S_{iRiver} to an estimate of S_{iCoast} for 1987–2006, where $S_{iCoast} = S_{iRiver} / (1 - F)$. These estimated S_{iCoast} values suggest that Erriff fish contributed about 1.3% to the total annual catch of salmon in the Irish drift net fishery. This value is somewhat uncertain, as F was derived from a subsample of the overall commercial catch (Ó Maoiléidigh et al. 2015). However, it is similar to independent estimates of the contribution of Erriff salmon to the drift net catch based on assigning captured fish to their natal river using a genetic signature. Genetic assignment suggested that the total drift net catch comprised 1.7% Erriff fish in 2005 and 2.5% Erriff fish in 2006 (Anon 2008).

(2) Total annual catch in the Killary draft net fishery S_{id} is recorded and can be allocated to 3 local rivers including the Erriff. These 3 rivers have salmon conservation limits (CLs) of 1383 (Erriff), 136 (Culfin) and 165 (Delphi), where CL is defined as the spawning stock level that produces long term average maximum sustainable yield as derived from the adult to adult stock and recruitment relationship, and is quantitatively derived for each river by the Irish Standing Scientific Committee for Salmon. The Erriff CL represents 82% of the summed CL for the 3 rivers in Killary; F for 2007–2016 was thus calculated as $0.82 \times S_{id} / (0.82 \times S_{id}) + S_{iRiver}$, and $S_{iCoast} = S_{iRiver} / (1 - F)$ as above.

Statistical analysis: estimating the lice effect on salmon returns

By observation i, the data consisted of (S_i, Y, L_i), where S_i is the number of Erriff salmon returning (to either the river or the coast) in sampling year $Y_{i\ 1,\ \ldots,\ 30}$ (1987–2016) and L_i is the estimated total number of sea lice on the Killary salmon farm (on-growing and smolt sites) in the previous year Y_{i-1} (no data for 1987–1990). Sea lice number was also interpreted as a categorical variable with 3 intensity levels (Low, Medium, High) in order to facilitate interpretation of lice impacts across (continuous) Y_i. Two approaches to categorizing L_i were tested: (1) $Lcat_i$ according to 0–25th, 25th–75th and 75th–100th percentiles of L_i, and (2) $Lcat2_i$ using natural divisions in L_i, which had groups of data points at 3 distinct levels (Fig. 2).

We developed statistical models to quantify possible effects of sea lice on each of S_{iRiver} and S_{iCoast} (1987 and 1992–2016), while accounting for an observed declining trend in salmon returns, and also for other

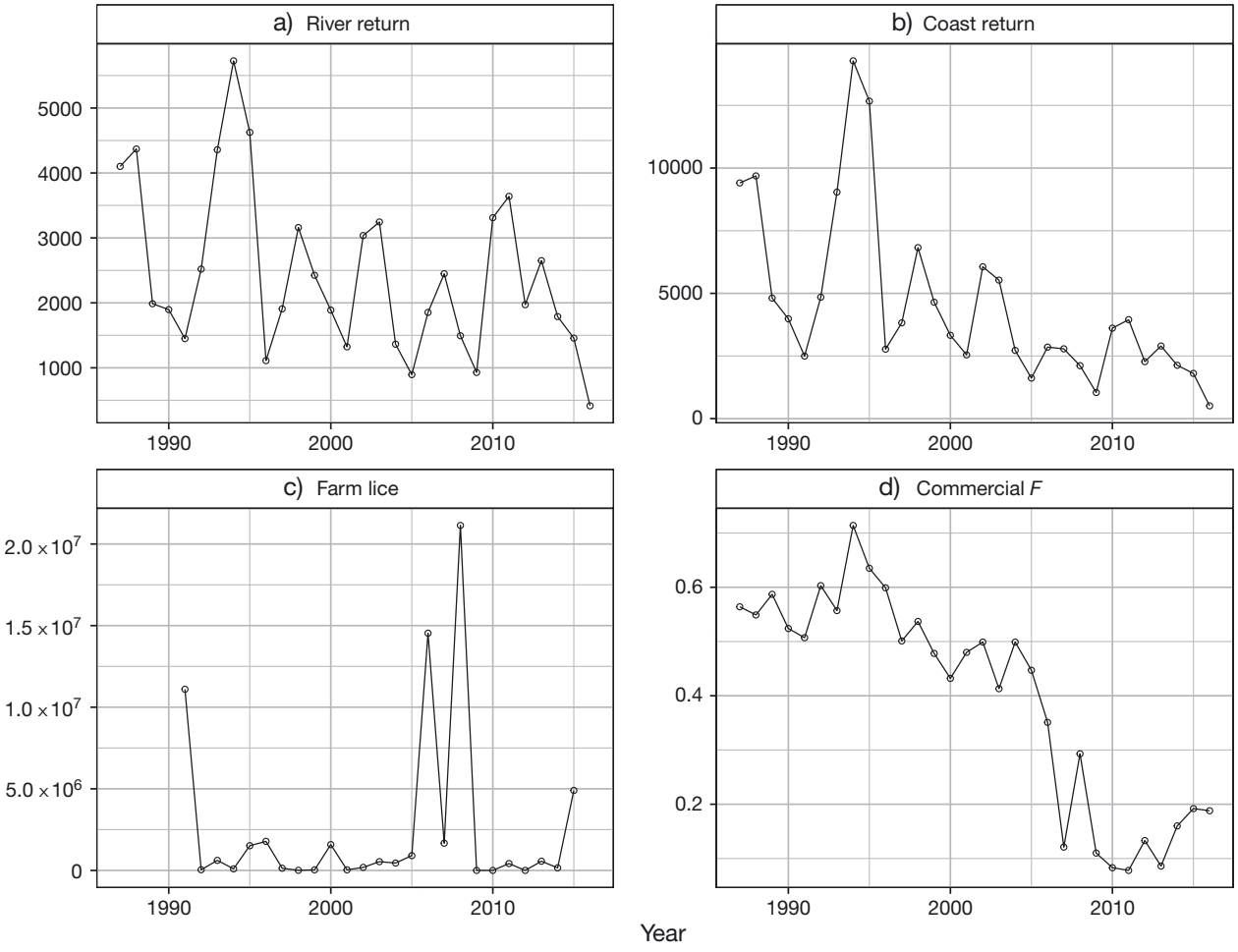

Fig. 3. Time series of returns of 1 sea-winter (1SW) Erriff River Atlantic salmon (a) to the river (S_{iRiver}) and (b) to the Irish coast (S_{iCoast}); (c) estimated number of sea lice in the Killary salmon farm; and (d) estimated commercial fishing mortality (F) for Erriff salmon

unexplained annually varying environmental drivers of marine mortality. The negative trend in Erriff salmon returns was incorporated by using standardised (subtracting the mean and dividing by the standard deviation) Y_i as a continuous fixed variable. Unexplained annual effects on salmon returns were incorporated by specifying year as a categorical variable $Ycat_i$, and including this variable as a random effect α_i on the intercept. $Ycat_i$ thus captures inter-annual effects on returns that cannot be accounted for by the lice and Y_i covariates (see Elston et al. 2001) and which are expected to largely comprise environmental variability. As an observation level random effect (OLRE), $Ycat_i$ also acts as a simple and robust means to account for overdispersion in count data (Harrison 2014). The 5 variables specified above (S_i, Y_i, L_i, $Lcat_i$, $Lcat2_i$) were used to specify a comprehensive set of 7 candidate models, all including $Ycat_i$ as a random effect α_i (Table 1).

The same modelling process was applied to each of S_{iRiver} and S_{iCoast} separately. In each case, the model set (Table 1) was fit using a Poisson GLMM (lme4 package in R, Bates et al. 2015). The full model had the form:

Table 1. Set of 7 candidate models of the number of 1 sea winter (1SW) Erriff River Atlantic salmon returning to the river and to the Irish coast (1987 and 1992 2016). Model parameters are defined in 'Materials and methods'

No.	Model
1	$\log(\mu_i) = Y_i + \alpha_i$
2	$\log(\mu_i) = \ln(L_i) + \alpha_i$
3	$\log(\mu_i) = \ln(L_i) + Y_i + \alpha_i$
4	$\log(\mu_i) = Lcat1_i + \alpha_i$
5	$\log(\mu_i) = Lcat1_i + Y_i + \alpha_i$
6	$\log(\mu_i) = Lcat2_i + \alpha_i$
7	$\log(\mu_i) = Lcat2_i + Y_i + \alpha_i$

$$S_i \sim \text{Poisson}(\mu_i) \quad (1)$$

$$E(S_i) = \text{var}(S_i) = \mu_i \quad (2)$$

$$\log(\mu_i) = L_i + Y_i + \alpha_i \quad (3)$$

$$\alpha_i \sim N(0, \sigma^2_{Ycat}) \quad (4)$$

Akaike's Information Criterion (AIC) was used to compare model fits, where any models within 2 AIC units of the best-fitting model would be considered to have similar fit to the data. Various diagnostics were used to explore model fit and statistical assumptions: (1) plots of standardised (Pearson) residuals were checked for homogeneity, (2) linearity in the relationships between salmon return and tested (continuous) covariates was evaluated by plotting Pearson residuals against each covariate in the model and fitting a GAM to visualize any non-linear patterns, and (3) temporal autocorrelation in model residuals was evaluated using the acf function in R.

Selected (lowest AIC) final models for both S_{iRiver} and S_{iCoast} included sea lice characterised as $Lcat2_i$. The effect on return of 1SW Erriff salmon of sea lice level ($Lcat2_i$: Low, Medium, High) in each of these selected models was visualized using the R package 'Effects', where other variables were held at average values (Fox 2003). The random effect of year $Ycat_i$ was plotted with 95% confidence intervals. Salmon returns at each lice level were also predicted (predict function in R) and plotted for each level of $Ycat_i$ (26 levels, i.e. years), considering 3 periods of the time series Y_i (Early, Mid, Recent) to show how the predicted (within year) lice effect on salmon returns compared to the (across year) random year effect (assumed to express annually-varying environmental effects on returns).

Predicting long-term salmon returns without sea lice

The models above predicted that the average return of 1SW Erriff salmon to the river is reduced by 18.6% following a year of Medium lice levels and 52.2% following a year of High lice levels; returns to the coast were predicted to be reduced by 2.3 and 49.6%, respectively. We used these lice impact levels and a fitted stock-recruitment relationship to estimate how annual salmon returns might have looked over the last 25 yr in the absence of a serious impact of sea lice from aquaculture:

(1) Observed annual salmon returns (each of S_{iRiver} and S_{iCoast}) were first 'lice-corrected' (multiplied up) according to the annual loss rates predicted from modelling; loss rates were expressed as the percentage difference between predicted salmon returns at each of Medium and High lice levels and the predicted return at Low lice levels in an average year. For example, the observed return to the river in 1992 was 2520 salmon, but because the lice level was 'High' during the smolt run in 1991, it is predicted that this run represents a 52.2% reduction compared to the run that would have happened in Low lice conditions (given average environmental conditions as expressed by $Ycat_i$). The lice-corrected return Se_i was thus calculated as $Se_{1992} = 2520 / (100 - 52.2) \times 100 = 5272$ salmon.

(2) To realistically estimate the cumulative impact of sea lice on long-term returns of Erriff salmon, it was then necessary to account for likely diminished recruitment associated with loss of potential spawners (hereafter 'missing spawners') that never returned to the river/coast because they suffered lice-related mortality as smolts. 85% of Erriff salmon migrate as 2 yr smolts, resulting in a 4 yr generation time (White et al. 2016). Adult-to-adult Ricker stock recruitment (SR) relationships were produced for each of river and coast returns (see Fig. 6), where S is the observed return S_i and R is the lice-corrected return 4 yr later, Se_{i+4}. These SR curves were used to estimate peak (asymptotic) recruitment Rp, and the peak stock Sp at Rp, for each of S_{iRiver} and S_{iCoast}. The number of 'missing spawners' Sm_i in each year was then estimated as $Sm_i = Se_i - S_i$, with Se_i being capped at Sp on the assumption that once Sp is exceeded, there is no further positive effect on subsequent recruitment.

(3) A lice-corrected adult-to-adult return rate, RR, was then estimated for each year Y_i in each of S_{iRiver} and S_{iCoast}, assuming that each S_i comprised 85% 4 yr and 15% 5 yr fish (White et al. 2016), such that RR_i is the weighted mean of (Se_i / S_{i-4}) and (Se_i / S_{i-5}) with weightings being 85 and 15, respectively. These RR_i are an estimate of the number of returning fish expected (given Low lice levels) from each parent fish. 85% of missing fish Sm_i were then allowed to contribute recruits Sr_i to the return 4 yr later according to the estimated return rate RR_i, where this contribution $Sr_{i+4} = (0.85 \times Sm_i) \times RR_{i+4}$. The remaining 15% of missing fish contributed to recruitment 5 yr later as $Sr_{i+5} = (0.15 \times Sm_i) \times RR_{i+5}$. To restrict un-quantified uncertainty in this process, missing fish were only considered to contribute recruits to a single generation.

(4) Finally, a total expanded return $Stot_i$, including the annual lice-correction and the associated (1 generation) effect on recruitment, was calculated as $Stot_i = Se_i + Sr_i$. Time series of S_i and $Stot_i$ were plotted together for visual comparison, with the first 4 yr of $Stot_i$ obviously not including any Sr_i as there were no lice data for their respective parent generations.

RESULTS

Estimating the lice effect

Model 7 (see Table 1) was the best fitting model for both S_{iRiver} (ΔAIC = 3.8) and S_{iCoast} (ΔAIC = 4.0). The model including only year as a continuous variable (Model 1, Table 1), had ΔAIC > 8.0 compared to Model 7 fitted to S_{iRiver}, and ΔAIC > 11.0 compared to Model 7 fitted to S_{iCoast}, indicating that $Lcat2_i$ strongly improved model fit. Diagnostic plots did not show important heterogeneity or non-linearity in residuals, and there was no significant temporal autocorrelation. There were significant negative effects of the continuous year variable Y_i on each of S_{iRiver} and S_{iCoast}, i.e. long-term declines in 1SW salmon returns (Table 2). There were also significant negative effects of High sea lice levels $Lcat2_i$ during the smolt outmigration on each of S_{iRiver} and S_{iCoast} in the following year (Table 2). Predicted returns were reduced at Medium and strongly reduced at High lice levels. For an average random year $Ycat_i$ and continuous year Y_i: the predicted S_{iRiver} (1394 fish) at High lice levels was 52.2% less than the predicted return (2919 fish) at Low lice levels (Fig. 4a); predicted S_{iCoast} (2226 fish) at High lice levels was 49.6% less than the predicted return (4419 fish) at Low lice levels (Fig. 4b).

The OLRE $Ycat_i$ captures any important patterns in the response variable that cannot be modelled by other terms in the model (Zuur et al. 2015). Strong variation in salmon returns across levels of $Ycat_i$ indicated considerable inter-annual variation in salmon returns to the river and coast (Fig. 5), probably reflecting environmental effects. However, the predicted 52.2% reduction in S_{iRiver} following 'High' lice levels is greater than the average year-to-year ($Ycat_i$ to $Ycat_{i+1}$) change in predicted returns (mean = 44.6%, range = 0.6% to 262.7%) for the Early value of Y_i, suggesting that the lice impact is meaningful in the context of background environmental forcing. This comparison showed similar results for S_{iCoast}.

Table 2. Results from selected models (Model 7, see Table 1) of annual returns of 1 sea-winter (1SW) Erriff River Atlantic salmon returning to the river (S_{iRiver}) and to the Irish coast (S_{iCoast})

River returns	Estimate	SE	*z*-value	p
(Intercept)	7.979	0.143	55.917	<0.001
Year	−0.277	0.081	−3.412	<0.001
Lice level Medium	−0.206	0.197	−1.045	0.296
Lice level High	−0.739	0.196	−3.772	<0.001
Coast returns				
(Intercept)	8.394	0.129	65.064	<0.001
Year	−0.551	0.073	−7.512	<0.001
Lice level Medium	−0.023	0.178	−0.128	0.898
Lice level High	−0.686	0.177	−3.871	<0.001

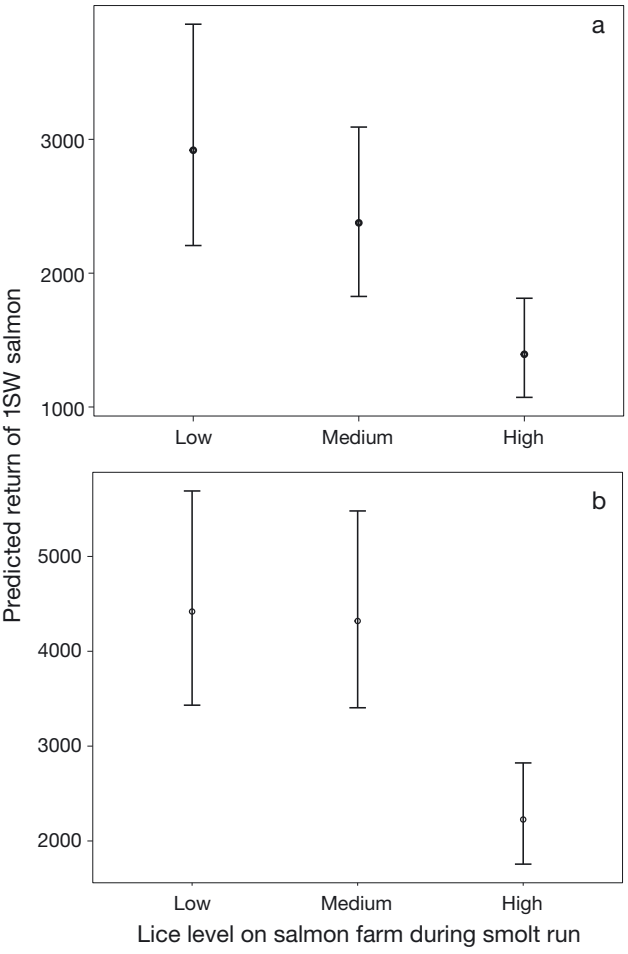

Fig. 4. Predicted return of 1 sea-winter (1SW) Erriff River Atlantic salmon to (a) the river (S_{iRiver}) and (b) the Irish coast (S_{iCoast}) at 3 levels of sea lice density at the Killary salmon farm during the smolt run. Error bars are 95% confidence intervals

Predicting the contribution of sea lice impacts to long-term returns of Erriff salmon

Adult-to-adult Ricker *SR* curves, assuming a 4 yr generation time, showed a (visually) reasonable fit for both river and coast returns, suggesting that estimates of *Rp* and *Sp* were acceptable (Fig. 6). Comparing observed salmon returns S_i with lice-corrected returns $Stot_i$ for S_{iRiver} and S_{iCoast} (Fig. 7) showed that while the sea lice effect can strongly reduce annual returns S_i, 'correcting' for this effect

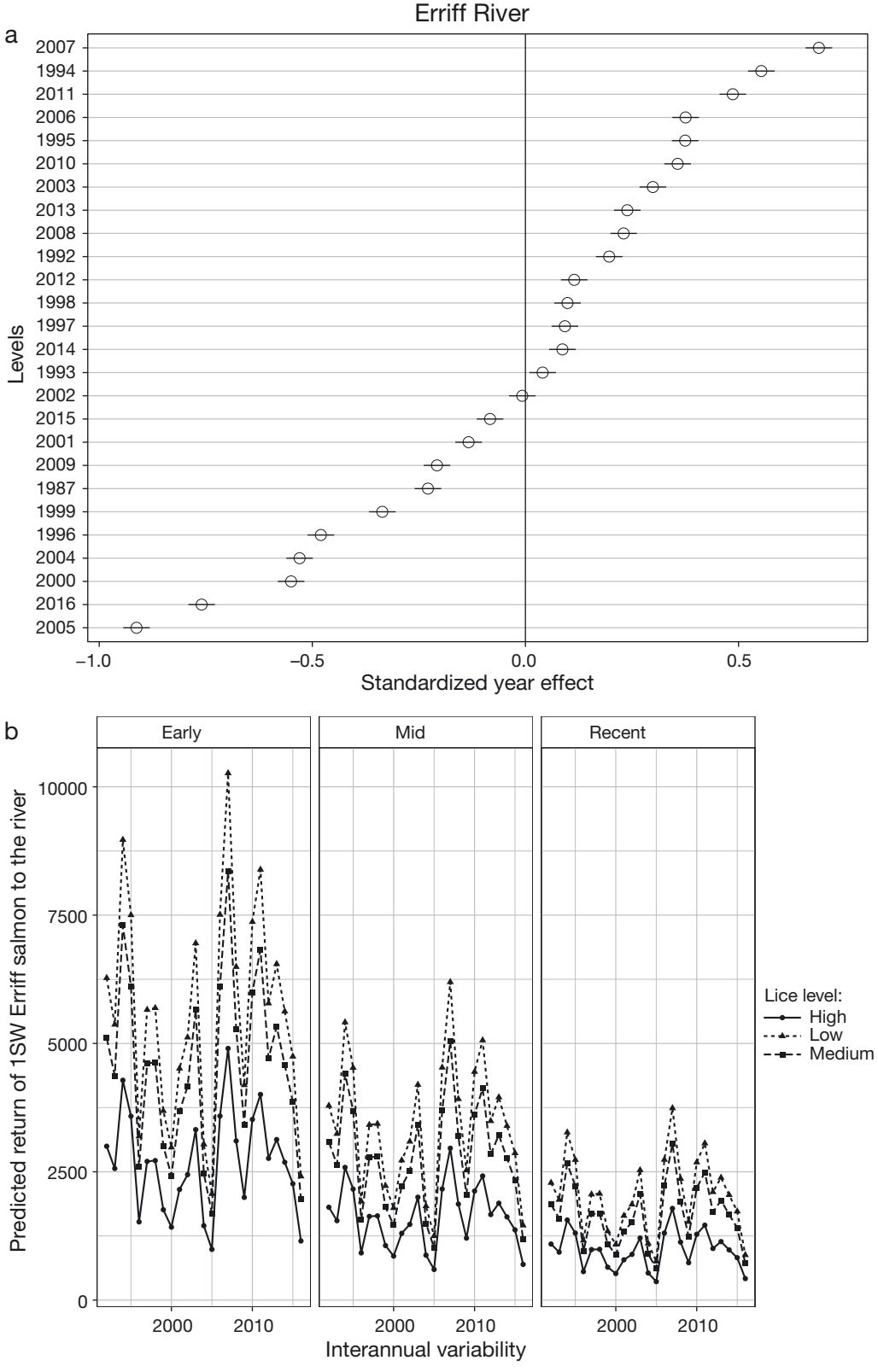

Fig. 5. (a,c) Random effects (with 95% CI) on the intercept of year ($Ycat_i$) from the selected model (Model 7, Table 1) of returns of 1 sea-winter (1SW) Erriff River Atlantic salmon to (a) the river (S_{iRiver}) and (c) the Irish coast (S_{iCoast}). (b,d) Random year effect (assumed to express unexplained inter-annual variability) on predicted returns of these salmon to (b) the river and (d) the Irish coast in 3 different periods of the return time series (Early, Mid, Recent) and at 3 levels of estimated salmon lice infestation ($Lcat2_i$) on the Killary salmon farm

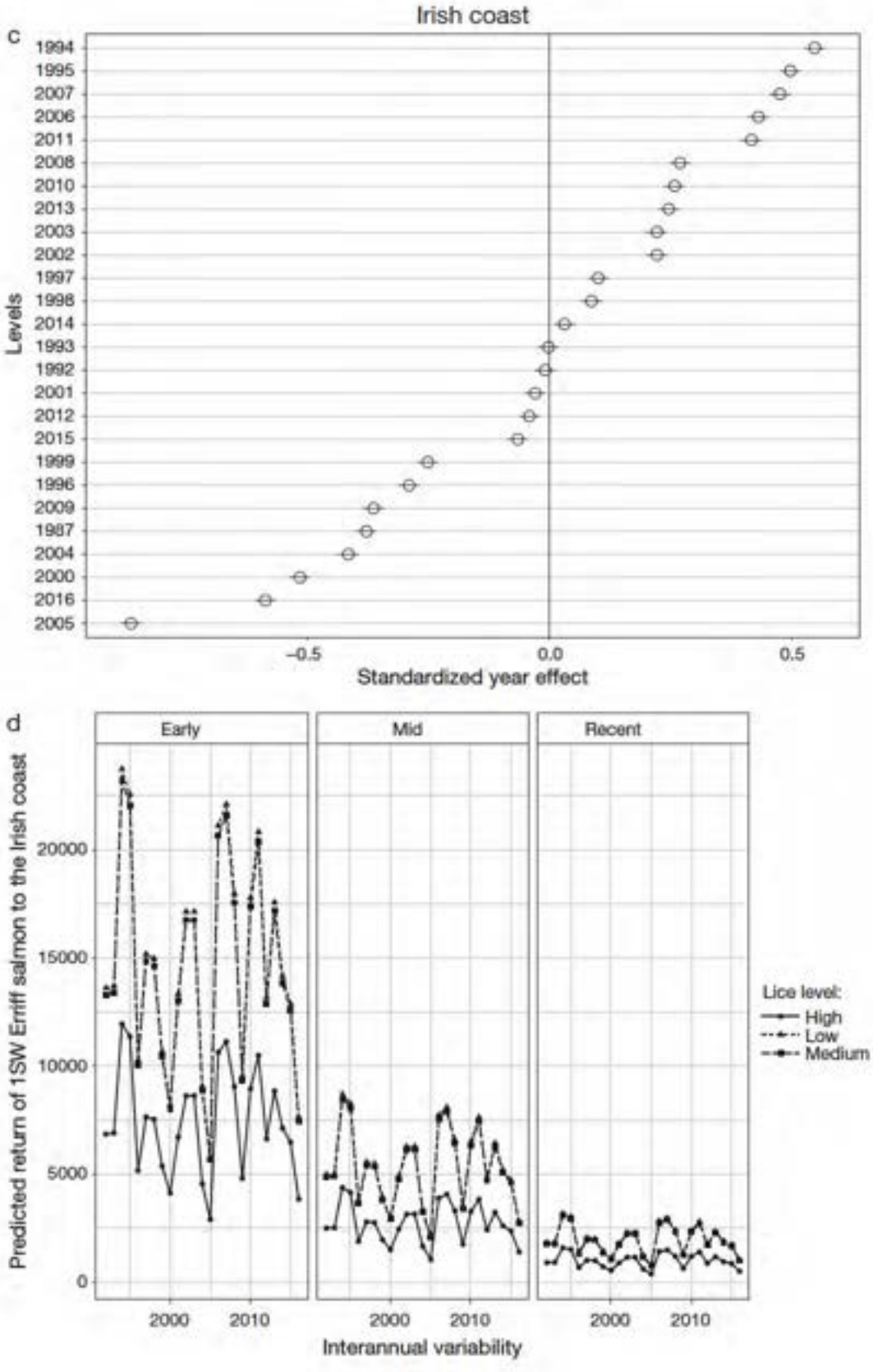

Fig. 5 (continued)

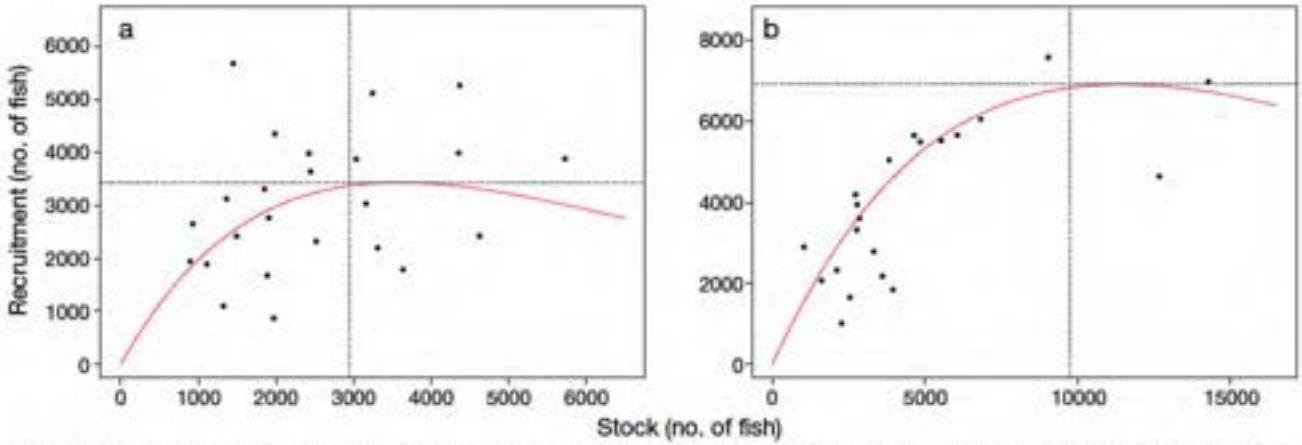

Fig. 6. Ricker stock recruitment curves fit to adult-to-adult stock-recruitment data for 1 sea-winter (1SW) Erriff River Atlantic salmon returns to (a) the river (S_{IRiver}) and (b) the Irish coast (S_{ICoast}). Stock values are observed returns; recruitment values are 'corrected' according to the annual lice effect estimated from statistical models. A 4 yr generation time (adult return to adult return) is assumed. The horizontal dashed lines are peak recruitment (*Rp*) and the vertical lines are peak stock (*Sp*)

does not remove the declining trend. The marked decline in the last 3 yr of both time series reflects relatively low estimated salmon return rates RR_i for these years (Table 3).

DISCUSSION

We analysed a 30 yr time series of returns of 1SW Erriff salmon, with 26 yr of corresponding estimated lice counts from the Killary salmon farm. Wild salmon returns were strongly reduced (>50%) following years when there had been high lice levels on the salmon farm during the smolt out-migration. This result accounts for the effect of unexplained among-year variation in returns, which probably reflects how marine survival varies naturally independent of lice-induced mortality (Vollset et al. 2016). 'Correcting' for the estimated lice effect predicted that Erriff salmon returns might now be twice as large without observed anthropogenic sea lice impacts, but would probably show a similar long-term decline.

Infectious disease is a contributing factor in 8% of cases where a species is listed by the IUCN as Critically Endangered (Smith et al. 2006). Peacock et al. (2013) estimated that the percentage mortality of pink salmon in the Broughton Archipelago of British Columbia, Canada, due to sea lice infestations ranged from 3.8% for returns in 2010 to 90.1% for returns in 2002; Bateman et al. (2016) estimated that lice-induced mortality in the same region was 9 to 39% in 2015. Our results demonstrate that sea lice infestation from coastal salmon aquaculture is likely to be an important contributor to observed decline in

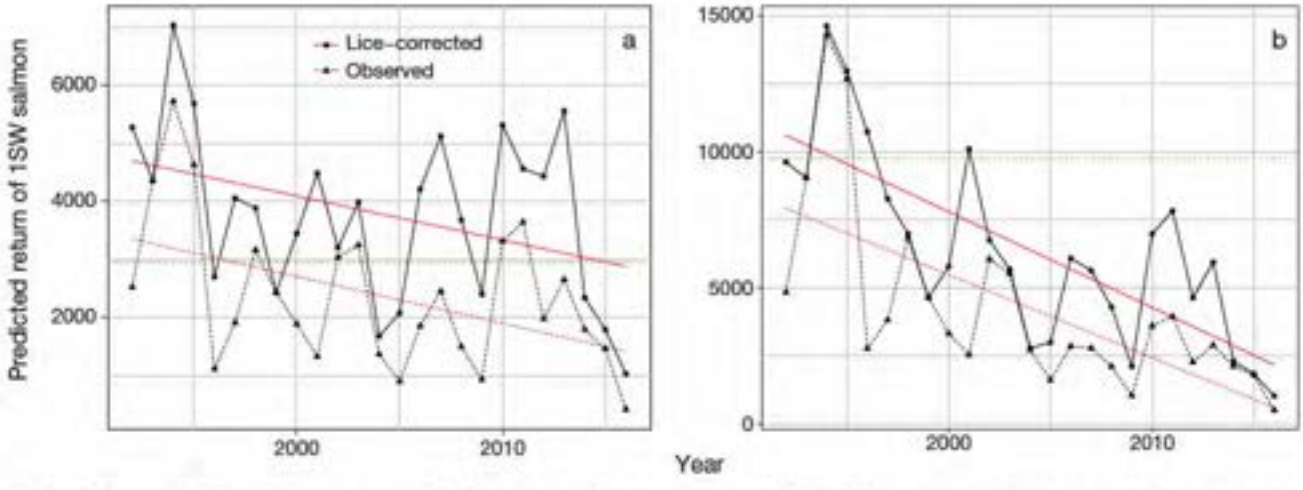

Fig. 7. Observed (S_i) and 'lice-corrected' (Se_i) returns of 1 sea-winter (1SW) Erriff River Atlantic salmon to (a) the river and (b) the Irish coast. The green line is peak recruitment (*Rp*) estimated from the Ricker curve (see Fig. 6)

Table 3. Estimated annual adult-to-adult return rates (RR_i) for 1 sea-winter (1SW) Erriff River Atlantic salmon to the river or the Irish coast

Year	River RR_i	Coast RR_i
1992	1.21	0.99
1993	2.01	1.74
1994	3.68	3.58
1995	3.78	4.91
1996	1.02	1.29
1997	1.02	0.95
1998	0.71	0.53
1999	0.51	0.36
2000	1.51	1.06
2001	1.61	1.40
2002	1.05	0.99
2003	1.58	1.16
2004	0.86	0.80
2005	0.79	0.63
2006	1.53	1.13
2007	1.60	0.99
2008	2.09	1.42
2009	2.06	1.20
2010	2.08	1.41
2011	1.56	1.41
2012	1.53	1.06
2013	2.69	2.57
2014	0.92	0.82
2015	0.50	0.48
2016	0.41	0.42

returns of wild Atlantic salmon to the Erriff River system. This finding upholds a substantial literature on the impacts of sea lice on salmonids, and successive experiments using anti-parasite lice treatments. A meta-analysis of differential survival between control and parasiticide-treatment groups of cultured Atlantic salmon showed that returns of treated fish were 39% greater (Krkošek et al. 2012). Our results for the Erriff predict that the return of 1SW salmon migrating in a high lice year may be reduced by more than 50% compared to the return from wild smolts that were not exposed to high levels of sea lice from salmon aquaculture during early out-migration.

Sea lice present during the spring smolt outmigration through Killary Harbour could have salmon farm and/or wild salmon sources. Gargan et al. (2012) found that the number of wild salmonids was very low during this period, and that sea lice abundance on local farmed salmon was 3 to 4 orders of magnitude greater than the estimate for wild salmonids. A study on the production of sea lice larvae from farmed and wild salmon and its relation to the infestation of wild sea trout found that farmed salmon contributed 95% of the total production of *L. salmonis* nauplii in the mid-west Irish coast region (Tully & Whelan 1993). These observations suggest that sea lice infestation pressure on wild Erriff smolts originates overwhelmingly from aquaculture.

Lice-induced mortality may have 2 components. Short-term mortality probably occurs when attached lice reach the pre-adult and adult life stages, causing severe osmoregulatory problems indicated by highly elevated plasma chloride levels and increased plasma osmolality (Bjørn & Finstad 1997, Dawson et al. 1998, Wells et al. 2006). A longer-term reduction in survival may be associated with impacts that impair on-going fitness during migration. The impact of sea lice seems to vary with baseline survival of salmon; a meta-analysis of studies using anti-parasite treatments on salmon smolts found that in groups with low recapture in the control group (low baseline survival), the effect of treatment was high, while in groups with high recapture in the control group (high baseline survival), there was no effect of treatment (Vollset et al. 2016). This result implies that the detrimental effect of lice is exacerbated in situations when the salmon smolts also have to cope with increased pressure from other causes of mortality, e.g. unfavourable environmental conditions. A post hoc plot of standardised salmon returns to the Erriff S_{iRiver} shows that observed returns approximately track the random year effect $Ycat_i$ (expressing environmental variability). However, the 4 lowest returns on record occurred when a high lice year coincided with poor baseline survival, while the only 2 occasions when a high lice year produced a greater than average run (1992 and 2007) were during high baseline survival (Fig. 8).

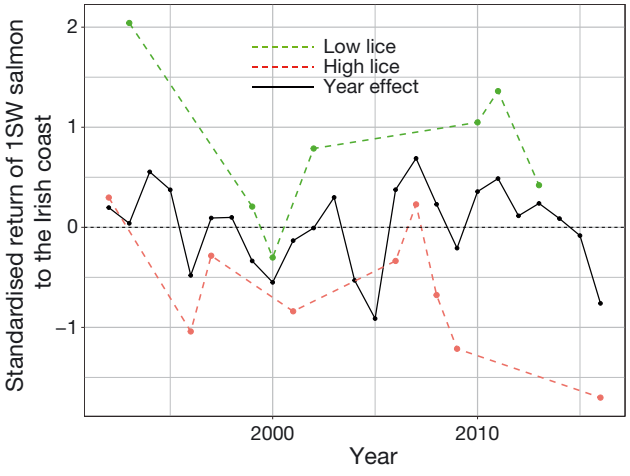

Fig. 8. Observed Erriff River Atlantic salmon returns to the river (S_{iRiver}), standardised to 0 for years estimated to have 'High' and 'Low' levels of sea lice (years of Medium lice level are excluded for clarity). Year effect is the random effect of year ($Ycat_i$), assumed to express environmental effects on salmon returns

Our results show very low return rates of Erriff salmon in the most recent years, corresponding to apparent declines in marine survival of Atlantic salmon (ICES 2016). Oceanic life stages of salmon are susceptible to climate forcing by the NAO and the AMO that drive SST and thus thermal habitat (Friedland et al. 1993, 2003, Jonsson & Jonsson 2004, Mills et al. 2013) and associated prey dynamics (Beaugrand & Reid 2012, Defriez et al. 2016). Recent studies suggest that warming SST has had a negative impact on oceanic growth and survival (McCarthy et al. 2008, Todd et al. 2008, Friedland et al. 2009) of Atlantic salmon, possibly mediated through productivity and trophic interactions (Beaugrand & Reid 2003, Mills et al. 2013). Hence, aquaculture sea lice impacts on wild Atlantic salmon are imposed upon possibly declining baseline survival.

The negative effect of sea lice from aquaculture may be unusually strong for the Erriff wild salmon population because the Killary salmon farm is located in the mouth of a long and narrow fjord. It is also the case that the 26 yr time series of salmon runs and lice counts, while valuable, still refer to only a single system. A preliminary analysis using these records attempted to identify specific environmental components of marine mortality in addition to sea lice. There were insufficient data for this exercise and so the simpler and more robust random year approach presented here was followed. Despite this limitation, it seems reasonable to expect important lice impacts in other systems where salmon farm(s) with high spring lice levels occur in bays and estuaries with rivers having wild salmon populations. A study of chemically treated and untreated salmon smolt releases in 3 west of Ireland bays (including Killary) found that lice-induced mortality of adult salmon can be significant, and that an increase in mortality of salmon smolts can be expected where farm lice levels are not maintained at sufficiently low levels in spring (Gargan et al. 2012). This observation is consistent with research on pink salmon (e.g. Bateman et al. 2016). A potential 50% lice-induced reduction in annual return of wild Atlantic salmon is likely to have serious general implications for long-term viability of populations in aquaculture areas. Natal homing in salmon results in a high level of genetic structuring, and smaller salmon rivers typically have a relatively low effective population size (Nikolic et al. 2009). As such populations decline, they are likely to become vulnerable to inbreeding and related demographic problems (e.g. Lande et al. 2003) that can erode future evolutionary potential of salmon populations (McGinnity et al. 2003) and lead to an extinction vortex (Fagan & Holmes 2006). Many Atlantic salmon populations are already under pressure from (possibly climate-mediated) reductions in marine survival. The addition of significant lice-related mortality during the coastal stage of smolt out-migration could be critical.

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ORIGINAL ARTICLE

WILEY Evolutionary Applications

Estimating the relative fitness of escaped farmed salmon offspring in the wild and modelling the consequences of invasion for wild populations

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Abstract

Throughout their native range, wild Atlantic salmon populations are threatened by hybridization and introgression with escapees from net-pen salmon aquaculture. Although domestic–wild hybrid offspring have shown reduced fitness in laboratory and field experiments, consequential impacts on population abundance and genetic integrity remain difficult to predict in the field, in part because the strength of selection against domestic offspring is often unknown and context-dependent. Here, we follow a single large escape event of farmed Atlantic salmon in southern Newfoundland and monitor changes in the in-river proportions of hybrids and feral individuals over time using genetically based hybrid identification. Over a three-year period following the escape, the overall proportion of wild parr increased consistently (total wild proportion of 71.6%, 75.1% and 87.5% each year, respectively), with subsequent declines in feral (genetically pure farmed individuals originating from escaped, farmed adults) and hybrid parr. We quantify the strength of selection against parr of aquaculture ancestry and explore the genetic and demographic consequences for populations in the region. Within-cohort changes in the relative proportions of feral and F1 parr suggest reduced relative survival compared to wild individuals over the first (0.15 and 0.81 for feral and F1, respectively) and second years of life (0.26, 0.83). These relative survivorship estimates were used to inform an individual-based salmon eco-genetic model to project changes in adult abundance and overall allele frequency across three invasion scenarios ranging from short-term to long-term invasion and three relative survival scenarios. Modelling results indicate that total population abundance and time to recovery were greatly affected by relative survivorship and predict significant declines in wild population abundance under continued large escape events and calculated survivorship. Overall, this work demonstrates the importance of estimating the strength of selection against domestic offspring in the wild to predict the long-term impact of farmed salmon escape events on wild populations.

KEYWORDS
aquaculture impacts, fish farming, introgression, population eco-genetic modelling, relative fitness, *Salmo salar*

1 | INTRODUCTION

The threat of invasion from domesticated Atlantic salmon (*Salmo salar*) into wild populations is of growing concern to management and conservation efforts (Clifford, McGinnity, & Ferguson, 1998a, 1998b; Forseth et al., 2017; Glover et al., 2012; Gross, 1998; Le Cam, Perrier, Besnard, Bernatchez, & Evanno, 2015). Farmed escapees often outnumber wild populations annually, and hybridization and genetic introgression between farmed and wild salmon have been detected throughout their native range (Bourret, O'Reilly, Carr, Berg, & Bernatchez, 2011; Glover et al., 2017). Mating of farmed and wild salmon may result in reduced genetic integrity of the wild population (Fleming et al., 2000; McGinnity et al., 2003; Skaala et al., 2012; Solberg, Dyrhovden, Matre, & Glover, 2016) and, under pressure from continual invasion, a loss of overall population fitness (Baskett, Burgess, & Waples, 2013; McGinnity et al., 2003). The degree of genetic impact on wild populations due to invasion is often population-specific (Baskett et al., 2013; Karlsson, Diserud, Fiske, & Hindar, 2016) and may be highly dependent on the selective pressures acting on invading individuals and their progeny (Thurman & Barrett, 2016).

Current methods of estimating these selective pressures or relative fitness of aquaculture offspring (i.e., hybrid and feral) under wild conditions are often family-specific (Skaala et al., 2012) and rely on laborious experimental approaches (McGinnity et al., 2003, 1997; Miller, Close, & Kapuscinski, 2004). Field experiments suggest that the relative fitness of hybrid and feral individuals may follow a pattern of additive genetic inheritance (Einum & Fleming, 1997; Fleming et al., 2000; McGinnity et al., 2003), although maternal environmental effects are potentially also influential in early life stages (Houde, Black, Wilson, Pitcher, & Neff, 2015). Due to the complexity of interactions and effects on individual fitness, estimating the strength of selection at the population or regional scale remains difficult. Namely, hybridization success and selection pressures can widely vary across even small spatial scales (Sylvester et al., 2018), and controlled experiments (Skaala et al., 2012) may not reflect the conditions of wild populations and landscapes (Fleming et al., 2000). Also, the impacts of invasion by farmed individuals have been shown to vary depending on the demography of the native population (Heino, Svåsand, Wennevik, & Glover, 2015; Wringe, Jeffery, et al., 2018) and the degree of relatedness between farmed salmon and the wild populations they invade (Baskett et al., 2013). Similarly, wild individuals straying from nearby rivers may buffer the impact of domesticated invasion in populations (Castellani et al., 2018). Given this inherent complexity, enhanced understanding of the relative fitness of domestic offspring at the population level in a range of natural environments is required to better predict and mitigate impacts of escaped farmed salmon on wild populations.

Here, we capitalize on a large escape event that occurred in 2013 in southern Newfoundland to explore how these changes may be monitored and applied to understand long-term consequences for wild populations. This single event resulted in the escape of 20,000 adult farmed salmon into a region supporting an approximately equal number of wild salmon. Previous work has documented widespread hybridization between wild and farmed escaped salmon following this escape event (Wringe, Jeffery, et al., 2018). By observing temporal changes in hybrid class composition after an influx of invaders into a system, the strength of selection against aquaculture-derived individuals may be directly estimated for a real-world system of invasion. As such, we aim to (a) monitor the changes in the proportion of wild, hybrid and feral parr over time, (b) use these data to estimate survivorship as a proxy of the strength of selection against feral and hybrid offspring, and (c) using these realistic estimates of selection, model the consequences for these populations over various invasion scenarios, exploring the sensitivity to the strength of selection. We build directly on previous work which developed genetic and analytical tools to identify hybrids (Anderson & Thompson, 2002; Wringe, Stanley, Jeffery, Anderson, & Bradbury, 2017a, 2017b; Wringe, Stanley, et al., 2018) and documented interbreeding between escaped farmed and wild salmon following this escape event (Wringe, Jeffery, et al., 2018). We expand on these studies and others (Clifford, McGinnity, & Ferguson, 1998a, 1998b; Fleming et al., 2000; McGinnity et al., 2003; Skaala et al., 2012) by estimating the strength of selection against domestic and hybrid offspring in the wild, and explore the importance of obtaining accurate estimates of relative survival for predicting long-term consequences of invasion.

2 | MATERIALS AND METHODS

2.1 | Sampling and genotyping

A total of 4,619 parr were collected by electrofishing across 19 rivers in southern Newfoundland, Canada (Figure 1), in the summers of 2014, 2015 and 2016. As emergence of alevins in southern Newfoundland generally occurs in early June, summer sampling allows for collection of newly emerged individuals, that is, individuals from the previous spawning season or young-of-year (YoY), as well as parr remaining in streams from earlier spawning seasons, generally up to 2–4 years in Newfoundland (Porter, 1975). Individuals were assigned to an age class based on length (YoY: 0–70 mm, 1+: 71–110 mm, 2+: >110 mm) and stored in 95% ethanol for later DNA extraction and analysis. In addition to these samples, 301 wild individuals (previously identified as pure wild with high certainty) and

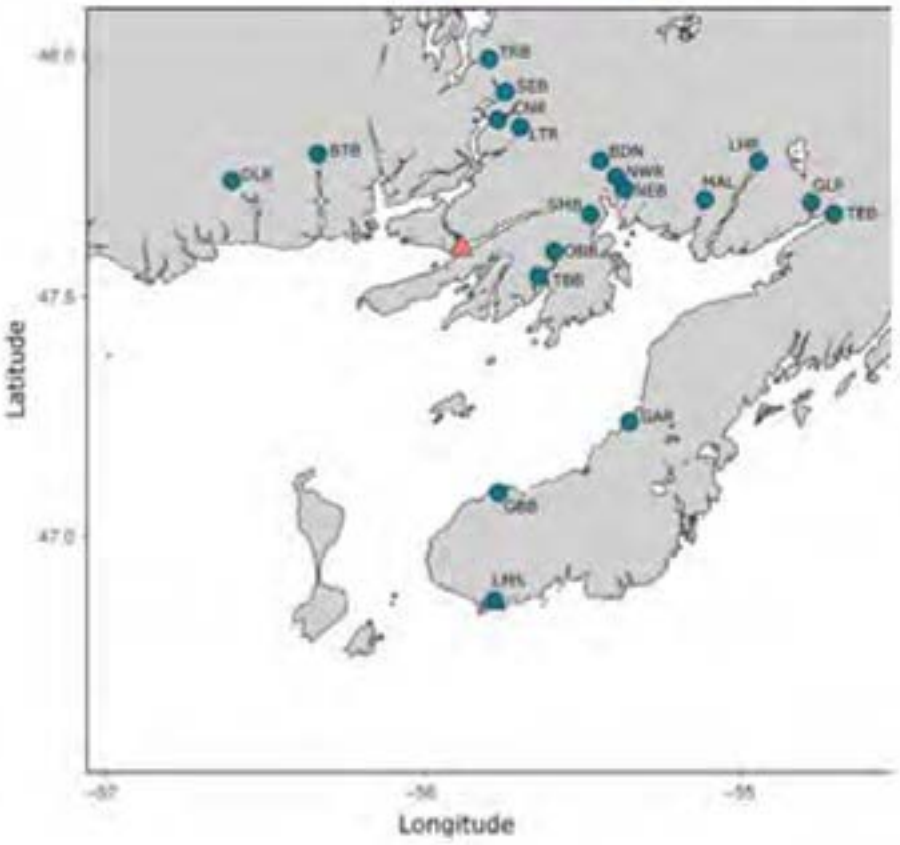

FIGURE 1 Sites in southern Newfoundland sampled in 2014–2016. Sample sizes per site and year can be found in Table 1. The location of the 2013 escape event is indicated by the pink triangle

156 farmed reference individuals were analysed as baseline samples. Farmed references were provided from three cage sites within Newfoundland and are likely representative of escapees sampled throughout the region as salmon cages in Atlantic Canada are presently stocked only with individuals from a single, non-local Saint John River population.

DNA was extracted using QIAamp 96 DNA QIAcube HT Kit (Qiagen, Toronto, Ontario, Canada) on a QIACube HT (Qiagen) following the manufacturer's protocol. Tissue samples were disrupted using a Tissue-Lyser II (Qiagen) mixing 2 × 10 s at 20 s^{-1}. DNA was eluted twice in 100 µl Buffer AE (Qiagen) preheated to 70°C. DNA extracts were quantified using QuantiT PicoGreen dsDNA Assay Kit (Thermo Fisher Scientific, Waltham, MA USA) and read on a FLUOStar OPTIMA fluorescence plate reader (BMG Labtech, Ortenberg, Germany). Individuals were genotyped using SNP Type assays (Fluidigm) following the manufacturer's protocols, targeting 95 SNPs previously established for the classification of farmed and wild salmon in Newfoundland (Wringe, Stanley, et al., 2018). At the applied posterior probability threshold (see below), this panel has been shown to assign individuals to a genetic class with over 90% accuracy, based on simulations in "hybriddetective" (Wringe, Stanley, Jeffery, Anderson, & Bradbury, 2017a; Wringe, Stanley, et al., 2018), with high congruency to genetic class assignment conducted using STRUCTURE (Pritchard, Stephens, & Donnelly, 2000; Sylvester et al., 2018). Each plate extraction included 10 redundant samples to detect processing errors. A total of 220, 190 and 214 samples from 2014, 2015 and 2016, respectively, were genotyped a second time to estimate the genotyping discordance rate which was used as a proxy for the genotyping error rate for each year (Pompanon, Bonin, Bellemain, & Taberlet, 2005).

2.2 | Statistical analyses

All analyses were run and figures created using R v. 3.4.1, and data manipulation and conversion conducted using "genepopedit" (Stanley, Jeffery, Wringe, DiBacco, & Bradbury, 2017). Wild and farmed baseline individuals were simulated and centred (see Karlsson, Diserud, Moen, and Hindar (2014)) from the actual baseline samples using the R package "hybriddetective" (Wringe et al., 2017a) to reduce the erroneous interpretation of naturally occurring inter-river genetic variation as evidence of introgression. Samples were classified into one of six genetic classes: pure wild, feral, first-generation hybrids (F1), second-generation hybrids (F2), backcross wild (BCW) or backcross feral/farmed (BCF) using NewHybrids (Anderson & Thompson, 2002). This approach implements a Bayesian Markov chain Monte Carlo approach for assignment, producing a posterior probability per class for each individual based on the provided baselines. NewHybrids was run using the R package "parallelnewhybrid" (Wringe, Stanley, Jeffery, Anderson, & Bradbury, 2017b) with a burn-in of 50,000 and 100,000 sweeps. All samples were pooled together by year, with samples from each river run independently to reduce bias, such that naturally occurring genetic differentiation between rivers was not misinterpreted as signals of introgression. We then filtered

TABLE 1 Sample size per sampling year and age class for each river, after filtering at a minimum posterior probability threshold in NewHybrids. Relative survivorship was estimated from a single cohort spanning all three years (2014 young-of-year (YoY), 2015 1+ and 2016 2+; see Methods)

	2014	2015		2016		
River	YoY	YoY	1+	YoY	1+	2+
BDN	0	49	60	45	51	16
BTB	16	3	17	29	1	22
CNR	364	19	0	77	26	3
DLR	18	20	20	54	15	10
GAR	193	52	50	96	109	21
GBB	39	15	25	3	75	26
GLP	102	9	51	4	34	2
LHR	124	41	84	44	72	3
LTR	120	0	0	68	23	6
LMS	40	89	11	59	56	7
MAL	10	28	49	0	26	13
NEB	103	0	15	46	46	0
NWR	41	0	10	76	4	4
OBB	14	0	0	34	39	17
SEB	14	0	11	0	2	52
SMB	62	20	49	76	5	6
TBB	111	0	0	1	20	1
TEB	71	3	0	25	24	1
TRB	37	2	33	35	25	13
Total	1,479	350	485	772	653	223

individuals at a minimum posterior probability of assignment to a single class of 0.8 (Wringe, Jeffery, et al., 2018), resulting in 3,962 assigned individuals (see Table 2 for a breakdown of final sample size by age class). Per-river class proportions were calculated for all parr, young-of-year (YoY) parr only and parr within a single cohort. Overall proportions were estimated after weighing by the axial length of each river (the distance along a straight line along the longest axis of the river; Porter, Riche, & Traverse, 1974) to reduce bias in sampled population size (Wringe, Jeffery, et al., 2018).

The relative fitness of wild, feral and first-generation (F1) hybrids was estimated using a single cohort of individuals (2014 YoY, 2015 1+, 2016 2+) for each age/time step (YoY to 1+, 1+ to 2+). Relative fitness estimates of second-generation hybrids (F2 and backcross) were not calculated due to restrictions in sample sizes. Traditional methods of estimating the relative fitness (or relative survival as a proxy of relative fitness; Hendry, 2017; Rice, 2004) of individuals of a known genotype (i.e., AA, Aa and aa) were applied to individuals of known genetic class (i.e., pure wild, F1 and pure feral; Thurman & Barrett, 2016). We calculated the proportional change in the population composition of a genetic class (P_{t+1}/P_t, where P is the class proportion at year t) within each river, then averaged across rivers and divided by the maximum proportional change (i.e., the average proportional change of the wild class) to obtain the relative, overall survivorship of each class across the region. Sites with fewer than 10 individuals per age class were removed from the calculation. Additionally, if the formula for the proportional change of a given genetic class at time t resulted in a denominator of 0, these rivers were removed for that time point calculation for that genetic class. This estimate of relative survivorship was interpreted as the relative fitness (w) of each genetic class.

TABLE 2 Estimated relative fitness (and standard error of estimates across rivers) for the first two years of development (young-of-year (YoY) to 1+, 1+ to 2+) based on changes in population composition of genetic class

	Wild	F1	Feral
YoY to 1+	1 (0.09)	0.81 (0.26)	0.15 (0.11)
1 + to 2+	1 (0.10)	0.83 (0.42)	0.26 (0.24)

2.3 | Individual-based modelling approach

We used an individual-based salmon eco-genetic model (IBSEM) developed by Castellani et al. (2015) to explore the possible long-term effects of various invasion scenarios and relative survival associated with the farmed genotype in southern Newfoundland. IBSEM models the outcome of Atlantic salmon populations in response to invasion of domesticated individuals. Duration of invasion and recovery, wild population size and number of invaders, environmental conditions, individual size and genotypic and phenotypic differences between individuals of farm and wild origin are considered to model population changes in abundance, genotype and individual size. Growth and survival are simulated by stochastic procedures that are influenced by genotype, fish size and age, temperature and population density at three life stages: embryo, juvenile and adult. The effects of the genetic make-up in the life history of the individuals are modelled through three independent sets of loci, one set for each life stage. The distribution of genetic effects across the 21 loci is modelled via an exponentially declining function, where the last locus has no effect and is used as a neutral marker. Through the influence of genotype, the differential between growth and survival of wild and feral individuals can be set and the consequences observed over time. Simulated loci are unlinked with possible gamete recombination and random inheritance (and are therefore influenced by drift), and a range of influences on phenotype and therefore suitability to the environment. The sum of the genetic effects is linearly related to phenotype, such that genotypic values approaching 1 are associated with growth and survival rates typical of wild salmon, and values approaching zero are associated with rates observed in farm escapees. Reproductive success of both wild and domestic individuals is sex-specific, with female fertility dependent upon weight, and male reproductive success dependent upon length, with the possibility of precocial sexual maturation. Farm escapees are given a reduced spawning success than fish of any genetic make-up that are born in the wild. We tested three temporal scenarios of invasion to

TABLE 3 Scenarios tested in an individual-based salmon eco-genetic modelling (IBSEM) approach (Castellani et al., 2015). All other parameters were consistent across scenarios and can be found in the Supporting Information. Each of the three values for number of invaders was modelled using each pair of relative survival parameters (low, calculated and high), resulting in nine models for each temporal scenario (see Figures 3 and 4)

Temporal scenario	Invasion time (years)	Number of invaders annually	Relative survival (parr0/parr1)
Scenario 1: Short-term	10	0, 500, 1,000	0.075/0.13, 0.15/0.26, 0.3/0.52
Scenario 2: Intermediate	50	0, 100, 200	0.075/0.13, 0.15/0.26, 0.3/0.52
Scenario 3: Long-term	100	0, 50, 100	0.075/0.13, 0.15/0.26, 0.3/0.52

investigate the impacts of consistent, annual invasion as a (a) short-term, large escape event over 10 years of invasion relative to an (b) intermediate invasion rate (over 50 years) and (c) long-term, trickle escapes (over 100 years; Table 3). For each temporal scenario, three levels of the magnitude of invasion were tested (no invasion, intermediate invasion and high invasion). Invasion levels were set such that the total number of invaders was equal across scenarios (i.e., 0, 500 and 1,000 invaders annually for 10 years; 0, 100 and 200 invaders annually for 50 years; and 0, 50 and 100 invaders annually for 100 years). Each temporal scenario and magnitude of invasion was tested at three levels of relative feral parr survival: our estimated value, low survival (half our estimate) or high survival (double our estimate). Our estimates of hybrid relative survival were not incorporated as IBSEM infers this based on additive genetic inheritance. It should be noted that the high survival scenario, while high relative to that estimated for southern Newfoundland populations, is still lower than most previous estimates of relative survival of feral parr (McGinnity et al., 2003, 1997). We compared the change in adult population abundance (both wild and escaped farmed fish) and sum of the genetic effects across the adult set of genes included in the simulation to observe changes in the genetic fitness of the population. All models were run for 100 years prior to invasion to ensure model stability and for 100 years after the invasion period ceased to assess time to recovery. All other parameters remained consistent across models. A full list of parameters, set to be representative of Newfoundland salmon and environmental conditions in the region

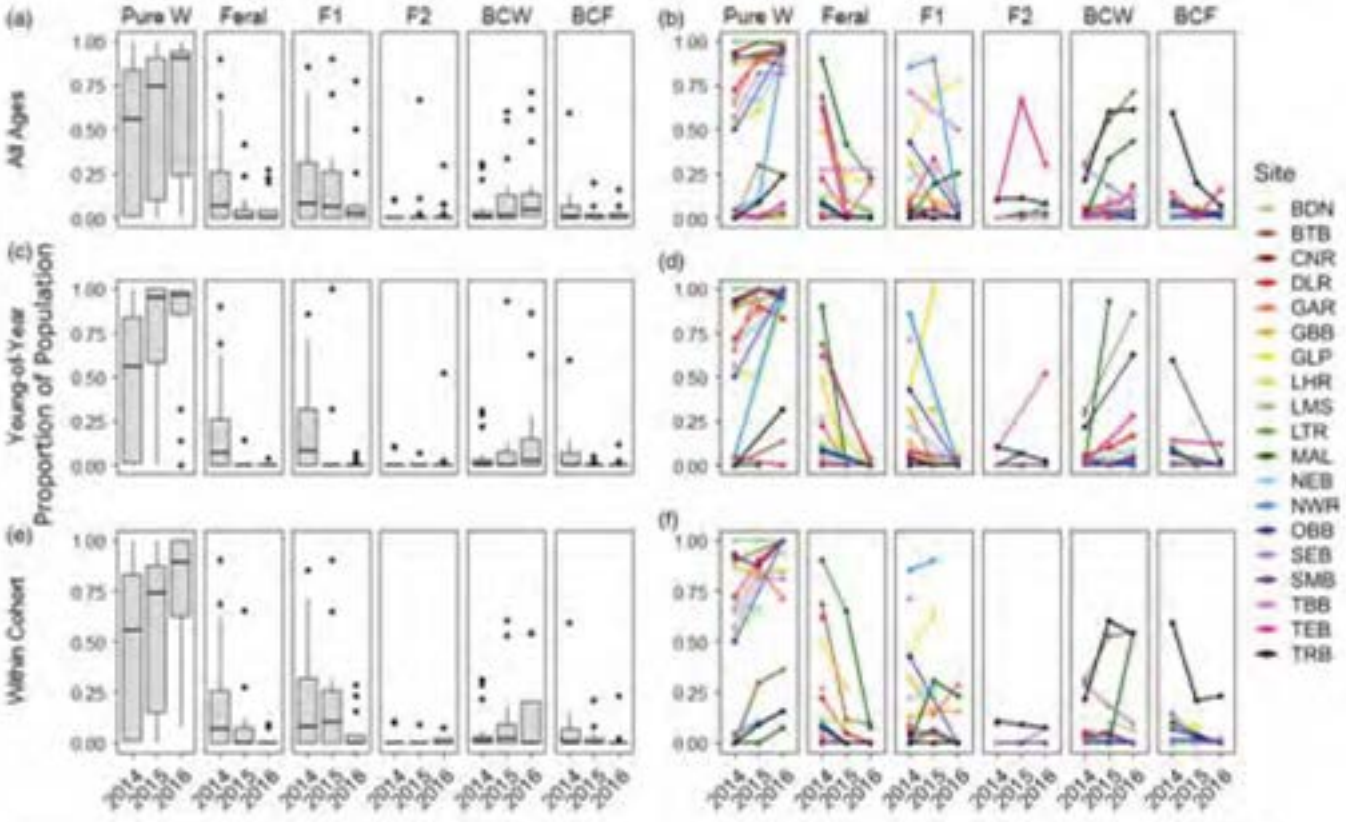

FIGURE 2 Per cent population composition by genetic class (pure feral, pure wild, F1, F2, backcross wild (BCW) and backcross feral (BCF)) across three sampled years including (row 1) individuals of all ages, (row 2) young-of-year (YoY) only and (row 3) within cohort (2014 YoY, 2015 1+, 2016 2+). Each panel comprises a single genetic class, including (a, c, e) boxplots of overall trends and (b, d, f) individual river proportion indicated by colour. Sites with fewer than 5 assigned samples were removed to avoid bias in river composition. Temporal fluctuations in within-cohort population composition were used to estimate relative survival as a proxy of relative fitness for pure wild, feral and F1 genetic classes (see Methods)

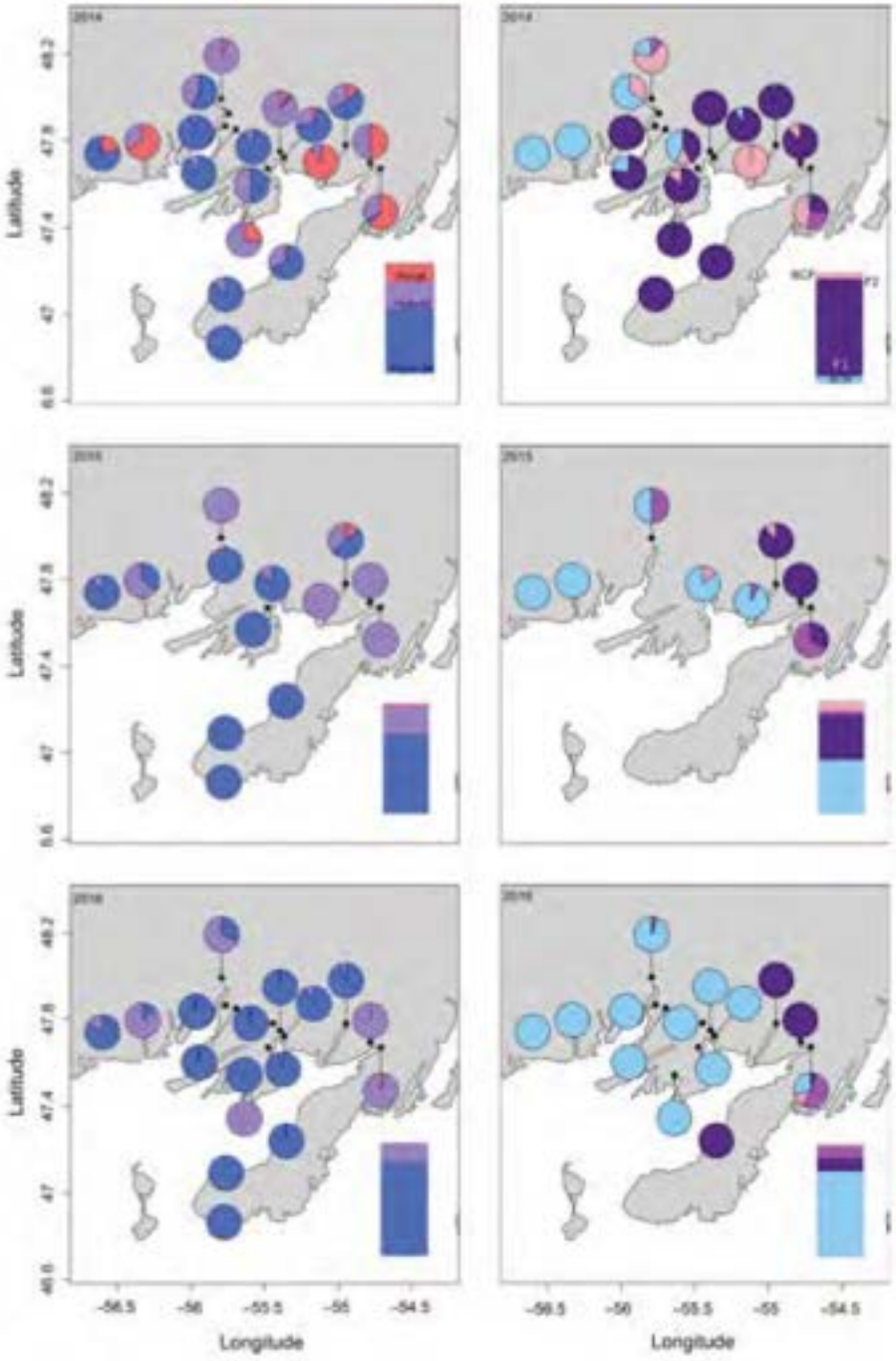

FIGURE 3 Genetic class (pure wild, pure feral, F1, F2, backcross wild (BCW) and backcross feral (BCF)) proportion for each sampled river as determined using NewHybrids for young-of-year (YoY) samples across all sampled years. Panels in column one convey proportions of wild, feral and hybrid parr (all hybrid classes combined) while panels in column two convey proportions of hybrid classes (F1, F2, backcross wild (BCW), backcross feral (BCF)) for each river with hybrid individuals detected in that year (row), as indicated in purple in column one. Bars in each panel represent overall proportions after standardizing by river size (axial length). Corresponding figures for other age classes can be found in Supporting Information Figure S1

(Veinott et al., 2018; correspondence with Dr. Brian Dempson) or set as default, can be found in the Supporting Information. Two parameters reflective of overall wild survival were selected by trial-and-error to achieve a consistent (stable) population size under a zero invasion scenario with all other parameters set as described in Supporting Information Table S1.

3 | RESULTS

A total of 4,619 parr were genotyped using the SNP panel. The genotype error rate was estimated to be 0.17%, 0.01% and 0.13% for 2014, 2015 and 2016, respectively. Of all samples, 86% of individuals were classified by NewHybrids above the posterior probability threshold of 0.8. Across age classes, pure wild parr were the most prevalent class, followed by hybrids and feral parr (Figure 2a,b), with few exceptions in particular rivers. After scaling by river size (axial length), wild population proportion increased overall (increasing by a factor of 1.05 and 1.16 in the first and second year, respectively), with a corresponding decline in feral (by a factor of 0.62, 0.33) and hybrid parr (by a factor of 0.93, 0.57; Figure 2). First-generation hybrids (F1) were the most common hybrid class in 2014, with a steady decline in most rivers (Figures 2 and 3) in subsequent years (by a factor of 0.68 and 0.25 in the first and second year, respectively).

Population proportion of backcross wild (BCW) parr increased during the first year (by a factor of 4.8), driven mostly by dramatic increases in BCW proportion in three rivers, MAL, BTB and TRB. BCW proportion remained generally constant (population proportion decreased by a factor of 0.91) in the second year of life. These trends were consistent within young-of-year (YoY) parr (Figures 2c,d and 3) and within a single cohort (Figure 2e,f). Increasing class proportions within a cohort suggest a higher relative fitness compared with those classes that are observed to decrease with time. We applied this reasoning to estimate relative survival as a proxy of fitness and strength of selection against classes that are seen to decrease over time, relative to wild types within a single cohort. As such, the relative fitness of the wild class was 1 for all estimates. Relative fitness was higher for F1 than for feral salmon and was slightly lower for both classes in the first year of development than the second year (Table 2). Variance (reported as standard error) in the relative survival of F1 parr was considerably higher than that of feral or wild individuals (Table 2). Although low within-river sample sizes at single time points limited our ability to estimate river-specific relative survival of genetic classes, we report these estimates in Supporting

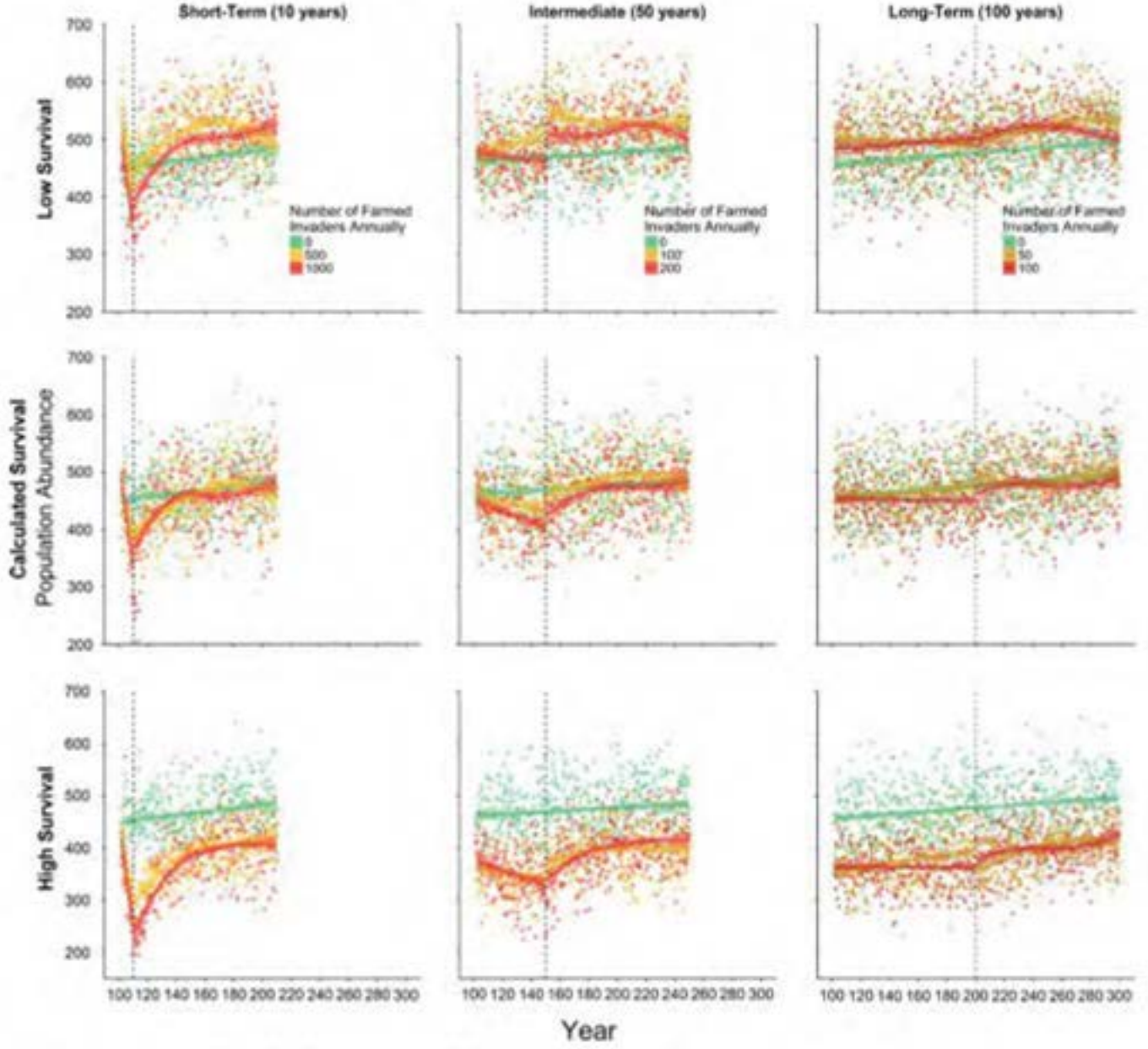

FIGURE 4 Adult population abundance as estimated using IBSEM (Castellani et al., 2015) for all tested scenarios (see Table 3). Three invasion scenarios (columns: short-term, intermediate and long-term) were each modelled at three levels of relative survival for feral parr (rows: half calculated relative survival, calculated relative survival for the study region (as shown in Table 2) and double calculated relative survival). Each of these nine scenarios was tested with three levels of invasion (number of farmed invaders) as indicated by colour. Invasion started after 100 years of settling; the time at which invasion ceased (duration of invasion) is indicated by a vertical dashed line. Loess curves are used for visualization of trends in the data

Information Table S2 to demonstrate variance in estimated relative survival across rivers.

Our estimates of average relative survival of individuals with feral genotypes (see Table 2) were incorporated into the individual-based modelling approach (IBSEM). We examined three temporal scenarios (Table 3), and three relative survival scenarios: our calculated relative survival, half and twice that value. These scenarios revealed differences in population response and recovery, affirming the importance of estimating relative survival in predicting population response to invasion. Severity of the population crash and time to recovery increased with increasing relative survival of feral parr and decreasing duration or increasing intensity of invasion (Figure 4). In calculated relative survival models, full recovery was observed after 30–40 years post-invasion in the short-term invasion scenario, less than 20 years in the intermediate scenario and immediately after invasion ceased in the low invasion scenario. High relative survival of farmed invaders and the short-term temporal scenario resulted in the greatest decrease in overall population abundance, to as few as 200 individuals after 10 years of invasion, from a stable population of approximately 475 under a zero invasion scenario (Figure 4).

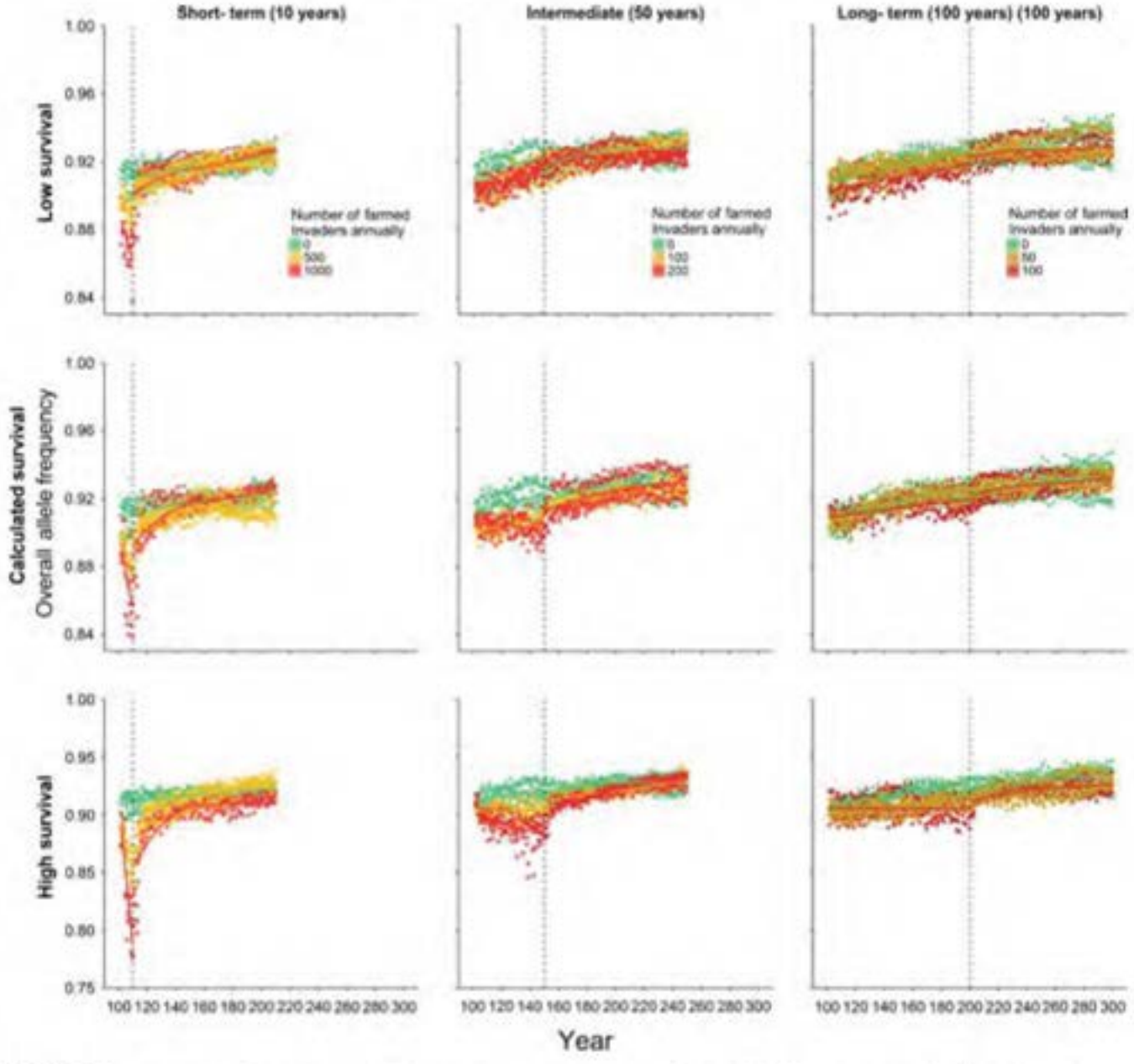

FIGURE 5 Average allele frequency across 21 simulated genotypes as estimated using IBSEM (Castellani et al., 2015) for all tested scenarios (see Table 3). Three invasion scenarios (columns: short-term, intermediate and long-term) modelled at three levels of relative survival for feral parr (rows: half calculated relative survival, calculated relative survival for the study region (as shown in Table 2) and double calculated relative survival). Each of these nine scenarios was tested with three levels of invasion (number of farmed invaders) as indicated by colour. Invasion started after 100 years of settling; the time at which invasion ceased (duration of invasion) is indicated by a vertical dashed line. Loess curves are used for visualization of trends in the data

Additionally, high relative survival tests did not fully recover after 100 years in any of the invasion scenarios. Overall, modelled allele frequencies shifted towards the farmed genotype in all temporal and survivorship scenarios following similar patterns as population abundance. That is, the severity of the change in allele frequency increased with increasing relative survival of feral parr and increasing intensity (decreasing duration) of invasion (Figure 5). Time to recover to allele frequencies comparable with the zero invasion models were similar and rapid across all scenarios, with the longest recovery time observed at approximately 50 years after ceasing invasion in short-term invasion models at high relative survival.

4 | DISCUSSION

With the continued growth of Atlantic salmon aquaculture, understanding and predicting the impacts of escape events of farmed Atlantic salmon are central to the persistence of wild populations across the species range (Forseth et al., 2017; Glover et al., 2017). The survival and fitness of farmed escapee Atlantic salmon relative to the wild populations they invade has the potential to ultimately determine the genetic impacts of invasion on wild populations, yet population- or region-specific relative survival of individuals of aquaculture ancestry in the wild is rarely estimated. Here, we build on previous work investigating the extent of hybridization following a large escape event in southern Newfoundland (Wringe, Jeffery, et al., 2018) and calculate relative survival and associated strength of selection against feral and hybrid parr using temporal changes in population composition. We demonstrate decreased survival of offspring of aquaculture escapees relative to pure wild individuals and explore how relative survival and rates of invasion may impact wild populations using an individual-based modelling approach (Castellani et al., 2015). This method of estimating survival and consequential long-term genetic impacts of farmed invasion provides a novel field-based approach that has previously generally been limited to controlled experiments that do not account for population or regional variation. By modelling various invasion and survival scenarios, we highlight the importance of considering region-specific relative survival when predicting population trajectories under various rates of invasion with the potential to inform approaches to population conservation and fisheries management.

We examined temporal changes in the proportion of hybrids within young-of-year (YoY) samples and within a single cohort to illuminate the factors influencing the presence and survival of farmed escaped offspring in the wild. Temporal changes in hybrid proportions within a cohort reflected continued declines in hybrids and feral parr with an increase in wild-type individuals over time as noted elsewhere (DFO, 2018; McGinnity et al., 2003, 1997; Skaala et al., 2012). Changes in the relative proportions of hybrid types also followed a similar pattern with a relative decrease in F1s and an increase in backcross wild individuals. This change in population composition over time suggests reduced relative fitness of feral and hybrid offspring compared to pure wild. Interestingly, our estimates of relative survival are considerably lower than some previous estimates for feral parr (ranging from 0.61 to 1.53, relative to pure wild survival of 1; Hindar, Fleming, McGinnity, & Diserud, 2006) but comparable to that of previous studies for F1 parr (Fleming et al., 2000; McGinnity et al., 2003, 1997; Skaala et al., 2012). Relative survival of feral individuals in the wild is likely related to the degree of genetic differentiation between wild and farmed strains. Our low estimates of feral survival may thus reflect high domestication selection or drift in domestic salmon (Glover et al., 2017; Gross, 1998) or pre-existing genetic differences between wild Newfoundland populations and the Saint John River lineage currently stocked in the region (Bradbury et al., 2015; Moore et al., 2014). Consequential genetic divergence may result in reduced suitability to conditions in southern Newfoundland (Vandersteen, Biro, Harris, & Devlin, 2011). Our observations of an increase in the proportion of wild backcrossed individuals (BCW) in some populations suggest comparable survival to pure wild individuals, consistent with previous findings of higher performance in backcross classes (Fraser, Cook, Eddington, Bentzen, & Hutchings, 2008; McGinnity et al., 2003, 1997).

In 2014, the year immediately following a large escape event, levels of hybridization were consistent with reported impacts elsewhere (Glover et al., 2017; Karlsson et al., 2016), with all but one of 18 rivers showing evidence of hybrid or feral parr presence. In subsequent years, the overall proportion of hybrids and of F1 hybrids decreased, suggesting that mating between farmed escapees and wild salmon was highest immediately following the escape, likely due to the large influx of farmed individuals. This decrease is consistent with reduced contributions from this escape event over time. The presence of second-generation hybrids throughout our sampling years indicates that hybrid individuals and therefore farmed invaders unrelated to the escape event are present in these rivers, indicative of continued low-level trickle invasion (Wringe, Stanley, et al., 2018). While general temporal trends are consistent across rivers, there is a large degree of spatial variation in genetic class proportion at a given time point, as previously reported by Sylvester et al. (2018), with consequential variation in relative survival estimates. Due to low within-river sample size at individual time points, we focus on average relative survival of parr in the sampled region. However, the approach for estimating relative survival applied here can be easily applied for river-scale estimates of relative survival when sample sizes are adequate.

The precocious maturation of hybrid or feral male parr may influence levels of hybridization and introgression and thus alter allele frequency (Gjerde, Simianer, & Refstie, 1994) and lower productivity. This phenomenon may increase the relative fitness of feral parr as evidence suggests that reproductive success of farmed precocious males may be higher than that of wild individuals (Garant, Fleming Ian, Einum, & Bernatchez, 2003). Genetic introgression may be exacerbated by feral precocial males contributing to the population as allele frequency shifts towards the farmed genotype to a greater extent in early life stages (Castellani et al., 2015). Although aquaculture breeding practices often select against early maturation, early maturation is also largely environmentally determined (Good

& Davidson, 2016; Jonsson, Jonsson, & Finstad Anders, 2012), and high rates of sexual precocity have been reported in wild southern Newfoundland populations (Dalley, Andrews, & Green, 1983; Myers, 1984). However, common garden experiments have revealed male parr maturation to be lower in farmed progeny than in wild parr (McGinnity et al., 2007), with F1 hybrids demonstrating an intermediate likelihood of precocial maturation. Estimates of rates of hybrid precocial maturation and fitness in the region would enhance the ability to predict rates of introgression between and wild and farmed salmon.

Our modelling results suggest that consequences of invasion of farmed salmon could vary dramatically with the magnitude and temporal scope of escape events. Repeated large pulses of invasion were more detrimental to wild population productivity than continued low-level escape events. Interestingly, previous modelling efforts have disagreed on the relative impact of low-level chronic or large pulse escape events. Hindar et al. (2006) and Hindar and Diserud (2007) suggest greater impacts following large pulses of escapees contrasting the results of Baskett et al. (2013) who suggest that low-level leakage may be more detrimental to wild populations due to a gradual shift towards the farmed genotype. This variation in results has been suggested to be due to the time period considered and equilibrium status of model simulations (Baskett et al., 2013). However, the temporal scenarios modelled here made very little difference to long-term population trajectories compared to the impact of the parr survivorship parameters. Under the most extreme scenarios (i.e., high relative survival), wild population abundance did not fully recover regardless of the temporal scenario even after 100 years of recovery, although overall genetic effects were not substantially different after 100 years of recovery, suggesting that recovery in population abundance is limited despite shifts in allele frequency towards the wild type after invasion has ceased. Decreasing population abundance with increasing relative survival of feral parr is likely due to an overall reduction in population productivity as a consequence of higher feral and hybrid presence and thus contribution to the gene pool, compared to models with lower relative survival of feral parr. In the models with our calculated relative survival rates for southern Newfoundland, population abundance and allele frequency recovered shortly after invasion ceased. In reality, however, southern Newfoundland wild populations continue to decline (COSEWIC, 2011; DFO, 2013). This suggests that farmed invasion may be ongoing or that other factors such as at-sea survival, habitat degradation or fishing pressures may be at play (Bourret et al., 2011; Vähä, Erkinaro, Niemelä, & Primmer, 2007), exacerbating large-scale population declines.

Although IBSEM is a comprehensive Atlantic salmon individual-based model, there are additional factors such as the introduction of disease, fishing pressure and river flow that may influence population response to invasion (Castellani et al., 2015). IBSEM implements a constant or random number or proportion of farmed invaders, and constant relative survival and reproduction. Consequently, more realistic escape scenarios such as constant low-level invasion combined with a large, single-year event or multiple invasions at infrequent intervals are not currently considered, possibly limiting our understanding of population response to domestic invasion. Relative survival in IBSEM is generally reflective of marine survival, as this is known to strongly influence overall survival rates (Jonsson, Jonsson, & Hansen, 2003; McGinnity et al., 2003). However, although we have attempted to parameterize to reflect conditions in southern Newfoundland, a paucity of available region-specific data (such as relative marine survival) may reduce the accuracy of these models. With ongoing investigations within the region, estimates of marine return may be informed by subsequent sampling, allowing for future modifications and improvements to these simulations. Despite the current limitations, simulations such as those conducted here allow an unprecedented opportunity to explore long-term population responses to invasion of farmed escaped salmon and can directly inform decisions regarding management practices and the conservation of wild populations.

Extending our survival estimates with the inclusion of numerous cohorts would provide additional support for our estimates; however, sample sizes of 2015 YoY individuals were insufficient to include this cohort in our analysis. Also, limiting the analysis to only the highly supported hybrid assignments by filtering individuals by posterior probability in NewHybrids may bias our results for some hybrid classes as individuals that do not reach this threshold are more likely to be second-generation hybrids, backcrosses or further introgressed individuals (Sylvester et al., 2018). However, as this bias is consistent across years, we expect temporal fluctuations in hybrid classes to be robust and with little to no effect on our parameter estimates as we did not estimate relative survival of second-generation hybrid classes.

Existing efforts to estimate relative fitness and, accordingly, strength of selection against feral or hybrid parr in wild Atlantic salmon populations invaded by farmed escapees are often labour-intensive, requiring experimental manipulation in the laboratory or in rivers, and do not consider how variation in landscape and susceptibility of a wild population to introgression may differentially impact survival of individuals of aquaculture ancestry. We present a novel approach utilizing genetic data following a large escape event to classify individuals to a genetic class (pure wild, pure feral, F1, F2, BCW, BCF) and infer relative fitness based on within-cohort changes to class composition, applied to a region of southern Newfoundland. These approaches may be easily applied at any scale with sufficient sampling. We further apply our estimates to demonstrate that survival of feral parr, relative to their wild counterparts, affects long-term levels of introgression, particularly under stochastic invasion conditions. Wild population abundance was greatly affected by the relative survival of feral parr without full recovery in all invasion scenarios (short-term, intermediate and long-term) at high relative survival of feral parr. These results indicate the importance of obtaining accurate estimates of region- or population-specific relative fitness to predict population response to farmed invasion. Incorporating this knowledge may allow a deeper understanding of possible impacts on wild populations and may inform management and conservation decisions accordingly.

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CONFLICT OF INTEREST

None declared.

DATA AVAILABILITY

Data for this study are available at the Dryad Digital Repository: Genotype data of 2014 and 2015 samples can be found at https://doi.org/10.5061/dryad.3k888n7. Genotype data of 2016 samples and field ages assigned to all individuals can be found at https://doi.org/10.5061/dryad.2kc5rh0.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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REVIEW

Effects of salmon lice *Lepeophtheirus salmonis* on wild sea trout *Salmo trutta*—a literature review

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ABSTRACT: Salmon farming increases the abundance of salmon lice, which are ectoparasites of salmonids in the sea. Here we review the current knowledge on the effects of salmon lice on wild sea trout. Salmon lice feed on host mucus, skin and muscle, and infestation may induce osmoregulatory dysfunction, physiological stress, anaemia, reduced feeding and growth, increased susceptibility to secondary infections, reduced disease resistance and ultimately mortality of individual sea trout. Wild sea trout in farm-free areas generally show low lice levels. In farm-intensive areas, lice levels on wild sea trout are typically higher, and more variable than in farm-free areas. Lice on wild sea trout are found at elevated levels particularly within 30 km of the nearest farms but can also extend to further ranges. Salmon lice in intensively farmed areas have negatively impacted wild sea trout populations by reducing growth and increasing marine mortality. Quantification of these impacts remains a challenge, although population-level effects have been quantified in Atlantic salmon by comparing the survival of chemically protected fish with control groups, which are relevant also for sea trout. Mortality attributable to salmon lice can lead to an average of 12–29% fewer salmon spawners. Reduced growth and increased mortality will reduce the benefits of marine migration for sea trout, and may also result in selection against anadromy in areas with high lice levels. Salmon lice-induced effects on sea trout populations may also extend to altered genetic composition and reduced diversity, and possibly to the local loss of sea trout, and establishment of exclusively freshwater resident populations.

KEY WORDS: Salmon lice · *Lepeophtheirus salmonis* · Sea trout · *Salmo trutta* · Parasite · Aquaculture · Salmon farming

Introduction

The salmon louse *Lepeophtheirus salmonis* is an external parasite of salmonids in the marine environment, and occurs naturally both in the North Atlantic and North Pacific Oceans. Salmon lice found in the Atlantic and Pacific oceans are regarded as 2 different sub-species (Skern-Mauritzen et al. 2014). From fishery management and conservation perspectives, the effects of salmon lice on wild salmonid populations are potentially problematic in areas with intensive Atlantic salmon *Salmo salar* aquaculture (Finstad et al. 2011). Since farmed salmonids act as hosts, open net cage farms can increase the local production of infective salmon lice larvae in coastal areas. The first outbreaks of salmon lice infestation oc-

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curred on Norwegian Atlantic salmon farms during the 1960s, soon after cage culture began (Pike & Wadsworth 1999). Similar outbreaks occurred in Scottish Atlantic salmon farms from the mid-1970s (Pike & Wadsworth 1999). In Ireland, between 1989 and 1991, heavily salmon lice-infested wild sea trout *Salmo trutta* in poor physical condition were recorded for the first time in areas with salmon farming (Whelan 1991, Tully et al. 1993b).

Amongst salmonids, sea trout—the anadromous form of brown trout—are perhaps especially vulnerable to salmon lice infestation because most sea trout remain feeding and growing in coastal waters where salmon farms are situated during their marine migration. Since the late 1980s and early 1990s, some sea trout populations in western European countries including Norway, Scotland and Ireland have suffered severe stock declines. Such population declines have been linked to the development of open net cage salmon farming in coastal waters and resultant salmon lice infestation on local wild sea trout stocks (Tully & Whelan 1993, Gargan et al. 2003, 2006a,b, Butler & Walker 2006, Skaala et al. 2014b).

Our aim is to summarize and review existing knowledge on the effects of salmon lice on sea trout. Our review will provide researchers, the aquaculture industry, and fishery managers with a comprehensive and updated overview of documented knowledge on the effects of salmon lice on sea trout. This includes physiological and pathological effects on individual sea trout in laboratory studies, verification of such effects from field studies, quantification of salmon lice levels in wild populations, and specifically, the impacts of salmon lice on sea trout populations. By contrast, previous reviews have largely focused on sea lice effects on salmonids in general, with particular attention given to Atlantic salmon (Pike & Wadsworth 1999, Todd 2007, Costello 2009, Finstad & Bjørn 2011, Finstad et al. 2011, Torrissen et al. 2013). Here, we aimed to provide a complementary and comparative appraisal of the literature pertaining particularly to sea trout, but with qualifying reference to relevant studies of Atlantic salmon. In this paper, we refer to 'smolt' and 'post-smolt' in relation to brown trout, unless we have specified that the reference concerns another salmonid species.

The biology of *Lepeophtheirus salmonis*

Salmon lice are marine parasitic copepods of the Family Caligidae. They are planktonic and free-living in the sea during the first, post-hatching, larval life-stages, before they encounter and attach externally to the surface of the host fish. The life cycle of salmon lice comprises 5 phases, namely the nauplius, copepodid, chalimus, preadult and adult phases (Johnson & Albright 1991b, Pike and Wadsworth 1999) (Fig. 1). Each phase comprises 1 or 2 life stages, and the life cycle has a total of 8 life stages. The life cycle was previously divided into 10 stages, but Hamre et al. (2013) found that there are in fact only 2 chalimus stages, and not 4 as previously reported.

The first phase of the life cycle is the free-swimming, and non-feeding, planktonic nauplius phase (2 stages). Nauplius I larvae hatch from the paired egg strings carried by the adult female and are released to the water column. Following the first moult to nauplius II, the larva then moults to the copepodid phase (comprising a single stage) in which it remains free-swimming and non-feeding. This is the infective stage when the salmon louse must find a host fish to survive. Once the copepodid has attached to a host fish, it moults to the chalimus phase (2 stages). The sessile chalimus remains attached to the fish by a frontal filament and feeding is restricted to the host skin around the attachment point. This phase is followed by the immature preadult phase (2 stages) and finally the adult phase (one stage). The louse becomes mobile from the first preadult moult onwards and can move over the body surface of the host fish. Preadults and adults can swim in the water column for short periods and perhaps successfully infest other fish. Attached copepodids, chalimus, preadults and adults use rasping mouthparts to feed on host mucus, skin and underlying tissue including blood (Brandal et al. 1976, Costello 2006).

The planktonic stages may last 1–2 mo (Heuch et al. 2005). In areas with strong currents, the free-swimming and infective stages may be widely dispersed from the release source (perhaps up to 100 km or more) (Asplin et al. 2011, 2014). The development rate is temperature-dependent (Wootten et al. 1982, Johnson & Albright 1991a, Stien et al. 2005), and salmon lice can develop into the infectious copepodid stage even during the colder winter months (Boxaspen 2006). Salmon lice are generally absent from sites of low salinity, but various life stages of salmon lice have different salinity tolerances, and this varies with water temperature (Johnson & Albright 1991a, Pike and Wadsworth 1999, Bricknell et al. 2006). In the laboratory at 12°C, copepodids would not develop at salinity lower than 30 (Johnson & Albright 1991a). Copepodids transferred to low salinity water, survived for less than 1 d in waters of salinity 10 or less, and between 2–8 d at salinities of 15–30. Salmon

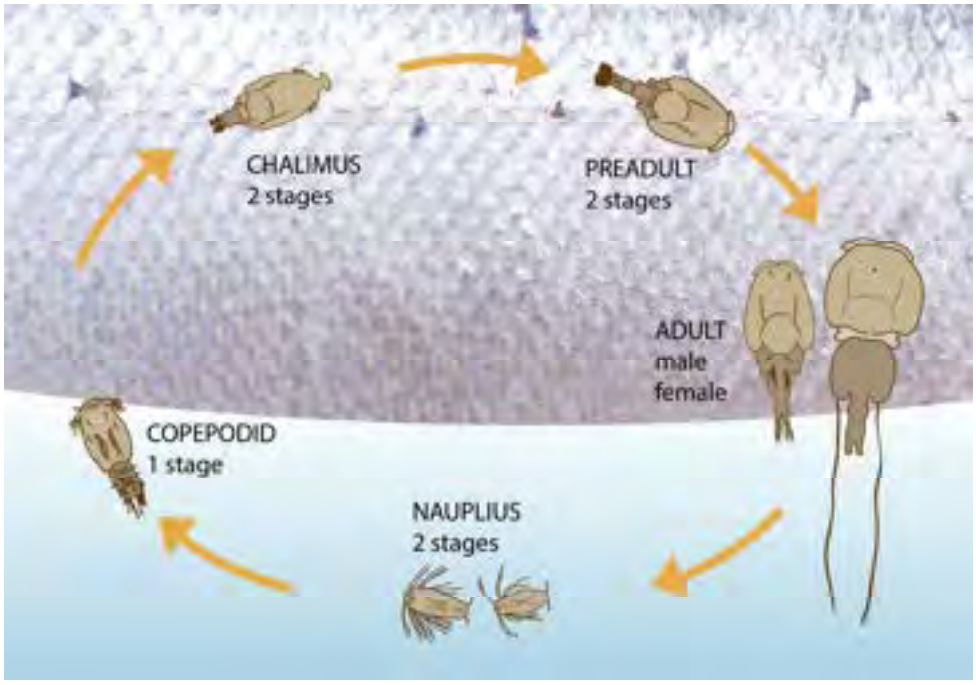

Fig. 1. The 5 phases of the salmon louse life cycle. Each phase comprises 1 or 2 life stages. The different phases are not shown scaled to size. Nauplius typically are of length ~0.5–0.6 mm, copepodids 0.7 mm, chalimi 1.1–2.3 mm, preadults 3.4–5.2 mm and adults 5–6 mm (males) and 8–12 mm (females). Graphic design: Kari Sivertsen, NINA

lice are shed by the host fish within a few days or weeks of fish re-entering freshwater (McLean et al. 1990, Finstad et al. 1995).

Parasitologists conventionally apply 3 distinct terms to define the frequency and abundance of lice on wild salmonids (Bush et al. 1997). 'Prevalence' is defined as the proportion, or percentage, of infested hosts in a sample. 'Abundance' refers to the mean number of parasites per host sampled, and 'intensity' is the mean number of parasites per infested host.

Effects of salmon lice on individual sea trout in laboratory studies

Mechanical damage of fish skin and tissue

In laboratory studies, copepodids tend to show an attachment preference for gills and fins, and especially the dorsal fin. Attachment to the gills may be a laboratory artefact (Wagner et al. 2008). Whilst the attached copepodid typically does not cause visible tissue damage at initial attachment, the damage to host tissues caused by the (sessile) chalimus stages can be visibly obvious but is usually relatively minor, except in dorsal fin areas where damage may be severe for heavily infested fish (Bjørn & Finstad 1998, Dawson 1998, Dawson et al. 1997, 1998, Wells et al. 2006, 2007). The most severe tissue damage arises from the feeding of the mobile preadult and adult stages and may cause mortality for heavily infested fish (Bjørn & Finstad 1998, Dawson 1998, Dawson et al. 1998, Wells et al. 2006, 2007).

Osmoregulatory problems and physiological stress responses

Anadromous fishes such as sea trout experience a physiologically challenging environmental shift when migrating from freshwater to seawater. In seawater, water is lost from the fish by osmosis, whereas salts tend to be gained. The fish would gradually become dehydrated if it did not compensate, which most fishes, including sea trout, achieve by drinking seawater and actively excreting the excess salts through the gills and kidneys (Evans 1979, Marshall & Grosell 2006).

The mechanical damage of the skin, mucus surfaces and dermal tissue caused by salmon lice impairs the barrier between the fish body and seawater, and results in increased leakage of water from the fish and thereby an osmotic and ionic imbalance (Bjørn & Finstad 1997). Reduced haematocrit (volume

percentage of red blood cells in blood) observed in infested and moribund fish (Bjørn & Finstad 1997, Wells et al. 2006) may be attributable to leakage of blood components (bleeding) due to mechanical damage of skin and tissue, possibly in combination with erythrocyte (red blood cell) shrinkage (dehydration) (Bjørn & Finstad 1997).

Salmon lice have been shown to induce primary, secondary and tertiary stress responses (Pickering 1981, Wendelaar Bonga 1997) in sea trout (Bjørn & Finstad 1998, Dawson et al. 1998, Wells et al. 2006, 2007). Salmon lice-infested sea trout typically show higher levels of plasma cortisol compared to uninfested control fish both in the early days post-exposure, and when the lice are at the attached chalimus developmental stages (Bjørn & Finstad 1997, Wells et al. 2006, 2007). Hence, either of the 2 attached chalimus stages, but particularly the mobile preadult and adult life stages, can cause a stress reaction in the fish as indicated by increased plasma cortisol levels.

Increased plasma chloride levels are indicative of osmoregulatory disturbance and have been observed by the time that the second chalimus stage has developed, with a more severe effect emanating from increasing chalimus densities (Bjørn & Finstad 1997). Hence, the second chalimus stage can cause minor osmoregulatory disturbance in heavily infested sea trout. Severe osmoregulatory problems, as indicated by highly elevated plasma chloride levels and increased plasma osmolality, have been demonstrated when the salmon lice develop to the preadult and adult stages and the lice become mobile (Bjørn & Finstad 1997, Dawson et al. 1998, Wells et al. 2006, 2007). Plasma chloride levels increased with increasing densities of preadult and adult lice, confirming that heavily infested fish were most affected, and moribund fish suffered from a complete osmoregulatory breakdown (Bjørn & Finstad 1997).

The osmoregulatory disturbance indicated by increased plasma chloride levels may be associated both with mechanical damage of the host skin and dermal tissues and with secondary stress responses on osmoregulation. Primary stress responses, such as release of catecholamines and cortisol, may cause structural changes in the gill tissues themselves. Osmoregulatory disturbance may therefore arise as a secondary response from such stress-mediated structural changes (Wendelaar Bonga 1997, Wells et al. 2007).

Given the energy demands related to stress responses, increases in metabolic rate can occur as a secondary stress response to acute and chronic stress. Thus, elevated plasma glucosis (hyperglycaemia), decrease in liver glycogen, and elevated plasma lactate have all been used as stress indicators in fish (reviewed in Wells et al. 2006, 2007). These measures can be further influenced by the metabolic status and feeding history of the host fish. When preadult and adult stages of salmon lice had developed on infested experimental fish, lice-induced elevation of plasma glucosis and plasma lactate (Wells et al. 2006, 2007), as well as depressed liver glycogen (Wells et al. 2007), were recorded.

Growth, behaviour and disease resistance

Salmon lice-infested sea trout have shown a reduced body mass and condition factor compared to control fish (Bjørn & Finstad 1997, Dawson et al. 1998), which may be due to adverse stress responses and dehydration (Pickering 1981, Bjørn & Finstad 1997, Wendelaar Bonga 1997, Wagner et al. 2008). Reduced feeding activity in salmon lice-infested fish has also been recorded, typically after the salmon lice had moulted to the preadult and adult stages (Dawson et al. 1998, Wells et al. 2006, 2007). However, in one study (Wells et al. 2006), this was noted within only 10 d of initial exposure and prior to the development of mobile salmon lice.

Salmon lice may also affect behavioural traits other than feeding. Wells et al. (2006, 2007) and Birkeland & Jakobsen (1997) noted that during the first 2–3 d of the infestation with copepodids, sea trout showed a distinct 'flashing' behaviour (lateral turning) or increasing leaping activity in experimental tanks. This behaviour ceased after 7 d, but was subsequently observed again when the salmon lice had reached the mobile stages. Such behaviour has also been described previously as a general response to sea lice infestation (Wootten et al. 1982).

Reduced disease resistance as a consequence of salmon lice infestations in sea trout has not been extensively studied. However, both the mechanical damage to the skin and the primary and secondary stress responses are indicative of a compromised immune system and thereby an increased risk of secondary infection. Bacterial or fungal infections of previously infested fish were recorded when fish were transferred from seawater to freshwater in the laboratory (Wells et al. 2007). Moreover, Bjørn & Finstad (1997) found a reduced lymphocyte-leukocyte ratio, indicative for reduced disease resistance.

Effects related to timing of seawater transfer and fish origin

Physiological effects, reduced feeding and skin damage caused by salmon lice have all been shown to be more severe for fish infested 2 wk after transfer from freshwater to seawater compared to those infested 6 wk after transfer (Dawson et al. 1998). This indicates that salmon lice may be more detrimental for sea trout smolts shortly after entry to seawater than when they have resided there for several weeks, possibly because physiological acclimation is itself a stressful process, and a simultaneous challenge from salmon lice infestation may constitute an additional stressor. Hatchery-reared Atlantic salmon and sea trout smolts may differ from wild fish in many traits and characteristics (Finstad & Jonsson 2001, Wells et al. 2006, 2007), and therefore experimental results from salmon lice exposure of hatchery-reared smolts may not always be representative of wild smolts. However, results from studies of salmon lice effects on wild and hatchery-reared sea trout smolts, and from those of seawater-adapted or newly transferred post-smolts, have been shown to be both comparable and similar (Bjørn & Finstad 1997, 1998, Dawson et al. 1998, Wells et al. 2006, 2007).

Mortality

Salmon lice-induced mortality of hatchery-reared (Bjørn & Finstad 1997, 1998) and wild (Wells et al. 2006, 2007) sea trout post-smolts was observed to commence within 10–20 d of exposure, by which time the salmon lice had reached the mobile preadult and adult life stages. Mortalities in these studies ranged between 25–46% for the infested fish (Bjørn & Finstad 1997, 1998, Wells et al. 2007). Wells et al. (2006) did not record final mortalities in their experiment, because they decided to euthanise the most heavily infested fish for animal welfare reasons. Salmon lice development rates are known to increase with increasing water temperatures (Wootten et al. 1982, Johnson & Albright 1991a, Stien et al. 2005), and fish mortality occurs earlier with increasing temperatures (Bjørn & Finstad 1998, Wells et al. 2006, 2007).

Critical threshold values for detrimental effects

Bjørn & Finstad (1997) showed that for hatchery-reared sea trout with an average mass of 91 g, the most heavily infested fish died as a result of infestation. The relative density of parasites found on moribund fish indicated that >1.0 lice per gram of fish body mass, or 50 preadult and adult lice per fish, may cause mortality in small (60 g) sea trout post-smolts. Given an average lice survival of 63%, a lethal relative density of approximately 1.6 chalimus per gram of fish mass, or >90 larvae for a small sea trout post-smolt (60 g), was suggested as a critical level (Bjørn & Finstad 1997, Finstad & Bjørn 2011). Furthermore, Wells et al. (2006) concluded that 12–13 preadult and adult (i.e. 'mobile') salmon lice per fish was a critical intensity which elicited sublethal stress responses in wild post-smolt sea trout (body mass range = 19–70 g). Hence, it has been suggested that a simple, conservative and precautionary approach to manage and protect wild sea trout populations would be to adopt a critical level of 10 mobile lice per fish for sea trout during their first year at sea (Finstad & Bjørn 2011, Finstad et al. 2011).

Recently, a classification system has been suggested for the expected salmon lice-induced mortality of first-time migrant sea trout based on existing knowledge (Taranger et al. 2015). This system predicts no additional mortality risk for sea trout with <0.1 lice per gram of fish body mass, 20% extra mortality for sea trout carrying 0.1–0.2 lice g^{-1}, 50% for sea trout with 0.2–0.3 lice g^{-1} and 100% mortality for sea trout with >0.3 lice g^{-1}. Studies on the effects of salmon lice on larger, veteran migrants and maturing sea trout are lacking, but a complementary study of Arctic char *Salvelinus alpinus* L. (Tveiten et al. 2010) suggested that the effects of salmon lice on maturing fish may be more severe than for first-time migrants. Based on that study, Taranger et al. (2015) assumed for veteran migrant and maturing sea trout no additional mortality risk for sea trout with <0.025 lice g^{-1} body mass, 20% extra mortality for sea trout with 0.025–0.05 lice g^{-1}, 50% for sea trout with 0.05–0.10 lice g^{-1}, 75% for sea trout with 0.10–0.15 lice g^{-1} and 100% mortality for sea trout with >0.15 lice g^{-1}.

The foregoing threshold level predictions are based on effects in relatively short-term laboratory experiments. Values should therefore perhaps be considered indicative, and not absolute, and require further verification and validation, especially if the objective is to determine critical parasite burdens to guide conservation and management criteria. For example, density dependent mortality of salmon lice developing on a fish may affect estimates of threshold values, and the assumption of a simple linear relationship between lice numbers and lice mortality may not be correct. In addition, fish mortality in the natural environment may be higher than that seen

in laboratory studies as a consequence of additive effects. The effects of salmon lice have, for example, been shown to be more severe for Atlantic salmon post-smolts impaired also by other influences such as suboptimal water quality (Finstad et al. 2007). Furthermore, compromised fish in the natural environment may experience an elevated mortality risk from predators (Thorstad et al. 2012). A reduced or compromised immune system (Bjørn & Finstad 1997) may incur additional mortality over a longer term, and yet other environmental effects may also exacerbate the effects of salmon lice and the critical threshold levels.

Effects of salmon lice on individual sea trout in field studies

Mechanical damage of fish skin and tissue

Field studies are important to verify the extent to which laboratory studies are representative of wild fish in natural systems. Similar to results from laboratory studies, fin erosion and haemorrhage at the base of the dorsal fin have been frequently recorded in wild-captured sea trout with heavy burdens of chalimus (McVicar et al. 1993, Dawson 1998, MacKenzie et al. 1998, Skaala et al. 2014a). The patterns reported from laboratory studies, with attachment of chalimi primarily to the dorsal fin and mobile stages present along the dorsal or more anterior body regions, are confirmed from numerous field studies (Tully et al. 1993a,b, Dawson 1998, MacKenzie et al. 1998, Marshall 2003, Urquhart et al. 2008). Cranial lesions and grazing marks on the gill opercula, and along the ventral body surfaces have also been described (McVicar et al. 1993, Tully et al. 1993b).

Osmoregulatory problems and physiological stress responses

Primary and secondary physiological stress responses to salmon lice infestation, as exemplified by elevated plasma cortisol, plasma chloride and blood glucose levels, have been documented in wild-captured sea trout, and the elevated cortisol levels were similar to those found in laboratory studies (Poole et al. 2000, Bjørn et al. 2001). Bjørn et al. (2001) concluded that the osmotic imbalance and need for mobilisation of energy stores may have been the result of the integrated stress response attributable to the infestation rather than a result of the mechanical damage caused by the salmon lice. This deduction was based on the observation that chalimus was the predominant life stage, and that only limited skin erosion was observed. Fish body sizes in these studies were <150 g body mass (Bjørn et al. 2001), or an average body length of 18 cm (Poole et al. 2000). For slightly larger fish carrying mobile salmon lice (mean fork length = 23 cm, body mass = 126 g), blood plasma showed a reduction in total protein, serum albumin, and cholesterol compared with sea trout lacking salmon lice or those with copepodids or chalimus stages only (Dawson 1998). Furthermore, plasma glucosis levels increased with lice numbers when all life-stages of salmon lice were pooled (Dawson 1998). The highest estimated cortisol levels in wild-captured sea trout occurred during the period when post-smolts had only recently entered the sea, affirming that post-smolts may be more vulnerable to salmon lice when physiologically adapting to seawater (Poole et al. 2000).

In a controlled experiment, downstream-migrating sea trout smolts were captured in freshwater and held in tanks; 1 group of fish was exposed to seawater (and thereby the natural concentration of lice larvae), whereas an unexposed control group was held in filtered seawater from which salmon lice larvae had been removed (Birkeland & Jakobsen 1997). Salmon lice-induced mortality commenced 11 d after exposure to unfiltered seawater, by which time some lice had developed to the preadult stage (water temperature = 17–20°C, mean abundance and intensity of salmon lice per fish = 59). Fish in the exposed group showed severe osmotic problems by this stage, with elevated plasma chloride levels and lower plasma total protein and albumin levels.

The direct observation of mortality is difficult to achieve for free-ranging individual fish in marine waters. Tully & Whelan (1993), Tully et al. (1993a,b) and Birkeland (1996) all reported direct observations of dead and moribund sea trout in estuaries linked to salmon lice infestations. However, fish in the marine environment may die from multiple causes, such as predation, before they may be lost as a direct result of a pathological disease or parasite infestation (Thorstad et al. 2013). Sea louse-infested hatchery-reared sea trout and Atlantic salmon smolts equipped with acoustic transmitters did not show increased mortality during fjord migration compared with uninfected control groups (Sivertsgård et al. 2007). However, the study extended only over a short time period, and during which period the salmon lice could develop only to the chalimus stage of the life cycle.

Growth

Growth patterns of sea trout in freshwater and seawater are generally complex and influenced by a number of environmental factors and characteristics of the fish. Selective salmon lice-induced mortality may mask other potential effects on sea trout growth. It is especially difficult to isolate the effects of salmon lice on fish growth from other possible effects in field studies, because multiple factors may change either independently or in concert over the observational period.

Notwithstanding this caveat, Fjørtoft et al. (2014) compared growth of sea trout from a river in western Norway during 1976–1982, in the absence of local salmon farming, and between 2000–2007 whilst farming was active, based on scale analyses. They demonstrated that fish growth was slower during both their first and second summers at sea during the observational period that salmon farming was active, but there was no difference in growth rate of the same individuals whilst resident in freshwater. The growth reduction after the first summer in the sea corresponded to a body mass reduction of 20–40%.

A gradual decrease in marine growth rates was also detected from scale analyses of sea trout from a Scottish river adjacent to salmon farms (data from 1980 to 1989–1990, 1992–1993, and 1997–2001) (Butler & Walker 2006). Thus, from 1980 to the period 1997–2001, maximum sea age was reduced from 11 to 5 yr. When comparing scale samples from 1926 and 1980, the sea age and marine growth rates did not differ markedly. Butler & Walker (2006) concluded that the decline in growth after 1980 was at least partly caused by salmon lice epizootics emanating from the fish farms established 4 and 7 km from the river mouth in 1987.

For the Burrishoole sea trout stock in Ireland, ratios of sea growth to freshwater growth showed no discernible trend until 1990, after which this ratio showed a marked decrease over the period 1990–1992 (Poole et al. 1996). A significant reduction in marine growth was most likely linked to premature return to freshwater of salmon lice-infested fish (Poole et al. 1996).

Behaviour and migration patterns — premature return to freshwater

Premature return to freshwater of sea trout carrying large numbers of salmon lice has repeatedly been recorded, and has been interpreted as an adaptive behavioural response to salmon lice-induced osmoregulatory dysfunction (Birkeland 1996, Birkeland & Jakobsen 1997, Bjørn et al. 2001, Wells et al. 2007). The return to freshwater may enable the infested sea trout to regain its osmotic balance and survive, because salmon lice have a low tolerance to hyposaline or freshwater conditions (Birkeland 1996). It should be noted that these impacts extended beyond those induced by the chalimus and mobile stages of salmon lice, because high levels of copepodids alone also caused premature freshwater return of sea trout (Birkeland & Jakobsen 1997). Birkeland (1996) concluded that the recorded high salmon lice levels indicated that the post-smolts that returned to freshwater would not have survived had they remained at sea.

The first reports of post-smolt sea trout returning to freshwater prematurely in poor physical condition and with heavy salmon lice infestations, within only a few weeks of their seaward migration, date from the late 1980s and early 1990s in Ireland (Whelan 1991, Tully & Whelan 1993, Tully et al. 1993a,b). Subsequent studies from Ireland, Norway and Scotland have reported similar observations (Birkeland 1996, Birkeland & Jakobsen 1997, Gargan 2000, Bjørn et al. 2001, Butler & Walker 2006, Hatton-Ellis et al. 2006, Pert et al. 2009, Gjelland et al. 2014). It was apparent from relatively early studies that premature return to freshwater may occur as soon as within the first few days, or the first 1–2 wk, at sea (Birkeland & Jakobsen 1997, Bjørn et al. 2001).

The timing of freshwater return was monitored by operating a fish trap in a Norwegian river (Birkeland 1996, Birkeland & Jakobsen 1997). Nearly half (41%) of prematurely returning post-smolts migrated to sea again that same summer, with a median freshwater residency of 38 d following their return to the river (Birkeland 1996). By the time of second descent, most fish had lost the salmon lice, but they also had lost one quarter of their body mass. Whereas the returning post-smolts carried mainly copepodid and chalimus stages of salmon lice, the older migrants showed a larger proportion of mobile preadult and adult salmon louse stages. Several older returning sea trout died. Within 1 wk, 20% of the older migrants were found dead in the river, and they had considerable skin lesions from salmon lice infestations that had become secondarily infected by fungi or bacteria.

Laboratory studies have confirmed that transfer from seawater to freshwater after initial exposure to salmon lice improves the physiological status of the fish and that mortality was reduced compared to fish maintained infested in seawater (Wells et al. 2007). However, secondary bacterial or fungal infection was recorded on a number of the infested fish following

Table 1. Summary of salmon lice levels found on wild sea trout in the current literature, showing the mean abundance of salmon lice per fish caught per sample (max. number of lice on an individual sea trout is given in parentheses where data available), mean (median where specified) intensity of salmon lice per infested fish in the sample, and percentage prevalence

Mean (max.) abundance	Mean intensity	Prevalence (%)	Lice life cycle stage	Study period
3.2	4.0	81	Not specified	1972–1973, month not specified
1.0–77.5 (325)	7.0–104.8	14.3–100	Dominated by chalimus stages	May 1992
5.0–8.0 (46)	5.0–10.7	75–100	20–26% chalimus	Jun–Aug, 1991 and 1992
7.0–63.9 (216)	7.0–63.9	75–100	0–79% chalimus, increasing proportion of chalimus with increasing lice abundance	Jun–Aug, 1991 and 1992
1.4–5.0 (11)	1.4–5.0	25–100	6–55% chalimus	Jun–Aug, 1991 and 1992
89.8–260.8 (1002)	103.0–272.4	87–96	Mainly chalimus	Jun–Jul 1992
53.5–623.0 (1179)	53.5–623.0	88–100	Mainly copepodids and chalimus	Jun 1992
4.66 (41), and 4.42 (55)	5.26 (range = 1–41) and 5.47 (range = 1–55)	96, both years	Mainly preadults and adults	Jun–Nov, 1992 and 1993
3.19 (12), and 1.17 (4)	3.96 (range = 1–12) and 1.75 (range 1–4)	67 and 81	Not specified	1972–1973, month not specified
42 (SE = 35)	Not available	82	Mainly chalimus	May 1996
71 (SE = 45)	Not given in publication and not calculated here because prevalence is not given separately per district, but for Ballinakill and Connemara Districts combined.	82	Mainly chalimus, but also preadults and adults	May 1996
0.5–10.9 (84)	2.7–26.7	20–85	Proportion between larvae and mobile stages varied among samples. Proportion of mobile stages always >30%	Aug–Oct 1992, May–Sep 1993
0–72.7 (207)	0–46.4	0–100	Mainly copepodids and chalimus, but increasing proportion of mobile stages from late May and onwards for many locations	Apr–Sep 1994
Not given	Median = <3–8	20–100	Mar–Apr mainly preadults and adults. Dominated by adults also the rest of the summer, but with chalimus appearing in Apr–Oct (never >15% chalimus)	Mar–Dec, 1992–1995
20.1 (253)	27.9	72	Information not given	May–Jun 1995
0–111	0–156 fish farming	0–100	Information not given	May–Jun, 1993–1997
49.3–194.9 (471)	53–203	89–96	Chalimus dominated during the entire period	Jun–Sep 1997
0.6–8.9 (36)	1–13	55–89	Chalimus dominated in Jun, but up to 50% preadults and adults later in summer	Jun–Sep 1997
0.1–23.6 (134)	0–29.5	0–95	Mainly chalimus	Jun–Aug, 1992–1993
0.2–13.0 (84)	0–17.3	0–83	Mainly chalimus in Jul and increasing amount of preadults and adults in Aug	Jun–Aug, 1992–1993
0.75 and 0.33 (11)	Median = 1–2	0–49	Majority preadults and adults	Oct 1998–Apr 1999, Oct 1999–Mar 2000
0–68.4 (500)	0–46.4	0–100	Mainly chalimus, but increased proportions of mobile stages in Jul–Sep	Mar–Oct, 1998–2001
0–6.8 (33)	0–8.6	0–81	Preadults and adults dominated in winter, chalimus in Sep–Oct	Mar–Dec 2001
0.1–3.6 (28)	1.0–4.7	6–80	Preadults and adults dominated in winter, chalimus in Sep–Oct	Mar–Dec 2001
1.3 (Jun) to 21.2 (Aug) (59)	6.4 (Jun) to 26.5 (Aug)	21 (Jun) to 88 (Aug)	Chalimus dominated in Jun–Jul, and preadults and adults started to occur in Aug	Jun–Aug 2000
0 (Jun) to 16.7 (Aug) (78)	0 (Jun) to 18.9 (Aug)	0 (Jun) to 80 (Aug)	Chalimus dominated in Jun–Jul, and preadults and adults started to occur in Aug	Jun–Aug 2000
7.8 (95% CI = 6.0–10.0)	7.8	100	Preadults and adults	May and Jun 2005
30 (69)	30	100	Only copepodid and chalimus stage	May 2007
0.82–7.87	0.24–7.87	29–100	Information not given	Jul–Dec, 2006–2007
0.03–0.37	0.00–0.09	3–23	Information not given	May–Aug 2005, 2006 and 2007
0.2–20.5 (186)	3.5–30.2	4–77	All stages in May, mainly chalimus in Jun, and increased proportion of adults again thereafter	May–Aug, 2003–2004
3.3–52.8 (130)	4.6–52.8	73–100	All stages in May, mainly chalimus in Jun and Jul, and subsequently increased proportion of adults	May–Aug, 2003–2004
0–8.1 (44)	0–12.0	0–83	Information not given	May–Aug, 2008–2012
0–106 (689)	1.8–114.8	0–100	Information not given	May–Aug, 2008–2012
2–254 (759)	6–254	13–100	All stages. Dominance of chalimus stages during epizootic outbreak in Mar 2014	Mar–Jun, 2013–2014

of salmon lice infested fish in the sample. Life cycle stage of lice, time of sample collection, capture methods used, sea trout size, study site and extent of fish farming in the area are also summarised. *denotes lice levels given for brown trout and Arctic char combined, because lice levels did not differ among the 2 species

Capture method	Fish size	Study site	Extent of salmon farming in area	Reference
Research fishing vessels	Mean = 520 mm	North Sea off Yorkshire (England)	No farms	Boxshall (1974)
Gill nets	Mean = 164–273 mm	Rivers Clifden, Costello, Gowla, Owengarve, Burrishoole, Newport, Inny River, Owenduff, Ballynahinch, Killary, Currane, Dowras, Drumcliffe, Argideen (west coast of Ireland)	Fish farming area, but variation among embayments covered in the study	Tully et al. (1993a)
Rod and line	Not given	River Eachaig and Argyll rivers, (1) west coast of Scotland,	Information not given	Sharp et al. (1994)
Rod and line	Not given	Rivers Morar, Ewe and Burn (northwest coast of Scotland)	Information not given	Sharp et al. (1994)
Rod and line	Not given	Rivers Don, Ythan and Hope (north and northeast coast of Scotland)	Information not given	Sharp et al. (1994)
Fish trap in lower part of river capturing prematurely returned trout	Post-smolts: mean total length = 174 mm, mean mass = 42.3 g; older migrants: mean total length = 374 mm, mean mass = 581 g	Lønningdalselven (Hordaland, Norway)	Intensive farming	Birkeland (1996)
Fish trap in lower part of river capturing prematurely returned trout	Mean total length = 160 mm, mean mass = 30 g	Lønningdalselven (Hordaland, Norway)	Intensive farming	Birkeland & Jakobsen (1997)
Gill nets and market	Mean fork length = 29–32 cm (range 25–64 cm)	East Anglia (England)	No farms	Tingley et al. (1997)
Not given	Not given	North Sea off Yorkshire (England)	No farms	Tingley et al. (1997). Data from 1972 were also published by Boxshall (1974)
Gill nets	Mean fork length = 228 mm, 126 g	Rivers Bunowen, Bundorragha, Erriff, Culfin, Dawros and Owenglin in Ballinakill District (Ireland)	Information not given	Dawson (1998)
Gill nets	Mean fork length = 206 mm, 82 g	Rivers Gowla, Invermore, Furnace and Cashla in Connemara District (Ireland)	Information not given	Dawson (1998)
Electrofishing in river mouth, and gill nets	Mean length = 245 mm (river), and 426 mm (fjord)	Akerselva and Oslofjord (southern Norway)	No farms	Mo & Heuch (1998)
Seine nets, rod and line, gill nets	Fork length = 101–559 mm	Locations on the west coast (n = 17), east coast (n = 2) and north coast (n = 1) of Scotland	Both from areas with and without intensive fish farming	MacKenzie et al. (1998)
Beach seine	Mean = 320 mm, 440 g	Skagerrak coast (southern Norway)	No farms	Schram et al. (1998)
Gill nets, electrofishing, and wolf trap	Not given	North Mayo, South Mayo, Galway and Kerry locations (total n = 10) (Ireland)	Information not given	Byrne et al. (1999)
Mainly gill nets. Some fish captured also by traps, draft nets, and electrofishing	Only fish <260 mm fork length were included in analysis	42 estuaries in Ireland	Both areas with and without intensive	Tully et al. (1999)
Gill nets	Mean = 119–209 g	Vesterålen (northern Norway)	Intensive farming	Bjørn et al. (2001)*
Gill nets	Mean = 119–464 g	Ofoten (northern Norway)	Low farming intensity	Bjørn et al. (2001)*
Gill nets	Not given (gill net mesh sizes = 19–35 mm)	Altafjord (northern Norway)	Intensive farming	Bjørn & Finstad (2002)*
Gill nets	Not given (gill net mesh sizes = 19–35 mm)	Lille Porsanger (northern Norway)	Low farming intensity	Bjørn & Finstad (2002)*
Gill nets	Mean = 328 g (SD 63)	Skagerrak coast (southern Norway)	No farms	Heuch et al. (2002)
Sweepnets	Not given	Laxford Bay (Sutherland, Scotland)	During fallow and production periods at nearby farm	Marshall (2003)
Gill nets	Mean = 668 g (SD 432)	Ranafjord (northern Norway)	No farms	Rikardsen (2004)
Gill nets	Mean = 340 g (SD = 314)	Balsfjord (northern Norway)	No farms	Rikardsen (2004)
Gill nets	Mean = 240 g	Løksefjord and Malangsbotn (northern Norway)	Low farming intensity	Bjørn et al. (2007)*
Gill nets	Mean = 170 g	Altafjord (northern Norway)	Intensive farming	Bjørn et al. (2007)*
Bag nets	Mean = 1.16 kg (SD = 0.32)	North Esk (east coast of Scotland)	No farms	Urquhart et al. (2008)
Electrofishing during return to freshwater	Mean = 155 mm, 35 g	River Shieldaig (Scotland)	Information not given	Pert et al. (2009)
Gill nets	Mean = 440–480 mm, 1.06–1.21 kg	Rivers Annan and Carron (west coast of Scotland)	Close to salmon farms	Urquhart et al. (2010)
Bag nets, sweepnets, gill nets	Mean = 221–308 mm, 0.16–0.31 kg	Upper Forth Estuary, North Esk, and Stonehaven Bay (east coast of Scotland)	No farming	Urquhart et al. (2010)
Gill nets	Mean = 97–383 g, 210–270 mm	Eresfjord in Romsdalsfjord (Norway)	Protection zone with low farm activity	Bjørn et al. (2011)
Gill nets	Mean = 364–490 g, 310–320 mm	Karlsøyfjord in Romsdalsfjord (Norway)	Intensive farming	Bjørn et al. (2011)
Gill nets	Mean mass = 131–457 g	Five large fjord areas in Norway with restrictions on fish farming (National Salmon Fjords)	>30 km to nearest farm	Serra Llinares et al. (2014)
Gill nets	Mean mass = 85–823 g	Five smaller fjord areas in Norway with some restrictions on fish farming activity (National Salmon Fjords)	<30 km to nearest farm	Serra Llinares et al. (2014)
Bag nets	Mean = 31–35 cm, 283–405 g	Sognefjord (Norway)	Intensive farming	Vollset & Barlaup (2014)

their transfer to freshwater. Although premature return migraton can reduce or eliminate the lice infestations on individual fish, it will also involve a fitness cost in terms of reduced growth opportunities (Birkeland 1996), and subsequently reduced resources for egg production, thus reducing female fecundity.

Salmon lice levels in samples of wild sea trout

Salmon lice levels in areas before, or without, salmon farming

Ideally, in order to evaluate whether or not salmon lice levels have become elevated in wild populations, and their possible association with salmon farming, baseline information on lice levels and their year-round population dynamics would be required for time periods preceding the development of fish farming, or from areas lacking fish farming. Historical salmon lice levels on sea trout prior to the industry (Boxshall 1974), and data for areas lacking fish farming (Tingley et al. 1997, Schram et al. 1998, Heuch et al. 2002, Rikardsen 2004, Urquhart et al. 2010), generally show a relatively high prevalence, but low intensity of salmon lice on sea trout (Table 1, Figs. 2 & 3). The natural intensity of salmon lice on sea trout in areas without fish farming may be as low as 0–3 lice per fish, and with a prevalence of 0–20% during late winter and spring (Schram et al. 1998, Heuch et al. 2002, Rikardsen, 2004). Intensities increased to a peak of up to 4–8 lice per fish with higher prevalences in the late summer and autumn (Tingley et al. 1997, Schram et al. 1998, Rikardsen 2004, Urquhart et al. 2010). In areas without fish farms, prevalence may range up to 100%, but is most often <70% (Table 1, Figs. 2 & 3). The peak in salmon lice levels on sea trout may occur 1–2 mo later at more northerly locations compared to more southern latitudes, perhaps reflecting seasonal contrasts in temperature and ontogenetic developmental rates for salmon lice. At more northern latitudes in Norway, the peak salmon lice level in sea trout has been noted during the period August–October (Bjørn & Finstad 2002, Rikardsen 2004), whereas at more southerly latitudes this may advance to June–August (Mo & Heuch 1998, Schram et al. 1998, Heuch et al. 2005).

Atlantic salmon, sea trout and Arctic char all are natural hosts of salmon lice and, due to the seasonality of their migrations, there are few of these wild hosts in coastal waters during the winter months. Atlantic salmon feed in the open ocean and traverse coastal areas relatively quickly during the outward migration in the spring (Thorstad et al. 2011). The

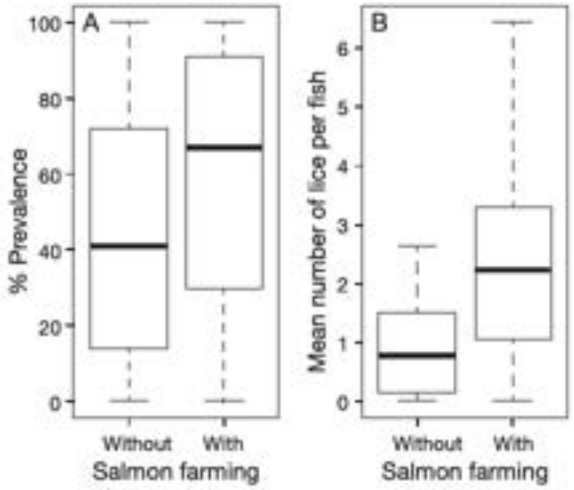

Fig. 2. (A) Prevalence and (B) mean number of salmon lice per sea trout sampled in areas where salmon farming was present ('with') and not present ('without'). Box plots show the median (line) and interquartile range (box length, IQR), whiskers extend to 1.5 × IQR. Data sourced from published studies: Boxshall (1974), Tully et al. (1993a,b, 1999), Sharp et al. (1994), Birkeland (1996), Birkeland & Jakobsen (1997), Tingley et al. (1997), MacKenzie et al. (1998), Mo & Heuch (1998), Bjørn & Finstad (2002), Marshall (2003), Rikardsen (2004), Bjørn et al. (2007, 2011), Urquhart et al. (2008, 2010), Serra Llinares et al. (2014), Vollset & Barlaup (2014). Mean numbers of lice are log-transformed

rate of transit of returning adult salmon through coastal waters also is typically rapid (e.g. Davidsen et al. 2013). These return migrations usually occur during May–September in Norway, but with more extended and variable timing in Scotland and other southerly regions of salmon distribution (Thorstad et al. 2011). By contrast, sea trout and Arctic char often spend weeks or months during the summer in coastal areas and the remainder of the year in freshwater, although a proportion of trout and char populations may reside at sea throughout the year (Jonsson & Jonsson 2011). Because salmon lice cannot survive long in freshwater, the persistence of the parasite population depends upon hosts at sea over the winter months. For wild host populations, these winter components therefore include Atlantic salmon feeding in the open ocean, and the small numbers of sea trout and Arctic char that remain in coastal areas (Klemetsen et al. 2003, Jonsson & Jonsson 2011). In areas without salmon farms, the salmon lice populations therefore have few available hosts and appear to encounter a host resource bottleneck in winter (Schram et al. 1998, Heuch et al. 2002, Rikardsen 2004).

The highest levels of salmon lice on sea trout reported for an area without fish farming was a mean abundance of 10.9 lice per fish sampled, and mean

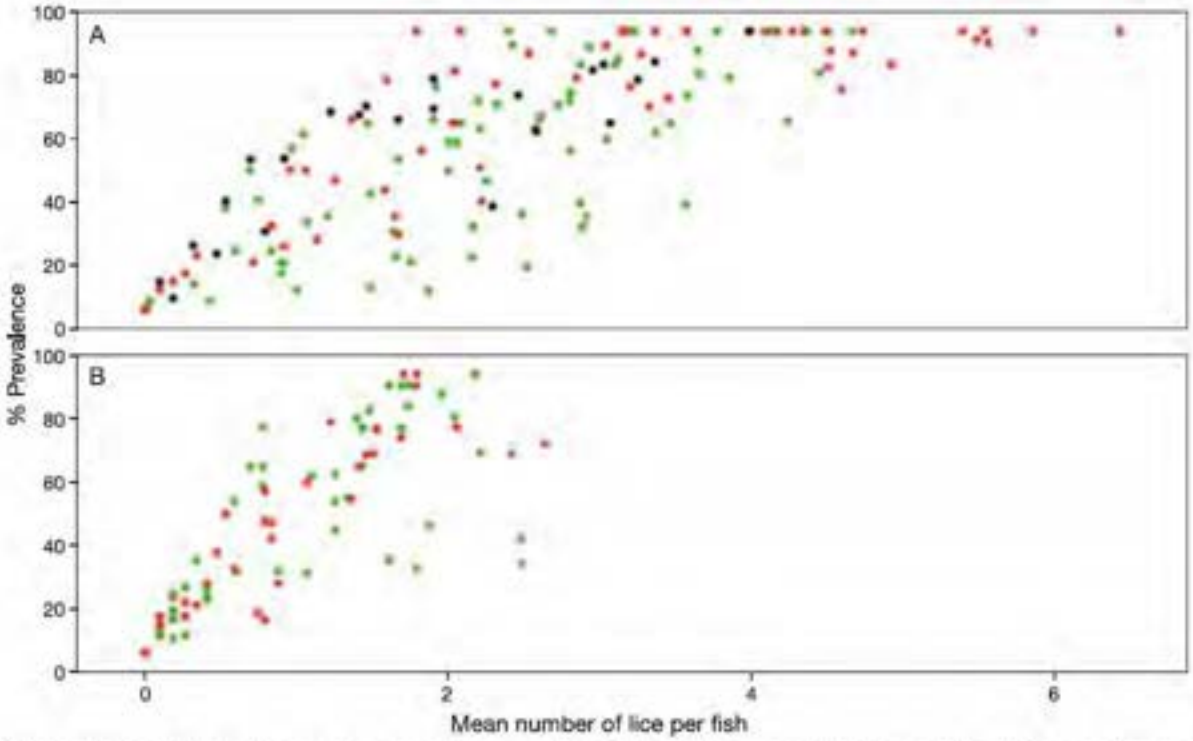

Fig. 3. Relationship between the prevalence and mean number of salmon lice per sea trout sampled in (A) areas where salmon farming was present and, (B) areas without salmon farming. Data sourced from published studies (see references in Fig. 2). Studies performed in Ireland, Norway and Scotland denoted by black, red and green circles, respectively. Mean numbers of lice are log-transformed

intensity of 11.6 lice per infested fish (Oslofjord, southern Norway) (Mo & Heuch 1998). However, most of the samples in that study showed abundances and median intensities in the range of 0.5–8 and 1.5–10 salmon lice per fish, respectively, with an overall prevalence of 51%. However, it is important to note that 4 heavily-infested individuals (of a total sample of 102 fish) each carried as many as 33–84 lice (of which 43–72% were adults). This shows that even in areas without fish farming a few individual sea trout may carry salmon lice levels that, on the basis of laboratory studies, will probably induce stress in the host fish.

To our knowledge, there are no published records of outbreaks of salmon lice epizootics on sea trout populations that pre-date the commencement of salmon farming. Nevertheless, it is important to emphasize that salmon louse epizootics were reported for Atlantic salmon and brook trout *Salvelinus fontinalis* (Mitchill, 1814) over the period 1939–1940 at Moser River in Nova Scotia on the Canadian east coast (White 1940, 1942). Notwithstanding a possible publication bias, the lack of known and reported epizootics in areas without salmon farming indicate that this is not a common phenomenon for salmon lice on wild sea trout or other salmonid populations.

Salmon lice levels in areas with salmon farming

Salmon lice levels reported for sea trout in farm intensive areas are generally higher and more variable than in areas without fish farming (Table 1, Figs. 2 & 3). High variation in salmon lice levels can be expected because studies differ in time of the year of the survey, the fish sizes collected, sampling methods, habitats sampled and sample sizes. Moreover, only fish that survived infestation will be caught. Fish captured in gill nets and seine nets may be subject to physical abrasion during capture and removal from the net, thereby resulting in the loss of some salmon lice. When fish are captured in bag nets or other gear where they are retained free-swimming without being killed, lice may move between individual sea trout (K. Vollset and S. Kålås pers. obs.). Furthermore, the place and time of sampling may not be representative of the local sea trout population, and the salmon lice level may be overestimated if only the most heavily infested trout that are returning prematurely to freshwater are caught. Conversely, salmon lice levels on fish captured in estuaries may be underestimated because sampling might be biased towards fish that have only recently arrived and have not been at sea for sufficient time for salmon lice to

attach. Underestimation of salmon lice intensities and abundances is also likely if sea trout have been resident in hyposaline waters at river estuaries for a sufficient period for the lice to become detached from the host fish. All of these caveats apply equally to samples from areas with or without salmon farming. Additionally in farm-impacted areas, there also is likely to be considerable variation among studies because fish number and biomass production in nearby farms, and the associated salmon lice levels, vary.

Studies in farming areas show that chalimus stages of salmon lice dominate in spring and early summer, and preadult and adult stages have been recorded on sea trout primarily in late summer and autumn (Tully et al. 1993a, MacKenzie et al. 1998, Bjørn et al. 2001, 2007, 2011, Bjørn & Finstad 2002). However, in areas with continuously high salmon lice levels, chalimi dominate throughout the summer and autumn season, and sea trout rarely carry adult lice (Tully et al. 1993a, Sharp et al. 1994, Birkeland & Jakobsen 1997, Gargan 2000, Bjørn et al. 2001, Butler 2002, Gargan et al. 2003). The predominance of chalimus in areas with high salmon lice levels may be explained by heavily infested fish dying at sea or returning prematurely to freshwater (and not being sampled) before lice had attained the adult stage (Tully et al. 1993a, Birkeland & Jakobsen 1997, Bjørn et al. 2001). In areas with fish farms, high levels of salmon lice have also been recorded during winter (Vollset & Barlaup 2014).

If the proportion of fish carrying potentially lethal levels of a parasite is known, the consequences of the parasite for the host population may be estimated. Bjørn et al. (2001) found that 32% of the sea trout post-smolts captured at sea in northern Norway exhibited relative densities of salmon lice above the level that caused mortality in laboratory studies. The corresponding estimate from sea trout that returned prematurely to freshwater was 47% (Bjørn et al. 2001). Even though it is not known to what extent threshold levels based on laboratory results are directly applicable to wild free-ranging fish, Bjørn et al. (2001) could conclude that excess mortality of the most heavily-infested post-smolts most likely occurred in that study area. Other studies also report increased salmon lice levels in areas with salmon farming. An Irish study of 4600 sea trout sampled at 15–52 sites over the period 1992–2001 (Gargan et al. 2003) showed that 3.4% of the sea trout in bays without farms had salmon lice levels above a critical threshold (Bjørn & Finstad 1997) of 0.7 chalimi per gram of fish mass. By contrast, in bays with farms, 31% of the sea trout carried salmon lice levels above that level.

Recent Norwegian studies have shown that the risk of mortality was elevated for 12 to 90% of the sampled fish at 1 or more sampling occasions in 5 fjord areas <30 km from the nearest farms (Serra-Llinares et al. 2014). Moreover, Taranger et al. (2015) found that of 109 stations investigated along the Norwegian coast for salmon lice infection, 67 locations indicated moderate-to-high mortality of wild sea trout. Finally, a large-scale study, with nearly 5000 sea trout sampled from 48 sites along the Scottish west coast and Outer Hebrides during 2003–2009 (Middlemas et al. 2013), showed that 13% of the fish carried salmon lice levels above the suggested critical threshold of 13 mobile lice (Wells et al. 2006).

Interactions between fish farming activity and salmon lice levels of sea trout in coastal areas

In coastal areas with intensive Atlantic salmon farming, the large disparity in abundance between cultured and wild hosts is such that local larval production of salmon lice most likely originates primarily from farmed salmon and not from wild fish, although all salmon lice hosts potentially cross-infest one another (Tully & Whelan 1993, Heuch & Mo 2001, Butler 2002, Todd et al. 2004, Heuch et al. 2005, Penston & Davies 2009, Jansen et al. 2012, Torrissen et al. 2013). Several studies of wild sea trout have shown increased salmon lice levels with decreasing distance to salmon aquaculture sites (Tully et al. 1999, Gargan 2000, Bjørn et al. 2001, 2011, Bjørn & Finstad 2002, Gargan et al. 2003, Middlemas et al. 2013, Serra-Llinares et al. 2014). Others show increased concentrations of salmon lice larvae in the water column with decreasing distance to salmon farms (Gillibrand et al. 2005, Penston et al. 2008a,b). Moreover, there is additional evidence of a correlation between the abundance of salmon lice larvae in the water column and the number of gravid salmon lice larvae produced by adjacent farms (Penston & Davies 2009). Hence, these studies support a link between salmon farms and salmon lice burdens in sea trout.

A correlation between salmon farming and lice production is even more apparent in farmed areas when farms synchronize their production cycles. During a synchronised 2-yr production cycle, the mean total biomass of fish, and thereby the potential for salmon lice larval production, increased over time (Butler 2002, Revie et al. 2002, Gillibrand et al. 2005). Several studies have shown a relationship between the production cycle in salmon farms and salmon lice

levels on wild sea trout, with higher lice levels on trout in the second year of the farm production cycle (Butler 2002, Marshall 2003, Hatton-Ellis et al. 2006, Middlemas et al. 2010, 2013). Biannual cycles of salmon lice epizootics have been observed only in areas with synchronised-year class production, whereas epizootics were observed every spring in areas with a mixed-year class production (Butler 2002).

Gargan et al. (2003), Middlemas et al. (2013), and Serra-Llinares et al. (2014) all included a large number of sampling sites during monitoring of salmon lice levels on wild sea trout over several years. In all 3 studies (respectively from Ireland, Scotland and Norway), the highest levels of salmon lice were found on sea trout sampled in coastal areas within 20–30 km of the farms. In Scotland, the distance to the nearest farm did not influence the probability of infestations above the critical level for physiological impact by salmon lice (based on Wells et al. 2006, Bjørn & Finstad 1997) beyond 31 km, although there was considerable uncertainty around this cut-off distance (95% confidence limits: 13–149 km) (Middlemas et al. 2013). Gargan et al. (2003) found reduced lice levels on wild sea trout recorded at distances >30 km from farms. Chalimus dominated at a distance to farms of <30 km, and preadult and adult lice stages dominated at distances >100 km. Furthermore, Serra-Llinares et al. (2014) found that 41% of the variance of the mean lice abundance on wild sea trout could be explained by the lice production in farms, in areas where active fish farms existed within a distance of <30 km. Elevated salmon lice levels on wild sea trout have, however, also been recorded at greater distances from farms (e.g. >25–30 km) (Bjørn & Finstad 2002, Bjørn et al. 2011).

The distance and directionality of salmon lice larval transport from their release source depend upon multiple variables, including their development rate, water temperature, currents and wind-driven circulation (Gillibrand et al. 2005, Asplin et al. 2011, 2014). Ontogenetic development rates of larvae increase with water temperature (Wootten et al. 1982, Johnson & Albright 1991a, Stien et al. 2005), and larval drift distance may thus increase with decreasing temperatures. Numerical models show that nauplii and copepodids may be transported up to 100 km from their source, although typical dispersal distances are up to ~25 km (Asplin et al. 2011, 2014, reviewed in Costello 2009). In addition, salinity influences salmon lice survival and behaviour (Heuch 1995), which also affects the density of salmon lice in a given area. Hence, although these various studies show potentially considerable variability in the effective dispersal of salmon lice, it is likely that the majority of lice larvae remain relatively close to their source.

Aggregation of salmon lice larvae may occur in certain areas, typically close to land and in embayments (Asplin et al. 2014), and larval distribution is commonly spatially and temporally patchy within a given area (Murray 2002). The movements of wild sea trout themselves will also contribute to variation in their risk of exposure to salmon lice. Fish may move between sites of variable infestation risk, and are not necessarily captured close to the site where they have been infested. Furthermore, premature return to freshwater could reduce the lice infestation rates. Hence, considerable variation in salmon lice levels on wild sea trout, as has been observed in rivers close to farms in Ireland (Gargan et al. 2003), is to be expected. Such complexities may underlie the occasional reports of the lack of a relationship between salmon lice levels and distance to nearby farms, or between lice levels in wild sea trout and those on a nearby fish farm (MacKenzie et al. 1998, Marshall 2003).

Population effects of salmon lice

Population level effects of salmon lice on marine survival and growth of sea trout

Brown trout populations in catchments, tributaries and river stretches accessible from the sea show genetic differentiation, and some of this variability is likely the result of local adaptation (Jonsson & Jonsson 2011). Within populations and rivers, there is little genetic differentiation between sea-migrating and resident individuals (Hindar et al. 1991, Charles et al. 2005, 2006), but, anadromy is a quantitative trait that is controlled by interactions between genetic and environmental factors (Jonsson & Jonsson 1993, 2006, 2011, reviewed by Ferguson 2006). Migrant and resident brown trout within rivers can spawn separately and form discrete populations, or they may spawn together successfully, and thereby constitute freely interbreeding fractions of a single spawning stock (Jonsson & Jonsson 1993, 2006, 2011). The advantages of marine migrations for sea trout include the opportunity of accessing more productive feeding conditions in order to enhance growth, fecundity and thereby evolutionary fitness (Jonsson & Jonsson 1993, 2006, 2011).

Anadromy evolves in response to trade-offs between the costs and benefits of migration compared with

residency, and these are balanced through their effect on fitness (Jonsson & Jonsson 1993, 2006, Bohlin et al. 2001, Ferguson 2006, Solomon 2006). A higher growth rate in freshwater, combined with an increase in the migratory cost, can result in a higher proportion of resident trout (Jonsson & Jonsson 2006). Changes in environmental conditions or genes can, therefore, result in a population shift in life-history strategy (Jonsson & Jonsson 1993, 2006, Ferguson 2006). The likelihood of moderate heritability of anadromy as a trait, in concert with the higher fecundity of larger sea trout, can result in substantial population changes occurring within perhaps only a few generations. Thus, increases in marine mortality and reduced growth of sea trout induced by salmon lice both can shift the selective balance in favour of the freshwater resident life history.

In contrast to the density-dependent freshwater mortality of sea trout that occurs especially during the earliest embryonic and post-emergence life stages, marine mortality seems not to be density-dependent. Mortality in the freshwater phase therefore can have a population regulating effect, whereas mortality in the marine phase (including that attributable to salmon lice) is not regulatory, but has a population reducing effect (see Milner et al. 2003, Einum & Nislow 2011). Elevated mortality during the freshwater phase can, to a varying extent, be compensated by increased growth and survival of the remaining juveniles, whereas there are no compensatory mechanisms for additional mortality in the marine phase. Hence, elevated rates of marine mortality, such as that induced by salmon lice, can result in a proportional reduction in the number of spawning adults. Because sea-run brown trout typically are females (Jonsson & Jonsson 2011), any additional marine mortality has the potential to affect recruitment even more negatively than would be the case for an equal sex ratio.

Reduced marine survival and growth as a result of increased salmon lice levels in farm intensive areas will likely lead to a decreased frequency of sea-run brown trout, as indicated by Gargan et al. (2006b). Catchments offering poor environmental conditions for brown trout during some periods of the year, for example, due to drought or freezing (Borgstrøm & Heggenes 1988, Järvi et al. 1996, Limburg et al. 2001), may be at risk of losing their brown trout populations if the marine mortality is chronically high. Larger catchments with more suitable year-round conditions for brown trout may not be at such risk, but severe reduction or loss of the sea-run migratory form can result in (1) altered genetic composition of populations (which may be regarded as the effective loss of a sea trout population and its replacement by a freshwater resident population with differing population genetic characteristics), (2) reduced genetic diversity, and (3) a greater uniformity in life history characteristics. The loss of access to the improved growth opportunities offered by the marine environment also will lead to a lower abundance of brown trout and reduced recruitment.

Population effects in Ireland

Data for salmon lice intensities on marine salmon farms and wild populations, in addition to observations of the incidence of premature return by sea trout, indicate that salmon lice from marine salmon farms was a significant factor in observed stock collapses in western Ireland in the late 1980s (Tully & Whelan 1993, Tully et al. 1999, Gargan et al. 2003). Data on upstream migration are available since 1970 from the Burrishoole upstream trap and 1985 for the Tawnyard (Erriff) sea trout kelt trap. Rod catch data and trap records from both fisheries indicate a stable sea trout population structure prior to 1989, dominated by a peak of finnock (sea trout that return to freshwater in the autumn, following a few months at sea), a second peak of maiden sea trout (fish that had spent the previous winter at sea), some older fish and previous return spawners (Poole et al. 1996, Gargan 2000). Subsequent to the 1989 sea trout stock decline in western Ireland there was a marked reduction in the number and proportions of sea age classes, and the stocks were characterised by low returns of finnock and fewer veteran sea trout in the older age classes (Whelan 1993, Poole et al. 1996, Gargan 2000, Poole et al. 2006). The number of ova deposited by sea trout in the Burrishoole system, estimated to range between 0.49 and 1.61 million before 1987, decreased to <60 000 by 2000 and showed a minimum of 27 500 in 2003 (Poole et al. 2006). O'Farrell et al. (1989) estimated that the percentage contribution to ova deposition of 0-sea age fish was 5.6%, whereas that of 1-sea age trout was 41% and 2-sea age fish and older contributed 54% to ova deposition. Hence, reduced marine survivorship of larger, older spawners that contribute disproportionately to overall egg deposition can exert considerable and rapid impacts at the population level.

Prior to the onset of marine salmon aquaculture in the Burrishoole system, western Ireland, the percentage of sea trout smolts that survived to return as 0+ sea age finnock in the same year ranged from 11 to

32%, with a historical mean of 21%. Throughout the 1990s (i.e. subsequent to introduction of salmon farming) there was a saw-tooth pattern of finnock return rates, whereby the mean return rate for this period (excluding 1999) was three times lower (6.8%) than the historical average (Poole et al. 2006). Data from 2 other trap facilities in western Ireland (Owengowla and Invermore) indicate a marine survival rate of <2% in the majority of the years during this period (Gargan et al. 2006b). The highest marine survival (19%) for these 2 traps was observed on the Owengowla in 1994, coinciding with whole-bay spring fallowing of salmon aquaculture. Although survival estimates under circumstances of local farm fallowing would require replication in multiple years and locations, these data strongly indicate that salmon lice from marine Atlantic salmon farms made an important contribution to the sea trout stock decline on Ireland's west coast (Tully & Whelan 1993 Gargan et al. 2003, 2006b, Poole et al. 2006).

Since 1974, the sea trout rod catch has been monitored for 18 west coast fisheries in the Connemara district (Fig. 4). The data show a decline during 1987–1988, from ~10 000 fish caught every year in the 1970s and and early 1980s, to only 240 fish caught in 1990 (Whelan & Poole 1996, Gargan et al. 2006a) (Fig. 4). Sampling of sea trout in estuaries was initiated in the Irish mid-west in 1990, and sea trout post-smolts were recorded in all rivers with high levels of predominantly juvenile salmon lice stages (Tully et al. 1993b). This documented decline in sea trout rod catch coincided with the development of salmon aquaculture in western Ireland during the mid-1980s, and has been linked to salmon lice infestation on sea trout (Tully & Whelan 1993, Tully et al. 1999, Gargan et al. 2003). However, in determining whether any reduction in rod catch is reflective of an overall reduction in sea trout stock size, it is important to consider catch per unit effort (CPUE) for the fishery. In this context, the 'catch and release' by-law introduced in western Ireland in 1990 may have affected angling effort for some fisheries. Based on analysis of sea trout rod catch and effort data (CPUE), Gargan et al. (2006b) found that the sea trout catch decline recorded between 1988 and 1990 was not related to reduced angling effort, but that a marked reduction in CPUE had indeed occurred.

Following a decline in sea trout stocks in 2 Irish fisheries, Gargan et al. (2006b) recorded that substantial sea trout smolt runs continued for a number of years despite the very small numbers of adult trout returning from the sea. Trend analysis indicated a reduction in sea trout smolt output from both fisheries over the study period, which suggested that although freshwater resident trout contribute significantly to sea trout smolt runs, a reduction in smolt output can be expected after a relatively short period of very poor marine survival. If the individuals that adopted the anadromous strategy had very low marine survival, there would be selection in favour of those with higher genetic propensity for freshwater residence. The declining numbers of smolts produced by the freshwater stock therefore could be explained by such selection against the anadromous life history strategy within a population (Gargan et al. 2006b).

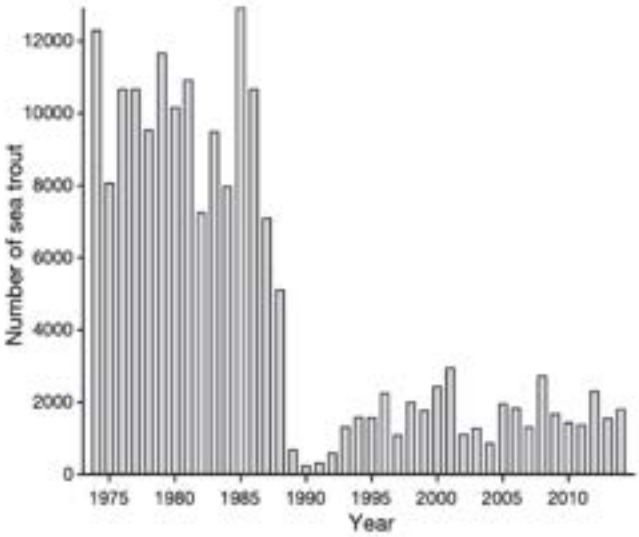

Fig. 4. Sea trout rod catches for 18 fisheries in the Connemara district in western Ireland during 1974–2014. Data from 1990 and onwards are based on catch and release angling

Population effects in Scotland

In Scotland, during the late 1980s, unprecedented declines in sea trout rod fisheries were recorded throughout the west coast region (Walker 1994, Northcott & Walker 1996). Butler & Walker (2006) reported a collapse and a marked shift in population structure of the River Ewe rod-caught sea trout beginning in 1988, linked to salmon lice epizootics following the establishment of salmon farms near the river mouth in the marine embayment of Loch Ewe. Between 1980 and the period 1997–2001, maximum sea age fell from 11 to 5 yr and marine growth rates declined. Butler (2002) further estimated that farmed salmon was probably the primary source of salmon lice (78–97% of parasites) on wild salmon and sea trout populations, and that aqua-

culture facilities comprised the major source of lice to emigrating smolts in springtime on the west coast of Scotland. Taken together, the changes in the River Ewe stock structure could be related to declines in marine growth and survival, which were deduced to have been at least partly attributable to salmon lice epizootics emanating from salmon farms in the adjacent coastal waters (Butler & Walker 2006). This contention was supported by Walker et al. (2006) in comparing contemporaneous catch data for east coast Scotland sea trout stocks. The east coast of Scotland has been essentially free of commercial salmon farming throughout the history of the industry, and sea trout stock structure there remained stable over the same period that the west coast collapses were reported. Further corroborative reports of contemporaneous collapses in other, smaller, sea trout fisheries in the west of Scotland include those for rivers draining into Loch Torridon (McKibben & Hay 2004).

Notwithstanding the clear contrasts in these sea trout stock assessments for east (non-farmed) versus west (farmed) coasts regions, it has to be acknowledged also that the presence or absence of salmon farming is not the only difference between these coastlines. Ideally, comparisons would be drawn between areas or rivers in farmed and non-farmed regions within the Scottish west coast itself; but the development history and extent of the industry is such that suitably large non-farmed, or 'non-impacted' areas are not present. Furthermore, even drawing comparisons among specific sea lochs within western Scotland is fraught with difficulty because of the problem of pseudoreplication — no two sea lochs are identical in terms of their size, depth or hydrography. The absence of extensive areas of western Scotland without salmon farming, and which might be designated as 'controls' for experimental comparison with salmon farm 'impacted' areas, has proven to be a major obstacle to scientists investigating the likely impacts of salmon farming on adjacent wild stocks of sea trout and salmon. The Scottish Salmon Producers Organisation does provide publicly available summary statistics on their website (www.scottishsalmon.co.uk/fish-health-management-report-january-to-march-2015) for the monthly average abundance of adult female parasites on farmed salmon stocks in Scotland. Whilst these summary data can be informative of the overall status of sea lice abundances on farmed stocks, they are summarised by geographic area for 30 regions and lack resolution. Furthermore, the lack of access for scientists to detailed data (e.g. lice levels, and number and size of fish held in particular fish farms) hampers analyses of the likely impacts of salmon farming on local wild sea trout and Atlantic salmon stocks.

Whilst no marine survival data exist for Scottish west coast rivers prior to the sea trout collapse in the late 1980s, low smolt-to-finnock marine survival rates of 0.8–8.1% and 1.0–4.6% were also recorded for the rivers Tournaig and Shieldaig, respectively, over the period 1999–2001 (Butler & Walker 2006) and have been related to salmon lice infestation. Butler & Walker (2006) noted an increase in the abundance of resident (non-anadromous) trout following the sea trout stock collapse in the River Ewe system in western Scotland. Given the reductions in egg deposition resulting from the collapse in adult sea trout abundances, it is possible that lack of competition, and related improvements in freshwater growth rates, might lead to a greater prevalence of freshwater-resident trout in some impacted populations (Butler & Walker 2006).

Population effects in Norway

Sea trout from the majority of sampled sites along the Norwegian coast from Hordaland to Finnmark had salmon lice levels that indicated moderate or high mortality in 2011–2013 (data from the national monitoring programme) (Taranger et al. 2015). The infection levels of salmon lice on anadromous brown trout in the central and outer regions of the intensively farmed Hardangerfjord are among the highest observed in Norway (Skaala et al. 2014b). From 2001 to 2011, all descending smolts and returning sea trout in River Guddalselva, in the central region of Hardangerfjord, were captured in traps, and the smolts were individually tagged (Skaala et al. 2014a). Samples of the emigrant smolt cohorts were treated with Substance EX to prevent early salmon lice infestation. The results show a very low marine survival rate of only 0.6–3.4% for tagged smolts, with the highest survival rates in years with the lowest registrations of farm salmon lice in springtime. The survival rates of Substance EX-treated smolts and controls were respectively 3.41% and 1.76%. Although both these levels of survival are low, they indicate the extent to which spawning abundances of adult sea trout may be reduced in local populations (i.e. in this case by almost one half).

Bjørn et al. (2001) quantified salmon lice levels on sea trout at 2 sites in northern Norway; one 'exposed' area subject to extensive salmon farming was compared with an 'unexposed' area with little

farming activity. At the exposed location, 47% of the fish caught in freshwater and 32% of those captured at sea carried salmon lice at intensities above the level that has been shown to induce mortality in laboratory experiments (Bjørn & Finstad 1997). Bjørn et al. (2001) concluded that excessive mortality of the most heavily-infested post-smolts most likely occurred in that study area and that high salmon lice levels may therefore have profound negative effects upon wild populations of sea trout.

Genetic differences among sea trout populations and effects of salmon lice

The effects of salmon lice on sea trout populations may vary according to the genetic structure of a target population. In this regard, Glover et al. (2001) recorded a clear difference in susceptibility to salmon lice between fish from a freshwater resident brown trout population and an anadromous population when exposed to salmon lice in a laboratory experiment, as measured by their respective salmon lice abundances. Subsequently, Glover et al. (2003) reported significant differences in the abundance, density, and development rate of salmon lice among 3 sea trout populations in southwest Norway. Their results suggest that the observed differences in salmon lice level among the 3 sea trout populations reflect host genetic differences. Also in Atlantic salmon, differences in infection level are observed among stocks, which may reflect genetic differences in their susceptibility to sea lice infestation (Glover et al. 2004).

Coughlan et al. (2006) sampled DNA from scales of sea trout in the Burrishoole River, in the west of Ireland, before and at intervals during aquaculture activities. Amongst these samples, allelic variation at a microsatellite marker (Satr-UBA), tightly linked to a locus critical to immune response, was compared with variation at 6 neutral microsatellite loci. No substantial evidence of the variability of a genetic signal for the immune response genes was observed at neutral microsatellite loci. A significant decline in allelic richness and gene diversity at the Satr-UBA marker locus, which preceded a severe sea trout stock collapse, does however appear to be associated with aquaculture activities. These data therefore suggest that salmon farming-mediated disease can indirectly affect the genetic structure of sympatric sea trout populations by reducing variability at major histocompatibility genes.

Population-reducing effects on Atlantic salmon: relevance to sea trout

Experimental studies have been conducted on the mortality of salmon lice on Atlantic salmon post-smolts, comparing fish chemically treated to provide protection from salmon lice with control groups of untreated fish. These field studies have been conducted with the presumption that salmon lice originating from local farm sources might confer increased mortality risk to the untreated control smolts, and that this effect will extend to the wild Atlantic salmon smolt population.

All these studies have found greater return rates of treated salmon smolts, but not in every location or in each year. The estimated average risk ratio of protected fish returning to their natal rivers to spawn compared to unprotected fish ranged from an average 1.14:1 to 1.41:1 (Jackson et al. 2011a,b, 2013, 2014, Gargan et al. 2012, Krkošek et al. 2013, 2014, Skilbrei et al. 2013, Vollset et al. 2014). Within any given release group, a risk ratio of 1.14–1.41:1 reflects that 12–29% fewer unprotected than protected fish ultimately are recaptured as adults. Skilbrei et al. (2013) also showed that grilse were 100 grams heavier when treated, suggesting that a proportion of the surviving individuals were infested with sublethal levels of salmon lice. The most recent study on releases of treated and untreated salmon smolts (Vollset et al. 2014) concluded that salmon lice effects may increase the sea age of returning salmon, either by influencing their age at maturity or by disproportionately increasing mortality amongst those fish that mature early.

These variations in survival estimates may reflect both the variation in treatment efficacy and the variation in actual exposure of the released fish to salmon lice (Skilbrei & Wennevik 2006, Gargan et al. 2012). Because the effect of such treatments is only temporary for the first few weeks of the marine migration, and the acquired dose of the active component will vary among individuals, it is likely that mortality for treated fish underestimates the impact of salmon lice. We should, therefore, be cautious in extrapolating data from single studies to a population level. Nonetheless, comprehensive meta-analyses, long-term studies, and similar results from an increasing number of experimental studies, support that mortalities caused by salmon lice in farm-intensive areas can be expected to result in 12–29% fewer returning Atlantic salmon adult spawners.

Atlantic salmon post-smolts migrate through farm-intensive areas in near-coastal areas only in spring-

time, and perhaps are present there for only a few days or weeks en route to ocean feeding grounds (Thorstad et al. 2011, 2012). The salmon louse-induced mortality impacts from studies of Atlantic salmon should therefore be regarded as minimum estimates for sea trout mortality, if protected and unprotected groups of sea trout were to be compared. Sea trout normally remain for extended periods (weeks, months or sometimes even a year or more) in near-coastal areas. If those coastal areas are characterised by high salmon lice levels, sea trout postsmolts are likely to be more affected by salmon lice than are Atlantic salmon. Sea trout typically migrate downstream and enter the sea for the first time as smolts in spring or early summer, and may return to freshwater in the autumn, following a few months at sea (Fahy 1978, Gargan et al. 2006a, Jonsson & Jonsson 2009). However, sea trout need not return to freshwater after their first summer at sea, but can remain continuously at sea during the summer and winter until they mature and return to freshwater for spawning the following year, or even several years later (Fahy 1978, Jonsson & Jonsson 2009, Skaala et al. 2014a). Since sea trout remain in coastal areas later in the spring and summer months than Atlantic salmon, they are exposed to seasonally higher risks of salmon lice infestation. Finally, sea trout can remain at sea for longer periods than the period of short-term protection provided by the chemical treatment. Accordingly, results from studies applying these kinds of experimental methods to sea trout (e.g. Skaala et al. 2014a) are most likely to be underestimates of the potential for salmon lice-induced mortality.

Knowledge gaps and research needs

The effect of salmon lice on sea trout is a well-studied subject, with a large number of published studies available, as shown in this review. The effects of salmon lice on individual sea trout are relatively well documented through both laboratory and field studies. The most important knowledge gaps are related to salmon lice impacts at the population level and in quantifying the reduction in wild sea trout populations arising from increased mortality and reduced growth attributable to salmon lice. The effects of salmon lice on life history traits, especially of sea trout population age structure and size at maturation, and selection against anadromous behaviour in favour of permanent freshwater residence also are not well understood. For robust and informed evaluation of the effects of salmon lice on sea trout populations, field experiments comparing survival and growth of fish released to the environment following prophylactic treatment against salmon lice should be undertaken. More information also is needed on how salmon lice planktonic larval stages may spread and be dispersed in coastal areas, and on the primary environmental factors that ultimately determine the resultant salmon lice levels on wild sea trout in a given area.

Wild sea trout populations have generally been poorly studied, monitored and mapped, although there is variation in this respect among catchments, regions and countries. With specific regard to the marine environment, the behaviour, migration routes and survival of sea trout are less well understood than for many other salmonid species. Such information is essential when interpreting salmon lice monitoring data on farmed and wild fish, in evaluating the likely efficacy of any adopted mitigation measures and in facilitating the formulation of appropriate and relevant scientific advice on possible mitigation measures.

Overall conclusions

The studies reviewed demonstrate that salmon farming increases the abundance of salmon lice in the marine habitat and there is extensive published evidence that salmon lice in intensively farmed areas have negatively impacted wild sea trout populations. The effects of salmon lice on sea trout include increased marine mortality, changes in migratory behaviour, reduction of marine growth of individual fish, and reduced population sizes. These conclusions are based on:

(1) Studies of salmon lice impacts on individual sea trout in laboratory and field studies documenting host tissue damage, osmoregulatory dysfunction and other physiological stress responses, reduced growth, and increased susceptibility to secondary microbial infections and reduced disease resistance;

(2) Documentation of premature return to freshwater of sea trout carrying high levels of salmon lice. Premature return may facilitate individual survival and recovery from infestation in the short term, but does compromise growth potential, and thereby future fecundity, as well as impairing the immune defence system;

(3) Catch statistics and routine population monitoring utilizing in-river traps that have indicated changes in population abundance, age structure and altered life history characteristics in association with the onset and development of salmon farming in the adjacent environment;

(4) Monitoring of salmon lice levels on wild fish in relation to spatiotemporal variation in salmon farming intensity and biomass producton;

(5) Indications of population-level effects on sea trout derived from monitoring of salmon lice levels on wild fish in relation to experimentally determined threshold levels known to induce physiological compromise and mortality of individual fish.

Because the brown trout is a partially migrating species, reduced marine growth and increased marine mortality will reduce the benefit of marine migrations for individuals in anadromous populations. Potentially, this could result in the loss of anadromous sea trout populations, and the possibility for anadromy is crucial in catchments with environmental conditions unsuitable for brown trout during some periods of the year. Large rivers and catchments with suitable year-round conditions may not be subject to a risk of total loss of brown trout, but a severe reduction in the incidence of the anadromous life history strategy may result in altered genetic composition of a trout population, the establishment of populations characterised by freshwater residency, and perhaps reduced overall genetic diversity with less variable life-history characteristics. The loss of the enhanced growth opportunities offered by the marine environment may also lead to a lower local abundance of brown trout, altered life-history traits, lowered recruitment and loss of the large veteran migrants popular among fishers. To sustain and enhance sea trout populations, and to ensure a harvestable surplus for fisheries, salmon lice levels need to be reduced in many farm-intensive areas compared to present levels.

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VIEWPOINT

Atlantic salmon in a rapidly changing environment—Facing the challenges of reduced marine survival and climate change

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Abstract

1. Atlantic salmon populations have declined in recent decades. Many of the threats to the species during its freshwater and coastal residency periods are known, and management approaches are available to mitigate them. The global scale of climate change and altered ocean ecosystems make these threats more difficult to address.
2. Managers need to be aware that promoting strong, healthy, and resilient wild populations migrating from rivers is the optimal approach currently to reduce the impacts of changing ecosystems and low marine survival. We argue that a fundamental strategy should be to ensure that the highest number of wild smolts in the best condition leave from rivers and coastal areas to the ocean. There is great scope for water quality, river regulation, migration barriers, and physical river habitat improvements.
3. Maintenance of genetic integrity and diversity of wild populations by eliminating interbreeding with escaped farmed salmon, eliminating poorly planned stocking, and reducing impacts that reduce population sizes to dangerously low levels will support the ability of Atlantic salmon to adapt to changing environments. Reducing the impacts from aquaculture and other human activities in coastal areas can greatly increase marine survival in affected areas.
4. As most of the threats to wild salmon are the result of human activities, a focus on human dimensions and improved communication, from scientific and management perspectives, needs to be increasingly emphasized. When political and social will are coupled with adequate resources, managers often have the tools to mitigate many of the threats to wild salmon.

KEYWORDS
aquaculture, catchment management, climate change, conservation evaluation, fish, habitat management, hydropower, ocean, river

1 | INTRODUCTION

The Atlantic salmon (*Salmo salar*) is native to European and North American catchments draining to the temperate and subarctic regions of the North Atlantic Ocean, Barents Sea, and Baltic Sea (Thorstad et al., 2011). It is one of the best studied and culturally valuable fish species in the northern hemisphere. Historically it has supported commercial, sustenance, and recreational fisheries throughout its range and is still a highly prized fish among anglers while also being used by indigenous peoples for food, social and ceremonial purposes. The value of the Atlantic salmon for biodiversity conservation is recognized through the formation of the inter-governmental North Atlantic Salmon Conservation Organization (NASCO) in 1983, which enables seven Parties (six countries and the European Union) that represent all countries in the North Atlantic producing wild Atlantic salmon to co-operate in its conservation. In addition, the Atlantic salmon is listed in Annexes II and V of the European Union Habitats Directive as a species of European importance (Council of the European Communities, 1992). Annex II lists species for which special areas of conservation should be designated by Member States, and Annex V lists species whose exploitation is subject to management measures. In the USA and parts of Canada, Atlantic salmon is listed as endangered under the US Endangered Species Act and Canada's Species at Risk Act.

The Atlantic salmon has a complex and diverse array of life histories. Most forms are anadromous with a juvenile phase in fresh water, followed by a migration of 1 to several years in the ocean for feeding and growth, and a return migration to fresh water for spawning (Klemetsen et al., 2003; Thorstad et al., 2011). Individual salmon return to spawn in their home river, and often even to the same part of the river where they were hatched. This has enabled the formation of genetically distinct populations, adapted to the local conditions among and within catchments (Garcia de Leaniz et al., 2007). As a result, guidelines agreed within NASCO state that management targets for the species should be set for each river, and that all stocks should be maintained above their conservation limits (NASCO, 1998). Conservation limits are defined as the stock level, in terms of number of spawners, that will achieve long-term average maximum sustainable yield (International Council for the Exploration of the Sea (ICES), 2020). Conservation limits can be developed at the tributary or river level, or at the level of a stock complex.

The Atlantic salmon has declined in large parts of its distribution during the last few decades, which has reduced or eliminated harvestable surpluses for fisheries, and in many extreme cases has severely reduced population abundance or resulted in extirpations (NASCO, 2019; ICES, 2020; Norwegian Scientific Advisory Committee for Atlantic Salmon Management, 2020). Human activities in catchments and near-coastal areas are generally well known and have directly contributed to population extirpations or declines. Among the many threats are impacts of hydropower production, other migration barriers (e.g. weirs and culverts), habitat alterations in rivers, multiple stressors from Atlantic salmon farming (e.g. escaped farmed domestic salmon, sea lice and transfer of other disease pathogens), invasive species, pollution, fisheries, and reduced water quality (Forseth et al., 2017; ICES, 2020). Some of these threats act at regional or larger scales. When political and social will are coupled with adequate resources, managers often have the knowledge and tools to mitigate many of the threats to wild salmon.

In the ocean, management actions for salmon have focused historically on controlling and reducing all fisheries to sustainable levels and eliminating fisheries where sustainable harvesting is not possible. However, a recently recognized and ill-defined compounding threat to Atlantic salmon is the multiple effects of climate change on the aquatic environments and ecological functioning of salmon. Multiple stressors are already having a major impact on Atlantic salmon productivity as evidenced by reduced survival during its marine migration (ICES, 2020; Olmos et al., 2020). Marine survival may even decline further as the effects of climate change become more pronounced. Climate change is also likely to have adverse impacts on the freshwater environment of salmon, as temperatures warm and precipitation patterns change. Consequently, mitigation and conservation actions based solely on controlling harvests will not be sufficient to reverse declines in Atlantic salmon populations.

So how can managers conserve, protect and enhance wild Atlantic salmon populations at local, regional, and international levels in the face of these overwhelming challenges? The aim of this article is to give an overview of current stressors on Atlantic salmon populations, including those brought by climate change and altered ocean ecosystems, discuss the challenges faced in addressing them, and provide our vision of how local, regional, national, and international managers can best use available scientific knowledge to address these challenges. There is debate at present in the conservation community about what the best focus should be to address the restoration of Atlantic salmon. We present here the case for concentrating on fresh water and the nearshore coastal zone.

2 | THE FRESHWATER PHASE OF THE ATLANTIC SALMON

Anadromous Atlantic salmon spawn in rivers from September to February (Thorstad et al., 2011). During spawning, the females dig and deposit their eggs in one or more redds in the gravel. The eggs hatch in the following spring. The juveniles (parr) remain in fresh water for 1–8 years, before they migrate to sea for feeding. When they migrate to the sea for the first time, they are termed smolts; these are only 10–20 cm long, so the bulk of growth occurs in the sea.

River-specific productivity is largely determined by water discharge and the quality, quantity, and distribution of suitable spawning and shelter habitat for the juveniles (Finstad et al., 2007; Finstad et al., 2010; Foldvik et al., 2017); hence, these are key factors to be considered in stream restoration and habitat classification. Habitat quality and quantity can be reduced by human activities such as water regulation, channelization, flood control, intensive agriculture, forestry, gravel extraction, and other activities causing substrate

removal or sedimentation. In addition, hydropower projects can alter the extent of wetted area and thermal regimes in rivers, which in turn may alter fish physiology, growth, and timing of important life history events such as hatching, emergence, smoltification, and smolt migration (McCormick et al., 1998; Finstad, Armstrong & Nislow, 2011; Enders & Boisclair, 2016; Harvey et al., 2020). Hydropower dams, weirs, and other migration barriers can affect the distribution both of juveniles and adult spawners in the river, and severely increase the mortality of downstream migrants (McCormick et al., 1998; Thorstad et al., 2012). There are also a host of damaging impacts associated with acid precipitation and a wide range of other freshwater contaminants derived from intensive agriculture, industry, and other human activities, which also affect Atlantic salmon in their freshwater phase (Rosseland & Kroglund, 2011).

3 | THE MARINE PHASE OF THE ATLANTIC SALMON

The marine feeding areas of Atlantic salmon cover large swathes of the North Atlantic Ocean, and marine survival rates have been shown to vary across time and space, declining considerably over the last 3 decades (ICES, 2020). Marine mortality rates have a large influence on the number of adult salmon returning to spawn (Nieland, Sheehan & Saunders, 2015). The lack of evidence for compensatory mortality in the marine environment (Milner et al., 2003; Einum & Nislow, 2011) means that increasing the number of smolts migrating to the ocean will not affect the marine survival of that cohort. Hence, any increase in the smolt output from a river is assumed to translate directly into an increase in the number of returning adults, assuming other factors influencing natural mortality in the ocean remain constant. It is hypothesized that early marine phase Atlantic salmon experience higher mortality rates than later phase migrants, but, for multi-sea-winter stocks, mortality during the second year can also be high (Chaput, 2012).

It is important to note that marine survival rates, from the point when salmon smolts leave the rivers until they return, are influenced by the condition and quality of the smolts when they leave fresh water (Russell et al., 2012), by human activities in coastal areas such as aquaculture (Thorstad et al., 2015), and by climate and other ecosystem changes in the sea (Beaugrand & Reid, 2012; Mills et al., 2013). Hence, there are human activities other than harvest controls that can be altered to help reduce marine mortality, indirectly by increasing freshwater survival to increase the number of smolts that reach the sea, and directly by reducing marine mortality caused by human activities in the coastal areas. Other natural resource managers and government sectors need to be engaged in salmon management to minimize the impact of human activities in rivers and coastal areas. However, mitigating marine mortality arising from climate and ecosystem stressors remains, for now, an intractable problem owing to the incomplete understanding of the marine phase of the salmon, the size of the habitat, and the complexity of the changing ecosystem.

Poor ocean conditions leading to reduced survival in the marine phase lowers the resilience of salmon populations to other human impacts. For example, sea lice spread from salmon farms has been shown to reduce growth of wild salmon during the first months at sea. This synergistic effect is particularly strong in years when general ocean survival is low (Vollset, Barlaup & Friedland, 2019). Interaction effects like this underline the importance of reducing human impacts as a strategy to conserve salmon populations in the face of low marine survival and a changing climate.

An incomplete understanding of the causes of ocean mortality is one of the biggest problems faced in predicting the long-term future of Atlantic salmon and in forecasting abundances for management use. Marine mortality is difficult to monitor given the large expanse of the marine range of the species and because dead fish disappear, rendering marine mortality largely invisible. Marine mortality may also result from cross-over effects, where the impacts of a stressor applied in one environment do not emerge until the fish has entered the new environment. For instance, pollution in fresh water may result in mortality after the salmon smolts have entered the sea, and mortality from sea lice acquired in coastal areas may not occur before the sea lice have developed to adult stages and the post-smolts have entered the open ocean. Disentangling the various components that contribute to overall mortality is difficult. As an example, a common technique for estimating marine mortality is to compare the number of out-migrating smolts with the number of adult returns; however, this estimate may unintentionally contain a portion of freshwater and estuarine mortality (Flávio et al., 2020). Disentangling the contributing mortality components will allow a more accurate estimate of the mortality attributable to the marine environment (Stevens, Sheehan & Kocik, 2019). Moreover, when data are collected from returning fish (e.g. from scale samples of adult salmon after they have returned from the ocean), only the surviving fish are studied, which needs to be accounted for when interpreting results.

Predation by mammals and fishes can be one source of marine mortality (Strøm et al., 2019). With declining Atlantic salmon populations, mortality from predation seems to be increasingly in focus. However, conclusions on marine mortality based on studies of predation should be drawn with care. Predation is a natural phenomenon and just because it is documented, it does not mean that it is a driver of current salmon population declines. Also, simply showing that a post-smolt is eaten by a predator does not necessarily mean that the fish would have survived if it had not been preyed upon. Predation often preferentially occurs on individuals that have been weakened by other stressors, and even in the absence of predation these individuals may have eventually died (Thorstad et al., 2013). For instance, a post-smolt with a deadly infestation of sea lice is likely to be eaten by a predator before it dies from the sea lice. This fish would have eventually died from the lice infestation even in the absence of predators. Predation impacts are primarily of concern when predation rates in the ocean are increased above

natural levels as a result of the variety of human-induced impacts, and when salmon populations are reduced because of other impacts and are close to critical lower limits.

In recent decades, reduced survival of Atlantic salmon during the feeding migration could be a cyclical phenomenon, and salmon productivity could increase again; however, human-induced climate change has been implicated as a cause. As temperatures continue to increase over the next century, the outlook for Atlantic salmon in the North Atlantic will result in significant challenges for managers to maintain all stocks above their conservation limits.

4 | ATLANTIC SALMON IN A CHANGING CLIMATE

4.1 | Climate alteration is changing ecosystems inhabited by salmon

Warming and its cascading effects in all ecosystems and habitats have put wild salmonids under pressure, which render them more vulnerable to other stressors. The greatest impacts experienced by Atlantic salmon are in the southern part of its range. At present, the northern populations have more scope for acclimatization, because temperature increases are not expected to force physiological status towards or beyond the species' upper thermal tolerance (Anttila et al., 2014).

Climate change is having a major impact on Atlantic salmon in fresh water and at sea, directly through changes in temperature, water flow, and other abiotic factors, and indirectly through ecosystem changes such as food availability and altered predator–prey dynamics. Under future climate scenarios, higher temperatures and increased hydrological variability are predicted to affect all components of freshwater systems (Schneider et al., 2013; Knouft & Ficklin, 2017). Precipitation is expected to increase in the Northern Hemisphere, with wet areas typically becoming wetter, but with increased variability such that the risk and intensity of floods and droughts will increase (Schneider et al., 2013). In northern Europe and North America, the climate is projected to have warmer, drier summers and milder, wetter winters with more precipitation falling as rain and less as snow, a decrease in ice-covered periods, and more frequent periods with extreme weather events (Intergovernmental Panel on Climate Change (IPCC), 2014; Hoegh-Guldberg et al., 2018; IPCC, 2018). Periods of extreme low water levels during summer and higher water temperatures must, therefore, be expected for many rivers. In addition, expected flash flooding events may lead to significant habitat damage and alteration of river beds. Marine ecosystems are also expected to continue to change. With rising ocean temperatures and acidity there will be concurrent shifts in circulation, stratification, nutrient input, and oxygen content, with potentially wide-ranging effects on ocean productivity, food web dynamics, and other ecosystem processes (Hoegh-Guldberg & Bruno, 2010; Doney et al., 2012).

4.2 | Impacts on Atlantic salmon

Scientists are projecting that conditions that foster healthy Atlantic salmon populations will deteriorate, both in fresh water and at sea, as a result of the ecosystem changes brought about by climate heating. The vulnerability of salmon to a rapidly warming environment is a known concern but with some uncertainty as to how well the species will be able to adapt. Wild Atlantic salmon populations from rivers in Europe have displayed similar plasticity in physiology and acclimation capacities in response to acute warming despite their different acclimation history in the wild (Anttila et al., 2014). This indicates that these populations have the capacity to acclimatize to increasing water temperatures up to their upper lethal limit. Although Atlantic salmon have some capacity to respond and potentially adapt to variations in environmental conditions (Garcia de Leaniz et al., 2007), there are limits to these capacities, especially over short time periods. Further research is needed to understand the extent by which individual populations can adapt to increasing temperatures, especially as annual average temperatures approach, and in some cases exceed, lethal upper limits.

4.2.1 | Hydrology

The predicted changes to river hydrology are likely to influence the population dynamics of Atlantic salmon (Jonsson & Jonsson, 2009; Hedger et al., 2013; Sundt-Hansen et al., 2018). The average annual water flow in many regions is expected to increase, but the flow pattern will tend towards the extremes with high flows in the autumn and winter and very low flows during the summer. Therefore, the wetted habitat area available for juveniles will vary greatly during the course of the year. Future periods of low river flow and high temperatures during summers may, therefore, be a potential bottleneck for Atlantic salmon production and survival in some areas.

4.2.2 | Temperature

Migratory fishes are particularly vulnerable to warming environments as the transitions between habitats are finely tuned to specific environmental cues (Crozier et al., 2008). The success of these transition periods has consequences for subsequent survival. Salmon are ectotherms and, as such, water temperature directly controls their physiology and metabolism. During spawning, eggs are laid in the gravel, and the timing of hatching and the rate at which fry consume the nutrients from the yolk sack before emerging is controlled by water temperature (Crisp, 1981; Jensen, Johnsen & Saksgård, 1989). With increased water temperatures, this process will be more rapid, leading to earlier fry emergence and possibly to a disconnect between the timing of fry emergence and food availability. When temperatures increase, the growth of juvenile salmon in the river will generally increase, and the juveniles may reach smolt size earlier. Studies have

shown that smolt age has decreased in the past decades, as water temperatures have increased (ICES, 2009; Russell et al., 2012).

Water temperatures in many rivers are expected to periodically exceed the upper thermal tolerance limit for salmonids, and during the summer many populations are already encountering water temperatures near or exceeding laboratory-derived lethal limits. Salmon, like other vertebrates, are most sensitive to high temperatures at the embryonic stage (16°C, Jonsson & Jonsson, 2009). For juvenile salmon in the river, growth is optimal from 16 to 20°C, and stops when the temperature approaches 23°C (Garside, 1973; Elliott, 1991; Jonsson & Jonsson, 2009). The incipient lethal temperature limit is estimated at 27.8°C and absolute mortality occurs at 33°C (Elliott, 1991; Jonsson & Jonsson, 2009). Although the lethal temperature for adult Atlantic salmon is expected to be lower than for juveniles (Breau, Cunjak & Peake, 2011), this information has not yet been published.

Warmer river temperatures earlier in spring appear to have influenced the timing of migration, with smolts migrating to the ocean earlier in the year (Otero et al., 2014). There is concern that the changed environmental conditions in the ocean are creating a mismatch between timing of smolt sea entry and favourable conditions at sea (Kennedy & Crozier, 2010; Hawkes, Sheehan & Stich, 2017).

Energy depletion at high temperatures before spawning has been shown to be greater in small than in large salmon, suggesting that smaller individuals may be more affected by high temperatures (Lennox et al., 2018). If so, long-term phenotypic change may be expected in salmon populations experiencing high temperatures. Exposure of female salmon to elevated water temperature prior to reproduction may also have detrimental effects on egg maturation, fertility, and survival (Pankhurst & King, 2010; Pankhurst et al., 2011).

Historically, research on climate effects on salmon in fresh water has focused on factors such as the changes in water temperature and flow, whereas research in the marine phase has examined temperature correlations with growth. Marine ecosystems have altered in response to climate change, which has influenced the food supply for Atlantic salmon in the marine phase (Beaugrand & Reid, 2012; Mills et al., 2013; Renkawitz et al., 2015). The spatial distribution of food and high-productivity areas will probably change, which may affect the ocean migration routes, distributions and marine survival of Atlantic salmon.

4.2.3 | Management options in freshwater

Changes in river hydrology, river temperatures and the marine environment due to climate change will radically alter the various habitats and environments on which Atlantic salmon rely. Some practical management options more directly related to the altered hydrology and water temperatures in the rivers are discussed here.

In freshwater areas where salmon encounter high water temperatures, suitable cold-water refuges can sometimes be found (Jackson et al., 2018; Jackson et al., 2020). The spatial heterogeneity of water temperatures in streams provides potential relief during warm water conditions if the animals can move to these cooler refugia. Atlantic salmon, as other salmonids, are known to thermoregulate behaviourally to maintain a body temperature close to optimal levels (Breau, Cunjak & Bremset, 2007) to minimize energetic costs associated with high temperature (Breau, Cunjak & Peake, 2011). Managers should prioritize promoting, protecting, and restoring cold-water refuges and habitat heterogeneity as it provides a range of thermal conditions for fish to select from based on their specific requirements. Managers should also prioritize maintaining and improving access to cold-water refuges, often located in headwater reaches, as these areas may be the most climate-resilient habitats within a catchment. This can be particularly challenging for salmon populations in rivers where there are problems with connectivity. It is expected that access to cold-water refuges will become increasingly important as more rivers experience extreme temperature events. A better understanding of habitat characteristics forming optimal cool water refuges is warranted if managers are to create artificial refuges. Removing dams, weirs, and other migration barriers, or constructing and improving road crossings and fishways to facilitate free movement of fish will improve river connectivity and access to cold-water refugia and the varied habitats that salmon need to survive. Removal of barriers also often facilitates increased production of salmon juveniles, by creating rearing habitats with faster flow.

The protection and restoration of native riparian shading and healthy forest cover are tangible local management actions that could mitigate some of the adverse effects of climate change. Riparian edges lower the water temperature of streams (Broadmeadow et al., 2011) and help to maintain cool water temperatures in thermal refuges (Breau, Cunjak & Bremset, 2007), as well as restoring access to the higher reaches, which are typically colder. Healthy forest cover composed of biodiverse native vegetation will also support a high abundance of good quality prey for salmon juveniles. Healthy forests throughout river catchments reduce problems related to flash flooding. These measures improve the overall river habitat for the benefit of all aquatic organisms, including salmon. This is particularly important for the southern populations that are already experiencing critically elevated water temperatures.

Hydropower production and other types of river regulation often have severe impacts on Atlantic salmon populations. In some instances, however, particularly when water is obtained from reservoirs, managers can try to ensure that the regulation scheme is adapted to counteract the impacts of climate change by applying water release strategies to avoid periods of extreme low flows and high water temperatures. Release of water from reservoirs also has the potential to regulate water temperatures, as taking water from various depths within the reservoir can influence the water temperature of downstream river stretches.

Catch-and-release fisheries have become common in many Atlantic salmon rivers as the populations have declined. There are differences among countries, regions and rivers in how catch-and-release angling is viewed and used by managers. Where catch-and-release is practised, it is important to recognize that the adverse

impacts and mortality of released fish increase at high water temperatures (Lennox et al., 2017; Van Leeuwen et al., 2020), and to prohibit catch-and-release angling when water temperatures exceed temperature thresholds. Where the fishing regulations are based on mandatory release of all fish or some groups of fish (e.g. females, large fish), this may imply that all angling for Atlantic salmon is stopped above these temperatures. One option is to establish river-specific environmental thresholds (i.e. water temperature, flow and oxygen level) at which closures of recreational Atlantic salmon fisheries should occur (Breau & Caissie, 2012). A strategy that is used in New Brunswick, Canada, is to close identified 'cold water pools' to angling when water temperatures increase above 20°C for two consecutive days. This is to protect salmon when they congregate in the cold-water areas. Enforcement might be increased, if necessary, in cold-water refugia to discourage poaching when fish congregate in these areas. Better education of anglers on the relationship between water temperature and mortality, and appropriate catch-and-release techniques, might also be important measures.

4.3 | Other stressors caused by human activities reduce resilience to climate effects

As the ecosystems and habitats of Atlantic salmon change because of the effects of altered climate, there are cascading effects and negative feedback loops that are only now being identified. Some human activities will amplify the stress caused by climate change and reduce the resilience of salmon and their ability to adapt to changing environments. Known stressors of high concern in relation to climate alteration, and possible management options linked to are described in 4.3.1–4.3.6.

4.3.1 | Escaped farmed salmon and sea lice

Farmed salmon are genetically different from their wild conspecifics and less adapted to the natural environment, particularly in a rapidly changing natural environment (Karlsson et al., 2016; Glover et al., 2017). Genetic introgression of escaped farmed salmon represents an existential threat to the viability of many wild salmon populations. For instance, in Norway there were indications of genetic introgression from escaped farmed salmon in the wild population in two-thirds of the screened rivers (150 of 225 rivers), of which 67 populations were severely affected (30% of the screened populations) (Diserud et al., 2019). Similar results have been demonstrated from eastern Canada (Wringe et al., 2018). Wild salmon populations suffer a loss of local adaptation when they interbreed with farmed salmon owing to the introgression of maladapted genotypes and life history traits (McGinnity et al., 2003; Bolstad et al., 2017). Genetic introgression of farmed salmon imposes an extra impediment to the natural process of adaptation and may reduce the ability of Atlantic salmon to adapt to rapid environmental changes. Beyond genetic introgression, sea lice from salmon farms can increase mortality substantially and reduce Atlantic salmon population sizes (Thorstad et al., 2015), which contribute to reducing population resilience to climate change.

There is a need to develop fish farm technologies and approaches for eliminating escapes, sea lice and other disease pathogens from farms that are influencing wild Atlantic salmon. This can be done by developing closed containment technologies for sea-based farms, using sterilized fish to avoid genetic introgression, and developing land-based technologies. Using sterilized fish will not by themselves solve all the problems related to escapes from farms, because farmed salmon also affect wild salmon through interference competition (Robertsen et al., 2019), and sterilized fish will not solve problems with sea lice and disease pathogens. Therefore, closed containment technologies in the sea or on land are needed. Beyond the technical challenges of conducting intensive and large-scale Atlantic salmon production on land, these operations require much space and potential conflicts may arise with other uses of these areas. Most salmon-producing countries have agreed, through NASCO, to the goals of eliminating escapes and the impacts of sea lice (NASCO, 2006), and there are several emerging technologies and approaches to address these issues; however, a lack of strong political will is preventing these goals from being realized.

4.3.2 | Habitat alteration

Atlantic salmon populations are sensitive to freshwater habitat loss and alteration from a range of human activities, including hydropower production, damming, intensive agriculture, river and flood control near towns and cities, transport, and forestry. Poorly executed land-use practices can result in reduced productive area, substrate quality and prey abundance, and can also have many other detrimental impacts on Atlantic salmon. Habitat alterations can interact with the effects of climate change through direct impacts on population size and indirect impacts through altered flow rates and thermal regimes. Any changes to salmon habitat that reduce population size or smolt quality have the potential to exacerbate changes caused by climate change and further erode population resilience.

Management options related to habitat alteration are diverse and often river specific. Examples of potential management actions to correct habitat alterations are removal of dams and other barriers, building and improving fish passes, liming to address low pH, substrate restoration, afforestation and forest tending, pollution control, repairing and replacing culverts, and creating settling ponds to capture sediment from agricultural lands. There is significant potential for improvements in many salmon rivers related to hydropower development and other habitat alterations that have yet to be explored and employed. Managers are encouraged to follow a process-based approach to river restoration aimed at addressing the root causes of habitat degradation in a sustainable manner (Beechie et al., 2010).

4.3.3 | Pathogen diseases

Elevated temperatures may increase the virulence of several disease pathogens in fishes (Marcogliese, 2001). Atlantic salmon will experience temperatures that are outside their optimal range, which may affect immunological and physiological functions necessary to combat diseases. Wild Atlantic salmon may be adversely affected by climate-induced effects of pathogens (Johnsen & Jensen, 2005; Sterud et al., 2007), both in their natural habitats and by pathogen transmission from salmon farms. Understanding and reducing the spread of pathogens from farms to wild fish is highly important. Preserving genetic diversity in wild populations is also essential so that they have the best chance of adapting to new and increased disease challenges as a result of projected climate warming and increased disease outbreaks in aquaculture farms (de Eyto et al., 2007; de Eyto et al., 2011).

4.3.4 | Artificial stocking of natural populations to augment abundance

Stocking is frequently used as an attempt to augment wild populations in response to declines. Although well intentioned, stocking often comes with a range of harmful consequences and may, overall, often be counterproductive (O'Sullivan et al., 2020). Such genetic consequences include reduction of effective population size and loss of genetic variation owing to a disproportionately large contribution of stocked individuals from a low number of broodfish; loss of local adaptation if using non-local broodfish; unintentional domestication selection in the hatchery; and epigenetic effects from being reared in the hatchery instead of in a natural river (Christie et al., 2012; Christie et al., 2016; Hagen et al., 2019). The consequences of poorly planned or inappropriate stocking will reduce the ability of Atlantic salmon to adapt to environmental change (McGinnity et al., 2003). Consideration should be given as to whether stocking is really needed to maintain a population. If a population is self-sustaining, or this goal can be reached through habitat improvements or other management actions, managers should consider these options before issuing permits for stocking. Stocking should be a last resort to preserve endangered populations, after other impacts on the populations have been tackled. If stocking is undertaken, proponents should have the obligation to monitor both its effectiveness and the consequences for wild salmon. General guidelines for stocking include the use only of local wild broodfish; stocking with the earliest possible life stages to minimize the risk of unintentional domestication selection and epigenetic effects; balancing the number of stocked fish with the number of broodfish and the number of naturally reproducing fish; avoiding using broodfish with genetic introgression from escaped farmed salmon; and ensuring that all hatchery-produced fish are traceable so that the effects of stocking can be evaluated (Karlsson et al., 2016; Hagen et al., 2019; Hagen et al., 2020).

4.3.5 | Selective fishing

Selective exploitation of early running fish or certain size groups may induce genetic and phenotypic changes in Atlantic salmon (Consuegra et al., 2005; Harvey et al., 2017), which in turn may reduce genetic variation and the ability of populations to adapt to climate change. Managers should, therefore, evaluate the risk of fishing imposing selective mortality in the different catchments. Potential problems caused by selective fishing can be counteracted by adjusting fishing regulations (timing, gears, etc.), and also by introducing mandatory catch-and-release of certain groups of fish or at certain times of the year.

4.3.6 | Invasive alien species

A range of introduced fishes and other organisms may affect Atlantic salmon as competitors, predators, vectors of new disease pathogens, or as plants that alter aquatic habitats. With increased temperatures, new species may invade rivers inhabited by Atlantic salmon, and some introduced species may increase in abundance. This may lead salmon to face additional competition for resources, increased predation, or other harmful ecological impacts. With the arrival of invasive species, the risks of exposure to new viruses, bacteria, protozoans, and multicellular parasites may also increase. A contemporary example of this stressor is the increased likelihood of the continued expansion and establishment of invasive pink salmon (*Oncorhynchus gorbuscha*) populations in rivers around the Atlantic Ocean (Sandlund et al., 2019; Hindar et al., 2020), given a warming ocean and other impacts of climate change.

Educational information on the damage that invasive organisms can do to native species, including salmon, should be a pillar of management actions to limit intentional releases. Once invasive species are present, they are cause for significant concern owing to the difficulty to control or eradicate them. However, if sufficient effort and resources are applied, progress can be made. For example, large or visible organisms such as invasive plants and fishes (e.g. rainbow trout and pink salmon) can be eliminated or reduced to a point where they pose significantly less threat by intensive fishing or harvesting. Elimination has also been possible for the small invasive parasite *Gyrodactylus salaris*, which nearly eradicated the Atlantic salmon in more than 50 rivers in Norway (Forseth et al., 2017). Eliminating the parasite from more than 40 of these rivers has cost 1 billion NOK, equivalent to about 110 million euros. This example shows that elimination or severe reduction of introduced organisms can sometimes be achieved, but it requires significant resources and political will for it to be accomplished. The education of the public and anglers on the adverse impacts of non-native fish on salmon should be done to limit intentional releases of fish in rivers. Resources and site- and species-specific knowledge are also needed to reduce potential harm to Atlantic salmon and other native species during mitigation.

5 | DISCUSSION

Global responses to reduce carbon emissions, which are beyond the scope of fisheries management, are needed to reduce planetary warming and its impacts. In the interim, we argue that managers must meet the challenges of maintaining and even increasing current wild Atlantic salmon populations by incorporating climate perspectives into decision making. This will demand a holistic view of salmon management and will require working across sectors, governments, and borders to effectively reduce human induced pressures on salmon.

5.1 | Ensuring strong, healthy, and resilient populations

At present, it is not possible to identify and implement direct management actions to counteract salmon declines resulting from climate and ecosystem alteration in the ocean. Given the challenges of managing threats in the ocean, an emphasis should be placed on freshwater ecosystems and salmon health during that period of their life cycle while also minimizing the undesirable impacts from salmon farming in coastal areas.

Fisheries managers, other natural resource and environmental managers and conservation organizations need to promote strong, healthy, and resilient populations of local wild salmonids in rivers and coastal environments. A fundamental strategy to achieve this is to optimize species productivity by ensuring that the greatest number of wild smolts in the best condition enter the ocean. Migration barriers, loss of rearing and spawning habitat, changes in prey base, introduction of non-native species, and poor water quality have contributed to declines and loss of populations in large parts of the range of Atlantic salmon. Improving or maintaining habitat quality, connectivity, ecological functioning, and water quality are front-line defences to mitigate the compounding effects of altered freshwater ecosystems. There is great potential within the Atlantic salmon range for improvements related to water quality, river regulation, migration barriers, refuges (e.g. cold water), and physical river habitats, which can increase production of Atlantic salmon, and improve the quality of juveniles entering the ocean from rivers and coastal areas.

Population-specific conservation limits and management targets that are based on biological reference points have been developed to evaluate attainment of conservation goals (NASCO, 1998). Varying methods to establish conservation limits and assess compliance with these limits and management targets have been developed and used in different countries. Developing conservation limits based on maximizing smolt output from the rivers, and including sufficient levels of uncertainty in the models used to establish the conservation limits and evaluate whether they are attained, will help to ensure that fisheries are not a primary cause for population decline. Research is needed on current conservation limits and the methods used to produce them in order to evaluate whether they provide a robust metric that reflects genetically and demographically healthy populations in the long term.

Maintaining genetic integrity, diversity, and life history variation in Atlantic salmon populations is vital to maximize their ability to adapt to altered environments. Escaped farmed salmon, poorly planned stocking, and all impacts that reduce effective population sizes contribute to undermining the resilience of salmon populations and to their ability to adapt rapidly to climate change. Eliminating interbreeding with escaped farmed salmon, reserving stocking activities to preserve endangered populations after other damaging population impacts have been mitigated, ensuring that genetic integrity and variation is maintained in any stocking programmes, avoiding selective fishing that may alter the genetic variation of populations, and reducing or eliminating other activities that lower effective population sizes are strategies that will maintain the ability of salmon populations to adapt to climate change.

5.2 | Importance of the human dimension

Conservation action (or inaction) is largely an expression of societal values towards wild salmon and their environment. Although most large-scale commercial Atlantic salmon fisheries have been closed or greatly reduced, wild salmon continue to display significant cultural, social, and economic values through indigenous fisheries, recreational fisheries, and tourism. These values, and the people who hold them, are vital for generating and maintaining public, political, and financial support for conservation, protection, and wise management of the resource. Furthermore, much of the practical work of salmon conservation (e.g. habitat restoration) is led by anglers, indigenous communities, and community-based non-governmental organizations, often in partnership with various levels of government. The need for such shared stewardship and meaningful stakeholder engagement is increasingly recognized in government policy (see, e.g. Canada's Wild Atlantic Salmon Conservation Policy (Fisheries and Oceans Canada, 2018). Thus, policies and decisions that disconnect people from wild salmon, or cause them to feel alienated from conservation efforts, can be counter-productive to long-term conservation goals. As salmon populations decline, conservationists and managers are faced with the very real challenge of finding solutions that balance the need to maintain or enhance the value of wild salmon to society against the need to meet biological targets. Simple or obvious solutions – such as the complete closure of indigenous or angling fisheries – may sometimes be less optimal than innovative solutions that allow people to maintain some level of resource use while aiming to increase community stewardship and engagement in conservation efforts to address the root causes of population decline. Developing such solutions will require knowledge of and ability to work with a diverse range of stakeholders.

Most or all of the problems facing wild salmon result, directly or indirectly, from human activities. In many cases, existing scientific knowledge of these issues is sufficient to develop solutions, but the inability to implement such solutions in a timely and effective manner

is often hampered by social–political–economic factors (Aas et al., 2018). These include conflict of interest, lack of consensus, mistrust of science and of other stakeholders, diversity of environmental values and ethics, ineffective governance, failure to consider alternative perspectives (e.g. indigenous perspectives), and difficulties in motivating governments, communities, and individuals to take appropriate action. These challenges lie in the realm of communications, education, community engagement, conflict resolution, consensus building, organizational behaviours, and agency culture. Conservationists and managers must, by necessity, expand their capacity to meet these non-biological challenges.

Thus, restoration and conservation of wild Atlantic salmon require attention to the human dimensions from the perspective both of science (i.e. understanding human values, attitudes, and behaviours) and management (i.e. applying a knowledge of human dimensions to develop and implement solutions). Managers, scientists, conservation organizations, and governments must recognize that people are a critical element and need to be deeply involved in the conservation process (Bennett et al., 2017), and that human dimensions must be integral in efforts to conserve, restore and enhance Atlantic salmon populations.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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ORIGINAL ARTICLE

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Selection against individuals from genetic introgression of escaped farmed salmon in a natural population of Atlantic salmon

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Abstract

The viability of wild Atlantic salmon populations is threatened by genetic introgression from escaped farmed salmon. Farmed Atlantic salmon are genetically improved for important commercial traits and a life in captivity but are poorly adapted to the natural environment. The rate of gene flow from escaped farmed to wild salmon depends on their spawning success and on offspring survival at various life stages. We here investigate relative survival of introgressed juvenile Atlantic salmon (parr) in a river in northern Norway. The studied population has experienced genetic introgression from farmed salmon for about four generations (20 years). We followed two cohorts of parr from the year of hatching (0+) to the age of 2 years (2+). Farmed genetic introgression was quantified at the individual level and on a continuous scale using diagnostic SNPs. Population-level genetic introgression decreased from 0+ to 2+ by 64% (2011 cohort) and 37% (2013 cohort). This change was driven by a 70% (2011 cohort) and 49% (2013 cohort) lower survival from age 0+ to 2+ in introgressed parr compared to parr of wild origin. Our observations show that there is natural selection against genetic introgression with a potential cost of lower productivity.

KEYWORDS
aquaculture, Atlantic salmon, farmed salmon, genetic introgression, *Salmo salar*, survival

1 | INTRODUCTION

Domesticated animals that escape from captivity or are released intentionally may hybridize with wild conspecifics, leading to unidirectional gene flow into wild populations. Examples of genetic introgression from domesticated animals into wild populations include mammals (Anderson et al., 2019; Kidd et al., 2009), birds (Brisbin & Peterson, 2007; Wu et al., 2020), fish (Letourneau et al., 2018) and insects (Seabra et al., 2019). Genetic introgression from domesticated animals alters the gene pool of wild populations and may constrain their viability and evolutionary potential (Glover et al., 2017; Naylor et al., 2005). Farmed domesticated animals are adapted to a captive environment and selected for characteristics that are of commercial importance. The same characteristics may reduce survival and reproductive success in the natural environment (Araki et al., 2007; Bertolotti et al., 2020). Domesticated animals may also originate from a limited set of founder populations and from a geographical range that does not reflect the genetic diversity of the species (Hindar et al., 1991). Reduced genetic diversity and non-native origin are also commonly found in captive-bred animals that

are intentionally released into the environment for the purpose of stocking wild populations (Kitada, 2018; Letourneau et al., 2018). Due to domestication selection and the origin of founder populations, genetic introgression from escaped farmed animals and intentionally released domesticated animals is expected to reduce genetic diversity and to interfere with local adaption of wild populations.

The fast-growing aquaculture industry commonly involves farming of fish species outside their natural distribution and farming of highly domesticated fish species (Bostock et al., 2010; Naylor et al., 2001). Escaped farmed fish threaten native species through the introduction of invasive species and through hybridization between domesticated individuals and wild conspecifics (Araki & Schmid, 2010). Among the best-documented examples of genetic introgression from farmed domesticated fish into wild populations is Atlantic salmon (*Salmo salar*) (Forseth et al., 2017; Glover et al., 2017; Karlsson et al., 2016; Wringe et al., 2018). Farmed Atlantic salmon in Norway originate from several wild founder populations from western Norway and have been selected for traits that are favourable in aquaculture since the 1970s (Gjedrem & Baranski, 2009; Gjedrem et al., 1991). They hold lower genetic variation compared to wild Atlantic salmon and differ in fundamental life-history traits such as growth and maturation (Bolstad et al., 2017; Glover et al., 2017). Farmed Atlantic salmon kept in aquaculture outnumber their wild conspecifics 1000-fold and escape events occur frequently (Fiske et al., 2006; Glover et al., 2019, 2020).

Escaped farmed Atlantic salmon may enter rivers and hybridize with wild Atlantic salmon, leading to unidirectional gene flow and genetic introgression (Glover et al., 2013; Karlsson et al., 2016). Hybrid and farmed offspring are able to survive to maturity in the wild and to return to freshwater for spawning (Fleming et al., 2000; McGinnity et al., 2003). Genetic introgression from escapees is thereby carried over to future generations and manifested in wild populations (Glover et al., 2013; Karlsson et al., 2016). Such farmed genetic introgression has been found in many geographic regions where wild Atlantic salmon co-occur with Atlantic salmon farming, including Canada, Ireland and Norway (Glover et al., 2017). There was large spatial and temporal variation in the incidence of escaped farmed salmon in rivers across Norway over a 25-year period (1989–2013), with average incidences ranging from ca. 8%–29% across geographical regions and with high incidences during the early 1990s and the early 2000s (Diserud et al., 2019). Hybridization of escaped farmed salmon with wild Atlantic salmon has resulted in an average level of farmed genetic introgression of 6.4% (range 0%–42%) in 109 rivers across Norway (Karlsson et al., 2016). These estimates are from adult salmon sampled after having spent their entire life in the wild. At the juvenile stages, the level of introgression is expected to be higher, but few comparisons exist (Karlsson et al., 2016).

Hybrids of farmed and wild Atlantic salmon are poorly adapted to the natural environment (Bolstad et al., 2017) and show lower survival and reproductive success than wild conspecifics (Fleming et al., 2000; McGinnity et al., 2003). Hybridization may thereby substantially reduce population-level fitness of wild Atlantic salmon populations. At the same time, reduced survival and reproductive success of hybrids may limit genetic introgression into wild populations (Glover et al., 2017; Hindar et al., 2006), as found for stocking of brook charr (*Salvelinus fontinalis*) (Letourneau et al., 2018) and for hybridization between native westslope cutthroat trout (*Oncorhynchus clarkii lewisi*) and invasive rainbow trout (*O. mykiss*) (Kovach et al., 2016). Knowledge of survival and reproductive success of farmed hybrids is therefore important for the prediction of both population-level fitness and genetic introgression in wild Atlantic salmon.

The early survival of hybrid Atlantic salmon from eggs to smolt in the wild has previously been studied in field experiments. Farmed and wild Atlantic salmon were allowed to interact and spawn freely in experimental rivers (Fleming et al., 2000), or eggs from crossings were planted into experimental rivers (McGinnity et al., 2003; Skaala et al., 2019). Juveniles sampled at later stages were genetically assigned to farmed and wild parents. Studies that quantified total survival in freshwater from eggs to out-migrating smolt uniformly reported a reduced survival of farmed and hybrid individuals (Fleming et al., 2000; McGinnity et al., 2003; Skaala et al., 2012, 2019). Field experiments that quantified survival from age 0+ to out-migrating smolt found variable survival of farmed and hybrid parr in Ireland (McGinnity et al., 1997, 2003), but little variation between groups in Norway (Fleming et al., 2000). A recent study used diagnostic SNPs to estimate the abundance of farmed and hybrid Atlantic salmon parr in a range of rivers in Canada, after a single large aquaculture escape (Sylvester et al., 2019; Wringe et al., 2018). From age 0+ to 2+, there was a reduction in the relative abundance of farmed parr and hybrids (Sylvester et al., 2019; Wringe et al., 2018). In summary, field studies uniformly reported decreased survival of hybrid and farmed offspring during the entire freshwater stage (eggs to smolt), while the evidence was mixed for parr survival (Fleming et al., 2000; McGinnity et al., 2003; Sylvester et al., 2019).

Earlier field studies on the survival of farmed and hybrid individuals in freshwater have focussed on first-generation offspring of farmed Atlantic salmon and their crossing with wild fish. Genetic introgression from escaped farmed salmon over many generations is expected to result in offspring of various hybrid classes. Field studies in Ireland found reduced parr survival (0+ to out-migrating smolt) for farmed and first-generation hybrids, while second-generation hybrids and second-generation backcrosses between hybrids and farmed or wild fishes had reduced survival at the egg stage but not as parr (McGinnity et al., 2003). Earlier studies also focussed on high proportions of farmed and hybrid offspring (ca. 25%–75%) and on scenarios of single large-scale introgression events (Fleming et al., 2000; McGinnity et al., 2003; Skaala et al., 2019; Wringe et al., 2018). The relative survival of hybrid parr may depend on whether they primarily compete with hybrid parr or with wild parr. Farmed parr show higher levels of aggression than wild parr (Einum & Fleming, 1997), and under constant density, the presence of farmed parr, but not the presence of wild parr, has been found to reduce the survival of wild parr (Robertsen et al., 2019; Sundt-Hansen et al., 2015). Knowledge of the survival of hybrid parr under moderate levels of genetic introgression is also important because the negative effects of genetic introgression on

wild populations may be more severe under low-level introgression over prolonged time than under rare large-scale introgression events (Baskett et al., 2013). The relative survival of farmed and hybrid Atlantic salmon parr has not been studied in rivers that have experienced genetic introgression over prolonged time and under moderate levels of genetic introgression.

Here, we study changes in genetic introgression over time in two cohorts (years of hatching) of Atlantic salmon parr in the River Alta in northern Norway. The Atlantic salmon in River Alta is part of the Barents–White Sea phylogenetic group, while all founder populations of the farmed strains are part of the Eastern Atlantic phylogenetic group (Bourret et al., 2013; Karlsson et al., 2016). Large genetic divergence between native River Alta Atlantic salmon and farmed escapees may increase maladaptation and thereby mortality of introgressed individuals (Baskett et al., 2013; Bolstad et al., 2017; Huisman & Tufto, 2012). Escaped farmed salmon have been recorded in River Alta since the late 1980s (Ugedal et al., 2016). The relative abundances of escaped farmed salmon in catches of adult spawners in the autumn ranged from 0% to 22% between 1991 and 2018 (Ugedal et al., 2016) (Table S1). The overall level of genetic introgression in parr of the studied cohorts was moderate. To study relative survival of introgressed and wild salmon in a natural population, we quantified genetic introgression in parr at the ages of 0+, 1+ and 2+ within two cohorts. We hypothesized that the level of genetic introgression would decrease as the cohort grew older. This study aims at understanding the ability of natural selection to reduce the level of genetic introgression of escaped farmed salmon. The results add new knowledge about the consequences of escaped farmed salmon in wild populations.

2 | METHODS

2.1 | River Alta

River Alta is an Atlantic salmon river in northern Norway (70°N 23°E) with an average discharge of 98.9 m^3/s and an average catch of salmon of 16 tonnes per year (Ugedal et al., 2016). The River Alta has been utilized for hydroelectric generation purposes since 1987 and the outlet of the water tunnel from the power plant is located at the upper end of the salmon producing section, which is limited to the lower 50 km of the 160 km long main stem (Ugedal et al., 2008).

The population level of farmed genetic introgression, measured in adult fish and with the same methods as used in this study (described under Statistical analysis), in River Alta varied between 0% and 5.4% from 2012 to 2016 (Karlsson et al., 2016). This study was conducted in the uppermost section of River Alta, called Sautso (Figure 1), which in many years had a higher proportion of escaped farmed Atlantic salmon than the lower parts of the river (Table S1). Tagging studies have shown that escaped farmed salmon have a higher propensity than wild salmon to migrate to the upper parts of River Alta (Heggberget et al., 1996).

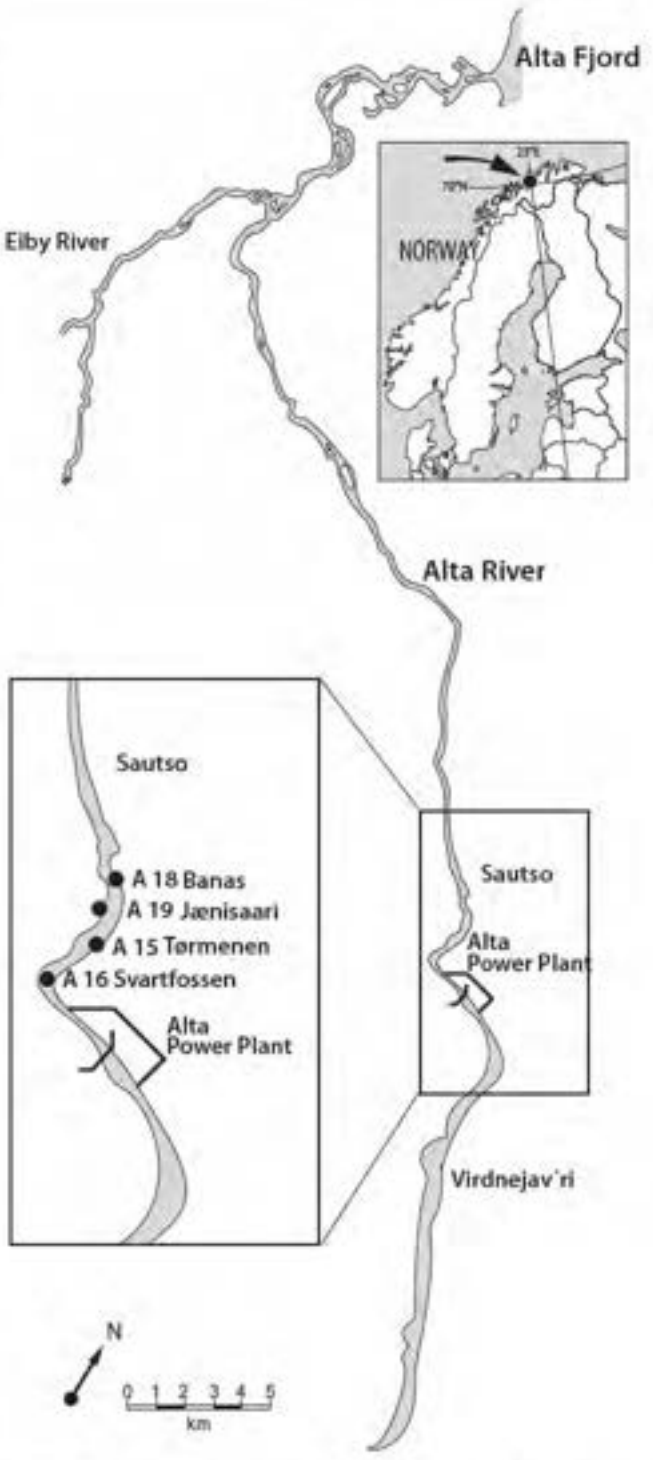

FIGURE 1 River Alta with the anadromous part of the river from the Alta Fjord to the hydropower station (Alta Power Plant). The study was conducted in the uppermost part of the river (Sautso), and samples were collected at four sampling locations (A16, A15, A19 and A18)

2.2 | Sampling of fish

In order to study changes in the level of farmed genetic introgression within cohorts of juvenile Atlantic salmon, fish were sampled at the year of hatching (0+), 1 year after hatching (1+) and 2 years after

hatching (2+). Samples were accordingly collected in 2012, 2013 and 2014 for breeding year 2011 and in 2014, 2015 and 2016 for breeding year 2013. Juveniles were collected by electrofishing at four sampling locations (Figure 1) and stored in ethanol (breeding year 2011: 0+, 1+, 2+; breeding year 2013: 0+) or frozen (breeding year 2013: 1+ and 2+). Juveniles were thereafter measured for fork length to the nearest mm, and measurements were back-calculated to live fork length using previously established relationships (Thorstad et al., 2007). Age of juveniles was determined from readings of scales and otoliths. Sampling took place between August and October, with, in most instances, two sampling days per age class and cohort (Table 1). The habitat at sampling location A19 is not suitable for 2+ parr and only a single and no fish of that age were caught at sampling location A19 for the breeding years 2011 and 2013, respectively (Table 1). We present results for the cohorts from the breeding years 2011 and 2013. For a third cohort (breeding year 2014), juveniles were collected at the ages of 0+ and 1+ and analysed for farmed genetic introgression. The level of genetic introgression in the 2014 cohort was marginal and not statistically significant at the age of 0+ (0.5%) and 1+ (1.5%). The data were therefore not suited to test for a change in the level of genetic introgression with increasing age.

2.3 | Genetic analysis

DNA was extracted from juvenile fish stored in ethanol using the DNEASY tissue kit (QIAGEN) and genotyped at 81 nuclear and 15 mitochondrial SNPs using a EP1™ 96.96 Dynamic array IFCs platform (Fluidigm). Forty-eight of the nuclear SNPs have been identified by Karlsson et al. (2011) as showing large genetic differences between Norwegian farmed and wild salmon regardless of farmed strain and wild population, and these were used for estimating wild and farmed ancestry of individual fish (Karlsson et al., 2014, 2016).

2.4 | Estimating genetic introgression

We estimated genetic introgression with the method described by Karlsson et al. (2014). The method uses the programme STRUCTURE (Pritchard et al., 2000) to estimate the likelihood of an individual to belong to a wild salmon reference sample versus a farmed salmon reference sample. We hereafter refer to this likelihood as *P*(*Wild*) (Karlsson et al., 2014). The wild reference sample is given by historical samples collected before the onset of commercial Atlantic salmon farming. River Alta belongs to the Barents–White Sea phylogenetic group (Bourret et al., 2013) and historical samples from a range of populations belonging to this phylogenetic group were used as wild reference (Karlsson et al., 2014, 2016). Samples from the Norwegian breeding kernels for farmed salmon were used as farmed salmon reference (Karlsson et al., 2014, 2016).

Genetic introgression on the population level (proportion of the genome being of farmed origin) was estimated from individual *P*(*Wild*) estimates. Individual *P*(*wild*) estimates range from zero to one, so wild reference samples will always have an average *P*(*wild*) estimate less than one, while the farmed salmon reference sample has an average *P*(*wild*) estimate larger than zero (Karlsson et al., 2014). When estimating genetic introgression on population level, the scale must therefore be calibrated by the respective average observed *P*(*Wild*) in the wild and farmed reference samples (Karlsson et al., 2014). Historical samples from River Alta collected in 1981 and 1982 (Karlsson et al., 2016) were used as wild reference sample for calibrating the scale of population-level genetic introgression. This procedure ensured unbiased estimation of genetic introgression for River Alta.

The power of quantifying introgression on individual and population level with the above methods has been explored in simulations (Karlsson et al., 2014). On population level, introgression was estimated with high precision; that is, the estimate was close to the simulated proportion of the genome being of farmed origin. On the individual level, introgression is estimated with larger uncertainty and *P*(*wild*) estimates for first-generation hybrids may cover the whole range from 0 to 1 (Karlsson et al., 2014).

2.5 | Statistical analysis

We tested whether population-level genetic introgression was significantly larger than 0 within each cohort and age class. This was done by testing if the observed mean *P*(*Wild*) was smaller than the mean *P*(*Wild*) of the historical sample from the River Alta (Karlsson

TABLE 1 Numbers of juvenile Atlantic salmon from two cohorts (breeding years 2011 and 2013) collected at four sampling locations in River Alta (A15, A16, A18, A19)

	2011			2013		
	0+	1+	2+	0+	1+	2+
A15	23 (0 + 23)	26 (25 + 1)	26 (11 + 15)	24 (24 + 0)	24	46 (18 + 28)
A16	26 (19 + 7)	25 (17 + 8)	24 (12 + 12)	25 (0 + 25)	29	24 (9 + 15)
A18	24 (0 + 24)	26 (26 + 0)	24 (2 + 22)	22 (22 + 0)	24	30 (14 + 16)
A19	22 (8 + 14)	23 (12 + 11)	1 (1 + 0)	22 (22 + 0)	18	0
Total	95 (27 + 68)	100 (80 + 20)	75 (26 + 49)	93 (68 + 25)	95	100 (41 + 59)

Note: Juveniles were sampled at the year of hatching (0+) and at the age of one and two years (1+ and 2+). When sampling of a given cohort and age class took place at two different occasions, numbers of fish sampled at the first and second sampling date, respectively, are given in brackets.

et al., 2016) with a two-sample t test. *P*(*Wild*) estimates (proportion data varying from zero to one) were logit-transformed before testing to achieve that transformed proportions are approximately normally distributed (Karlsson et al., 2014). Tests assumed equal variance of samples (Karlsson et al., 2014, 2016).

A linear model was used to test for a temporal change of genetic introgression within cohorts, that is an effect of age on *P*(*Wild*). The model was fitted with *P*(*Wild*) as response variable and with age (continuous variable) and cohort (factor) as explanatory variables. In graphical exploration, there was no indication for a difference in slopes among the two cohorts and the model was fitted without an interaction between age and cohort. Sampling of juveniles of a given cohort and in a given year was carried out at two dates (Table 1), with 25–70 days in between. Changes in genetic introgression within cohorts may occur over time between years and between sampling dates within years, and age was therefore entered into the model as a continuous variable. Age was included in the model as the number of days counted from August 14 (the earliest date 0+ juveniles were sampled) in the year 0+ samples of the respective cohort were collected. On this scale, the age of 0+ samples was 0–77 days, the age of 1+ samples was 365–395 days, and the age of 2+ samples was 730–800 days. *P*(*Wild*) is a likelihood estimate measured on a scale from zero to one and was logit-transformed prior to analysis and residuals inspected for deviation from normal distribution.

We also tested for a temporal change in the proportion of introgressed individuals within cohorts, classifying individuals as introgressed or wild depending on their *P*(*wild*). In contrast to the above-described analysis of changes in *P*(wild), results from this analysis can be more directly related to earlier studies on the relative survival of introgressed parr, which reported abundances and relative survival of wild and hybrid parr (Fleming et al., 2000; McGinnity et al., 2003; Wringe et al., 2018). We used a generalized linear model with a binomial distribution to test for an effect of age on the likelihood of juveniles to be of wild origin. The response variable in the model was the classification of each juvenile as wild (entered as value one) or introgressed (entered as value zero) and explanatory variables were age (continuous variable) and cohort (factor). In graphical exploration, there was no indication for a difference in slopes among the two cohorts and the model was fitted without an interaction between age and cohort. There was indication for overdispersion of the residuals and the model was therefore fitted with a quasibinomial error distribution.

We classified juveniles as introgressed that had a *P*(*Wild*) below a given threshold. The threshold was based on the *P*(*Wild*) distribution of historical samples of Atlantic salmon from the Barents–White Sea phylogenetic group in Finnmark county, Norway (N = 1000). Those historical samples were collected before the onset of commercial Atlantic salmon farming and did therefore not include introgressed individuals. Using a threshold from the lower end of the *P*(*wild*) distribution of those historical samples ensured that juveniles from River Alta that were classified as introgressed were unlikely to be of pure wild origin. We analysed the data using three alternative lower percentiles of the historical distribution: the 5 percentile (*P*(*Wild*) = 0.8315), the 3 percentile (*P*(*Wild*) = 0.7420) and the 1 percentile (*P*(*Wild*) = 0.5528). Because of moderate levels of genetic introgression in the studied cohorts, a large proportion of juveniles were classified as wild. The number of truly wild juveniles expected to be wrongly classified as introgressed (1%–5% depending on the threshold) was therefore relatively high compared to the number of truly introgressed juveniles and highest when using the 5 percentile threshold. When the 1 percentile was used, few juveniles were classified as introgressed, increasing uncertainty in the statistical estimation of the proportion of introgressed individuals. We therefore present results based on the 3 percentile in the main text. Results for all three considered percentiles are presented in Table S2.

Classification of juveniles into wild and introgressed was also used to calculate survival of introgressed juveniles relative to survival of wild juveniles. Relative survival of introgressed juveniles was calculated for the two cohorts separately and for the entire time period (0+ to 2+ age), as well as for the time periods from 0+ to 1+ and from 1+ to 2+ separately. Relative survival of hybrid juveniles was calculated by dividing the ratio of introgressed to wild individuals in the first sample (e.g. 0+ age) by the ratio of introgressed to wild individuals in the second sample (e.g. 2+ age).

The fact that a substantial proportion of juveniles classified as introgressed was likely truly wild renders our analysis of a temporal change in introgression within cohorts conservative, because potential differences in survival between wild and introgressed juveniles are partly masked. This effect is expected to be stronger under a higher *P*(*Wild*) threshold.

Parr mortality may be related to body size which again can be related to genetic introgression (Fleming et al., 2000; Reed et al., 2015; Solberg et al., 2013). The effect of genetic introgression on growth is a potential route for reduced survival in hybrid parr, and we therefore tested for an effect of *P*(*Wild*) on fork length. The effect was tested with separate linear models for each age class (0+, 1+, 2+). Models were fitted with fork length (continuous variable) and cohort (factor) as explanatory variables. There was no indication for a difference in slopes among the two cohorts and the model was fitted without an interaction between fork length and cohort.

3 | RESULTS

We detected moderate farmed genetic introgression in juvenile Atlantic salmon from River Alta. Estimated population-level genetic introgression ranged from 0.02 to 0.10 within a cohort and age class (Figure 2) and between 3% and 16% of the sampled parr were classified as hybrids (*P*(*Wild*) < 0.7420; Figure 3). Population-level genetic introgression was significantly higher than 0 for all age classes in the 2013 cohort (all $p < 0.001$) and for the 0+ and 1+ age classes in the 2011 cohort (both $p < 0.05$) (Figure 2).

Estimated population-level genetic introgression decreased with the age of parr within cohorts. In the 2011 cohort, genetic introgression (the estimated proportion of the genome being of farmed origin) changed from 0.050 at 0+ age to 0.018 at 2+ age (64% reduction).

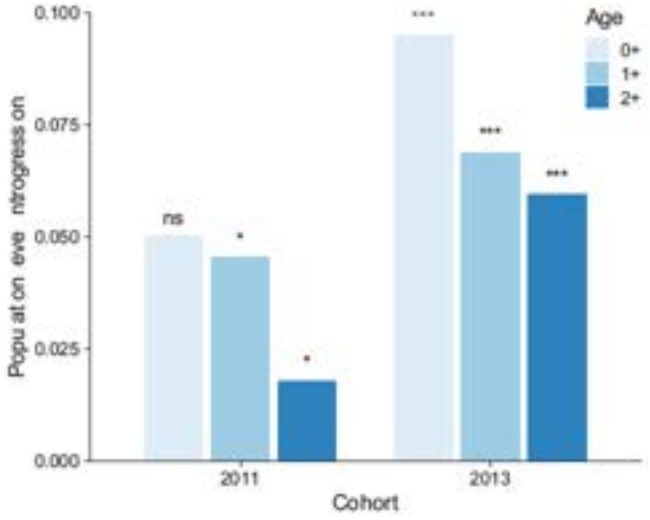

FIGURE 2 Estimated population-level farmed introgression (proportion of the genome being of farmed origin) in juvenile Atlantic salmon from River Alta of two cohorts (2011 and 2013) at the age of 0+ to 2+. Symbols above bars indicate whether introgression was statistically significantly higher than 0 (*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, ns $p > 0.05$)

In the 2013 cohort, genetic introgression changed from 0.095 at 0+ age to 0.059 at 2+ age (37% decrease) (Figure 2). We tested for a temporal change in individual *P*(*Wild*) (the probability of belonging to the wild reference sample) within cohorts and there was a statistically nonsignificant trend for an increase with parr age (slope ±SE: 0.000459 ± 0.000260 logit *P*(*wild*)*day^{-1}; $F = 3.1$, $p = 0.078$; Figure 4a). Overall the level of genetic introgression was higher in the 2013 than in the 2011 cohort, with a lower *P*(*Wild*) (intercept: $F = 11.1$, $p < 0.001$; Figure 4a).

The proportion of juveniles that were classified as wild (*P*(*Wild*) ≥ 0.7420) increased with the age of parr (slope: $\chi^2 = 4.8$, $p = 0.029$; Figure 4b; Table S2). An overall lower proportion of juveniles was classified as wild in the 2011 cohort than in the 2013 cohort (intercept: $\chi^2 = 5.2$, $p = 0.023$; Figure 4b). The estimated temporal increase in the proportion of individuals classified as wild was stronger when a lower *P*(*wild*) threshold was used and weaker when a higher *P*(*wild*) threshold was used (Table S2).

The survival of introgressed juveniles from 0+ to 2+, relative to the survival rate of wild parr, was estimated at 0.30 (0+ to 1+: 0.82; 1+ to 2+: 0.36) and 0.51 (0+ to 1+: 0.55; 1+ to 2+: 0.93) in the 2011 and 2013 cohorts, respectively. The proportion of juveniles

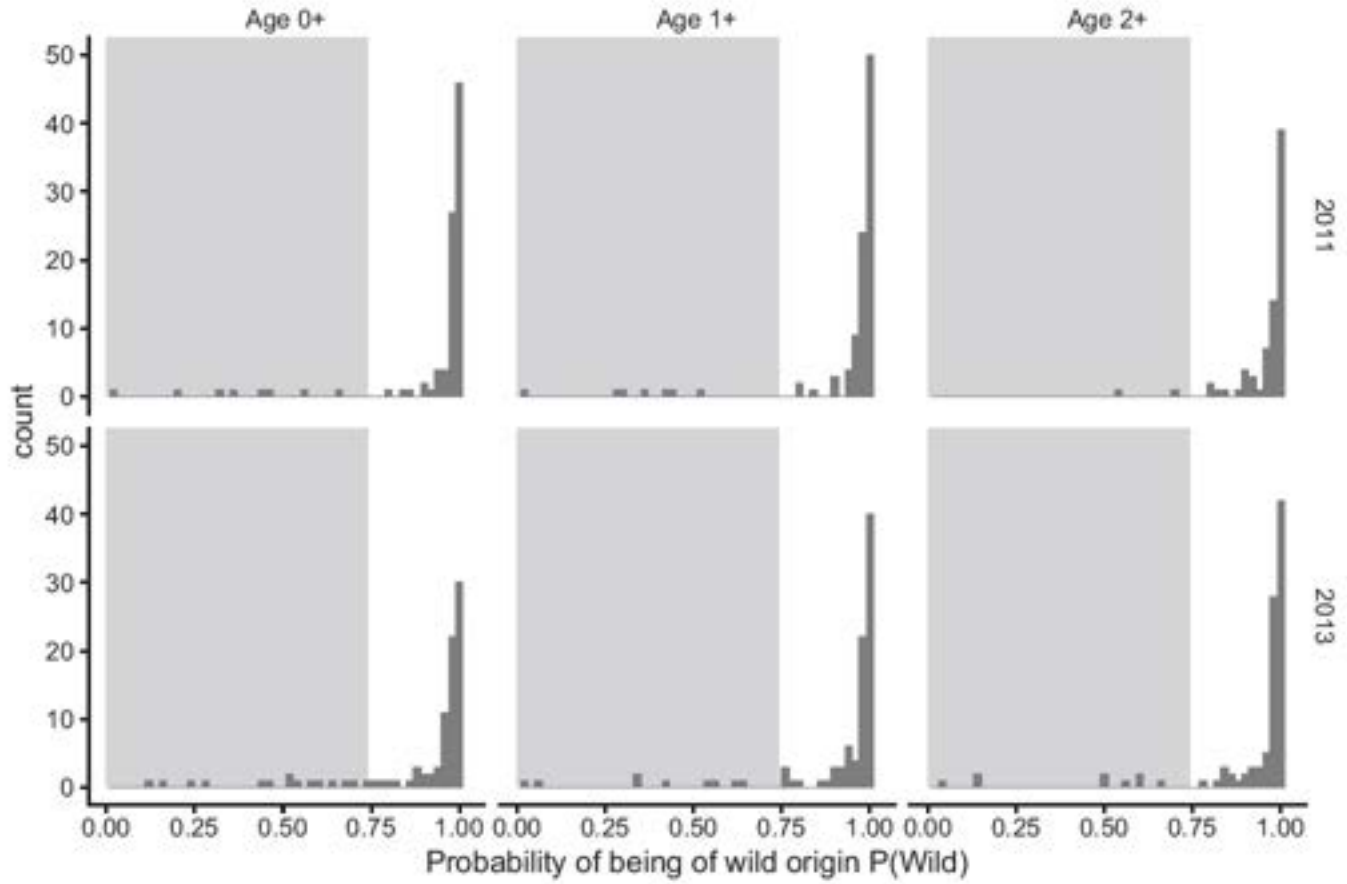

FIGURE 3 Distribution of individual *P*(*Wild*) estimates (probability of belonging to a wild reference sample) in juvenile Atlantic salmon from River Alta of two cohorts (2011 and 2013) sampled at the age of 0+, 1+ and 2+. Shaded areas indicate the range within which individuals were classified as farmed or hybrid offspring based on a *P*(*wild*) threshold of 0.7420

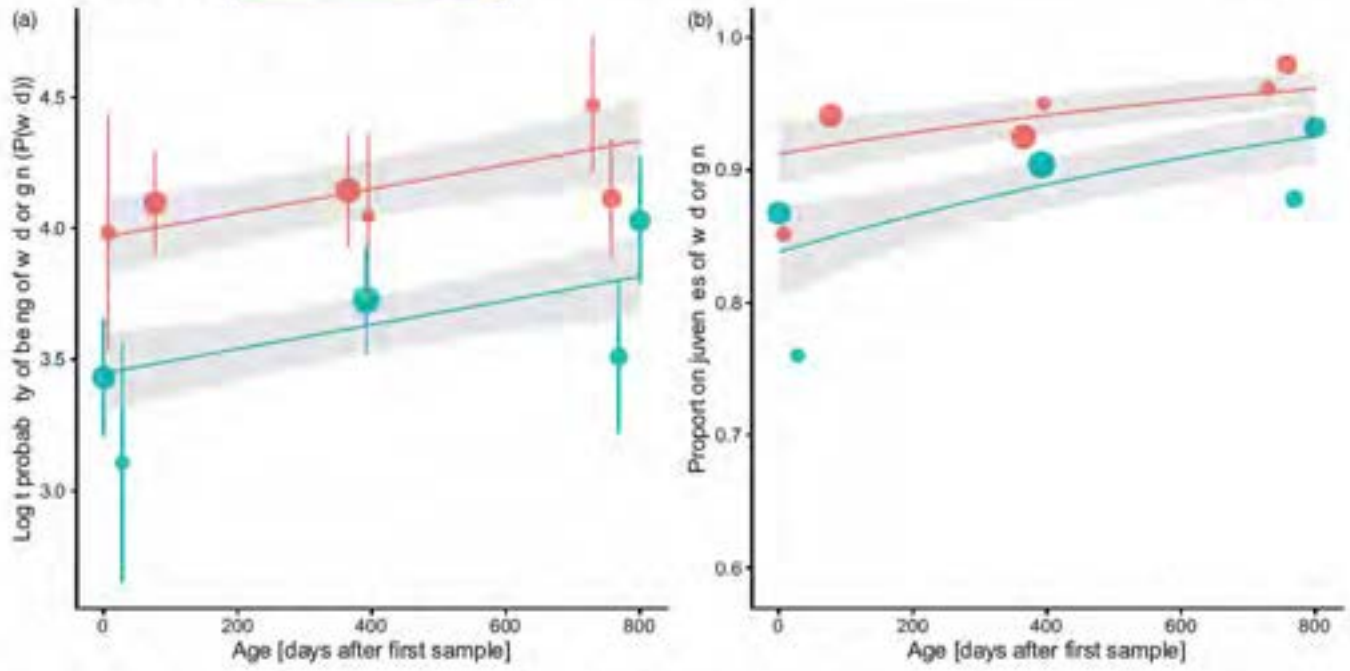

FIGURE 4 Effect of age on genetic introgression in two cohorts (red =2011, green =2013) of juvenile Atlantic salmon from River Alta. Age is given as the number of days after first sampling in the year of hatching for each cohort: 0+ (0–77 days), 1+ (365–395 days) and 2+ (730–800 days). Genetic introgression is given as (a) logit-transformed probability of being of wild origin *P*(*Wild*) and (b) the classification of individuals as being of wild origin or as introgressed, based on their *P*(*Wild*). Points indicate means per sampling date and the size of points indicates sample size (*N* between 20 and 94 individuals). For (a) *P*(*Wild*), bars indicate one standard error. Lines indicate effects estimated in (a) a linear model and (b) a generalized linear model, with shaded areas indicating one standard error

that were classified as introgressed varied widely among sampling localities, but decreased with age in most sampling localities within breeding years (Figure S1).

Parr length was significantly negatively associated with *P*(*Wild*); that is, introgressed parr were larger than wild parr, at the age of 1+ ($F = 5.0$, $p = 0.027$; Figure 5b), and there was a statistically nonsignificant trend for such a relationship at the age of 2+ ($F = 3.6$, $p = 0.06$; Figure 5c), but not at the age of 0+ ($F = 6.7$, $p = 0.55$; Figure 5a). At the age of 1+, there was an estimated decrease of 7.2% in fork length between the fish of lowest *P*(*Wild*) (2011 cohort: 78.3 mm) and the individual of highest *P*(*Wild*) (2011 cohort: 72.7 mm). In the model of length at 1+ age, cohort and logit *P*(*Wild*) together explained approximately 8% of the variation in fork length.

4 | DISCUSSION

Farmed genetic introgression decreased over the first 2 years after hatching in two cohorts of Atlantic salmon. The results show that introgressed parr had a lower survival than wild parr in River Alta. Survival of introgressed parr in the wild has previously been studied in Canada after a major escape event (Wringe et al., 2018) and in field experiments in Norway and Ireland (Fleming et al., 2000; McGinnity et al., 2003; Skaala et al., 2012, 2019). In line with our results, introgressed parr had a lower survival than wild parr in Canada (Sylvester et al., 2019; Wringe et al., 2018). Temporal changes in the relative abundance of introgressed parr differed widely in strength and direction among the thirteen rivers studied, but on average the relative abundance of farmed and hybrid parr was halved from the age of 0+ to 2+ (Sylvester et al., 2019). Reduced survival of farmed parr and first-generation hybrids was also found in Ireland (0+ to out-migrating smolt), after eggs from crossings of farmed and wild Atlantic salmon were planted into an experimental river (McGinnity et al., 1997, 2003; Reed et al., 2015). Fleming et al. (2000) released wild and farmed adult Atlantic salmon into an experimental river in Norway and quantified breeding success and offspring survival. Breeding success and early survival were lower for farmed than for wild fish, but there was no evidence for reduced survival of introgressed offspring in the parr stage (0+ in autumn to out-migrating smolt). Our results add to previous evidence of reduced survival of farmed and hybrid parr from Canada and Ireland.

We found reduced relative survival of introgressed parr in a river with moderate levels of genetic introgression. In the studied cohorts, population-level genetic introgression was only 5%–10% at the age of 0+, with 9%–16% of parr detected as introgressed. Despite moderate levels of genetic introgression, there was a substantial decrease in introgression from 0+ to 2+ (37%–64%) and the relative survival of introgressed parr was 0.30–0.51 across the same time period. There was considerable uncertainty in estimating those changes, as expected when analysing low rates of introgression and low proportions of introgressed parr. With a sample size of approximately 300 individuals per cohort, we detected significant introgression in both cohorts and a significant reduction in introgression with age, but the statistical significance was around the 0.05 acceptance

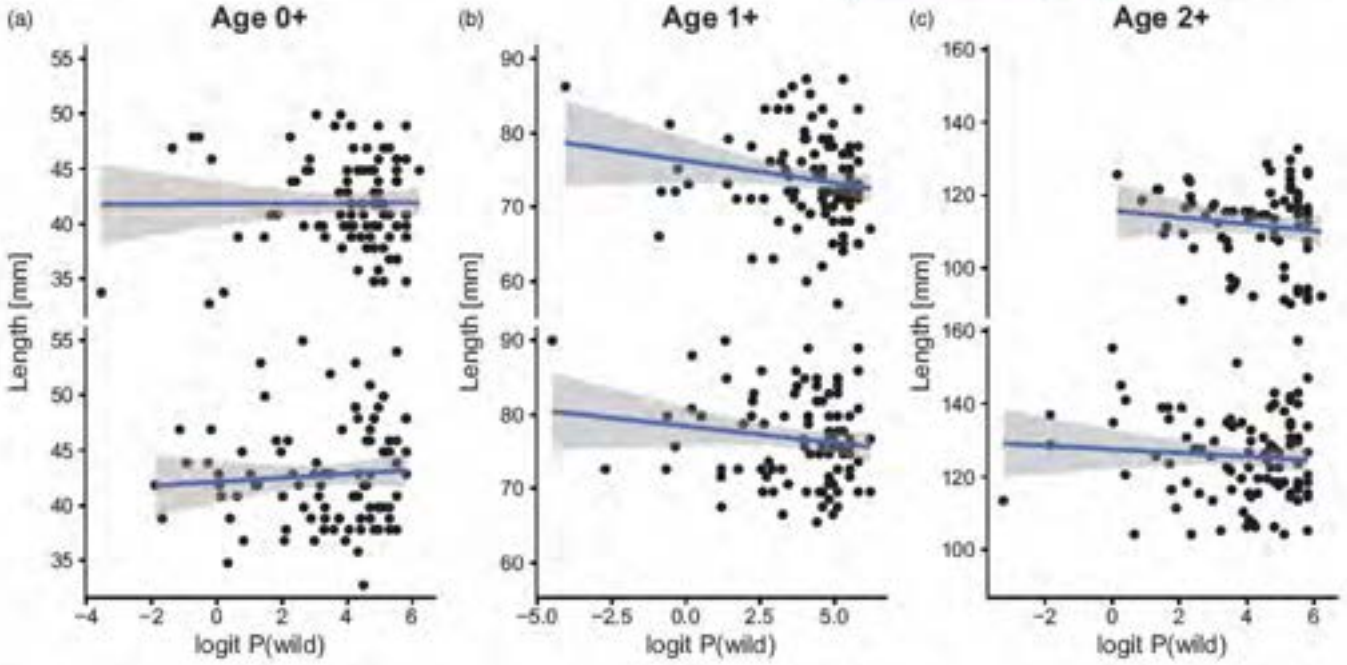

FIGURE 5 Relationship between genetic introgression (logit *P*(*Wild*)) and fork length [mm] in two cohorts (2011 upper panel and 2013 lower panel) of Atlantic salmon parr sampled in River Alta at the age of 0+ (a), 1+ (b) and 2+ (c). Blue lines indicate relationships estimated by linear regression, and grey shades indicate 95% confidence intervals. Note that scales on both axes differ among panels

threshold in several tests. Despite those uncertainties, our study shows that introgressed Atlantic salmon parr show reduced survival under moderate levels of introgression. Reduced survival of introgressed parr under moderate levels of introgression is a finding that complements earlier studies that quantified the survival of hybrid parr under higher relative abundances in field experiments (Fleming et al., 2000: >25%; McGinnity et al., 2003: 75%) and in Canadian rivers (median relative abundance 50%; Wringe et al., 2018). Our results also show that the survival of hybrid parr is reduced in rivers experiencing moderate levels of genetic introgression over prolonged time periods. This result is important for predicting the effect of genetic introgression on the viability of wild populations, which is expected to be stronger under constant low-level genetic introgression than under rare events of strong genetic introgression (Baskett et al., 2013). Together with previous studies, our results show reduced survival of introgressed parr across a range of levels of genetic introgression.

Our study considered introgression resulting after about 20 years of varying abundances of escaped farmed salmon (Table S1) (Diserud et al., 2019). Introgressed parr were therefore expected to belong to various hybrid classes, resulting from backcrosses between farmed, wild, and hybrid parents over several generations. Previous experimental studies followed survival of first-generation (Fleming et al., 2000; McGinnity et al., 1997) or first- and second-generation hybrids (McGinnity et al., 2003). This was also primarily the case in the observational study in Canada, where a large-scale escape event affected previously little introgressed rivers (Sylvester et al., 2019; Wringe et al., 2018). This may affect results because the effects of genetic introgression on survival and fitness vary among the different backcross types, and variation does not necessarily follow an additive manner (Debes et al., 2013; McGinnity et al., 2003; Wringe et al., 2018). McGinnity et al. (2003) found reduced survival for farmed parr and first-generation hybrids, but not for second-generation hybrids or backcrosses, which experienced increased relative mortality only at the egg stage. Our study did not detail survival rates for specific hybrid and backcross types. Instead, our results show that the total level of genetic introgression within cohorts decreased in parr over 2 years in a population that had experienced interbreeding with escaped farmed salmon over several generations.

Variation in the relative survival of introgressed parr may ultimately have a strong effect on the rate of gene flow into wild populations. In models based on experimental studies from Norway and Ireland, 0+ to smolt was the life stage at which differences in survival rates among experiments affected genetic introgression the most (Hindar et al., 2006). The relative survival of introgressed parr in our study was at the lower end of the range considered in those models, implying a reduction in population-level introgression under a given abundance of farmed escapees (Hindar et al., 2006). Relative survival of introgressed parr was also found to largely affect the rate of gene flow in models based on observations made after a large-scale escape of farmed salmon in Canada (Sylvester et al., 2019). Variation in the effects of introgression on survival has also been observed at other life stages, including early development and from smolt to returning adults (McGinnity et al., 2003; Robertsen et al., 2019; Sundt-Hansen et al., 2015). In consequence, the relative contribution of those effects to the relative fitness of introgressed Atlantic salmon varied. Together, those effects are likely to contribute to the elusive factors explaining

the relationship between abundances of escaped farmed Atlantic salmon and resulting genetic introgression across Norwegian rivers (Karlsson et al., 2016).

The negative impact of genetic introgression may not only depend on the rate of gene flow but also on genetic divergence between farmed and wild strains (Baskett et al., 2013; Castellani et al., 2015; Glover et al., 2017; Huisman & Tufto, 2012). Larger genetic divergence is expected to result in larger genetic impact on locally adapted wild populations. At the same time, larger genetic divergence may increase mortality of introgressed Atlantic salmon and thereby slow down the rate of gene flow. The negative impact may therefore be more severe under moderate genetic divergence (Baskett et al., 2013; Huisman & Tufto, 2012). Genetic divergence is largest when farmed strains originate from other phylogenetic groups than the local wild populations belong to. This is the case for Irish populations, but also for wild populations in northern Norway, which are part of the Barents–White Sea phylogenetic group, while all founder populations of the farmed strains are part of the Eastern Atlantic phylogenetic group (Bourret et al., 2013; Karlsson et al., 2016). Genetic introgression has therefore a potentially large genetic impact in River Alta and other populations of the Barents-White Sea phylogenetic group. Bolstad et al. (2017) found a significant effect of genetic introgression on sea-age and size at maturity and that this effect was different, and for some comparisons larger, in the Barents–White Sea phylogenetic group compared to the effects in the Eastern Atlantic phylogenetic group. The high relative mortality of introgressed parr found in this study is in line with the expectation that high genetic divergence leads to more pronounced maladaptation and mortality of introgressed individuals.

Possible mechanisms for the effect of introgression on survival are related to the faster growth rate of introgressed Atlantic salmon parr. Differences in growth rate between farmed and wild parr are substantial under farming conditions (Glover et al., 2009, 2018), but reduced under semi-natural (Einum & Fleming, 1997; Sundt-Hansen et al., 2015) and natural conditions (Fleming et al., 2000; Reed et al., 2015; Solberg et al., 2013). We found a moderate increase in body length (ca. 8%) with the level of introgression at age 1+ and 2+, but introgression explained only a small part of size variation. Increased growth rates under natural conditions may be an overproportional investment into growth at the cost of fat reserves, which may in turn increase winter mortality (Finstad et al., 2004). Overproportional investment into growth may be particularly costly in terms of survival in northern regions, where local populations show adaptations to long winters (Finstad et al., 2010; Finstad & Forseth, 2006) and due to the negative energy balance fat reserves may be important for their survival throughout the winter (Næsje et al., 2006). Farmed Atlantic salmon parr may be maladapted to such northern conditions as a result of selection for commercially important traits and because of their origin from western Norwegian populations (Gjedrem & Baranski, 2009).

An alternative mechanism for the effect of introgression on survival is predation, as farmed parr have been shown to be more risk-prone than wild parr (Einum & Fleming, 1997). Recently, an experimental study found that predation of brown trout (*S. trutta*) on Atlantic salmon juveniles could explain the markedly lower survival of farmed and hybrid offspring, whereas the same experimental groups had similar survival in the absence of trout (Solberg et al., 2020).

Temporal changes in genetic introgression may not only have been affected by the relative survival of introgressed parr but also by movement of parr between the studied upper part of River Alta and lower parts of the river. Larger introgressed parr may be superior in ecological competition with wild parr and have been found to displace wild parr under semi-natural conditions (Sundt-Hansen et al., 2015) and in natural rivers (McGinnity et al., 2003). Displacement of wild parr by introgressed parr cannot be excluded in our study, but would render our analysis conservative, given that survival of displaced wild parr would result in a stronger decrease in population-level introgression within cohorts.

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CONFLICT OF INTEREST

None declared.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Dryad at https://doi.org/10.5061/dryad.9kd51c5gm.

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SUPPORTING INFORMATION

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Extensive hybridization following a large escape of domesticated Atlantic salmon in the Northwest Atlantic

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Domestication is rife with episodes of interbreeding between cultured and wild populations, potentially challenging adaptive variation in the wild. In Atlantic salmon, *Salmo salar*, the number of domesticated individuals far exceeds wild individuals, and escape events occur regularly, yet evidence of the magnitude and geographic scale of interbreeding resulting from individual escape events is lacking. We screened juvenile Atlantic salmon using 95 single nucleotide polymorphisms following a single, large aquaculture escape in the Northwest Atlantic and report the landscape-scale detection of hybrid and feral salmon (27.1%, 17/18 rivers). Hybrids were reproductively viable, and observed at higher frequency in smaller wild populations. Repeated annual sampling of this cohort revealed decreases in the presence of hybrid and feral offspring over time. These results link previous observations of escaped salmon in rivers with reports of population genetic change, and demonstrate the potential negative consequences of escapes from net-pen aquaculture on wild populations.

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The process of domestication results in genetically based phenotypic divergence from wild populations, through both intentional and unintentional selection[1–3]. Modern genomic data in both plant[4–6] and animal systems[3,7,8] have revealed that recurrent hybridization and gene flow between cultured and wild populations can occur, not only during the early stages of domestication[9], but throughout the entire period of culture[10]. Repeated episodes of hybridization between cultured and wild populations can be detrimental for wild populations, resulting in the introduction of non native alleles[6], erosion of adaptive diversity in the wild[4,11], and ultimately a loss of wild population viability[12,13]. The management and conservation of wild populations, confronted with domesticated conspecifics, requires the accurate quantification of potential genetic and ecological impacts to inform risk assessment and mitigation strategies.

The Atlantic salmon, *Salmo salar*, is of considerable socio economic value both in culture and in the wild. Domestication of Atlantic salmon was initiated in 1969 in Norway[14], and separately in 1979 in Eastern Canada[15]. Despite this short period, the pro cess of domestication has resulted in genetic differences between cultured and wild Atlantic salmon[16–18] which are likely mala daptive, and lead to lower relative survival of cultured salmon in the wild[19]. Domesticated Atlantic salmon exhibit lower relative fitness and spawning success compared to wild Atlantic salmon[13,19–21], and interbreeding can impart lasting, heritable, population level reductions in fitness to wild populations[12]. Escapes from Atlantic salmon net pen aquaculture are a regular occurrence[22], and the number of escapees can equate to an appreciable fraction of, or exceed, wild census size[23,24]. As such, genetic changes in wild populations consistent with introgression from domesticated salmon have been detected in nearly all regions where salmon aquaculture and wild populations co occur, including: Norway[25,26], Ireland[27,28], Northern Ireland[29,30], and Canada[31]. Furthermore, methodological and theoretical improvements[32,33] have allowed the degree of hybridization within a single river[29,30] or the cumulative impact of introgres sion at large spatial scales (i.e., >100 populations in Norway[34,35]), to be resolved. Nonetheless, the unequivocal quantification of the magnitude and geographic scale of domestic wild hybridization associated with single escape events across a broad landscape of wild salmon populations has remained elusive.

Here we quantify the presence and magnitude of hybridization between wild and escaped domestic individuals following an escape of ~20,000 sexually mature, domestic Atlantic salmon from a single aquaculture net pen in southern Newfoundland, Canada. This event occured on September 18, 2013, just prior to the natural spawning period for salmon in this region (Fig. 1a). The southern Newfoundland region is analytically favorable for the detection of hybrids; because the domestic broodstock cur rently in use originates from a single non local source (Saint John River, New Brunswick, Canada), the magnitude of industry production in the region has been limited until recently, and finally estimates of the abundance of wild salmon throughout southern Newfoundland (~20,000 individuals) are approximately equal to the magnitude of the escape[22,36]. Juvenile salmon were collected from the region and screened using 95 single nucleotide polymorphisms (SNPs) to identify hybrids, hybrid classes, and feral individuals present following this escape event. Next, we evaluated factors influencing the distribution of hybrids, and the magnitude of hybridization. Finally, using repeated temporal sampling, we examined and compared relative changes in the abundance of various hybrid classes over time. We report wide spread evidence of hybridization (27.1% and hybrids detected in 17/18 rivers) following this escape event. Hybrids were observed in higher frequency in smaller rivers, and repeated annual sampling revealed decreases in the presence of hybrid and feral offspring over time. These results demonstrate the potential genetic consequences of a single escape event from net pen aquaculture on wild Atlantic salmon populations.

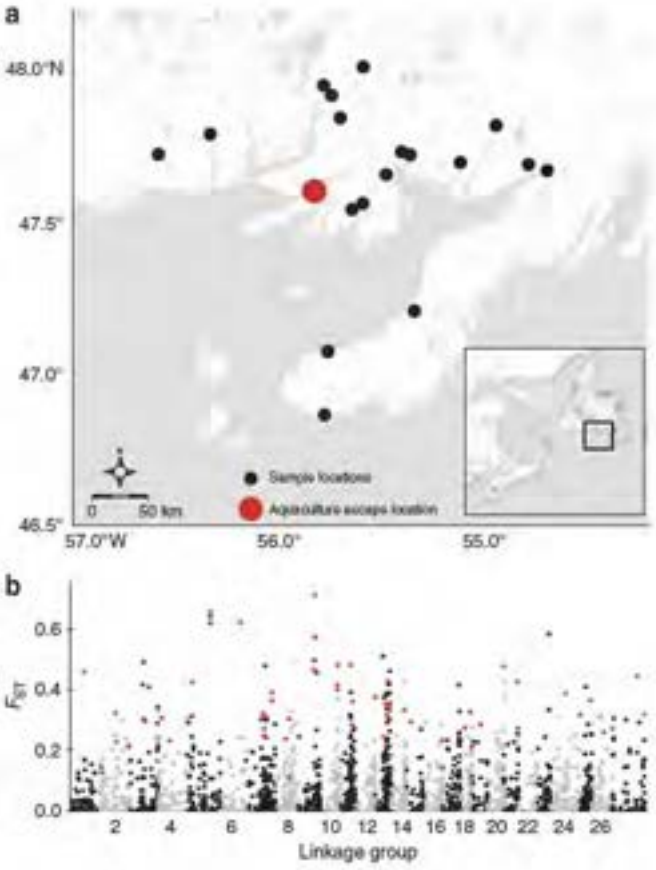

Fig. 1 Geographic distribution of sampling relative to aquaculture escape event and genome-wide comparison of wild and domestic salmon. **a** Map of southern Newfoundland, location relative to eastern Canada shown in inset. Black dots represent rivers surveyed; the red dot denotes the location of the 2013 aquaculture escape event. **b** Manhattan plot illustrating the genome-wide genetic differentiation (F_{ST}) between the wild and aquaculture baseline samples (Supplementary Table 7) used in the validation of the SNP panel accuracy. The red circles indicate the loci included in the 95 SNP collectively diagnostic panel. Linkage positions are from Brenna-Hansen et al.[64]

Results

Hybrid identification and genomic-based screening. In 2014, we collected 1704 young of the year (YoY; i.e., fertilized the fall of the year of the escape, and hatched in the spring of the year of sampling) salmon from 18 rivers in the area adjacent to the escape event (Fig. 1; Table 1). Samples were again collected in 2015 ($n = 836$ of YoY and the 2014 cohort as 1+ juveniles; Table 1). All samples were screened using 95 genome wide SNPs that were selected to maximize hybrid identification power and accuracy (Fig. 2; see Methods for further details as well as Sup plementary Figures 1 3).

Our panel of 95 highly informative genome wide SNPs identified 27.1% of the sampled YoY in 2014 as being of aquaculture ancestry based on a posterior probability assignment >0.80 (i.e., any of feral, F_1, F_2, and backcrosses, Fig. 3a). Hybrids were detected in 17 of the 18 rivers sampled (Fig. 3a, b), and feral

Table 1 Sample sizes of the juvenile Atlantic salmon screened for hybridization and introgression, the river from which they were collected, and the location of the river mouths

River name	Abbreviation	2104 YoY	2015 1+	2015 YoY	Lat (°N)	Long (°W)
Bottom Brook	BTB	32	33	0	47.765	56.322
Conne River	CNR	370	0	20	47.866	55.765
Dollard's Brook	DLR	25	24	22	47.708	56.555
Northwest Brook	FBN	41	0	0	47.720	55.393
Garnish River	GAR	199	50	56	47.239	55.353
Grand Bank Brook	GBB	42	26	15	47.104	55.754
Grand LaPierre	GLP	118	76	14	47.674	54.781
Long Harbour River	LHR	137	94	49	47.780	54.948
Salmonier Brook	LMS	40	22	89	46.865	55.775
Little River	LTR	130	0	0	47.809	55.743
Mal Bay Brook	MAL	17	70	36	47.669	55.131
Northeast Brook	NEB	115	19	0	47.723	55.367
Old Bay Brook	OBB	18	0	0	47.563	55.593
Southeast Brook	SEB	31	19	0	47.920	55.750
Simm's Brook	SMB	69	53	30	47.641	55.458
Taylor Bay Brook	TBB	120	0	0	47.543	55.637
Terrenceville Brook	TEB	120	0	0	47.671	54.711
Tailrace Brook	TRB	80	50	0	47.940	55.772

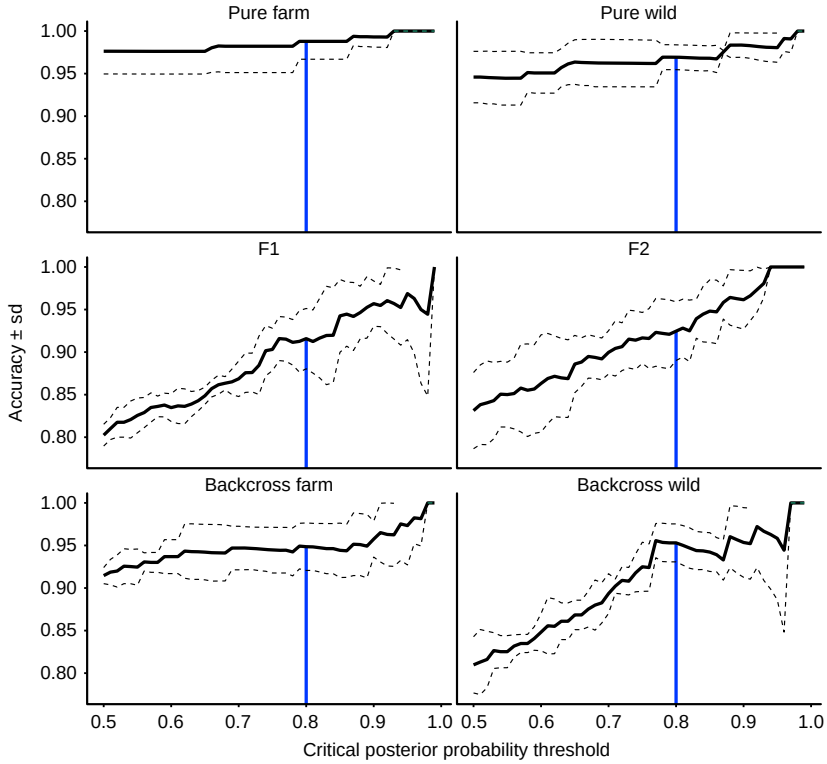

Fig. 2 Accuracy of detection of each of the genotype frequency classes across a range of critical posterior probability thresholds for the 95 SNP panel used in this study. The black line represents the mean of three replicate analyses of each of three independently simulated datasets and the dotted lines are the standard deviation. The vertical blue line is meant to highlight the critical posterior probability of assignment threshold (>0.8) used in this study

(i.e., offspring of two domestic salmon) offspring were detected in 13 rivers (Fig. 3a), revealing that the impacts of this escape event were substantial and region wide. F_1 hybrids were the most common hybrid class detected in 2014, but F_2 and backcross individuals were also present (Fig. 3b). Observations of post F_1 hybrids (i.e., F_2 and backcrosses) in 2014 YoY reveals that escape events had occurred prior to 2013, and that genetic introgression was occurring in some rivers. Observations of feral offspring indicative of successful reproduction among escapees has not been previously reported to our knowledge within the natural range of Atlantic salmon[18]. However, the potential for the establishment of feral populations remains unclear. Sibship

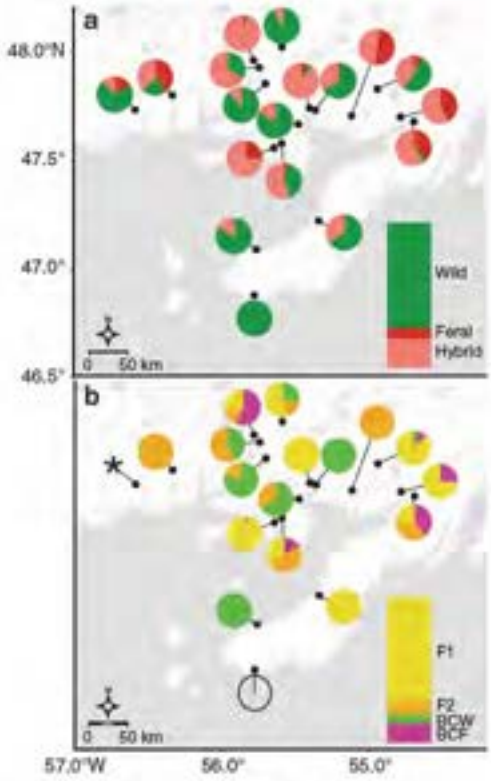

Fig. 3 Distribution and extent of hybridization following a large escape event of domestic Atlantic salmon. **a** Geographic distribution of wild, feral, or hybrid young-of-the-year Atlantic salmon across sample locations in 2014. **b** River-specific proportions of hybrid young-of-the-year salmon partitioned by hybrid genotype class (i.e., F_1, F_2, backcross wild (BCW), and backcross farm (BCF)). The open circle indicates a sample in which no hybrids were found, the asterisk signifies a location where accurate assignment to hybrid class was not possible. Bar graphs represent the overall proportions of each class in the entire sampling range, taking into account the varying sizes of the sampled populations (i.e., weighting by the axial distance, the distance along a straight line along the longest axis of the river), and colors therein are used as the legend

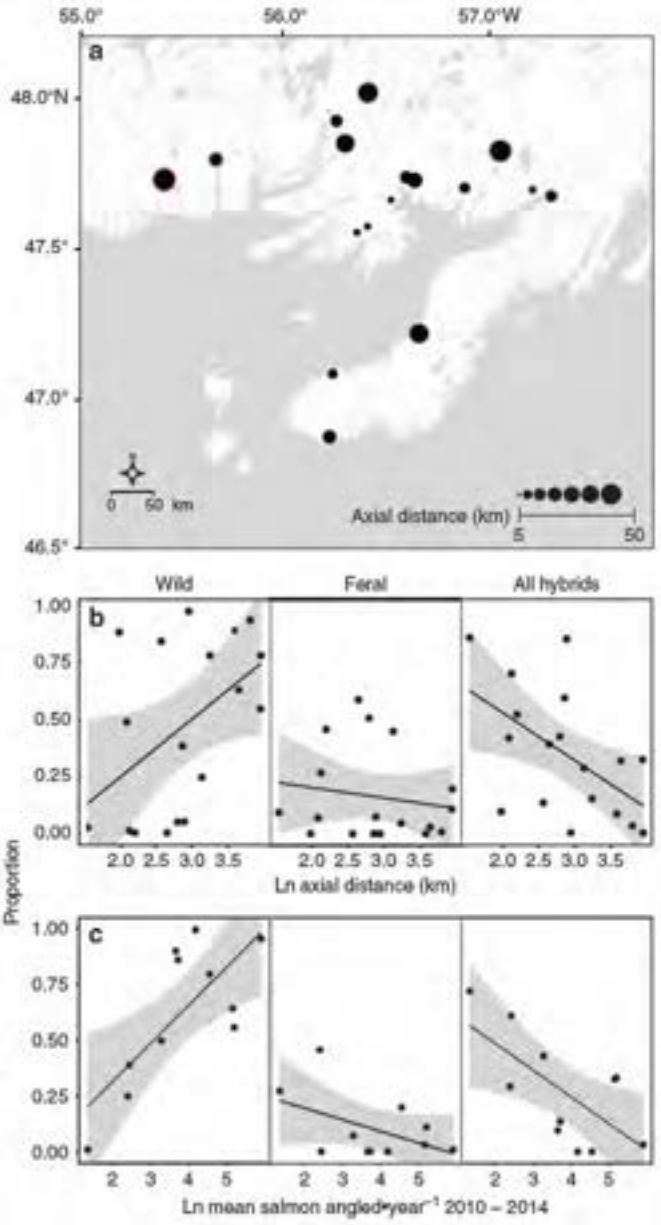

Fig. 4 Association between wild population size and levels of hybridization. **a** River axial distance (i.e., the distance along a straight line along the longest axis of the river). **b** Relationship between river axial distance and the proportions of wild, feral, and all hybrid (i.e., sum of proportions of F_1, F_2, BC wild, and BC farm) young-of-the-year Atlantic salmon sampled in 2014. **c** Relationship between mean number of salmon angled (2010–2014) and the proportions of wild, feral, and hybrid young-of-the-year Atlantic salmon sampled in 2014. The gray shading is the 95% CI of the prediction of the linear models. See Supplementary Table 4 for model parameter estimates

reconstruction revealed multiple unique parents for the hybrid and feral individuals in each river, suggesting that the over representation of a few families did not skew the river specific estimation of hybrid proportion (Supplementary Tables 1 3).

Factors influencing hybridization. Levels of hybridization detected in 2014 were significantly associated with wild popula tion size (Fig. 4; Supplementary Table 4). This was evident in significant associations between levels of hybridization and two proxies for salmon population size: river axial distance (i.e., the length of a straight line along a river's path), and average annual angling harvest (2010 2014), which correlate with salmon population size in this region (see Methods and Supplementary Figure 4). The proportion of hybrid YoY was negatively related to axial distance (Fig. 4b) and average annual angling (Fig. 4c); whereas, the opposite was true of the proportion of wild YoY (both $p < 0.001$, Supplementary Table 4). However, there was no statistical relationship between the proportion of feral YoY and either axial distance or average annual angling harvest (both $p > 0.10$; Supplementary Table 4). There was no evidence that dis tance between the location of the large escape event and river mouths influenced the proportion of wild, feral, or hybrid offspring detected in the year following the escape event (all $p > 0.28$; Supplementary Table 5).

Temporal variation. To explore changes in the relative propor tion of hybrids within the 2014 cohort over time, YoY and 1 +juvenile salmon were sampled and analyzed in 2015 from across the region. In comparison to the YoY sampled in 2014, these 2015 YoY samples revealed an almost complete absence of feral

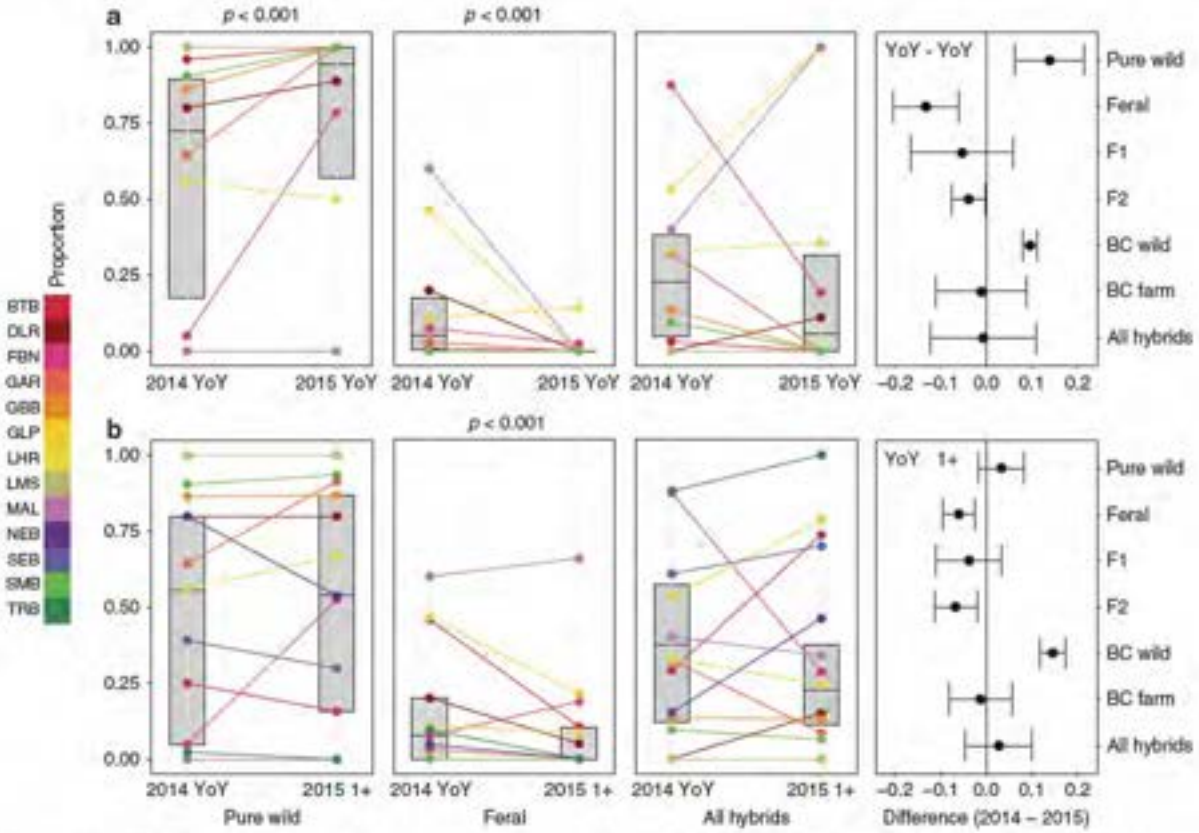

Fig. 5 Temporal variation (2014–2015) in levels of hybridization. **a** River specific and overall trends in the proportion of wild, feral, and hybrid young-of-the-year Atlantic salmon between 2014 and 2015. Gray shaded boxplots illustrate the overall proportions across all rivers, midline represents the medians, the upper and lower bounds the interquartile ranges, and the whiskers extend to 1.5 times the interquartile range. Black dots represent the mean difference (±SE) between 2014 and 2015 in the proportion of each pure and hybrid class present. All hybrids is the sum of proportions of F_1, F_2, BC wild, and BC farm. **b** River specific and overall trends in the proportion of wild, feral, and hybrid young-of-the-year and one year old (1+) Atlantic salmon sampled in 2014 and 2015, respectively. Gray shaded boxplots illustrate the overall proportions across all rivers. The midline represents the medians, the upper and lower bounds the interquartile ranges, and the whiskers extend to 1.5 times the interquartile range. Black dots represent the mean difference (±SE) across rivers between 2014 and 2015 in the proportion of each pure and hybrid class present of young-of-the-year and 1-year-old individuals. All hybrids is the sum of proportions of F_1, F_2, BC wild, and BC farm. See Fig. 1 and Table 1 for location information, and sample sizes

individuals and declines in the prevalence of most hybrid classes. This is likely reflective of overall lower numbers of escapees in 2014, a year in which no escape events were reported (Fig. 5a). The decline in feral individuals was significant ($p < 0.001$), as was the consequent increase in the proportion wild ($p < 0.001$). However, whereas most hybrid classes were found to decrease, the change in the overall proportion of hybrids was offset by the increase in backcross wild individuals resulting in no significant difference between years ($p = 0.56$; Fig. 5a).

Potential offspring from the 2013 escape event (1+individuals in 2015), showed that the proportion classified as feral declined significantly after a single year of selection in the wild ($p < 0.001$; Fig. 5b). There was no significant difference in the proportion of wild individuals ($p = 0.06$) and while there were decreases in most hybrid groups, the increase in backcross wild individuals muted any consistent statistical trend between years and among rivers and hybrid classes ($p = 0.20$; Fig. 5b). This decrease in the prevalence of offspring with part or full domestic ancestry is consistent with the reductions in relative hybrid survivorship observed in experimental studies[13,21,37] and expected selection against these individuals in the wild. Nonetheless, the continued presence of F_2 and backcross individuals, as well as the observed increases in prevalence of wild backcross individuals indicates introgression is occurring.

Discussion

We report unambiguous landscape scale evidence of interbreed ing between wild and escapee Atlantic salmon resulting from a single escape event, and of particular note, the first documented instance of which we are aware of feral offspring within the native range of Atlantic salmon[18]. The combination of a highly infor mative panel of genome wide SNPs with a large escape event of non local domestic individuals into largely pristine wild popula tions allowed unprecedented resolution of the magnitude and geographic scale of hybridization following a single escape event. Hybrid and feral offspring were widespread geographically, occurring at distances of up to100 km from the escape event, and accounted for ~27% of juvenile salmon surveyed. Moreover, the detection of F_2 and backcross individuals, presumably resultant from previous escape events, strongly supports the continued survival and reproductive viability of some hybrids, as well as the potential for significant demographic and genetic change as reported elsewhere[18].

Our results demonstrate a clear association between the size of wild populations and the degree of hybridization (Fig. 4) sug gesting that smaller salmon populations are at greater risk of hybridization and introgression with escaped domestic indivi duals as noted in Norway[34,38]. This relationship is consistent with the dilution of domestic individuals in larger wild populations, as

well the consequences of increased competition between wild and domestic individuals both on the spawning grounds and at juvenile stages[26,34]. Although, we lack actual estimates of wild population census size for many of the rivers included, the two correlates used here (river axial distance and annual angling harvest) are highly associated with population size on monitored rivers within the region (Supplementary Figure 5) and likely reflective of spatial trends in population census size.

Our results provide evidence consistent with declines in the proportion of offspring with domestic ancestry (e.g., hybrid, and feral) over time following the escape event. Comparison of the hybrid class composition of 1 year old individuals sampled in 2015 relative to young of the year sampled in 2014, revealed decreases in most hybrid classes, with only wild and wild back crosses increasing in prevalence (Fig. 5b). Reduced wild domestic hybrid survivorship for Atlantic salmon has previously been reported in experimental studies[13,21,37], but, we believe this is the first documentation following a single escape event in the wild (Fig. 5b). The observed loss of feral and hybrid individuals over time is consistent with expected selection against these indivi duals in the wild. Interestingly, hybrid class composition of young of the year sampled in 2015 revealed an almost complete absence of feral individuals and declines in the prevalence of most hybrid classes. This is consistent with an absence of reported escape events in 2015 and a reduced influence of the 2013 escape event. Despite evidence of declines in the proportion of domestic offspring or hybrids over time, the continued presence of F_2s and backcrosses is clear evidence of introgression and that significant genetic change is occurring in these wild populations[39].

The identification and quantification of introgression and hybridization between domestic and wild Atlantic salmon is a critical first step toward understanding, predicting, and managing the genetic impacts of net pen salmon aquaculture on wild populations. Our clear resolution of hybridization and intro gression between escapee and wild Atlantic salmon in the Northwest Atlantic is the first to our knowledge, and is consistent with observations of genetic perturbation from aquaculture escapees[31] both in the Canadian Maritimes and in Europe[14,34,35]. Our results link previous observations of escapes of domesticated Atlantic salmon with reports of population level genetic changes[31,35] and regional declines of Atlantic salmon popula tions[36]. Moreover, these results further demonstrate the potential consequences of escapes from net pen aquaculture on wild Atlantic salmon populations.

Methods

Development of collectively diagnostic SNP panel. The collection of wild samples used for the development of our single nucleotide polymorphism (SNP) panel has been previously detailed in Bradbury et al.[40]. Briefly, juvenile Atlantic salmon ($n = 260$, 0+ to 3+ years of age), were collected via electrofishing during the summers of 2008 2010 (sample sizes are found in Supplementary Table 6; genetic differentiation between populations are described in Supplementary Table 7). All wild collections were conducted under the auspices of Fisheries and Oceans collection permits. Aquaculture samples ($n = 156$) were obtained from two cage sites located within the region shown in Fig. 1. No effort was made to screen for or remove potential sibs from these baseline groups[41]. These baseline individuals were first screened using a 5568 SNP-locus panel developed by the Centre for Integrative Genomics (CIGENE, Norway[42,43]) as per Bradbury et al.[44]. Locus calls were visually confirmed and loci were retained if call rates were >0.85 and with overall minor allele frequencies >0.01 or a minor allele frequency >0.05 in either population[44]. The loci retained after quality control filtering were ranked by Weir and Cockerham's[45] F_{ST} between the two pooled reference groups (wild and domestic salmon), and the 95 most informative loci for which suitable assays could be developed were incorporated into the custom Fluidigm EPI array (see below). Linkage disequilibrium was not considered explicitly, however, the final panel provided genome-wide coverage (Fig. 1).

For each candidate locus, sequences from identified targets were downloaded from GenBank (SNP database, www.ncbi.nlm.nih.gov) and submitted to D3 Assay Design application (www.d3.fluidigm.com) for SNP Type assay design (Fluidigm, San Francisco, CA, USA). Assays were tested on samples with known genotype and the selection criteria for inclusion in the final panel included: correct genotypes for known samples and positive controls (see below); genotypes being reproducible across multiple chip runs; the ranking of the target SNP in the prioritized list; and assays not requiring the STA (Specific Target Amplification) step. Positive controls consisted of normalized solutions of synthesized double stranded DNA (gBlocks (Integrated DNA Technologies, Coralville, IA, USA))[46]. SNP genotyping was performed using SNP type assays (Fluidigm) per the manufacturer's protocols, without the STA (Specific Target Amplification) step, using 96.96 genotyping Integrated Fluidic Circuits (IFC) and read on an EP1 (Fluidigm) and analyzed using SNP Genotyping Analysis software (Fluidigm). Each 96-well plate extraction included 10 samples that were repeated on the plate (redundants) to detect processing errors (row or plate reversal) and ensure consistent clustering interpretation. The setup for each IFC also included positive controls (see above for details). To calculate the genotype error rate, 11.3% of the samples were reanalyzed from the original tissue where tissue samples were permitted. Based on Pompanon et al.[47], the genotype error rate was calculated to be 0.01%.

Hybrids. We used the R[48] package hybriddetective[49] to simulate pure wild, farmed, and hybrid populations to evaluate the power of this panel to identify hybrids and hybrid classes. Using hybriddetective we simulated multigenerational (viz. pure wild, pure farm, F_1, F_2, and backcrosses to wild and farm) hybrid datasets based on the genotypes of our wild and farmed baselines at the 95 SNPs in our panel. A random subset of 90% of the individuals from the wild and farmed baselines was first taken. A centered wild baseline was created by randomly sampling two alleles per locus from those of the randomly sampled subset without replacement. The same was done to create a centered farmed baseline. Centering was done following Karlsson et al.[33] and has the effect of removing linkage and deviances from Hardy-Weinberg equilibrium that may have been present in a pooled sample of populations. Next, using the centered baselines, individuals in generation $t+1$ were created by randomly sampling without replacement one allele per locus from each of the parental populations (i.e., wild baseline subsample and farmed baseline subsample) at time t[49]. Three independently simulated datasets were each in turn analyzed three times in parallel using NewHybrids[32] and the R package parallelnewhybrid[50], with a burn-in of 50,000 followed by 100,000 sweeps. NEWHYBRIDS calculates the posterior probability that an individual belongs to each of, in our case, six hybrid classes[32]. The results of the analyses of these simulated datasets were used to determine the efficiency and accuracy[51] of our 95 SNP panel.

To evaluate the efficacy of our panel, two metrics were considered: the panel's accuracy and its efficiency. For both these measures, we use the definitions provided by Vähä and Primmer[51]. First, accuracy is the proportion of all individuals that were assigned to a hybrid class that truly belong in that hybrid class (i.e., number of individuals correctly assigned to a hybrid class divided by the total number of individuals assigned to that class), and is calculated independently for each hybrid class. Efficiency, is also calculated independently for each hybrid class, and measures the proportion of individuals that are known a priori to belong to a hybrid class that were assigned to that class (i.e., number of individuals correctly assigned to a hybrid class divided by total number of individuals known a priori to belong to a class). The accuracies and efficiencies calculated from the analyses of these simulated datasets across a range of posterior probability of assignment thresholds are shown in Fig. 2 and Supplementary Figures 1-3. From Fig. 2 (and also Supplementary Figure 3) it can be seen that the proportion of simulated individuals correctly assigned as either pure wild or feral are the highest across all posterior probability of assignment thresholds, while F_1, F_2, and backcross wild were comparatively lower. However, at all posterior probabilities of assignment shown, the accuracy for all hybrid classes was >80%, suggesting the potential impact of miss-assignments is low. Similarly, efficiencies (Supplementary Figures 1 and 2) were above 90% for posterior probability of assignments thresholds between 0.5 and 0.8 (used in this analysis), suggesting the majority of individuals were assigned. Taken together, the high accuracy indicates that of those individuals assigned to a given class the majority were assigned correctly (i.e., little false assignment bias), while the high efficiency suggests that most individuals were assigned. A posterior probability of assignment threshold of 0.8 for individual classification was chosen based on the simulations and calculation of efficiency and accuracy (Fig. 2, Supplementary Figures 1-3). Individuals which did not meet the 0.8 posterior probability threshold for any hybrid class were considered only for the assignment as wild, farmed, or hybrid, and excluded from analyses focusing on specific hybrid classes. Convergence of the MCMC chains in NewHybrids was also confirmed using *hybriddetective*[49].

We evaluated both assignment to each of the six genotype frequency classes (Fig. 2 and Supplementary Figure 1), and pooled hybrid class identification (Supplementary Figures 2 and 3) separately, and accepted individual assignments to a class if their posterior probability of assignment to that class met, or exceeded a threshold of 0.8. We chose the threshold of 0.8, which is more conservative than what is typically used (e.g., 0.5[51,52]), because, we wanted to maximize the accuracy of assignments (Fig. 2 and Supplementary Figures 3).

Sample collection and analysis. On 18 September 2013, 20,000 sexually mature Atlantic salmon weighing between 4.5 and 7 kg (10 15 lbs) escaped from an open

cage culture facility in southern Newfoundland, Canada. A number of these escapees were subsequently detected and captured in nearby rivers by technicians working for Fisheries and Oceans Canada (DFO). Gross morphological examination, in addition to necropsies conducted by DFO employees, showed that the recovered salmon were sexually mature, and in spawning condition. In 2014, the year following the large escape event, young-of-the-year (YoY) salmon were collected by electrofishing stream and river habitats in the 18 rivers shown in Fig. 1. Sampling included both rivers with historical records of established salmon populations (Conne River, Little River, Garnish River) and smaller streams lacking prior information on the presence or status of Atlantic salmon populations. With the exception of a few monitored rivers, information on the status of the wild populations in these rivers is largely lacking[36]; what information does exist suggests recent declines in abundance.

Individuals were approximately age-binned based on an expected size size age distribution from 200 K aged Newfoundland parr with a 97.5% accuracy in YoY identification. All YoYs captured were euthanized and stored whole in 95% ethanol for later DNA extraction. Sample sizes by year for each river are listed in Table 1. Sampling was repeated in 2015 using the same methodology, with the exception that both YoY and 1+individuals were retained. The 1+individuals collected in 2015 belong to the same cohort of fish that were spawned following the escape event in 2013, and collected as YoY in 2014. Conversely, the YoY collected in the 2015 sampling were spawned in 2014, a year in which no escape events were reported in Newfoundland, and are thus expected to be reflective of the background rates of hybridization and introgression.

DNA was isolated from tissue samples using QIAamp 96 DNA QIAcube HT Kit (Qiagen, Toronto, ON, Canada) on a QIACube HT (Qiagen) per the manufacturer's protocol with some modifications. Tissue samples were manually disrupted using a Tissue Lyser II (Qiagen) mixing 2 × 10 s at 20 s^{-1}. DNA was eluted twice in 100 µL buffer AE (Qiagen) pre-heated to 70 °C. DNA extracts were quantified using Quant-iT PicoGreen dsDNA Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA) and read on a FLUOStar OPTIMA fluorescence plate reader (BMG Labtech, Ortenberg, Germany). All individuals were screened using the custom Fluidigm SNP panel and NEWHYBRIDS was used to quantify the proportion of individuals from different genotype frequency classes present in a river sample[32]. Samples from each river, and each year, were run independently. Prior information on allele frequencies of baseline farm and wild salmon were also provided to NEWHYBRIDS during analyses by including simulated pure farm and pure wild individuals (i.e., the same individuals used in the testing of the accuracy and efficiency of the panel described above). The known class (i.e., pure wild and pure farm) were indicated to NEWHBYRIDS, as well as the fact that they were not to be included as part of the mixture[53]. Like in the determination of the efficacy of our panel described above, NEWHBYRIDS was run with a burn-in of 50,000 followed by 100,000 sweeps, which was found to be sufficient to ensure convergence during the panel testing. Proportions assigned to the various hybrid classes are shown in Supplementary Tables 8-10.

COLONY[54] was used to simultaneously infer the parentage and sibships of the YoY sampled in 2014, and the YoY and 1+individuals sampled in 2015. Each river, sampling year, and year class was analyzed separately in COLONY and parents were assigned an ancestry (wild, farm, or F_1) based on the hybrid class in question (i.e., if an individual was feral, both parents must be farmed, if an individual was an F1, one parent must be farmed and the other wild, if an individual is an F2, both parents must themselves have been F1s, etc.). For each river, sampling year, and year class, locus-specific allelic dropout rates were estimated using the "missing" function in PLINK[55,56], and these were provided to COLONY. Allele frequencies were estimated by COLONY from the data provided. In running COLONY, because all samples were wild caught, no information about numbers of candidate males or females provided. Both sexes were assumed to be polygynous, and "long" runs with "VeryHigh" precision were used. Because, we were not attempting to assign parentage, merely estimate the number of families present in each sample, and show that the proportions of hybrid classes detected was not the result of over representation of one, or a few families, the full-sib grouping for each individual with the highest probability was accepted. It should also be noted that because no parental genotypes were provided to COLONY, it was unable to meaningfully assign sexes to parents. Therefore, the total number of parents are presented.

Statistical analyses. All statistical analyses were conducted in R version 3.4[48]. The proportion of wild, feral, and hybrid at each location was explored for associations with wild population size; in this case two proxies were used (axial river distance and average annual harvest). For the Newfoundland region, wild population size[57] is associated with river axial distance[58] (the distance along a straight line along the longest axis of the river; linear model, $R^2 = 0.6944$, $F_{1,8} = 18.18$, $p < 0.01$; Supplementary Figure 4) and as such, axial distance was used as a proxy for population size. We also used average annual harvest (2010 2014) as a proxy of population size; because, the two were related (linear model, $F_{1,8} = 40.47$, $R^2 = 0.835$, $p < 0.001$). Harvest statistics are collected annually by Fisheries and Oceans Canada[59], and counts of population size and estimates of annual harvest were available for 10 rivers (Supplementary Table 11).

Exponential models for effect of distance from the escape event were used because straying of farmed salmon generally follows a negative exponential distribution[60]. The relationship between the proportion feral, wild, and hybrids detected in each river and the distance between the river mouths and the site of the escape were tested and fit using the R function *nls*. No significant relationships were found for distance from the escape event (all $p > 0.28$; Supplementary Table 5), so this factor was not considered further. The impact of the relative size of the native salmon populations in respective rivers on proportions was tested using linear models with the R function *lm*. The proportion wild, the proportion feral, and the proportion hybrid were tested separately as a function of axial distance, and then average annual angling harvest between 2010 and 2014.

We tested for differences in proportion of wild, feral, or hybrid individuals between years within the same cohort (i.e., the YoY collected in 2014 and the 1+collected in 2015), and between years with and without reported large escape events (i.e., YoY collected in 2014 and YoY collected in 2015) using binomial mixed-effects models with river as the random effect using the R function *glmer*[61]. The proportions of wild, feral, and hybrid were tested with separate models, and *p*-values were adjusted using the false discovery rate[62].

Data availability. Genotype, river characteristic, salmon and angling count data for this study are available in the Dryad Digital repository[63] at: https://doi.org/10.5061/dryad.3k888n7

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Author contributions

B.F.W., I.R.B., C.G., and I.A.F. conceived of and designed the study. Statistical and genomic analyses were conducted by B.F.W., E.C.A., and L.C.H. Figures were produced by B.F.W., N. W.J., R.R.E.S., and I.R.B. All authors wrote and approved the final draft of the manuscript.

Additional information

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Competing interests: The authors declare no competing interests.

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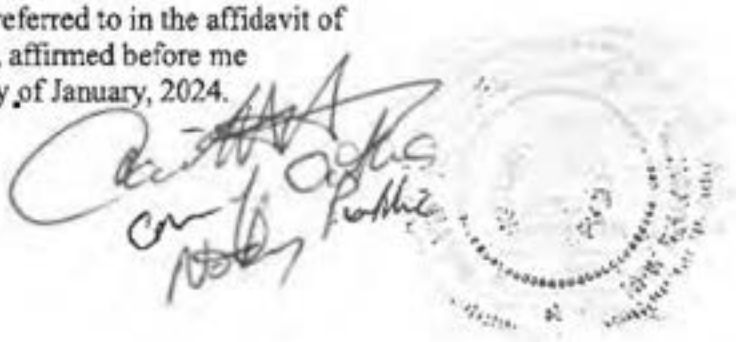
This is **Exhibit "B"** referred to in the affidavit of
Jon W. Carr, affirmed before me
this 19th day of January, 2024.



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Quinn B., Trudel M., Wilson B., Carr J., **Daniels J.**, Haigh S., Hardie D., Hawkes J., McKindsey C., O'Flaherty-Sproul M., Simard É., and Page F. 2021.*Modelling the effects of currents and migratory behaviours on the dispersal of Atlantic salmon (Salmo salar) post-smolts in a coastal embayment.* Canadian Journal of Fisheries and Aquatic Sciences. 79(12): 2087-2111. https://doi.org/10.1139/cjfas-2021-0316

Daniels, J., Brunsdon, E.B., Chaput, G., Dixon H. J., Labadie H., Carr J. 2021.*Quantifying the effects of post-surgery recovery time on the migration dynamics and survival rates in the wild of acoustically tagged Atlantic Salmon Salmo salar smolts*. 2021. Animal Biotelemetry 9, 6 https://doi.org/10.1186/s40317-020-00228-6.

Technical Reports

Carr, J. 1997. Downstream movements of presmolt in the Tobique River: 1996 tracking summary, Atlantic Salmon Federation: 74pp.

Carr, J.W. 1998. Initial Results: 1997 Downstream Movement of Presmolt in St. John River Drainage. Atlantic Salmon Federation: 4pp.

Carr, J.W. & Whoriskey, F.G. 1998. Atlantic salmon (*Salmo salar*) in the Magaguadavic River New Brunswick 1992-1997. Atlantic Salmon Federation: 45pp.

Whoriskey, F.G., Carr, J.W., Lacroix, G.L. & Stokesbury, M. 1998. A review and update of aquaculture impact studies carried out on the Magaguadavic River, Southern Bay of Fundy, New Brunswick. Presented at a Dept. of Fisheries & Oceans Workshop On Interactions of Wild & Farmed Atlantic Salmon Moncton, NB, 1998: 49pp.

Whoriskey, F. G. & Carr, J.W. 1999. Homing of escaped aquaculture Atlantic salmon to the Magaguadavic River, New Brunswick. Atlantic Salmon Federation, 12pp.

Carr, J.W. & Whoriskey, F.G. 2000. A review of aquaculture impact studies carried out on Southwestern New Brunswick outer Bay of Fundy Rivers, with emphasis on the Magaguadavic River. Atlantic Salmon Federation, 57pp.

Carr, J.W. 2000. Summary of Downstream Migration of Juvenile Salmon in the Tobique River System, 1999- Atlantic Salmon Federation, 4pp.

Carr, J.W. 2001. A review of downstream movements of juvenile Atlantic salmon (*Salmo salar)* in the dam-impacted Saint John River drainage. Atlantic Salmon Federation, 85pp.

Carr, J.W. 2001. Downstream Migration of Juvenile Salmon in the Tobique River System 2000 Summary Report. Atlantic Salmon Federation, 5pp.

Whoriskey, F. G. & Carr, J.W. 2001. Interactions of escaped farmed salmon, and wild salmon, in the Bay of Fundy region. Atlantic Salmon Federation, 11pp.

Carr, J.W. & Whoriskey, F.G. 2002. Assessment of Atlantic salmon in Southwestern New Brunswick outer Bay of Fundy Rivers, with emphasis on the Magaguadavic River, 1992-2001 project report. Atlantic Salmon Federation, 46pp.

Carr, J.W. 2002. Western Fundy Atlantic Salmon Recovery Program, Progress Report October 2002. Atlantic Salmon Federation, 2pp.

Whoriskey, F. G., O'Reilly, P., & Carr, J.W. 2002. Reversal of the recent decreasing trend of escaped farmed Atlantic salmon entering the Magaguadavic River, New Brunswick, Canada, and genetic evidence for the presence of escaped juvenile and adult farmed salmon of European ancestry. Atlantic Salmon Federation and Department of Fisheries and Oceans, 11pp.

Carr, J.W. & Whoriskey, F.G. 2003. 'Rearing Magaguadavic Broodstock at Huntsman Marine Science Center' Final Report. Atlantic Salmon Federation, 5pp.

Carr, J.W. & Whoriskey, F.G. 2003. Western Fundy Atlantic Salmon Recovery Program Final Report. Atlantic Salmon Federation, 34pp.

Carr, J.W. & Whoriskey, F.G. 2003. Magaguadavic River Monitoring and Recovery Program. Atlantic Salmon Federation, 24pp.

Carr, J.W. & Whoriskey, F.G. 2004. Restoration of Western Fundy Atlantic Salmon- Project Report. Atlantic Salmon Federation, 46pp.

Carr J.W. 2004. Feasibility of using landlocked salmon in anadromous salmon recovery program. Atlantic Salmon Federation, 34pp.

Carr, J.W. & Whoriskey, F.G. 2005. Fish Passage Effects Monitoring Program for St. George Hydroelectric Redevelopment. Atlantic Salmon Federation, 25pp.

Carr, J.W. 2005. Restoration of Western Fundy Atlantic Salmon- Final Report. Atlantic Salmon Federation, 34pp.

Carr, J.W. 2006. Status of rainbow trout in New Brunswick watercourses. Atlantic Salmon Federation, 47pp.

Carr, J.W. 2006. Multi Species Recovery Program with emphasis on Atlantic salmon- Final Report. Atlantic Salmon Federation, 34pp.

Carr, J.W. 2008. Magaguadavic River Anadromous Fish Restoration and Monitoring Program. Atlantic Salmon Federation, ETF and NBWTF Final Report. 36pp

Carr, J.W. 2009. Outer Bay of Fundy Atlantic Salmon and Alewife Recovery Program. Project No. F308-037. Submitted to the New Brunswick Wildlife Trust. 26pp.

Carr, J.W. 2010. At Sea Interactions of Atlantic Salmon Kelt and Smolt from Bay of Fundy Region. Project No. F309-026. Submitted to the New Brunswick Wildlife Trust. 19pp.

Carr, J.W. 2011. Salmon Smolt Tracking and Assessment. Submitted to the Atlantic Salmon Conservation Foundation. 9pp.

Carr, J.W. 2010. 2009 Summary Report on Monitoring Times, Locations, and Fish Handling in Outer Bay of Fundy Rivers. License 322376. Submitted to Department of Fisheries and Oceans. 6pp.

Carr, J.W. 2010. Magaguadavic River Exotic Fish Species Inventory and Public Awareness. Project No. F309-050. Submitted to the New Brunswick Wildlife Trust. 2pp.

Carr, J.W. 2011. 2010 Summary Report on Monitoring Times, Locations, and Fish Handling in Outer Bay of Fundy Rivers. License 322376. Submitted to Department of Fisheries and Oceans. 5pp.

Carr, J.W. 2011. Magaguadavic River Recovery Program. No. F300-031. Submitted to the New Brunswick Wildlife Trust. 6pp.

Carr, J.W. 2012. Salmon Smolt Tracking and Assessment. Submitted to the Atlantic Salmon Conservation Foundation. 12pp.

Carr, J.W. 2013. ASF Tracking Research – 2013. Submitted to the Atlantic Salmon Conservation Foundation. 10pp.

Summerfelt, Steven, Waldrop, T., Good, C., Davidson, J., Backover, P., Vinci, B., Carr, J. 2013 Freshwater Growout Trial of St. John River Atlantic Salmon in a Commercial Scale Land-Based Closed Containment System. Freshwater Institute, January 2013. https://www.conservationfund.org/images/projects/files/FI-ASF_Final-Report_March-20131.pdf.

Carr, J., Trial, J., Sheehan, T., Gibson, J., Giffin, G., Meerburg, D. 2015. Summary of Proceedings of the Symposium: What Works? A Workshop on the Wild Atlantic Salmon Recovery Programs. Atlantic Salmon Federation, St. Andrews, New Brunswick, Canada, 24 pp.

Sheehan, T., Carr, J. Chafe, G., Renkawitz, M.,Robertson, M., Lyberth, B., and Bradbury, I. 2019. Update on Pop-off satellite tagging Atlantic salmon at Greenland. ICES-2019/30.

Popular Articles

Carr, J. 2013. Making it work - ASF co-hosts an international symposium summit on land-based fish farming. Atlantic Salmon Journal, Winter 2013, Vol. 62, No. 4, 2pp.

Carr, J. 2017. A Two-way Street. Atlantic Salmon Journal, Spring 2017, Vol 66, No. 1.

Carr, J. 2018. Breaking the Ice. Atlantic Salmon Journal, Spring 2018, Vol 67, No. 1.

Carr, J. 2016. Using Telemetry as a Tool to Help Unravel the Mystery of Salmon Lost at Sea. SPAWNER 2018.

Carr, J. 2018. Friends in Far Places. Atlantic Salmon Journal, Winter 2018, Vol 67, No 4.

PROFESSIONAL MEMBERSHIPS

American Fisheries Society

Aquaculture North America

Canadian Aquaculture Association

Fisheries Society of the British Isles

IntraFish Magazine

PROFESSIONAL APPOINTMENTS AND SERVICES

Expert Witness in a salmon poaching case, on scale determination to differentiate between wild and escaped farmed salmon, Court of Queen's Bench, Saint John NB Canada. 13 February 2002.

Consultant and training, Workshop for age determination and discrimination between wild and farmed Atlantic salmon. St. Andrews, NB. July 2002.

Co-developed and implemented Effects Monitoring Program (fish passage studies) at Magaguadavic River hydroelectric dam, 2004-2010.

Consultant and Training, Workshop for age determination and discrimination between wild and farmed Atlantic salmon. St. Andrews, NB. February 2007.

Board Member, Eastern Charlotte Waterways. 2010-2020.

Member, Huntsmen Marine Science Center Committee, 2010 -

Member of Restigouche River Watershed Science Committee, 2010 –

External Reviewer of COSEWIC Assessment and Status report on the Atlantic Salmon. 2010

Honorary Research Associate in School of Graduate Studies, University of New Brunswick, 2011-

Organizer, Didymo (invasive species) workshop in Plaster Rock, NB in 2011.

Member of ICES Working Group on Age Determination of Salmon (WKADS), 2011.

Consultant and Training, Workshop for age determination and discrimination between wild and farmed Atlantic salmon. St. Andrews, NB. July 2011.

External; Reviewer of DFO CSAS Sea lice monitoring and non-chemical treatment methods Process. 25-27 September 2012.

Co-Organizer, Salmon Land-Based Closed Containment Workshop. St. Andrews, NB. October 2012.

Co- Organizer, Salmon Farming in Land-Based Closed Containment Systems. Halifax, NS. October 2012.

External Reviewer of CSAS Science National Review Process: Potential effects surrounding the importation of European origin cultured Atlantic salmon to Atlantic salmon populations and habitats in Newfoundland. March 2013.

Co-Organizer, Aquaculture Innovation Workshop. Shepherdstown, WV. September 2013.

C0-Organiser, Symposium: What works? A workshop on wild Atlantic salmon recovery programs. September 2013.

Consultant and Training, Workshop for age determination and discrimination between wild and farmed Atlantic salmon. St. Johns, NL. October 2013.

Provided advice and consultation on development and implementation of effects monitoring program (fish passage studies) at Howland Dam Bypass Facility, Maine, 2014-2015.

Member, Aquaculture Association of Nova Scotia Codes of Containment Committee. 2014-2016

Co-Organizer, Salmon Containment Workshop. St. Andrews, NB. April 2014.

Chaired special session on Life History, Molecular Ecology and Evolution of Salmonids at the 2nd International Conference on Integrative Salmonid Biology, Vancouver BC, June 2014.

Consultant and Training, Workshop for age determination and discrimination between wild and farmed Atlantic salmon. Conner River, NL. July 2013.

Co-Organizer, Aquaculture Innovation Workshop. Vancouver, BC. October 2014.

Co-Organizer, Aquaculture Innovation Workshop. Shepherdstown, WV. October 2015.

Co-Organizer, Aquaculture Innovation Workshop. Roanoke, WV. August 2016.

Member of Atlantic Salmon Research Joint Venture Science Committee, 2016 -

Member of ICES Advisory Committee to address the North Atlantic Salmon Conservation Organization (NASCO) request for advice on possible effects of salmonid aquaculture on wild Atlantic salmon populations in the North Atlantic (WKCULEF), 2016.

Member, Nova Scotia Aquaculture Salmon Traceability Committee, 2016 -

Co-Chair of New Brunswick Aquaculture Containment, 2016 – 2018

Co-Organizer, Aquaculture Innovation Workshop. Vancouver, BC. November 2017.

External Reviewer of CSAS Science National Peer-Review Process: Environmental and Indirect Human Health Risk Assessment for the Manufacture and Production of Sterile AquAdvantage Salmon at a Land-Based and Contained Facility near Rollo Bay, PEI. December 2018

External Reviewer of CSAS document “Mortality of Atlantic salmon after catch and release angling: assessment of a recreational Atlantic salmon fishery in a changing climate. 2019.

Expert technical review of DFO's draft Framework for Aquaculture Risk Management. 7 February 2019

Member, Steering Committee, Environmental Studies Research Fund: Atlantic salmon in the Eastern Canadian offshore regions – assessing timing, duration, and effects of environmental variability and climate change. 2020-

External Reviewer of Pre- COSEWIC Assessment for Atlantic Salmon in Newfoundland and Labrador, Maritimes, Gulf and Quebec Regions. 2020/2021.

Expert witness. Submitted affidavit to provide expert opinion on impacts of salmon aquaculture on wild salmon in response to application by Kelly Cove Salmon Ltd. for a boundary amendment to marine finfish license and lease AQ#1039 in the Annapolis Basin, Digby County. Testified before the Nova Scotia Aquaculture Review Board. 17 November 2021.

MAJOR RESEARCH GRANTS

As Principal Investigator

2017-2018 Atlantic Salmon Post-Smolt Trawl and Troll Survey in the Strait of Belle Isle and Current Status of Knowledge, Data, and Research Efforts on Atlantic Salmon at Greenland. Atlantic Salmon Research Joint Venture. $171,200.

2018-2019 Mapping Spatial and Temporal Distribution of Atlantic Salmon Mixed Stocks in the North Atlantic. Atlantic Salmon Research Joint Venture. $166,980.

2019-2020 Mapping Spatial and Temporal Distribution of Atlantic Salmon Mixed Stocks in the North Atlantic. Atlantic Salmon Research Joint Venture. $146,280.

As Co-Investigator

2015-2015 Establishment of a water temperature monitoring network for Atlantic salmon: phase1., Natural Sciences and Engineering Research Council of Canada, $24,000.

2014-2017 Evolutionary and ecological impact of the escape of farmed salmon": policy and mitigation strategies. Natural Sciences and Engineering Research Council of Canada, $602,000.

2020-2025 Assessing the timing, duration, and effects of environmental variability and climate change for Atlantic salmon in the Eastern Canadian offshore regions. Environmental Studies Research Fund, $11,840,000.

STUDENTS SUPERVISED

2013 - 2020: External supervising committee for graduate student, UPEI.

Sept 2013 - Jan 2014: Supervised graduate student from Agro Campus, Rennes, France.

Sept 2012 - Jan 2013: Supervised graduate student from Agro Campus, Rennes, France.

May 2011- Sept 2011: Supervised graduate student from Netherlands.

Feb 2009 - May 2009: Supervised undergraduate student from University of Zeeland, Netherlands.

PRESENTATIONS AND SEMINARS

1998

Invasions of escaped cultured salmon and exotic smallmouth bass into wild salmon riffle habitats. 1998.

2001

A Review of Downstream Movements of Juvenile Atlantic Salmon in the Dam-Impacted Saint John River Drainage. Presented at the Canadian River Heritage Conference. June 2001.

2002

Scale Reading. Used for Discrimination Between Wild and Aquaculture Salmon. July 2002.

Genetic evidence of European Ioci in Atlantic salmon from a North American river. Presented at the AIC meeting. September 2002.

2003

Sea lice infestation rates on wild and escaped farmed Atlantic salmon entering the Magaguadavic River, New Brunswick. Presented at the 6ht International Sea Lice Conference. 2003

Magaguadavic River Smolt Monitoring Program. February 2003.

Seaward migration of landlocked Atlantic salmon: Implications for anadromous salmon recovery program. Presented to the American Fisheries Society. Quebec, City, QC. August 2003.

2004

Atlantic Salmon: What do you want to know? Presented to the NB Womans Institute. May 2004.

Efficacy of releasing captive reared broodstock into an imperiled wild salmon population as a recovery strategy. Presented at the Fisheries Society of the British Isles Annual International Conference. 22 July 2004.

Efficacy of releasing captive reared broodstock into an imperiled wild salmon population as a recover strategy. Presented at the 6th Bay of Fundy Workshop as part of the Bay of Fundy Ecosystem Partnership. September 2004.

Efficacy of releasing captive reared broodstock into an imperiled wild salmon population as a recovery strategy. Presented to the Atlantic International Chapter American Fisheries Society 30th Annual Meeting. Fairlee, Vermont. September 2004.

Efficacy of releasing reared broodstock into an imperiled wild salmon population as a recovery strategy. Presented at the Bay of Fundy Ecosystem Partnership 6th Bay of Fundy Workshop. September 2004.

2005

Magaguadavic River Salmon Restoration: The Dam Challenge. Presented at the Atlantic International Fisheries Society 31st Annual Meeting. Rangeley, ME, USA. 26 September 2005.

The escape of juvenile salmon from hatcheries into freshwater streams in New Brunswick, Canada. Presented at the ICES/NASCO Symposium. Bergen, Norway. 18 – 21 October 2005.

2006

Fisheries Management Applications: Magaguadavic River Studies. Presented at the College of Atlantic, Bar Harbour, ME, USA. 12 May 2006.

Effects Monitoring Program for St. George Hydroelectric Redevelopment. Progress Report. Presented to JDI. 7 April 2006.

2007

Scale Reading. Workshop. February 2007.

Magaguadavic River Atlantic Salmon Restoration Program. Presented to St. Stephen Middle School. 18 April 2007.

Migration of American Eels, *Anguilla rostrata*, past a Hydroelectric Dam and Through a Coastal Zone. Presented at the 7th International Fish Telemetry Conference. Silkeborg, Denmark. 21- 24 June 2007.

Using Acoustic Telemetry to Track the Movements of Adult Alewives, *Alosa pseudoharengus*, in a Freshwater and Coastal Zone. Presented at the 33rd Annual Meeting of the Atlantic International Chapter of the American Fisheries Society. French Village, NB. 23 – 25 September 2007.

Magaguadavic River Atlantic Salmon Restoration Program. Presented at the NBSGA Technical Session. 14 December 2007.

2008

Downstream Fish Passage by Hydroelectric Dams. Presented at the Renewable Energy Projects & Their Interactions with Wildlife Atlantic Society of Fish & Wildlife Biologists. Mount Allison University, Sackville, NB. 16 April 2008.

The Early Days. Vemco Days. September 2008.

Migration of American Eels, *Anguilla rostrata*, past a Hydroelectric Dam and Through a Coastal Zone. Meeting with JDI. 8 September 2008.

Migration of American Eels, *Anguilla rostrata*, past a Hydroelectric Dam and Through a Coastal Zone. Presented at an AIC Meeting. Digby, NS. 21- 23 September 2008.

2009

Sonic Tracking of Atlantic salmon smolts to sea: correlates of survival and lesson on the migration pathway. Maine. 10 January 2009.

Atlantic Salmon and Smallmouth Bass Interaction in the Magaguadavic River, New Brunswick. Presented at the Gulf Region Science Advisory Process. Moncton, NB. 28 January 2009.

Using acoustic telemetry to track the movements of river herring (*Alosa pseudoharengus*) in a freshwater and coastal zone. Presented at the 8th International Fish Telemetry Conference. Umea, Sweden. 14 – 18 September 2009.

Dam Delays. JDI Meeting. 27 October 2009.

2010

Magaguadavic River Atlantic Salmon Restoration Program. Presented at Inner Bay of Fundy & Atlantic Salmon Forum and Workshop. 27 October 2010.

ASF Research and Environment Department Report. ASF Fall Board Meetings. New York City, NY. November 2010.

2011

Atlantic Salmon Federation: Strategic Initiatives. Presented to Aquaculture. April 2011.

Sonic Tracking of Atlantic Salmon: lessons on the migration pathways, mortality points, and social dynamics. Presented in Restigouche. 2 April 2011.

ASF Research and Environment Department Report. Spring ASF Board Meetings. April 2011.

Outstanding research challenges for ASF. Fall Board Meetings. New York City, NY. November 2011.

Sonic Tracking of Atlantic salmon smolts and kelts to sea. ASF Fall Board Meetings. New York City, NY. 9 November 2011.

Sonic Tracking of Atlantic Salmon and Kelts to Sea. Presented in Quebec City, QC. 16 November 2011.

Sonic Tracking of Atlantic Salmon and Kelts to Sea. Presented at Fish Friends Summit in Doaktown, NB. 1 December 2011.

2012

Sonic tracking of Atlantic salmon: lessons on the migration pathways, mortality points, and social dynamics. Presented at the Nova Scotia Agricultural College, Truro, NS. 18 January 2012.

Sonic tracking of Atlantic salmon: lessons on the migration pathways, mortality points, and social dynamics. Restigouche Science Meeting, Campbellton, NB. 25 January 2012.

Sonic telemetry of Atlantic salmon smolts and kelts to sea: lessons on the migration pathways and mortality points. University of New Brunswick, Fredericton, NB. 27 January 2012.

Serpentine Atlantic Salmon Run in the St John River: Does it still exist? Miramichi Salmon Association Symposium, Boston, MA. 4 February 2012.

Sonic telemetry of Atlantic salmon smolts and kelts to sea: lessons on the migration pathways and mortality points. University of New Brunswick, Saint John, NB. 28 February 2012.

Salmon at Sea: Scientific advances and their implications for management. Nova Scotia Salmon Association Annual General Meeting, Halifax, NS. 3 March 2012.

Sonic telemetry of Atlantic salmon smolts and kelts to sea: lessons on the migration pathways and mortality points. Luncheon Speaker at Ocean Reef, Florida. 29 March 2012.

Update on Atlantic Salmon Growout Trial in Freshwater Closed- Containment System. Inner Bay of Fundy Recovery Team, Amherst, NS. 17 April 2012

Atlantic Salmon Federation Research & Environment Report. ASF Board Meetings, Toronto, ON. 25 April 2012.

Update on Atlantic Salmon Growout Trial in Freshwater Closed- Containment System, ASF Board Meetings, Toronto, ON. 25 April 2012.

Sonic telemetry of Atlantic salmon smolts and kelts to sea: lessons on the migration pathways and mortality points. Somerset Hills Country Club
Bernardsville, NJ. 15 May 2012.

Penobscot River Project : Not just a salmon project – leveraging resources. Somerset Hills Country Club, Bernardsville, NJ. 15 May 2012.

Update on Atlantic Salmon Grow out Trial in Freshwater Closed- Containment System. Aquaculture Canada 2012 Conference: New Frontiers – Bridging Technology and Economic Growth. Charlottetown, Prince Edward Island. 27-30 June 2012.

Sonic telemetry of Atlantic salmon smolts and kelts to sea: lessons on the migration pathways and mortality points. Luncheon speaker at Wilfred Carter Conservation Center, St. Andrews, NB. 1 August 2012.

Atlantic Salmon Federation Research & Environment Report. ASF Board Meetings, New York, NY. 14 November 2012.

Salmon Aquaculture: A Roadmap to Sustainability. Seminar presented to University of New Brunswick undergraduate students. Atlantic Salmon Federation, Chamcook, NB. 27 November 2012.

Summerfelt, S., Waldrop T., Good C., Davidson J., Backover P., Vinci B., Carr J. 2013. Freshwater Growout Trial of St John River Strain Atlantic Salmon in a Commercial-Scale, Land-based, Closed-Containment System. The Conservation Fund's Freshwater Institute. 17 pp.

2013

Escape Management From an NGO Perspective. Prevention and Management of Fish Escapes from Sea Cage Aquaculture in Atlantic Canada workshop. Halifax, NS. 23 January 2013.

Sonic Tracking of Atlantic Salmon. Restigouche River Science Meeting. Campbellton NB, 24 January 2013.

Freshwater Aquatic Invasives in New Brunswick. 2nd Annual New Brunswick Lakes Conference. Mactaquac Resort and Conference Center, Mactaquac, NB. 26 January 2013.

Salmon Aquaculture: A Roadmap to Sustainability. Miramichi Salmon Association Boston Symposium, Burlington, MA. 2 February 2013.

Salmon Aquaculture: A Roadmap to Sustainability. LaHave Salmon Association Annual General Meeting, Bridgewater, NS. 7 April 2013.

Farmed Salmon Escapee Management: An NGO Perspective. Inner Bay of Fundy Recovery Team Meeting. Amherst, NS. 17 April 2013.

Atlantic Salmon Federation Research & Environment Report. ASF Board Meetings, Montreal, QC, 1 May 2013.

Sonic telemetry of Atlantic salmon smolt and kelt to sea: lessons on the migration pathways and mortality points. Seminar given to Société Cascapédia, St. Jules Quebec. 23 May 2013.

Sonic telemetry of Atlantic salmon smolt and kelt to sea: lessons on the migration pathways and mortality points. Salmon Guides Night, Matapedia, QC. 23 May 2013.

Salmon Aquaculture: A Roadmap to Sustainability. Hammond River Angling Association Meeting, Hampton, NB. 10 June 2013.

Restoration of Alewives in Maine and New Brunswick Rivers. International St. Croix River Watershed Board Public Meeting, St. Stephen, NB. 17 June 2013.

One step forward two steps back: Obstacles to salmon recovery in the Magaguadavic River. What works? A Workshop on Wild Atlantic Salmon Recovery Programs, St Andrews, NB. 18 September 2013.

Scale Reading Used for Discriminating Between Wild and Aquaculture Salmon Workshop on Atlantic Salmon Scale Reading. Northwest Atlantic Fisheries Centre Fisheries and Oceans Canada St. Johns, NL. 22 October 2013.

Escape Management From an NGO Perspective. Workshop on Atlantic Salmon Scale Reading. Northwest Atlantic Fisheries Centre Fisheries and Oceans Canada St. Johns, NL. 22 October 2013.

Salmon Aquaculture: A Roadmap to Sustainability. Miawpukek First Nation 7th Annual General Staff Meeting Se't A'newey School Conne River, NL. 7 November 2013.

Atlantic Salmon Federation Research & Environment Report. ASF Board Meetings, New York, NY. 13 November 2013.

What Works: A Workshop on Wild Atlantic Salmon Recovery Programs. IBIS/AST Salmon Stocking Conference, Glasgow City, UK. 27 November 2013.

Salmon Aquaculture: A Roadmap to Sustainability Aquaculture Review Panel Target Meeting, Halifax, NS. 3 December 2013.

2014

Lessons on the migration pathways and mortality points of salmon at sea. Atlantic Salmon Ecosystems Forum, University of Maine, Orono, ME 8 January 2014

What Works: A Workshop on Wild Atlantic Salmon Recovery Programs Atlantic Salmon Ecosystems Forum, University of Maine, Orono, ME 9 January 2014

Smolt and Kelt Tracking Update. Miramichi Salmon Association Board of Directors

Meeting, Boston, MA. 31 January 2014.

Atlantic Salmon Smolt and Kelt Tracking Update. Restigouche River Watershed Management Committee Science Committee Meeting, Campbellton NB 4 February 2014.

Lessons on the migration pathways and mortality points of salmon at sea, VEMCO Workshop, St. Andrews, NB 26 March 2014.

Atlantic Salmon Federation Research & Environment Report. ASF Board Meetings, St. Andrews, NB. 21 May 2014.

Scale Reading Used for Discriminating Between Wild and Aquaculture Salmon Workshop on Atlantic Salmon Scale Reading**.** Miawpukek First Nation, Conne River, NL. 26 May 2014.

Escape Management From an NGO Perspective. Workshop on Atlantic Salmon Scale Reading. Miawpukek First Nation, Conne River, NL. 26 May 2014.

Salmon Aquaculture: A Roadmap to Sustainability. Kedgwick Salmon Lodge. 21 June 2014.

Sonic telemetry of Atlantic salmon smolt and kelt to sea: lessons on the migration pathways and mortality points. Kedgwick Salmon Lodge. 21 June 2014.

Lessons on the Migration Pathways of Atlantic Salmon Smolt and Kelt at Sea. 144th Annual Meeting American Fisheries Society, Quebec City. 21 August 2014.

Bayesian hierarchical modeling of 11 years of inter-stage survival rates of wild Atlantic salmon smolts and post-smolts from three rivers of eastern Canada. ICES Annual Science Conference. A Coruna, Spain. 15 September 2014.

Lessons on the Migration Pathways of Atlantic Salmon Smolt and Kelt at Sea. Ecology of Estuarine Fishes Course, Anderson House, St Andrews, NB. 23 September 2014.

The Atlantic Salmon Federation: The Wild Atlantic Salmon and Our Conservation Role. UNB Fredericton Marine Ecology Course, St. Andrews, NB. 25 September 2014.

SoBI Summary for Derek Simon Nunatukavut Consultation. Sonically Tagged Smolts & Kelts. 29 September 2014.

Lesson on the Migration Pathways of Atlantic Salmon Smolt and Kelt at Sea. Nunatukavut Meeting. 1 October 2014.

Aquaculture Issues Escapees & Code of Containment. ASF Research and Environment Meeting. University Club, 1-54th Street West, New York, NY. 12 November 2014.

Closed Containment Projects. ASF Board Research and Environment Meeting. University

Club, 1-54th Street West, New York, NY. 12 November 2014.

ASF Research & Environment Report on Low Marine Survival ASF Board Research and Environment Meeting. University Club, 1-54th Street West, New York, NY. 12 November 2014.

What happened to salmon in 2014? ASF Board Meeting University Club, 1-54th Street West, New York, NY. 13 November 2014.

ASF Research & Environment Report. Aquaculture Issues. University Club, 1-54th Street West, New York, NY. 15 November 2014.

Regulations of Aquaculture: Current Challenges and Future Prospects for Industry in Canada. Senate Committee Briefing, Moncton NB. 20 November 2014.

Lessons on the Migration Pathways of Atlantic Salmon Smolt and Kelt at Sea. Salmon Summit: Stewardship and Sustainable Management of Atlantic Salmon in the Gespe'gewa'gi, Listuguj, Quebec. 25 November 2014.

2015

What Works: A Workshop on Wild Atlantic Salmon Recovery Programs. Miramichi Salmon Association Symposium. Boston Marriott, Burlington, MA. 31 January 2015.

Using Telemetry to Unravel the Mystery of Atlantic Salmon Migration at Sea. Deborah Pratt Dawson Conservation Symposium. 14 March 2015.

Summary of NASCO's International Atlantic Salmon Research Board's Telemetry Workshop. Shangri-La Hotel, Toronto, Ontario. 29 April 2015.

ASF Research & Environment Report. Low Marine Survival. ASF Board Meetings. Toronto, Ontario. 29 April 2015.

ASF Research & Environment Report. Aquaculture Issues. Shangri-La Hotel Toronto, Ontario. 29 April 2015.

North American Wild Atlantic Salmon Recovery Strategy. Atlantic Salmon Federation Board Meeting. Shangri-La Hotel. Toronto, Ontario. 30 April 2015.

Using Telemetry to Unravel the Mystery of Atlantic Salmon Migrations at Sea. The Mount Royal Club. Montreal Dinner. Montreal, Quebec. 6 May 2015.

Using Telemetry to Unravel the Mystery of Atlantic Salmon Migrations at Sea. The Country Club. Chestnut Hill, MA. Boston Dinner. 13 May 2015.

Aquaculture Issues Escapees & Code of Containment. Governor General's Leadership Conference. ASF's Wild Salmon Center, Chamcook, NB. 25 May 2015.

Using Telemetry to Unravel the Mystery of Atlantic Salmon Migration at Sea. Cascapédia Société. Cascapédia St. Jules, Quebec. 4 June 2015.

Bayesian modeling of Atlantic salmon smolt inter-stage survival from Canadian rivers. 3rd International Conference on Fish Telemetry. Halifax, Nova Scotia. 13 – 17 July 2015.

Using Telemetry to Unravel the Mystery of Atlantic Salmon Migrations at Sea. CAST Presentation. UNB. 9 September 2015.

The Wild Atlantic Salmon and Our Conservation Role. UNB Fredericton Marine Ecology Course. St. Andrews. 5 October 2015.

Using Telemetry to Unravel the Mystery of Atlantic Salmon Migrations at Sea. Canadian Museum of History (Montreal Dinner). Gatineau, QC. 7 October 2015.

Low Marine Survival. ASF Board Meetings. University Club. 1-54th Street West, New York, NY. 11 November 2015.

Research & Environment Proposed Budget 2015 – 2016. ASF Board Meetings. University Club. 1-54th Street West, New York, NY. 11 November 2015.

Aquaculture Issues. Closed Containment Update. ASF Research & Environment Meeting. ASF Board Meetings. New York, NY. 11 November 2015.

Aquaculture Issues. Escapees & Code of Containment. ASF Board Meeting. University Club. 1-54th Street West, New York, NY. 11 November 2015.

North American Wild Atlantic Salmon Recovery Strategy. Listiguj Salmon Summit. Listiguj, Quebec. 19 November 2015.

Smolt & Kelt Tracking Program. Listiguj Salmon Summit. Listiguj, Quebec. 19 November 2015.

2016

Wild Atlantic Salmon Recovery Planning: What Works? Atlantic Salmon Ecosystems Forum. Orono, Maine. 6 January 2016.

Sonic telemetry of Atlantic Salmon Smolts and Kelt to Sea: Lesson on the Migration Pathway and Mortality. River Dee Tracking Workshop. Banchory Lodge Hotel, Aberdeenshire, Scotland. 12 February 2016.

Salmon Aquaculture in Newfoundland: A Roadmap to Sustainability. CAN: & ASF Meeting with Hon. Steve Crocker, NL Minister of Fisheries & Aquaculture. St. John's, NL. 23 February 2016.

Aquaculture Issues. ASF Research & Environment Meeting. Montreal Board Meetings. 27 April 2016.

Collaboration for Atlantic Salmon Tomorrow (CAST). ASF Board Meeting. Montreal, Quebec. 27 April 2016.

Using Telemetry to Unravel the Mystery of Atlantic Salmon Mortalities at Sea. ASF 24th Annual Fredericton Dinner. Fredericton, NB. 12 May 2016.

North Atlantic Salmon Marine Tracking Studies. National Fish and Wildlife Foundation. Washington, DC. 25 May 2016.

Using Telemetry to Unravel the Mystery of Atlantic Salmon Mortalities at Sea. New Derreen Camp, Cascapedia, Quebec. 24 June 2016.

Using Telemetry to Unravel the Mystery of Atlantic Salmon Mortalities at Sea. Joint Venture Meeting. 30 June 2016.

Using Telemetry to Unravel the Mystery of Atlantic Salmon Mortalities at Sea. ASF Staff Forum. Chamcook, NB. 17 August 2016.

Presentation to the Standing Committee on Fisheries and Oceans. Subject: Wild Salmon in Eastern Canada. Kinsmen Centre, Miramichi, NB. 29 September 2016.

Low Marine Survival. ASF Fall Board Meetings. University Club. 1-54th Street West, New York, NY. 9 November 2016.

2017

Using telemetry to Unravel the Mystery of Atlantic Salmon Mortalities at Sea. Annual Montreal Dinner. Club Saint James. 26 April 2017

Aquaculture Issues. Research & Environment Meeting. ASF Spring Board Meeting Saint Andrews, NB. 17 May 2017.

Tracking Salmon: Unravelling the Mystery of Where our Fish are Dying at Sea. Atlantic Salmon Trust 50th Anniversary Gala Celebrations. Science Symposium. Syon House, United Kingdom. 25 May 2017.

Tracking Salmon: Unravelling the Mystery of When and Where our Fish are Dying at Sea. Scotland's Salmon Festival Science Conference. Inverness College. 30 August 2017.

Tracking Salmon: Unravelling the Mystery of When and Where our Fish are Dying at Sea. 14th Annual Toronto Benefactor Dinner. Toronto Club. 12 October 2017.

Research and Environment Committee meeting. ASF Fall Board Meeting. University Club. 1-54th Street West, New York, NY. 8 November 2017.

Low Marine Survival. Atlantic Salmon Telemetry Planning Meeting: Expanding the tracking network into the North Atlantic. Halifax NS. 5 December 2017

2018

Tracking Salmon: Unravelling the Mystery of Where our Fish are Dying at Sea. Atlantic Salmon Conservation Fund Webinar. 21 February 2018.

Using Telemetry to Explore Atlantic Salmon Marine Mortality. Fisheries and Oceans Atlantic Salmon Assessment Meeting. St. Johns NL. 28 February 2018.

Science Update: The State of the Stocks. Managing the Challenges. New Approaches and New Technologies. North Atlantic Salmon Fund – The Orri Fund Salmon Summit. Reykjavik, Iceland. 21 & 22 March 2018.

Using Telemetry to Explore Atlantic Salmon Marine Mortality. ICES, 7 April 2018.

Research and Environment Committee Meeting. ASF Spring Board Meetings. King Edward Hotel. 37 King St., Toronto, ON. 2 May 2018.

Low Marine Survival. Research and Environment Committee Meeting. ASF Spring Board Meetings. King Edward Hotel. 37 King St., Toronto, ON. 2 May 2018.

Status of Tracking Salmon in the Ocean: Overview of Acoustic Tracking. ROAM Workshop. Woods Hole, MA. 7 June 2018.

Research and Environment Update. Staff Forum, Saint Andrews, NB. 9 August 2018.

ASF Research Update. New Derreen, Cascapédia, QC. 14 August 2018.

Atlantic Salmon Federation Research and Environment Committee Meeting. University Club, 1-54th St West, New York, NY. 7 November 2018.

2019

Using Telemetry to Unravel the Mystery of Atlantic Salmon Migrations at Sea. Mont Joli, QC. 28 January 2019.

Atlantic Salmon Federation Research Update. Restigouche River Watershed Management Committee Science Advisory Meeting. Campbellton, NB. 30 January 2019.

Tracking the Marine Migrations of Atlantic Salmon. Fisheries and Oceans Assessment of Atlantic Salmon in Newfoundland and Labrador. St. Johns, NL. 5 March 2019.

Using Telemetry to Map the Spatial and Temporal Distribution of Atlantic Salmon in the Ocean. Atlantic Salmon Ecosystem Forum. Quebec City, QC. 13 March 2019.

Using Telemetry to Map the Spatial and Temporal Distribution of Atlantic Salmon in the Ocean. ASF Director Dinner. St. James Club, Montreal, QC. 23 April 2019.

Low Marine Survival. Research and Environment Committee Meeting. Le Windsor

Ballroom, 1170 Peel St, Montreal, QC. 24 April 2019.

West Greenland Atlantic Salmon Telemetry Program. 23rd Annual Boston Dinner, Boston MA. 8 May 2019.

Using Telemetry to Map the Spatial and Temporal Distribution of Atlantic Salmon in the Ocean. ICES Working Group: WKSalmon. Copenhagen, Denmark. 24 June 2019.

Using Telemetry to Map the Spatial and Temporal Distribution of Atlantic Salmon in the Ocean. Atlantic Salmon Conservation Schools Network. ASF Conservation Center. Chamcook, NB. 31 July 2019.

Using Telemetry to Map the Spatial and Temporal Distribution of Atlantic Salmon in the Ocean. World Salmon Forum. Fairmont Olympic Hotel. Seattle, WA. 22 August 2019.

Using Telemetry to Map the Spatial and Temporal Distribution of Atlantic Salmon in the Ocean. Greenland Fisheries License Control Authority. NUUK, Greenland. 16 September 2019.

RAFOS Ocean Acoustic Monitoring (ROAM) Tag. SAMARCH International Salmonid Coastal and Marine Telemetry Workshop. Southhampton, UK. 5 November 2019.

Using Telemetry to Map the Spatial and Temporal Distribution of Atlantic Salmon in the Ocean. SAMARCH International Salmonid Coastal and Marine Telemetry Workshop. Southhampton, UK. 5 November 2019.

Low Marine Survival. ASF Research and Environment Committee Meeting. University Club, 1-54th St West, New York, NY. 13 November 2019.

2020

A molecular assessment of infectious agents carried by Atlantic salmon at sea and in three eastern Canadian rivers, including aquaculture escapees and North American and European origin wild stocks. Atlantic Salmon Ecosystems Forum. Orono, Maine. 14 January 2020.

Mapping Mixed Stocks in the North Atlantic. Atlantic Salmon Research Joint Venture Meeting. Moncton, NB. 12 February 2020.

Telemetry and the Atlantic Salmon Workshop: Next Steps from Estuary to the North Atlantic Ocean. Atlantic Salmon Research Joint Venture Meeting. Moncton, NB. 12 February 2020.

Atlantic Salmon Federation Research and Environment Update. ASF Board Information Session. Virtual Meeting. 14 May 2020.

Using Telemetry to Unravel the Mystery of Atlantic Salmon Mortalities at Sea. Salmon – Great Leap for a Future. The Explorers Club Public Lecture Series, New York, NY.

Virtual. 24 August 2020.

Using Telemetry to Unravel the Mystery of Atlantic Salmon Mortalities at Sea. Salmon – Great Leap for a Future. The Explorers Club Public Lecture Series, New York, NY. Virtual. 24 August 2020.

Using Telemetry to Unravel the Mystery of Atlantic Salmon Mortalities at Sea. Salmon – Great Leap for a Future. Chicago Fund Raising 'Virtual' Dinner. 7 October 2020.

Atlantic Salmon Federation Research and Environment Update. ASF Board Meeting. Virtual. 11 November 2020.

2021

Atlantic Salmon Federation Research and Environment Committee Meeting. 25 March 2021.

This is **Exhibit "C"** referred to in the affidavit
of **Jon W. Carr,** affirmed before me
this 19th day of January, 2024.

CURRICULUM VITAE

STEPHEN GORDON SUTTON

(January 2024)

PERSONAL

Address:	Atlantic Salmon Federation [redacted] Chamcook, NB Canada [redacted]
Telephone:	[redacted]
Email:	[redacted]
Place of Birth:	St. John's, Newfoundland, Canada
Citizenship:	Canadian, Australian

EDUCATION

Degrees and Qualifications

Ph.D.	2001	Texas A&M University College Station, TX	Wildlife and Fisheries Science
M.Sc.	1997	Memorial University St. John's, NL	Biology
B.Sc. (Hons.)	1994	Memorial University St. John's, NL	Biology (Ecology)
Grad. Cert. Educ.	2013	James Cook University Townsville, QLD	Tertiary Teaching

Theses

Ph.D.	Understanding Catch-and-Release Behaviour of Recreational Anglers.
M.Sc.	The Mystery Fish of Bonavista North: A Multidisciplinary Approach to Research and Management of a Unique Recreational Salmonid Fishery in Newfoundland.
B.Sc. (Hons.)	Spatial and Temporal Variability in the Fat Content and Condition of Juvenile Atlantic Salmon, *Salmo salar*, in a Newfoundland River System.

PROFESSIONAL WORK EXPERIENCE

August 2015 – Present
Director of Public Policy
Atlantic Salmon Federation
Chamcook, New Brunswick, Canada

September 2002 – December 2014
Research Fellow/Principal Research Fellow/Associate Professor
School of Earth and Environmental Sciences
James Cook University, Townsville, Queensland, Australia

September 2001 – September 2002
Biologist, Department of Fisheries and Oceans Canada
St. John's, Newfoundland, Canada

September 1997 – May 2001
Graduate Research Assistant, Department of Wildlife and Fisheries Sciences
Texas A&M University, College Station, Texas, USA

PUBLICATIONS

Edited Volumes

Beard, T.D., R. Arlinghaus, and **S.G. Sutton**, Editors. 2011. The Angler in the Environment: Social, Economic, Biological, and Ethical Dimensions. American Fisheries Society, Bethesda, Maryland.

S.G. Sutton, A. Danylchuk, K. Freire. 2016. Proceedings of the 7th World Recreational Fisheries Conference. Fisheries Management and Ecology 23 (3/4), 177-333

Journal Articles

Smith, W.E, G.T. Kyle, **S.G. Sutton**. 2023. Using a styles of participation self-classification measure to characterize highly specialized anglers, Human Dimensions of Wildlife, 28:1, 36-52

Smith, W.E, G.T. Kyle, **S.G. Sutton**, R. Dunlap. 2023. Characterizing style of participation among Texas inshore recreational fishing guides, Human Dimensions of Wildlife, 28:1, 18-35.

Smith, W.E., G.T. Kyle, and **S.G Sutton**. 2021. Displacement and associated substitution behavior among Texas inshore fishing guides due to perceived Spotted Seatrout declines. Marine Policy 131: 104624.

Thorstad, E., D. Bliss, C. Breau, K. Damon-Randall, L. Sundt-Hansen, E. Hatfield, G. Horsburgh. H. Hansen, N. Ó'Maoiléidigh, T. Sheehan, **S.G. Sutton**. 2021. Atlantic salmon in a rapidly changing environment - facing the challenges of reduced marine survival and climate change. Aquatic Conservation: Marine and Freshwater Ecosystems.

Bower, S.D., Ø. Aas, R. Arlinghaus, T. D. Beard, I. G. Cowx, A. J. Danylchuk, K. M. F. Freire, W. M. Potts, **S.G. Sutton**, S. J. Cooke. 2020. Knowledge Gaps and Management

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SD den Haring, **SG Sutton.** 2019. Comparing intended, self-reported, and observed behavior of snorkelers in the Mombasa Marine Park and Reserve, Kenya. Tourism in Marine Environments 14 (1-2), 1-17.

Oh, C., **S.G. Sutton.** 2019. Comparing the Developmental Process of Consumptive Orientation Across Different Population Groups, Leisure Sciences, 41 (3), 167-185

Wynveen, C.J., **S.G. Sutton.** 2017. Engaging Great Barrier Reef Stakeholders: Mediation Analyses of Barriers Among the Antecedents of Pro-Environmental Behavior. Human Dimensions of Wildlife, 22 (2), 126-141.

Bergseth, B.J., D.H. Williamson, G.R. Russ, **S.G. Sutton**, J.E. Cinner. 2017. A social–ecological approach to assessing and managing poaching by recreational fishers. Frontiers in Ecology and the Environment, 15 (2), 67-73.

van Riper, C.J., G.T. Kyle, B.C. Sherrouse, K.J. Bagstad, **S.G. Sutton**. 2017. Toward an integrated understanding of perceived biodiversity values and environmental conditions in a national park. Ecological Indicators 72, 278-287.

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Farr, M.N., Stoeckl, and **S.G. Sutton**. 2014. Recreational fishing and boating: Are the determinants the same? *Marine Policy,* 47: 126-137.

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van Riper, C.J., G.T. Kyle, **S.G. Sutton**, J.I. Yoon, and R.C. Tobin. 2013. Australian residents' attitudes toward pro-environmental behavior and climate change impacts on the Great Barrier Reef. *Journal of Environmental Planning and Management*, 56:494-511.

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van Riper, C.J., G.T. Kyle, **S.G. Sutton**, M. Barnes, and B.C. Sherrouse. 2012. Mapping outdoor recreationists' perceived social values for ecosystem services at Hinchinbrook Island National Park, Australia. *Applied Geography* 35: 164-173.

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Lédée, E.J.I, **S.G. Sutton,** R.C. Tobin, and D.M. De Freitas. 2012. Responses and adaptation strategies of commercial and charter fishers to zoning changes in the Great Barrier Reef Marine Park. *Marine Policy* 36:226-234.

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Cardona-Pons, F., B. Morales-Nin, and **S.G. Sutton**. 2010. Scientists and recreational fishers: Communication manners and its efficiency. *Fisheries Research*, 106:575-578.

McCook, L.J, T. Ayling, M. Cappo, J.H. Choat, R.D. Evans, D.M. De Freitas, M. Heupel, T.P. Hughes, G.P. Jones, B. Mapstone, H. Marsh, M. Mills, F. Molloy, C.R. Pitcher, R.L. Pressey, G.R. Russ, **S.G. Sutton**, H. Sweatman, R. Tobin, D.R. Wachenfeld, D.H. Williamson. 2010. Adaptive management and monitoring of the Great Barrier Reef reserve network: A globally significant case study in marine conservation. *Proceedings of the National Academy of Sciences* 107(43):18278-18285..

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Sutton, S.G., J.R. Stoll, and R.B. Ditton. 2001. Understanding anglers' willingness to pay increased fishing license fees. *Human Dimensions of Wildlife* 6:115-130.

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Book Chapters

Schratwieser, J. **S.G. Sutton**, and R. Arlinghaus. 2011. Introduction. Pages 1-9 *in* T. D. Beard, Jr., R. Arlinghaus, and S. G. Sutton, editors. The Angler in the Environment: Social, Economic, Biological, and Ethical Dimensions. American Fisheries Society, Bethesda, Maryland.

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Sutton, S.G. 2000. Local knowledge and community watershed management of recreational fisheries resources in Newfoundland. In: Neis, B. and L. Felt (eds.) *Finding our Sea Legs: Linking Fishery People and Their Knowledge With Science and Management*. ISER Press, St. John's.

Conference Proceedings

van Riper, C.J., Kyle, G.T., **S.G. Sutton,** Tobin, R., & Stronza, A. 2012. Place meanings among resource and recreation managers of the Great Barrier Reef Marine Park, Australia. In S. Weber (Ed.), *Rethinking Protected Areas in a Changing World Proceedings of the 2011 George Wright Society Conference on Parks, Protected Areas, and Cultural Sites* (pp. 344-349). Hancock, MI: The George Wright Society.

van Riper, C.J., Kyle, G.T., Yoon, J.I., and **S.G. Sutton**. 2010. Australian community members' attitudes toward climate change impacts at the Great Barrier Reef. In C. E. Watts & C. L. Fisher (Eds.), Proceedings of the 2010 Northeastern Recreation Research Symposium. General Technical Report. Newtown Square, PA: U.S. Department of Agriculture, Forest Service, Northern Research Station.

Smith, W.E., Kyle, G., & **S.G. Sutton.** 2010. Angler segmentation using perceptions of experiential quality in the Great Barrier Reef Marine Park. In C. E. Watts & C. L. Fisher (Eds.), Proceedings of the 2010 Northeastern Recreation Research Symposium. General Technical Report. Newtown Square, PA: U.S. Department of Agriculture, Forest Service, Northern Research Station.

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Sutton, S.G, E. J. Lédée, R.C. Tobin, D. M. De Freitas. 2010. Impacts of the 2004 Rezoning of the Great Barrier Reef Marine Park on Commercial Line, Trawl and Charter Fishers. Report to the Marine and Tropical Sciences Research Facility. Reef and Rainforest Research Centre Limited, Cairns, Australia.

Tobin, RC, **Sutton, SG** and Penny, AL. 2010. Towards Evaluating the Socio-economic Impacts of Changes to Queensland's Inshore Fishery Management. Fishing and Fisheries Research Centre Technical Report No. 9., James Cook University, Townsville.

Tobin, A, Schlaff, A, Tobin, R, Penny, A, Ayling, T, Ayling, A, Krause, B, Welch, D, **Sutton, S.G,** Sawynok, B, Marshall, N, Marshall, P and Maynard, J (2010). Adapting to Change: Minimising Uncertainty about the Effects of Rapidly-Changing Environmental Conditions on the Queensland Coral Reef Fin Fish Fishery, Fishing and Fisheries Research Centre Technical Report No. 11, James Cook University, Townsville, Australia.

Tobin, RC, **Sutton, SG,** Penny, A, Williams, L, Maroske, J and Nilsson, J. 2010. Baseline Socio-Economic Data for Queensland East-Coast Inshore and Rocky Reef Fishery Stakeholders. Part A: Commercial Fishers, Technical Report No. 5. FRDC Project No. 2007/048, Fishing and Fisheries Research Centre, Townsville, Australia.

Morgan C, **Sutton SG** and Tobin R. 2010. What drives fishers to comply with regulations, and how do you measure it? A literature review. Report to the Marine and Tropical Sciences Research Facility. Reef and Rainforest Research Centre Limited, Cairns.

Tobin, RC, Beggs, K, **Sutton, SG**, Penny, A, Maroske, J and Williams, L. 2010. Baseline Socio-Economic Data for Queensland East Coast Inshore and Rocky Reef Fishery Stakeholders Part B: Charter Fishers, Technical Report No. 6. FRDC Project No. 2007/048, Fishing and Fisheries Research Centre, Townsville, Australia.

Tobin, RC, Beggs, K, **Sutton, SG** and Penny, A. 2010. Baseline Socio-Economic Data for Queensland East-Coast Inshore and Rocky Reef Fishery Stakeholders. Part C: Recreational Fishers, Technical Report No. 7. FRDC Project No. 2007/048, Fishing and Fisheries Research Centre, Townsville, Australia.

Tobin, RC, Beggs, K, **Sutton, SG** and Penny, A. 2010. Baseline Socio-Economic Data for Queensland East-Coast Inshore and Rocky Reef Fishery Stakeholders. Part D: Seafood Consumers, Technical Report No. 8. FRDC Project No. 2007/048, Fishing and Fisheries Research Centre, Townsville, Australia.

Nilsson, J.A., **S.G. Sutton**, and R.C Tobin. 2009. A survey of climate change and the Great Barrier Reef. Report to the Marine and Tropical Sciences Research Facility. Reef and Rainforest Research Centre Limited, Cairns, Australia.

Lynch, A.M, **S.G. Sutton**, and R.C Tobin. 2009. A review of community perceptions of climate change: Implications for the Great Barrier Reef. Report to the Great Barrier Reef Marine Park Authority, Townsville, Australia.

Tobin R.C., Simpfendorfer C.A., **Sutton S.G.**, Goldman B., Muldoon G., Williams A.J., Ledee E. 2009. A review of the spawning closures in the Coral Reef Fin Fish Fishery. A Report to Queensland's Primary Industries and Fisheries, Queensland, Australia.

Sutton, S.G. 2008. Recreational fishers' perceptions about the costs and benefits of the 2004 Great Barrier Reef Marine Park Zoning Plan. Report to the Marine and Tropical Sciences Research Facility. Reef and Rainforest Research Centre Limited, Cairns (44pp.)

Little, L.R., G.A. Begg, B. Goldman, N. Ellis, B.D. Mapstone, A.E. Punt, A. Jones, **S.G. Sutton**, and A. Williams. 2008. Modelling multi-species targeting of fishing effort in the Queensland Coral Reef Fin Fish Fishery. Fishing and Fisheries Research Centre Technical Report No. 2. James Cook University, Townsville.

Sutton, S.G. 2006. An assessment of the social characteristics of Queensland's recreational fishers. Report prepared for the CRC Reef Research Centre, Townsville.

Shields, J.D., D.M. Taylor, **S.G. Sutton**, P.G. O'Keefe, D.W. Ings, and A.L. Pardy. 2004. Aspects of the epidemiology of bitter crab disease (hematodinium sp.) in snow crabs, *Chionocetes opilio* from Newfoundland, Canada. Canadian Science Advisory Secretariat Resarch Document 2004/077.

Sutton, S.G., and R.B. Ditton. 2001. Understanding an urban fishery: Braunig Lake and Calaveras Lake, San Antonio, Texas. Report prepared for Texas Parks and Wildlife Department. Texas A&M University, College Station.

Ditton, R.B., D.K. Anderson, B.L. Bohnsack, and **S.G. Sutton**. 2000. The 1999 Texas International Fishing Tournament: participants' characteristics, participation in fishing, attitudes, expenditures, and economic impacts. Human Dimensions of Fisheries Laboratory Technical Report to Texas International Fishing Tournament, Inc. Texas A&M University, College Station, Texas.

Ditton, R.B., D.K. Anderson, J.F. Thigpen III, B.L. Bohnsack and **S.G. Sutton**. 2000. The 1999 Pirate's Cove Big Game Tournaments: participants' characteristics, participation in fishing, attitudes, expenditures, and economic impacts. Human Dimensions of Fisheries Laboratory Technical Report. Texas A&M University: College Station, TX.

Sutton, S.G., R.B. Ditton, J.R. Stoll, and J.W. Milon. 1999. A cross-sectional study and longitudinal perspective on the social and economic characteristics of the charter and party boat fishing industry of Alabama, Mississippi, Louisiana, and Texas. Human Dimensions of Fisheries Laboratory Technical Report to the National Marine Fisheries Service. Texas A&M University, College Station, Texas.

Ditton, R.B., and **S.G. Sutton.** 1998. A social and economic study of Fort Hood anglers. Human Dimensions of Fisheries Laboratory Technical Report to the U.S. Fish and Wildlife Service. Texas A&M University, College Station, Texas.

Sutton, S.G. and R.S. Pritchett. 1995. Assessment of the 1995 winter fishery on Paddy's Pond and Cochrane Pond using a roving creel survey. Report submitted to the Department of Fisheries, Food and Agriculture, Government of Newfoundland and Labrador.

CONFERENCE PRESENTATIONS

Sutton, S.G., D. Gledhill, A.J. Hobday, D. J. Welch, M. J. Lansdell, M. Koopman, A.Jeloudev, A. Smith and P. R. Last. 2014. A collaborative approach to using historical spearfishing competition records to identify climate-induced environmental changes. 7th World Recreational Fishing Conference, Campinas, Brazil, September 1-4, 2014.

Sutton, S.G. 2013. Engaging the recreational fishing community in conservation of Australia's marine resources. 26th International Congress on Conservation Biology, Baltimore Maryland.

Sutton, S.G., and R.C. Tobin. 2012. Understanding commercial fishers' compliance with go-slow zones in the Great Sandy Marine Park. Pathways to Success 2012: Integrating Human Dimensions into Management of Fisheries and Wildlife. Breckenridge, Colorado.

Sutton, S.G. and L. Hunt. 2011. Human dimensions of recreational fisheries: Challenges, opportunities, and emerging research needs. Keynote Address, 6th World Recreational Fishing Conference. Berlin, Germany, August 1-4, 2011.

Sutton, S.G. 2010. Human Dimensions of Recreational Fisheries Research in Australia: Opportunities and Constraints. Pathways to Success 2010: Integrating Human Dimensions into Management of Fisheries and Wildlife, Estes Park, Colorado.

Sutton, S.G., R. Tobin, C. Simpfendorfer, B. Goldman, G. Muldoon, A. Williams. 2010. Reviewing Spawning Closures for Queensland's Coral Reef Finfish Fishery: A Multi-disciplinary Approach. 16th International Symposium on Society and Resource Management, Corpus Christi, Texas.

Sutton, S.G., J. Nilsson, and R. Tobin. 2009. Constraints on community engagement in Great Barrier Reef climate change action strategies. Paper presented at the 15th International Symposium on Society and Resource Management. Vienna, Austria.

Sutton, S.G., J. Nilsson, and R. Tobin. 2009. Constraints on community engagement in Great Barrier Reef climate change action strategies. Paper presented at the 23rd Annual Meeting of the Society for Conservation Biology. Beijing, China.

Sutton, S.G., J. Nilsson, and R. Tobin. 2009. Constraints on community engagement in Great Barrier Reef climate change action strategies. Paper presented at the Paper presented at the 3rd Annual Marine and Tropical Research Facility Conference, Townsville, Australia.

Sutton, S.G. and R. Tobin 2008. Recreational fishers perceptions of the costs and benefits of rezoning Australia's Great Barrier Reef Marine Park. Paper presented at the 5th World Recreational Fisheries Conference, Dania Beach, Florida, USA.

Sutton, S.G. and R. Tobin. 2008. Recreational fishers perceptions of the costs and benefits of rezoning Australia's Great Barrier Reef Marine Park. Paper presented at the 11th International Coral Reef Symposium, Fort Lauderdale, Florida, USA.

Sutton, S.G. and R. Tobin**.** 2008. Recreational fishers perceptions of the costs and benefits of rezoning Australia's Great Barrier Reef Marine Park. Paper presented at the 14th International Symposium on Society and Resource Management. Burlington, Vermont, USA.

Sutton, S.G. and R. Tobin**.** 2008. Recreational fishers perceptions of the costs and benefits of rezoning Australia's Great Barrier Reef Marine Park. Paper presented at the 2008 Australian Society for Fish Biology Conference, Sydney, Australia.

Sutton, S.G. 2008. Recreational fishers perceptions of the costs and benefits of the 2004Great Barrier Reef Marine Park Zoning Plan. Paper presented at the 2nd Annual Marine and Tropical Research Facility Conference, Cairns, Australia.

Sutton, S.G., and W. Sawynok. 2007. CapReef: Engaging the community in Great Barrier Reef science. Poster presented at the 13th International Symposium on Society and Resource

Management. June 17-21, Park City, Utah, USA.

Sutton, S.G. 2006. Towards effective engagement of recreational fishers in the marine protected area planning and management process. Paper presented at the Recfishing Research National Seminar on Marine Protected Areas. November 3-4, Brisbane, Australia.

Sutton, S.G., 2006. Understanding the effects of marine protected areas on recreational fishers. Paper presented at the Recfishing Research National Seminar on Marine Protected Areas. November 3-4, Brisbane, Australia.

Sutton, S.G. 2006. Why do people drop out of recreational fishing? A study from Queensland, Australia. Paper presented at the 12th International Symposium on Society and Resource Management. June 3-8, Vancouver, Canada.

Sutton, S.G., R. Pears, S. Bushnell, D. Williams. 2005. Does scientific evidence support popular beliefs about artificial reefs in the Great Barrier Reef Marine Park? Paper presented at the Rainforest Meets Reef Conference. November 22-34, Townsville, Australia.

Sutton, S.G. 2005. Understanding Recreational Fishers' Participation in Public Consultation Programs in Queensland, Australia. Paper presented at the 11th International Symposium on Society and Resource Management. June 16-19 2005, Ostersund, Sweden.

Sutton, S.G. 2005. Understanding Constraints on Recreational Fishing Activity in Queensland, Australia. Paper presented at the 4th World Recreational Fisheries Conference. June 12-16, Trondheim, Norway.

Mapleston, A., D. Welch, G. Begg, I. Brown, A. Butcher, I. Halliday, J. Kirkwood, M. McLennan, W. Sumpton, B. Sawynok, **S. Sutton.** 2005. Increasing the survival of released line caught fish: Australian tropical and sub-tropical species. Paper presented at the 4th World Recreational Fisheries Conference. June 12-16, Trondheim, Norway.

Sutton, S.G. 2004. Factors influencing satisfaction of anglers fishing in Australia's Great Barrier Reef Marine Park. Paper presented at the 10th International Symposium on Society and Resource Management, June 2004, Keystone, Colorado.

Sutton, S.G., B. Goldman, G. Begg, and B. Mapstone. 2004. Using social science to inform models of the Great Barrier Reef Line Fishery. Paper presented at the 10th International Symposium on Society and Resource Management, June 2004, Keystone, Colorado.

Sutton, S.G., and K.M. Hunt. 2000. Measuring the importance anglers place on catch-related aspects of the angling experience. Paper presented at the 130th Annual Meeting of the American Fisheries Society, August 2000, St. Louis, Missouri.

Sutton, S.G., J.R. Stoll, and R.B. Ditton. 2000. Contingent valuation methodology predicts consequences of fishing license fee increases. Poster presented at the 8th International Symposium on Society and Resource Management, June 2000, Bellingham, Washington.

Sutton, S.G., and R.B. Ditton. 1999. Understanding catch-and-release behavior among U.S.

Atlantic bluefin tuna anglers. Paper presented at the National Symposium on Catch and Release Angling in Marine Fisheries, December 5-8 1999, Virginia Beach, Virginia.

Sutton, S.G., R.B. Ditton, J.R. Stoll, and J.W. Milon. 1999. Economic importance of the charter and party boat fishing industry to Texas, Louisiana, Mississippi and Alabama. Paper presented at the International Workshop on Evaluating the Benefits of Recreational Fishing, June 1-3 1999, Vancouver, British Columbia.

Sutton, S.G., and R.B. Ditton. 1998. Comparison of three methods of estimating the effects of non-response bias on angler surveys. Paper presented at the 128th Annual Meeting of the American Fisheries Society, August 1998, Hartford, Connecticut.

Sutton, S.G., and R.B. Ditton. 1998. Understanding tagging behavior among Atlantic bluefin tuna anglers. Paper presented at the 128th Annual Meeting of the American Fisheries Society, August 1998, Hartford, Connecticut.

Sutton, S.G. 1998. The Southwest Pond Trout: Local knowledge and recreational fisheries management in Newfoundland. Paper presented at the International Workshop on Local Knowledge and Fisheries Management, May 1998, St. John's, Newfoundland.

Sutton, S.G., R.L. Radtke and R.L. Haedrich. 1997. Use of otolith microchemistry to describe life history variation in a unique population on Atlantic salmon (*Salmo salar*) in Newfoundland, Canada. Paper presented at the 127th Annual Meeting of the American Fisheries Society, August 1997, Monterrey, California.

Sutton, S.G, and R.L. Haedrich. 1995. Atlantic salmon parr lose fat in the fall. Poster presented at the 75th Annual Meeting of the American Society of Ichthyologists and Herpetologists, June 1995, Edmonton, Alberta.

MAJOR RESEARCH GRANTS

As Principal Investigator

2012-2013	Predicting the impacts of climate change on the recreational fishing industry in northern Australia. Fisheries Research and Development Corporation, $30,000
2010-2011	Recreational fishing of sharks in the Great Barrier Reef Marine Park: Species composition and catch-and-release stress. Marine and Tropical Sciences Research Facility, $22,840.
2010-2010	Evaluating science communication in Great Barrier Reef fisheries. $22,466.
2008-2009	Understanding community perceptions of the impacts of climate change on the Great Barrier Reef. Marine and Tropical Sciences Research Facility, $50,000
2006-2010	Incorporating stakeholders and their values in management of the Great Barrier Reef Marine Park. Marine and Tropical Sciences Research Facility, $612,000
2005-2007	Measuring the impacts of Great Barrier Reef Marine Park rezoning on recreational fishers. CRC Reef Research Centre, $179,000

2003-2005 Understanding the social characteristics of recreational fishers in Queensland, Australia. CRC Reef Research Centre, $86,000

As Co-Investigator

2013-2015 Adapt or fail: Risk management and business resilience in Queensland commercial fisheries. Fisheries Research and Development Corporation, $350,000 (PI: Renae Tobin, JCU)

2011-2013 Handling practices that reduce mortalities of sharks in commercial fisheries. Caring for our Country, $ 299,930 (PI: Barry Bruce, CSIRO)

2011-2013 Identification of climate-driven species shifts and adaptation options for recreational fishers: learning general lessons from a data rich case. National Adaptation Research Program, $150,000. (PI: Daniel Gledhill, CSIRO)

2010-2010 Investigating the motivations of commercial and recreational fishers to comply with Great Barrier Reef Marine Park zoning. Marine and Tropical Sciences Research Facility, $67,500 (PI: Renae Tobin, JCU).

2009-2011 Whose fish is it anyway? Investigation of co-management and self-governance solutions to local issues in Queensland's inshore fisheries. Fisheries Research and Development Corporation. $300,000, (PI: Daryl McPhee, Bond University)

2007-2010 Towards evaluating the socio-economic impacts of changes to Queensland's inshore fishery management. Fisheries Research and Development Corporation, $220,552. (PI: Renae Tobin, James Cook University)

2009-2010 Adapting to Change - Exploring the response of the GBR Coral Reef Fin Fish Fishery to a major environmental event (Cyclone Hamish) Marine and Tropical Sciences Research Facility, $23,000. (PI: Renae Tobin, James Cook University)

2007-2008 Review of Spawning Closures in the Coral Reef Fin Fish Management Plan. Queensland Department of Primary Industries and Fisheries, $93,620. (PI: Ashley Williams, James Cook University)

STUDENTS SUPERVISED TO COMPLETION

Doctor of Philosophy

Owen Li (JCU, 2016) – Employing informal learning theory and network analysis to improve the way we communicate scientific information to fisheries stakeholders. (Principal Supervisor).

Monica Gratani (JCU, 2015) – Promoting the inclusion of indigenous knowledge in natural resource management: a case study from the wet tropics of Queensland, Australia. (Principal Supervisor).

Sander den Haring (JCU, 2015) - Effective interpretation for recreational marine resource use in the Mombasa Marine Park and Reserve, Kenya. (Principal Supervisor).

Leanne Currey (JCU, 2015) - Movement of an exploited coral reef teleost across spatial and temporal scales. (Associate Supervisor).

Ruth Kamrowski (JCU, 2014) - Coastal light pollution in Australia: Insights and implications for marine turtle conservation. (Co-Supervisor).

Marina Farr (JCU, 2014) - Economic values assigned to boating and fishing trips in the Great Barrier Reef Marine Park. (Co-Supervisor).

Carena van Riper (Texas A&M, 2014) - Understanding and mapping values for ecosystem services among visitors to protected areas. (External Associate Supervisor).

Kathryn Larsen (JCU, 2013) - A social-ecological systems analysis of the potential for Indigenous Protected Areas on Cape York Peninsula, Australia. (Co-Supervisor/Principal Supervisor).

Alyson Lankester (JCU, 2013) - The influence of identity and learning on engagement in sustainability behaviour among extensive graziers in north Queensland, Australia. (Co-Supervisor).

Jillian Grayson (JCU, 2012) - Characteristics of traditional dugong and green turtle fisheries in Torres Strait: Opportunities for management. (Associate Supervisor).

William Smith (Texas A&M, 2012) - Using specialization theory to understand diversity among Texas recreational fishing guides. (External Co-Supervisor).

Sarah Busilacchi (JCU, 2011) – The subsistence reef fish fishery in the Torres Strait: monitoring protocols and assessment. (Co-Supervisor/Principal Supervisor).

Chris Bartlett (JCU, 2010) – Emergence, evolution, and outcomes of marine protected areas in Vanuatu: Implications for social-ecological governance. (Co-Supervisor).

Debora DeFreitas (JCU, 2010) - The role of public participation, spatial information and GIS in natural resource management of the dry tropical coast, northern Australia. (Co-Supervisor).

Chris Wynveen (Texas A&M, 2009) – Place meaning and attitudes towards impacts on marine environments in the Great Barrier Reef. (External Associate Supervisor).

Renae Tobin (JCU, 2006) - The effectiveness of recreational only fishing areas in north Queensland estuaries for reducing conflict and improving recreational catches. (Co-Supervisor/Principal Supervisor).

Joshua Cinner (JCU, 2006) - How socioeconomic factors influence traditional coral reef management in Papua New Guinea. (Co-Supervisor/Principal Supervisor).

Nadine Marshall (JCU, 2006) – A conceptual and operational understanding of social resilience in a primary resource industry – insights for optimizing social and environmental outcomes in the management of Queensland's commercial fishing industry. (Co-Supervisor/Principal Supervisor).

Master of Science

Roxanne Crossley (Imperial College, 2013) - Public perception and understanding of shark attack mitigation measures in Australia.

Natasha Szczecinski (JCU, 2012) - Catch susceptibility and life history of barred javelin in north eastern Australia.

Fernanda De Faria (JCU, 2012) - Catch composition and post-release stress of recreationally caught sharks in the Great Barrier Reef.

Master of Applied Science

Eline Kjoerven (JCU, 2014) - How anthropomorphism can influence perception of similarity to self, and its potential as a conservation tool.

Brian Gilmore (JCU, 2014) – Recreational fishers' attitudes towards a recreational fishing license in Queensland, Australia.

Lan Nguyen Hong (JCU, 2014) – Assessing the vulnerability of Vietnam's coastal aquaculture sector to climate change.

Andres Ramirez-Yaksic (JCU, 2014) - Recreational and commercial fishers in the Great Barrier Reef, what they value, perceive and their climate change beliefs.

Jensi Sartin (JCU, 2013) - Evaluating fishers' perception towards MPAs development and its impacts: case study in Bali, Indonesia.

Shwetha Dona (JCU, 2014) - Influence of risk perceptions on public participation in Environmental Impact Assessment

Imron Rosyidi (JCU, 2011) - An alternative mechanism to tuna fishing quota allocations among parties of the commission for the conservation of southern bluefin tuna case study: Southern bluefin tuna fishery in Indonesia .

Adityo Setiawan (JCU, 2010) - The perceived impact of the Panglima Laot system on community welfare in Nangroe Aceh Darussalam (NAD), Indonesia.

Charlotte Morgan (JCU, 2010) – Analysis of fisheries compliance data for the Great Barrier Reef Marine Park.

Adrian Arias (JCU, 2009) – Using random response theory to measure compliance of recreational fishers to zoning in the Great Barrier Reef Marine Park.

Chai-Yen Cheong (JCU, 2008) - Recreational fishers' motivations for participating in the CapReef community-based monitoring program in Queensland, Australia.

Roger Beeden (JCU, 2006) - A content analysis of attitudes to recreational fishing in Queensland, Australia.

Rima Jabado (JCU, 2006) – Attitudes and knowledge of Great Barrier Reef area residents towards sharks.

Kiri Peat (JCU, 2005) - Demographic and social influences on recreational fishers' social value orientations in Queensland, Australia.

Nicola Doss (JCU, 2005) - Influence of Socio-Economic Factors on Perceptions and Awareness of Marine Ecosystems and Management: A Case Study of Kepulauan Karimun Jawa Marine National Park, Indonesia.

Amy Smith (JCU, 2004) – The role of a flagship species in the formation of ecological intentions.

Honours

Ana Wegner (JCU 2011) – Recreational fishers' participation in public consultation programs.

Owen Li (JCU 2008) – Communicating scientific information to recreational fishers.

Ann-Maree Lynch (JCU 2007) – Implications of recreational fishing for elasmobranch conservation in the Great Barrier Reef Marine Park.

Alana White (JCU 2005) – Boaters' perceptions of speed guidelines introduced for dugong conservation: Use of the theory of reasoned action as a guiding tool to understand non-Compliance

Kara Dew (JCU 2004) - Land-based fishing activities and the importance of fishing to Traditional Owners of Girringun Country, North Queensland.

CURRENT STUDENTS

Amy Smith (PhD) - Using flagship species to motivate conservation behaviour of zoo visitors. (Principal Supervisor).

TEACHING EXPERIENCE

James Cook University

2010-2014 Foundations of Natural Resource Management. One of four lecturers responsible for designing and delivering the course to undergraduate and graduate students across two campuses. Served as course coordinator (Townsville Campus) in 2011.

2012-2014 Human Dimensions of Environment, Nature, and Conservation. Course designer, coordinator, and principal lecturer.

2012-2014 Managing Tropical Fisheries. One of two lecturers responsible for designing, coordinating, and delivering the course.

Texas A&M University

2001 Principals of Fisheries Management. Designed and taught the laboratory and field component.

Memorial University

1994-1997 Laboratory tutor for multiple undergraduate courses in the Department of Biology, including Ichthyology, Vertebrate Biology, and Quantitative Methods in Biology.

PROFESSIONAL SERVICE

Journal editorial board membership

Transactions of the American Fisheries Society
Associate Editor 2008-2015

Human Dimensions of Wildlife: An International Journal
Editorial Board 2010-2024
Associate Editor 2015-2024

Conference organizing committee membership

5th World Recreational Fisheries Conference
Organizing committee member 2007-2008 and Proceedings co-editor (AFS Books)

6th World Recreational Fisheries Conference

Organizing committee member 2008-2011

7th World Recreational Fisheries Conference

International advisory board member 2011-present

Research committee membership

Capricorn Community Based Fisheries Monitoring Program (CapReef)

Steering committee member 2005-present

Fisheries Research and Development Corporation Recfishing Research Committee

Steering committee member 2005-2013

Fisheries Research and Development Corporation Social Sciences Coordination Program

Technical reference committee member 2009-2011

This is **Exhibit "D"** referred to in the affidavit of **Jon W. Carr,** affirmed before me this 19th day of January, 2024.

CURRICULUM VITAE

Heather Aminata Evelynne Perry

(January 2024)

PERSONAL

Address: Atlantic Salmon Federation
[redacted]
Chamcook, NB Canada
[redacted]
Telephone: [redacted]
Email: [redacted]

Place of Birth: Toronto, Ontario, Canada

Citizenship: Canadian

EDUCATION

Degree

B Sc. (Hons.)	2019	Dalhousie University Halifax, NS	Marine Biology & Ocean Sciences

Thesis

B Sc. (Hons.) Examining energy trade-offs in alternative reproductive strategies of male Atlantic salmon (*Salmo salar*)

PROFESSIONAL WORK EXPERIENCE

July 2021 – Present

Biologist, Atlantic Salmon Federation

Chamcook, New Brunswick

June 2020 – July 2021

Fisheries Observer, Atlantic Catch Data

Yarmouth, Nova Scotia

May 2018 – August 2018

Research Assistant, Atlantic Salmon Federation

Chamcook, New Brunswick

January 2018 – April 2018

Compliance Promotion Intern, Environment and Climate Change Canada

Dartmouth, Nova Scotia

January 2017 – April 2017

Labratory Technician, Environment and Climate Change Canada

Moncton, New Brunswick

PRESENTATIONS

2024 Repeat Spawning Dynamics of Atlantic salmon kelt in the Gulf of St. Lawrence. *Atlantic Ecosystems Forum*

2023 Atlantic salmon Federation Telemetry Program. *Atlantic Society of Fish and Wildlife Biologists Spring Seminar*

Smolt Tracking on the Miramichi. *Miramichi Science Day*

2022 West Greenland Satellite Tracking of Atlantic Salmon. *Restigouche Science Advisory Committee meeting*

2019 Examining energy trade-offs in alternative reproductive strategies of male Atlantic salmon (*Salmo salar*). *Cameron Conference & Atlantic Science Forum*