2023 **NSARB-2023-001**

Nova Scotia Aquaculture Review Board

IN THE MATTER OF: Applications made 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.**

Kelly Cove Salmon Ltd.

APPLICANT

-and-

Minister of Fisheries and Aquaculture

PARTY

-and-

Kwilmu'kw Maw-Klusuaqn Negotiation Office (KMKNO)

FIRST INTERVENOR

-and-

Queens Recreational Boating Association (Brooklyn Marina)

SECOND INTERVENOR

22 Fishermen of Liverpool Bay

THIRD INTERVENOR

Region of Queens Municipality

FOURTH INTERVENOR

Protect Liverpool Bay Association

FIFTH INTERVENOR

Reply Affidavit of Jessica Feindel

I, Jessica Feindel, of Shelburne, Nova Scotia, affirm and give evidence as follows:

1. I am the Manager of Aquaculture Operations in the provincial Department of Fisheries and Aquaculture (the Department).

2. I have personal knowledge of the evidence affirmed in this Affidavit except where otherwise stated to be based on information and belief.

3. I state, in this Affidavit, the source of any information that is not based on my own personal knowledge, and I state my belief of the source.

4. In this Affidavit I address issues raised in the Reports filed by Dr. Peter Cranford and Dr. Inka Milewski.

Ultraviolet Spectrophotometric (UV Spec) method

5. Dr. Cranford is a proponent of the ultraviolet spectrophotometric (UV spec) method for measuring benthic total free sulfides.

6. Currently, the Department uses the Ion Selective Electrode (ISE) method for measuring benthic total free sulfides. ISE is also used federally by the Department of Fisheries and Oceans (DFO) and other provincial regulators. I am not aware of any other aquaculture regulator in Canada that has adopted the UV spec method.

7. The Department is in the process of assessing this technology.

8. In 2019, the Department purchased a UV spectrophotometer and associated equipment to enable the Department to evaluate this method.

9. In February 2021, the Department, in collaboration with the CMAR (Centre for Marine Applied Research), drafted a submission to DFO's Canadian Science Advisory Secretariat (CSAS) requesting a peer-review of the UV spec method for measuring benthic sulfides to support regulatory compliance. As of December 2023, this submission had not yet been prioritized and scheduled for a CSAS peer review.

10. In July 2021, the Department engaged Dr. Cranford to provide UV spec training to the Department's and CMAR's staff.

11. In 2021, the Department began testing the UV spec method to measure sulfide from marine sediments at finfish farms in Nova Scotia for the purpose of method exploration and assessment. The Department has not yet tested the UV Spec method at each marine finfish site in Nova Scotia.

12. The DFO CSAS held a National Peer Review meeting virtually on May 10-12, 2022 with respect to evaluating the factors impacting the ISE method. The Department had a

representative at this meeting. Proceedings (2022/039) and a Science Advisory Report (2022/049) have been published and a Research Document(s) is expected but has not yet been published.

13. The Department remains interested in exploring the UV spec method, along with other methodologies for measuring benthic impacts.

Environmental Indicators

14. Dr. Cranford's report purports to reproduce a table from Nova Scotia's Environmental Monitoring Program Framework for Marine Aquaculture in Nova Scotia (2021) at Table 1 of his report. The Table presented by Dr. Cranford omits a number of rows that were contained in the original table, which can be found in the Framework attached to my original Affidavit as Exhibit C at p. 35. For ease of reference, the complete table is reproduced below:

Table 1. Environmental Quality Definitions

	Sediment Classification		
Measurement	**Oxic**	**Hypoxic**	**Anoxic**
Sediment colour	Tan to depth > 0.5 cm	Tan to < 0.5 cm with some black sediments at surface	Surface sediments black
Microbial presence	No *Beggiatoa*-like bacteria present	Patchy *Beggiatoa*-like bacteria	Widespread *Beggiatoa*-like bacterial mats
Macrofaunal Assemblage	Wide array of infauna and epifauna	Mixed group of mostly small infauna	Small infauna only
Sulfide, µM	**≤ 749 (A)** **750 to 1499 (B)**	**1500 to 2999 (A)** **3000 to 5999 (B)**	**≥ 6000**
Redox (Eh), mVNHE	>100 (A) 100 to -50 (B)	-50 to -100 (A) -100 to -150 (B)	< -150
Organic matter, %	<= reference*	1.5 to 2X ref.	> 2X reference
Porosity, %	<= reference*	1 to 10X ref.	> 10X reference

Modified from the *Design of the Environmental Monitoring Program for the Marine Aquaculture Industry in Nova Scotia* (Smith et al 2002) and *Towards a Classification of Organic Enrichment in Marine Aquaculture* (Hargrave et al. 2008a)

15. This table is important because it shows that the Department does not rely on the ISE method alone for assessing sediment conditions. The Department relies on a number of other, well-established environmental indicators to assess benthic conditions which contribute to site-specific management responses.

Standard Operating Procedures

16. In Inka Milewski's report she states that no explanation is provided for the change from "hard bottom" designation of AQ#1205 in 2016 to a "soft bottom" designation in 2017.

17. Starting in 2016 to present, the '*Standard Operating Procedures for the Environmental Monitoring of Marine Aquaculture in Nova Scotia'* included a requirement that sediment sampling devices collect a minimum depth of 5 cm in order for sediment to be sub-sampled for analysis. A copy of the *Standard Operating Procedures for the Environmental Monitoring of Marine Aquaculture in Nova Scotia* is attached to this Affidavit as **Exhibit "A"**.

18. Prior to 2016, the Standard Operating Procedures did not prescribe a minimum sediment depth within a sediment sampling device.

19. In 2016, the sediment sampling device used at AQ#1205 was not able to collect sediment that met the minimum 5 cm depth requirement. As such, the hard-bottom visual monitoring protocol had to be used to evaluate the lease.

20. In 2017 a new, and heavier, grab sampler was used at AQ#1205 to collect sediment and was able to meet the minimum sediment depth requirement of 5 cm and thus the soft bottom protocol was used. The heavier sediment sampling device has continued to be used at AQ#1205 since 2017, allowing for the soft bottom protocol to be employed.

21. The sampling devices used at AQ#1204 from 2011 to present are listed in the chart below:

Year	**Sediment Sampling Device**
2011	Ekman
2012	Ekman
2013	Ekman
2014	Petite Ponar
2015	Petite Ponar
2016	Standard Ponar
2017 to present	Van Veen

STANDARD OPERATING PROCEDURES FOR THE ENVIRONMENTAL MONITORING OF MARINE AQUACULTURE IN NOVA SCOTIA

July 2021

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Standard Operating Procedures for Environmental Monitoring of Marine Aquaculture Sites in Nova Scotia

1 INTRODUCTION

The *Standard Operating Procedures for Environmental Monitoring of Marine Aquaculture Sites in Nova Scotia* describes the monitoring and laboratory methodologies for the Nova Scotia (NS) Environmental Monitoring Program (EMP). Both marine finfish and marine shellfish farms in NS are required by the Nova Scotia Department of Fisheries and Aquaculture (NSDFA) to comply with the EMP as outlined in the *Aquaculture Management Regulations* under authority of the *Fisheries and Coastal Resources Act*. Provided in this document are monitoring instructions, laboratory guides, field templates, and reporting requirements designed to assist those conducting environmental monitoring on a marine aquaculture lease. This document and methodologies described within will be reviewed yearly to include changes and innovations to field methods, laboratory techniques, technologies, and regulatory approaches.

This EMP Standard Operating Procedure (SOP) originated in 2002 as part of the document titled, *Design of the Environmental Monitoring Program for the Marine Aquaculture Industry in Nova Scotia* (Smith et al., 2002). Several revisions have been made to the EMP SOPs and the *Environmental Monitoring Program Framework for Marine Aquaculture in Nova Scotia* (PNS 2021A) to incorporate the latest advancements in science and technology. This helps to ensure that the EMP is up-to-date, relevant, and effective. The EMP is a mandatory requirement, and integral part of the leasing and licensing process. Marine finfish and shellfish farm operators are responsible to adhere to this program, coordinate monitoring as instructed and provide results to NSDFA as required. The SOPs in this document cover the requirements for baseline monitoring as well as the annual monitoring required as part of the program.

Should readers of this document have any questions, please contact EMPSupervisor@novascotia.ca or (902) 875-7436.

2 BASELINE MONITORING

Baseline data collection is required in the following situations:

- The establishment of new site;
- Reactivation of a lease that has been fallow/inactive;
- Amendment of the boundaries of an existing, active lease; and,
- The establishment of an experimental site.

Prior to completing a baseline monitoring event, NSDFA should be contacted to ascertain if any additional data collection is required. NSDFA must provide an approval for a baseline monitoring plan.

Collection of appropriate and complete baseline data ensures that ongoing environmental monitoring data can be compared with the initial condition of the site. The following sections outline the information to be collected and methodologies used in order to comply with NSDFA requirements for baseline monitoring. An electronic copy of the baseline monitoring event and corresponding video files can be sent to Aquaculture Operations via the secure file transfer system upon request. Requests can be made by email to EMPSupervisor@novascotia.ca.

OR

A physical copy of the baseline monitoring event and video files can be sent to the attention of Aquaculture Operations at the following mailing address:

Aquaculture Operations
Nova Scotia Department of Fisheries and Aquaculture, Aquaculture Division
1575 Lake Road
Shelburne, Nova Scotia
B0T 1W0

2.1 Baseline Monitoring Requirements

2.1.1 Finfish Requirements

For a typical marine finfish site, baseline monitoring will consist of the following:

- Video collection at each vertex of the proposed lease boundary;
- Video collection at the center of the proposed lease boundary;
- Video collection at a reference station located between 100 and 300 meters from the proposed lease boundary in the direction of the dominant current;
- Sediment collection and analysis at the above monitoring stations;
- Video collection along a transect running through the centre of the entire length of the proposed lease, or center of the proposed expansion area;
- Collection of current speed and direction measurements for 30 days; and
- A bathymetry survey of the proposed lease area.

Baseline information for marine finfish sites is also required by the Department of Fisheries and Oceans Canada (DFO) under the *Aquaculture Activity Regulations* (AAR 2021). For more information, please visit their website or contact DFO's Aquaculture Management office. Sites

which have been subject to a fish and fish habitat survey, as described in the *Aquaculture Activities Regulations*, may be exempt from the video collection requirements specified in this section.

2.1.2 Shellfish Requirement

For the most recent iteration of the requirements for shellfish baseline refer to the *Standard Operating Procedures for Baseline Monitoring of Marine Shellfish Aquaculture in Nova Scotia* (PNS 2021B).

2.2 Video Collection

Video collection that is carried out at static baseline monitoring stations (i.e. lease vertexes, center and references) is to be conducted in a manner which satisfies the methodology and quality criteria presented in Section 4.1.

Collection of benthic video for a required transect through a proposed lease may be conducted via surface deployed drop camera, handheld, diver operated video camera or remotely operated vehicle. Transect video is required to meet the methodology and quality criteria presented in Section 4.1 as well as the following:

- Recordings will be conducted along a transect extending through the center and running the entire length of the proposed lease;
- If utilizing a surface deployed drop camera, video stations will be established along the length of the transect, at 100 m intervals;
- A 360º panorama is only required at the beginning of each individual recording (i.e. if the camera system is recording continuously throughout multiple video stations of a transect a panorama is only required prior to submersion at the first station);
- If utilizing a diver-held or ROV mounted camera to conduct a continuous transect, a weighted drop line or other visual guide must be used to mark the transect line as well as the individual video stations at 100 m increments; and
- A diver or ROV conducting a continuous transect will do so at a speed and height above bottom which allows for the clear observation and identification of macro flora and fauna within 1 meter to either side of the established transect line.

For all video collected as part of baseline monitoring, detailed observations, as outlined in Section 4.3, must also be recorded. High quality copies of the original, unedited footage as well as their associated observations are to be provided to NSDFA (Aquaculture Division).

2.3 Sediment Collection

Benthic sediment collection is required at the vertexes, center and reference station associated with any finfish aquaculture lease undergoing baseline monitoring and may be performed using surface deployed equipment (e.g. grab, gravity corer) or manually operated core tube (diver-held or ROV). Collection, storage, and transport of these samples is required to meet all the methodology and quality criteria presented in Section 5 which apply to the chosen sampling method.

For each successful sediment retrieval attempt which meets the appropriate methodology and quality criteria, detailed observations, as outlined in Section 5.4, must be recorded and submitted to NSDFA.

If sediment consolidation or composition has resulted in five (5) failed collection attempts before three acceptable samples can be retrieved, the station will be considered a 'hard bottom station' for that monitoring event.

2.4 Sediment Analysis

All sediment samples collected as part of baseline monitoring are required to undergo laboratory analysis for free sulfide concentration, redox potential, percent organic matter, and porosity. Sediment analysis is required to meet the methodology and quality guidelines presented throughout Section 6. Additionally, sediment grain size analysis is required for the AAR's and the methodology can be found in the program documents (AAR 2021).

2.5 Current Speed and Direction

Where measurements of the current are required for baseline monitoring, a detailed profile of speed and direction must be collected at the centre of the proposed lease using an Acoustic Doppler Current Profiler (ADCP) of appropriate specification for the location. Profiles of the entire water column, in bins of no greater than 1 meter, are to be recorded at intervals of 30 minutes or less, for a minimum of 30 days. Speed and direction data from each profile will be composed of a sufficient number of individual measurements (pings), averaged over an appropriate interval such that the expected standard deviation of reported current measurements is <1 cm/s. The instrument to be used must be correctly calibrated as per manufacture's specifications and the results submitted to NSDFA along with details of the unit's configuration setup and all raw data resulting from the deployment.

2.6 Bathymetry Survey

A bathymetric survey must be conducted in order to generate contours of depth, relative to chart datum, with a minimum resolution of 10 m across the entire lease area. A bathymetric chart from the Canadian Hydrographic Service that includes depth profile contours in 10 m increments may be used instead of conducting a bathymetric survey.

3 DETERMINATION OF MONITORING STATIONS

This section provides guidance on determining the number and position of monitoring stations required for EMP. The following criteria are to be considered in making these determinations:

- Level of the monitoring event being conducted (Level I, II or III);
- Maximum number of fish onsite during the current production cycle (Table 1);
- Prevailing current direction relative to the shoreline
- Biomass contained within each cage at the time of monitoring (Figure 1);
- Water depth at cage edge;
- Bottom type and site conditions; and
- Historical environmental performance.

Site-specific conditions may prevent the positioning of monitoring stations exactly as described in this SOP. If the operator or third-party operator is aware of conditions that may prevent a station from being located in the correct position, they must notify NSDFA and receive approval for any deviations from the SOP prior to the monitoring event. Any deviations from the SOP that could not be pre-determined may be approved by NSDFA but must be submitted in the final report.

3.1 Pre- Monitoring Submissions

At a minimum of two (2) weeks prior to an anticipated monitoring event, the operator or the third-party conducting sampling are required to submit the following information to NSDFA for review:

- A detailed site diagram or aerial image indicating:
    - Biomass contained in each cage, in kilograms;
    - Proposed location of all monitoring stations;
    - Proposed alternative monitoring locations for all stations located on cage-edge;
    - Location of any assigned historic high stations (if applicable); and
    - Location of reference station to be sampled.
- Anticipated monitoring date
- Monitoring Equipment that will be used, including:
    - Sediment sampler (see **Appendix A1**)
    - Video camera system
- Details regarding any requested deviations from the monitoring methods specified by this SOP

3.2 Number of Finfish Monitoring Stations

The minimum number of monitoring stations required for each finfish aquaculture lease is based on the maximum number of fish on site during the current production cycle (Table 1). A minimum of two monitoring stations are required for sites containing a maximum of 1-200,000 finfish. The number of required monitoring stations will increase by one for every additional 100,000 finfish stocked. If more than one cage array is found within the same lease, each array will be treated individually. For example, if one lease has a maximum of 700,000 fish, and the first array contains 250,000 fish and the second array contains 450,000 fish. The first array would require three (3) monitoring stations and the second array would require five (5) monitoring stations.

In addition to the monitoring stations specified in Table 1, historic high monitoring stations must also be sampled as part of the EMP. Historic high monitoring stations are those soft bottom monitoring stations whose mean sulfide levels have previously exceeded 3,000 µM, or those hard bottom stations where hard-bottom indicators are identified at 70% or more of the video collection locations along a video transect. These stations must be resampled annually, until the mean sulfide level for that station decreases below 1,500 µM, or the hard-bottom indicators are identified at ≤70% of the video collection locations, respectively. Historic high stations must be located within 10 m of the original station coordinate. If samples are collected at a distance greater than 10 m from the original coordinates, the results will be considered invalid for determining the recovery status of the station. In cases where multiple historic high stations are located within 10 m of one another, NSDFA may consider reducing the number of stations required for re-monitoring upon request. Additionally, for historic high stations where attempts to sample have been made in two consecutive monitoring years; however, access to the station coordinate was not possible due to gear obstruction, the requirement to sample will be suspended.

For sites that are inactive at the time of the anticipated monitoring event, operators or third-party organizations, should consult the *Policy for Monitoring Inactive Sites* to determine the appropriate requirements.

Table 1. Number of Monitoring Stations Required for Level I Sediment and Video Collection

Maximum number of fish within cage site array during production cycle	Number of sampling stations (not including reference stations)	Number of samples (3 samples/station for soft bottom sites)
1-200,000	2	6
200,001-300,000	3	9
300,001-400,000	4	12
400,001-500,000	5	15
500,001-600,000	6	18
600,001-700,000	7	21
700,001-800,000	8	24
800,001-900,000	9	27
900,000-1,000,000	10	30

*Contact NSDFA if more than 1,000,000 finfish are stocked and when number of sampling stations exceeds number of cages

3.3 Position of Finfish Monitoring Stations

The position of monitoring stations for Level I and Level III EMP will be determined using the following criteria:

- position of the cage array relative to the shoreline;
- direction of the prevailing water current;
- current speed;
- cage biomass;
- water depth at cage edge;
- bottom type; and
- site conditions.

The application of these criteria in selecting monitoring locations is further outlined below. Examples of prioritized selection of monitoring locations are provided for sites with generally linear current flow (Figure 1) and for those with generally curving flow (Figure 2). All such monitoring stations will be located at cage edge, along the outside perimeter of the array. Where multiple sediment samples are to be collected, samples must be taken from three separate locations along the outer perimeter of the cage (Figure 1). The samples must be collected at a distance far enough apart to ensure that samples are not taken from a location that was disturbed by a previous monitoring attempt. If the sediment samples are not able to be collected from the station originally proposed (due to sediment consistency) then the samples must be taken from the alternate station (Figure 3) submitted in the pre-monitoring submission (Section 3.1)

Accurate recording of monitoring station locations is crucial for the efficacy of the program and ensures the consistency and repeatability of a monitoring event. As such, vessels are required to be moored to cages while conducting monitoring activities associated with a cage edge. Mooring is not required for stations that are not located at cage edge (e.g., historic high and reference stations). However, an appropriate method to remain within 10 m of the assigned station coordinates must be employed. If surface deployed monitoring equipment is being used to sample a cage edge monitoring station, this equipment must be deployed no more than 3 m away from cage edge. A GPS waypoint must be recorded at every monitoring station using the NAD83 CSRS datum and submitted to NSDFA in decimal degrees (Appendix A2).

When sediment samples are collected using a surface deployed grab sampler, the depth of the station must be recorded. The station water depth can be recorded using either a weighted drop line or equipment found on the vessel. If a weighted drop line is used, it should be deployed after sample collection.

Where samples are collected by a SCUBA diver, a weighted drop line will be used to assist in locating the sampling location on the seafloor and the DGPS coordinates must be recorded. Care must be taken to ensure sample locations have not been disturbed by the impact of the drop line anchor on the seafloor. All required samples will be collected in similar substrate within 1 m of the drop line anchor. If sediment cannot be retrieved from this area, divers may move to the closest undisturbed sediment for sample collection. Such deviation must be noted in the report along with an estimate of distance from the drop line anchor.

In situations where site infrastructure or other obstructions prevent access to a proposed monitoring station location, a revised monitoring location must be established. The revised monitoring station must be located as close to the cage with the highest biomass, without risking entanglement of equipment. As with any other monitoring station, a GPS waypoint (using NAD83 CSRS datum) must be logged at the new location. If a reference or historic high station cannot be monitored, then the operator or third-party must record the distance and direction of the revised station from the target monitoring waypoint. If a monitoring station location is revised, coordinates of the new monitoring location and an explanation of the spatial variation must be provided in the final report.

Generally linear, with moderate or high speed, currents

Station 1 and 2: Opposite ends of the array in alignment with the prevailing water current direction and at the cages nearest the shoreline. If an identified cage is empty, the station is positioned at the next stocked cage closest to the shoreline at time of monitoring.
For any remaining stations, if multiple cages meet the criteria for station selection, monitoring is to be carried out at the cage where water depth is the shallowest.

Station 3: Positioned approximately perpendicular to the prevailing current on the shoreward side of the array, and at the edge of the cage with the highest biomass at time of monitoring which has not yet been sampled.

Station 4: Positioned approximately perpendicular to the prevailing current on the seaward side of the array, and at the edge of the cage with the highest biomass at time of monitoring which has not yet been sampled.

Stations 5,6,7, and 8: Positioned on the same sides of the array as stations 1,2,3 and 4 respectively, and at the edge of the cage with the next highest biomass at time of monitoring which has not yet been sampled.

See Figure 1 as an example.

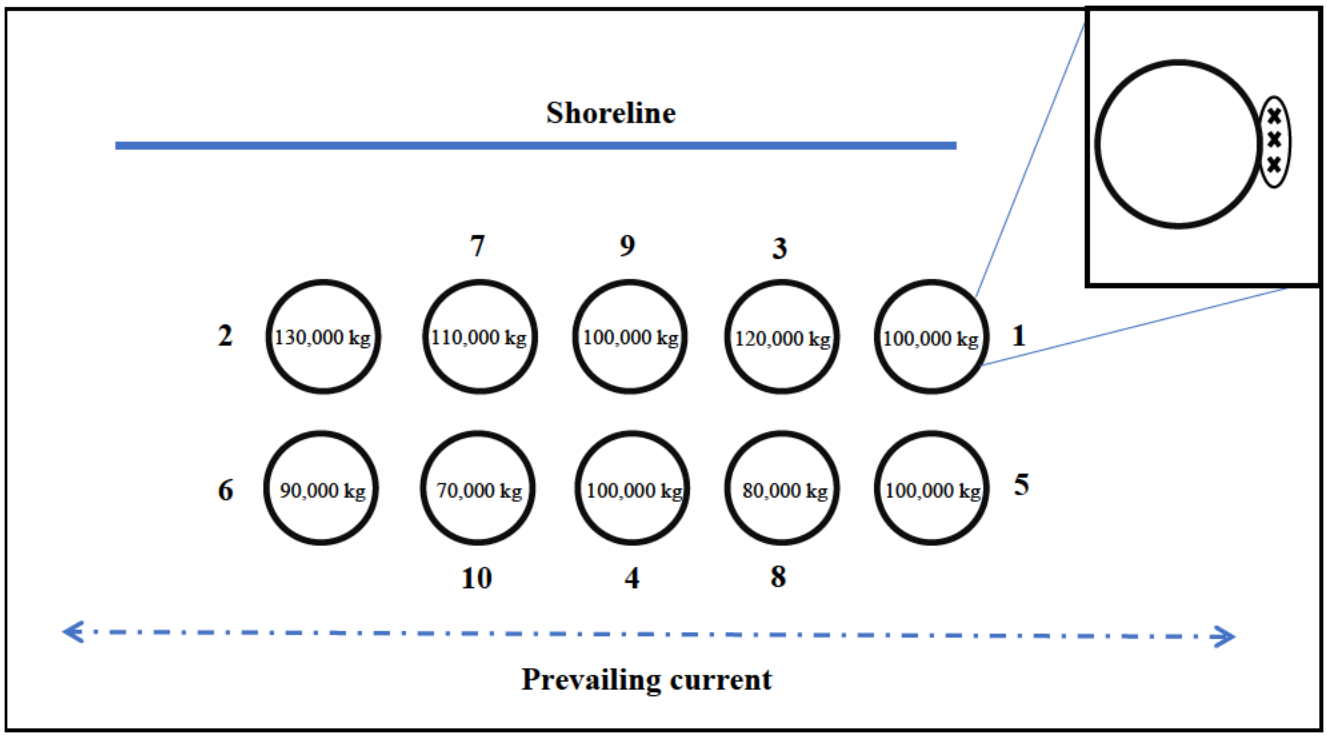


Figure 1: Example of monitoring station positioning at sites with generally linear water current patterns, where arrays contain varying biomass per cage. Inset shows where sediment samples should be taken on cage edge stations.

Generally curving, or low current speeds

If multiple cages meet the criteria for station selection, monitoring is to be carried out at the cage where water depth is the shallowest.

Stations 1 and 2: Positioned on adjacent sides of the array, with one on the shoreward side and the other aligned on the edge of the cage nearest to shore with the highest biomass at the time of monitoring.

Station 3: Positioned on the site opposite to station 2 at the cage nearest to shore. If an identified cage is empty, the station is positioned at the next stocked cage nearest to shore.

Station 4: Positioned on the seaward side of the array, on the edge of the cage with the highest biomass.

Station 5, 6, 7, and 8: Positioned on the same side as station 1, 2, 4 and 3, respectively, at cages with the next highest biomass at time of monitoring which have not yet been sampled.

See Figure 2 as an example

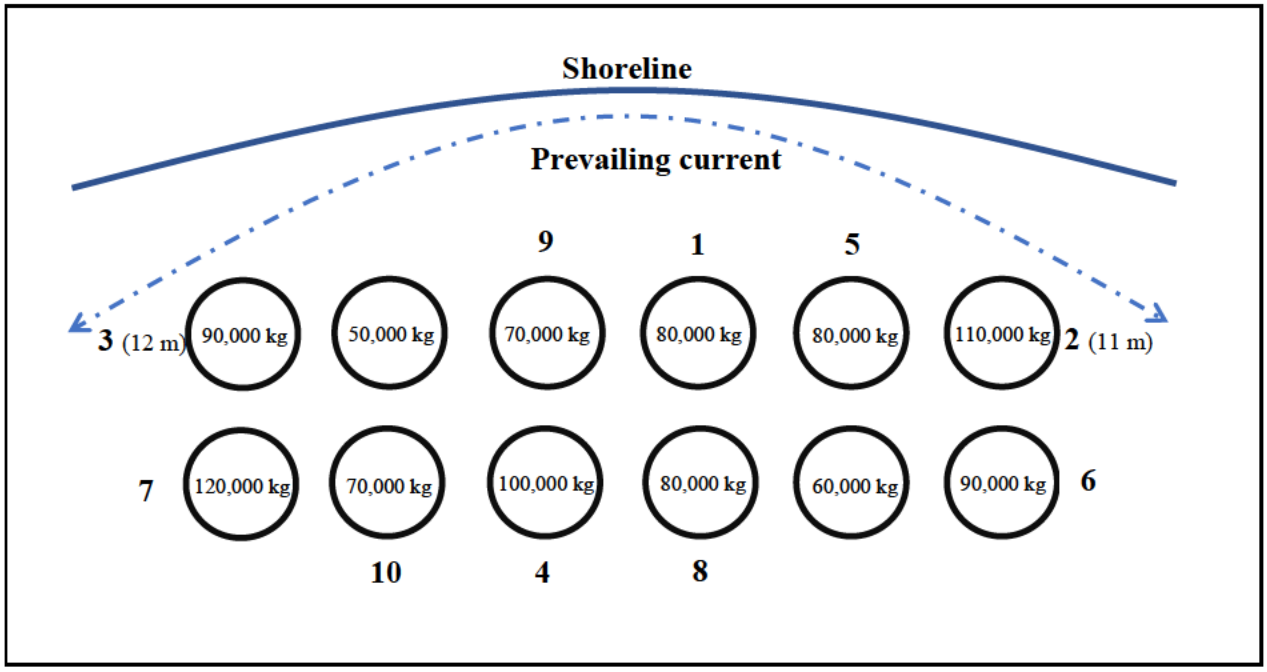


Figure 2: Example of monitoring station positioning at sites with generally curving water current patterns, where arrays contain varying biomass per cage.

Alternate stations

If sediment consolidation or composition has resulted in five (5) failed collection attempts before three (3) acceptable samples can be retrieved, an alternate station is to be used. The alternate station is established at the cage on the same side of the array which contains the next highest biomass and which is not already a proposed monitoring station (Figure 3). If an alternate station can not be established which meets the above criteria, visual monitoring, as described in Section 4.2.2, will be conducted in lieu of sediment monitoring at the initially selected location.

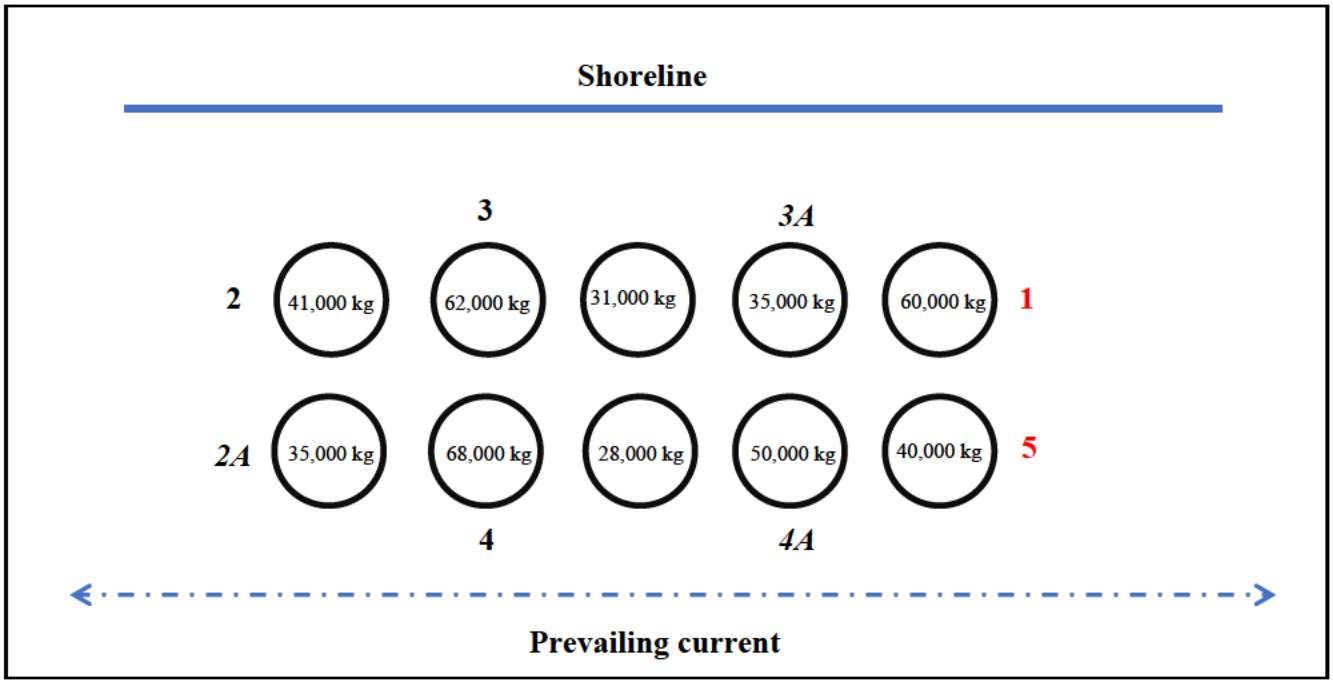


Figure 3: Example of the selection of monitoring stations (2, 4), and their respective alternate stations (2A, 4A), at sites where the array contains varying biomass per cage. In this example, stations in red have no viable alternate and would be subject to visual monitoring.

3.4 Shellfish Monitoring Stations

In addition to the baseline monitoring requirements outlined in the *Standard Operating Procedures for Baseline Monitoring of Marine Shellfish Aquaculture in Nova Scotia* (PNS 2021B), shellfish aquaculture sites may be required to conduct environmental monitoring while in operation.

The requirement for environmental monitoring will be determined by DFO and NSDFA prior to the establishment of the shellfish aquaculture site. Environmental monitoring for shellfish aquaculture sites will be scaled to the level of risk associated with the operation and will consider the following: production level, percent of bay volume, potential for impact to fish and fish habitat, and historical environmental performance.

Environmental monitoring requirements may include, but are not limited to, benthic sediment collection and video monitoring, video monitoring only, or monitoring at extended spatial and temporal intervals.

Alternative levels of monitoring may be proposed for shellfish aquaculture sites that have repeatedly shown no or limited potential for impact.

3.5 Reference Stations

Each marine aquaculture lease undergoing environmental monitoring requires that a minimum of one reference station be sampled. Reference stations are established during baseline monitoring. Reference stations must be located between 100 and 300 meters from the lease boundary, in the direction of the dominant current. Reference stations must be positioned in an area with a similar depth and sediment type to what is found at stations sampled within the lease boundary. If the required distance criterion cannot be achieved, reference stations should be positioned in an area with similar characteristics to the monitoring stations within the lease boundary (water depths and sediment type, etc.).

If acceptable sediment samples cannot be collected at a previously established reference station, a new reference station should be established. A new reference station can only be established after a minimum of five (5) unsuccessful attempts are made to collect sediment at the original reference station. A new reference station must meet the distance, depth and sediment type criteria detailed above and the new coordinate must be submitted in the final report to NSDFA. If a new, soft-bottom, reference station cannot be established which meets these criteria, a 200-meter video transect, in the direction of the dominant current, starting approximately 100 meters from the lease boundary and ending approximately 300 meters from the lease boundary will be conducted. Video collection is to be conducted as described in Section 4, with drop-camera video stations located at 50-meter intervals.

3.6 Monitoring Levels

Level I EMP events are conducted annually, between July 1 and October 31, and are the primary means of monitoring conducted at active aquaculture sites in Nova Scotia. Determination of the positioning and number of required stations for Level I monitoring is outlined in Sections 3.1 to 3.5. Historic high station results are included in the analysis for classification.

Level II monitoring events are required when the results of annual Level I monitoring classify a lease as Hypoxic B, Anoxic, having failed based on the mixed or hard bottom classification protocol, or as determined to be required through an audit. In such cases, a consistent rationale for additional monitoring will be applied based on the following monitoring objectives:

a) Better define the outer limits of the impacted area. This will include the establishment of cage-edge monitoring stations at all cages immediately adjacent to Level I monitoring stations with mean sulfide concentrations $\geq$ 3,000 µM or which failed to meet the Environmental Quality Objectives (EQO) for hard bottom stations (Framework Section 2.2.3.2).

b) Better define the outer limits of the zone of influence. Monitoring stations will be established at the four (4) corner compensator buoys of the array as well as additional perimeter compensator buoys at no more than 200 m spacing along the outer edge of the array. If compensator buoys are not utilized as part of the system design, contact NSDFA for monitoring guidance.

An example of appropriate monitoring is shown in Figure 4. Level II monitoring events do not require the inclusion of a reference or historic high stations.

A site will be initially classified using the results from the Level I monitoring event. If Level II monitoring is required for a site, then the final site classification will be based on the results from this monitoring event. The classification of the site, or the audit result indicating the higher level of environmental impact, will dictate the most appropriate site management responses for each aquaculture site. These site management responses can include things such as follow up monitoring and/or the implementation of mitigation measures. Please see Section 2.0 of the *Environmental Monitoring Program Framework for Marine Aquaculture in Nova Scotia* for more detail on classification of sites and management responses (PNS 2021A).

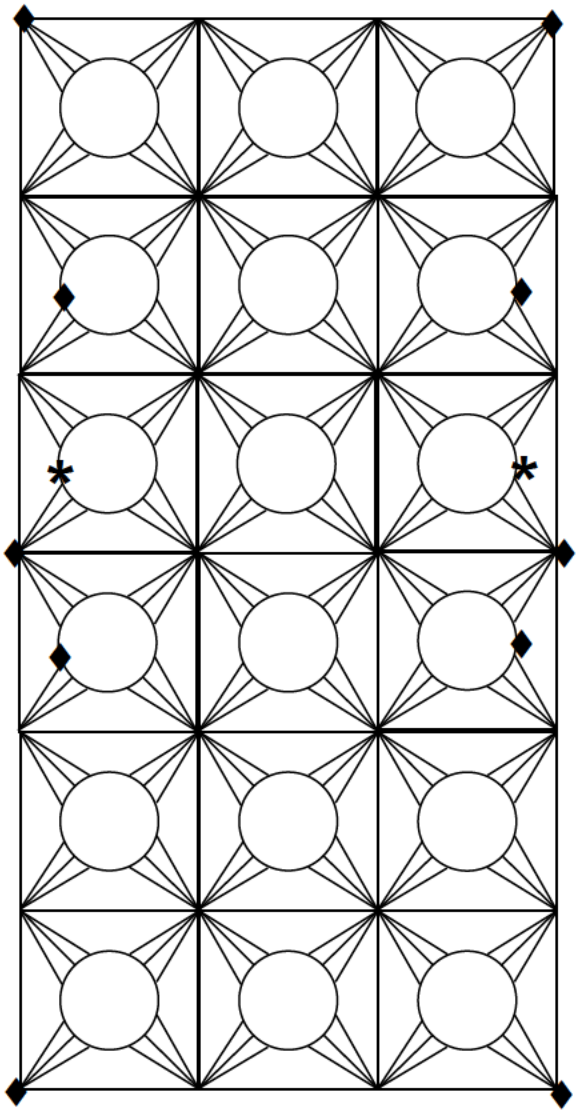


Figure 4. Example of Level II monitoring station placement (diamonds) relative to stations where average free sulfide concentrations were found to be ≥ 3,000 µM (stars).

Level III monitoring is required when a site consistently fails to meet oxic conditions, when the results of annual Level I monitoring classify a lease as Anoxic or otherwise severely impacted, or at the discretion of NSDFA. This monitoring is used to capture seasonal variation on a lease and is used to closely monitor impacted areas within the lease boundaries through increased temporal monitoring intensity. Level III monitoring events will take place between March 1st and May 31st of the year following the triggering results. Monitoring will target all stations visited during the previous Level I monitoring event and may also include additional requirements as determined by NSDFA in discussion with the site operator.

If Level III monitoring is required on an aquaculture lease, the results from both Level I and Level II monitoring will be evaluated to determine the state of the benthic environment within the lease, however the site will remain classified from the Level II results. Level III results will be used by regulators to provide site-specific recommendations for any remedial action that is required by the operator.

3.7 Timing of Monitoring

All Level I and Level II monitoring events must be completed annually between July 1st and October 31st. If Level III monitoring is necessary for a site it must be completed between March 1st and May 31st of the following year. All attempts must be made to complete any monitoring event in a single day. If it is anticipated that more time will be required to complete the monitoring event a request may be made for an approved deviation. In such cases, a maximum of two (2) consecutive days will be allowed for completion of sampling.

A monitoring extension may also be granted if unavoidable circumstances or equipment malfunction prevent an ongoing monitoring event from being completed in the approved monitoring timeframe. If an extension is required to complete the monitoring event, NSDFA must be consulted to request an approved deviation. If the remaining monitoring can not be completed within a five (5) day period, the results of this monitoring event will not be accepted by NSDFA and the entire monitoring event will have to be repeated.

Extensions will not be granted as a result of inclement weather. Those conducting required monitoring must plan appropriately to ensure that weather will not prevent monitoring from being completed within the prescribed timeframes.

4 BENTHIC VIDEO COLLECTION

4.1 Video Recording Methodology

Benthic video footage must be collected at every monitoring station during all levels of monitoring. Video may be recorded via surface deployed drop camera, hand-held diver operated video camera, or remotely operated vehicle and must be collected prior to sediment sample collection. The criteria for acceptable video recording are described below:

- Video overlay and/or a placard containing relevant video station details (date, time, coordinates, lease number and station ID) must be presented at the beginning of each video recording**,** prior to submersion;
- A 360º panorama (or as close as possible) of the water surface view plane must be recorded at each video station prior to submersion;
- The video must include continuous footage of the initial descent, impact with the seafloor, camera ascent and retrieval on deck;
- The field of view must include a visible reference scale. If measurements are not indicated on the reference scale, the measurements must be submitted to NSDFA as part of the video submission;
- Surface deployed camera video must include a digital overlay detailing real time latitude and longitude of the monitoring station. The latitude and longitude should be formatted using the NAD83 CSRS datum and submitted in decimal degrees;
- Hand-held, diver collected video must include a view of the current coordinate location on a sufficiently accurate DGPS unit both before and after entering the water;
- Once near bottom, the camera's descent will halt above the seafloor. Demonstration of benthic consistency will then take place via camera or diver contact with the sediment;
- Video lighting and resolution must be sufficient to allow for the characterization of sediment conditions, identification of macro flora and fauna, and accurate interpretation of the presented reference scale;
- A minimum 2 minutes of seafloor footage are required at each video station; and
- Video for each monitoring station must cover a minimum area of 5 m^2.

If any video submitted to NSDFA does not meet the video quality criteria listed above, it may render the monitoring event invalid.

4.2 Video Station Locations

4.2.1 Soft Bottom Monitoring Stations

At monitoring stations that are determined to be soft bottom, (Section 2.2.1 of the *Environmental Monitoring Program Framework for Marine Aquaculture in Nova Scotia*), a single video station will be completed at the identified cage edge or within 10 m of the assigned target coordinate. Recording at such video stations are to be carried out as specified in Section 4.1.

4.2.2 Hard Bottom Monitoring Stations

At any monitoring station that is determined to be hard bottom, of the *Environmental Monitoring Program Framework for Marine Aquaculture in Nova Scotia,*), video recording is to be conducted in accordance with the Department of Fisheries and Oceans Canada (DFO) *Aquaculture Activities Regulations* (AAR, 2021) and the following criteria:

- Recordings will be conducted along a transect extending away from the monitoring station in a direction perpendicular to the edge of the cage array on which the monitoring station lies;
- A total of six (6) video stations will be established along the length of the transect, at 0 m, 10 m, 20 m, 30 m, 40 m and 50 m from cage edge;
- Recordings at all video stations are required to meet the methodology and quality criteria outlined in Section 4.1 unless otherwise directed;
- A 360º panorama is only required at the beginning of each individual recording (i.e. if the camera system is recording continuously throughout multiple video stations of a transect a panorama is only required prior to submersion at the first station.);
- If utilizing a diver-held or remotely operated underwater vehicle (ROV) mounted camera to conduct a continuous transect, a weighted drop line or other visual guide must be used to mark the transect line as well as the individual video stations at 10 m increments; and
- A diver or ROV conducting a continuous transect will do so at a speed and height above bottom which allows for the clear observation and identification of macro flora and fauna within 1 meter to either side of the established transect line.

If any video submitted to the NSDFA does not meet the video quality criteria listed above it may render the monitoring event invalid.

4.3 Video Observation Requirements

For each established video station visited during a monitoring event, detailed observations are to be made, recorded, and submitted to NSDFA. **Appendices A3** and **A4** are provided as sample templates for the recording of field observations. Observations at each video station should include, but are not limited to:

- Video Station Details:
    - Waterbody
    - Aquaculture lease number
    - Station ID
    - Distance along established transect (where applicable)
    - Date and time of recording commencement
    - Water depth
    - Latitude and longitude
    - Distance and direction from assigned station (where applicable)
- Video Observations:
    - Sediment Description
        - Colour at surface and subsurface
        - Composition (e.g. sand, cobble, boulder, etc.)
        - Consistency/Consolidation (e.g. soft, hard, easily disturbed etc.)
    - Benthos Description
        - Macrofauna observed
        - Macroflora observed
        - Presence and relative abundance of uneaten finfish feed
        - Presence and relative abundance of finfish faeces
        - Presence and relative abundance of other organic detritus
        - Presence of gas bubbles released from sediment
        - Presence and approximate % coverage of *Beggiatoa*-like bacterial mats
        - Presence and approximate % coverage of polychaete complexes
        - Presence and approximate % coverage of barrenness
        - Anthropogenic debris observed
    - Biophysical conditions at depth
        - General visibility
        - Relative current speed
        - Relative abundance of suspended particulate matter

In the case of a continuous video transect collected by a diver or ROV, the above observations must be recorded at a minimum of 10 m intervals with note made of any significant changes occurring in the interim. The presence of any macroflora, macrofauna, or significant environmental indicators observed at any point throughout the transect must be recorded.

5 SEDIMENT COLLECTION

Samples of benthic sediment are required to be collected at each station during all levels of monitoring. The goal of sediment collection is to retrieve representative samples of the current benthic environment to assess relative levels of health and impact. Depending on the level of monitoring, collected sediments will be analyzed for oxidation-reduction potential, total dissolved sulfide concentration, porosity, and sediment organic matter. Although sulfide concentration is the main regulatory indicator used to classify an aquaculture lease, the other variables are used to validate and confirm accuracy of sulfide results via the empirical relationships of measured variables (Hargrave, 2010) and the Benthic Enrichment Index (BEI) (Hargrave, 1994).

Sediment samples may be collected via surface deployed equipment (e.g. grab, gravity corer) or manually operated core tube (diver-held or ROV). Selection of the appropriate sampling method and equipment to be utilized at a station will depend largely on site-specific conditions such as sediment composition and consolidation, water depth and current speed. A decision tree is provided in **Appendix A1** to serve as guidance in the selection of appropriate equipment. Proposals for the use of alternate methods or equipment not listed in this SOP should be submitted to NSDFA prior to monitoring for review and approval.

Sample collection must take place in a consistent and repeatable manner in order to maintain the integrity of the subsequent analysis results. All samples, regardless of collection method, are required to meet the following methodology and quality criteria to be considered acceptable:

- Triplicate samples are to be collected at all stations;
- Triplicate samples are to be sub-sampled from discrete sediment collection events. (i.e. a single sub-sample collected from each of three (3) grabs or three (3) separate core tubes);
- Each collection will have a minimum sediment depth of 5 cm;
- Sub-samples will be collected or directly analyzed from the top 2 cm of the collected sediment;
- Overlying water must be present over the entire sample surface at time of retrieval;
- The interface between the sediment surface and overlying water is relatively flat and undisturbed;
- Sediment sampling equipment must not be overfilled;
- All efforts must be made to collect sediment from seafloor that has not been disturbed as a result of previous sample or video collection;
- Accurate GPS coordinates are to be recorded at the location of each sample collection; and
- Photographic record of the results of each sample collection attempt are to be collected with overlying water and again once overlying water is removed, but prior to sub-sample extraction.

Additional, equipment-specific, quality criteria are provided in the following sections.

5.1 Surface Deployed Sampling

Surface deployed sediment collection equipment may be a core or grab, and should be selected, based on site and environmental conditions, in order to best satisfy the methodology and quality criteria of this section.

- Grab samplers must have sufficient opening to access and observe the entire surface of a collected sample;
- All sampling equipment is to be operated as per the manufacturer's specifications. Any modifications made must be approved by the NSDFA prior to use;
- The speed at which the sampler descends through the water column must minimize the disturbance of benthic surface sediment due to the force of water being displaced;
- Sediment collection equipment must descend and ascend vertically to ensure the sampler connects evenly with the seafloor and that the collected sample is not shifted during retrieval;
- Retrieval of the sampler should start by slowly lifting from the seafloor and then steadily raising it to the surface at a target speed of 30 cm/s or less (Environment Canada 1994). Sample retrieval speed must be calculated and included within the submitted report to NSDFA;
- If equipment uses covering flaps to protect samples during retrieval (e.g. Ponar or Van Veen grabs), flap position should remain down throughout deployment;
- If an acceptable sediment sample has not been successfully collected after three (3) consecutive attempts, uncontrolled descent (free-fall) of the sampling equipment will be permitted, and it should be highlighted in data submissions that this method was used. In such cases, the sampler should be allowed to free fall for no more than a few meters above the bottom;
- Sampler jaws must be fully closed upon retrieval (i.e. rocks or shells should not be holding bottom open);
- Collection attempts will only be considered unsuccessful if the failure is due to characteristics of the sediment composition or consolidation, including the presence of excess shell debris and macrophyte beds. Failure resulting from the presence of site debris or other anthropogenic factors will not count towards the number of unsuccessful attempts;
- Overlying water will be removed via siphoning before sub-sampling occurs;
- Photographs of the entire sample surface are to be collected before and after the removal of overlying water and before sub-sampling occurs;
- Sub-samples should be collected using plastic syringe cores with a rubber-tipped plunger and mL increments (e.g. Becton-Dickson 5 mL, Fisher # 14-823-35) with the tapered tip removed;
- Sub-samples collected from successful retrievals must be extracted from a minimum of three (3) different locations within the sample;
- Any subsamples collected must contain a minimum of 5 mL of sediment and be clearly labeled with a station and sample ID
- Sediment remaining after subsampling must be discarded away from subsequent monitoring locations;
- Sampling equipment must be rinsed thoroughly between deployments;
- If sediment consolidation or composition has resulted in five (5) failed collection attempts before three (3) acceptable samples can be retrieved, an alternate station is to be established

at the cage on the same side of the array which contains the next highest biomass and which is not already a proposed monitoring station;

- If sediment consolidation or composition, and presence of excess shell debris and macrophyte beds, has resulted in five (5) failed collection attempts before three (3) acceptable samples can be retrieved at the alternate location, the station will be considered a 'hard bottom station' for that monitoring event. Visual monitoring, as described in Section 4.2.2, will be conducted in lieu of sediment collection.
- Acceptable and unacceptable grab samples are presented in Figure 5

Any sample collection that fails to meet these methodology and quality criteria may not be accepted by NSDFA.

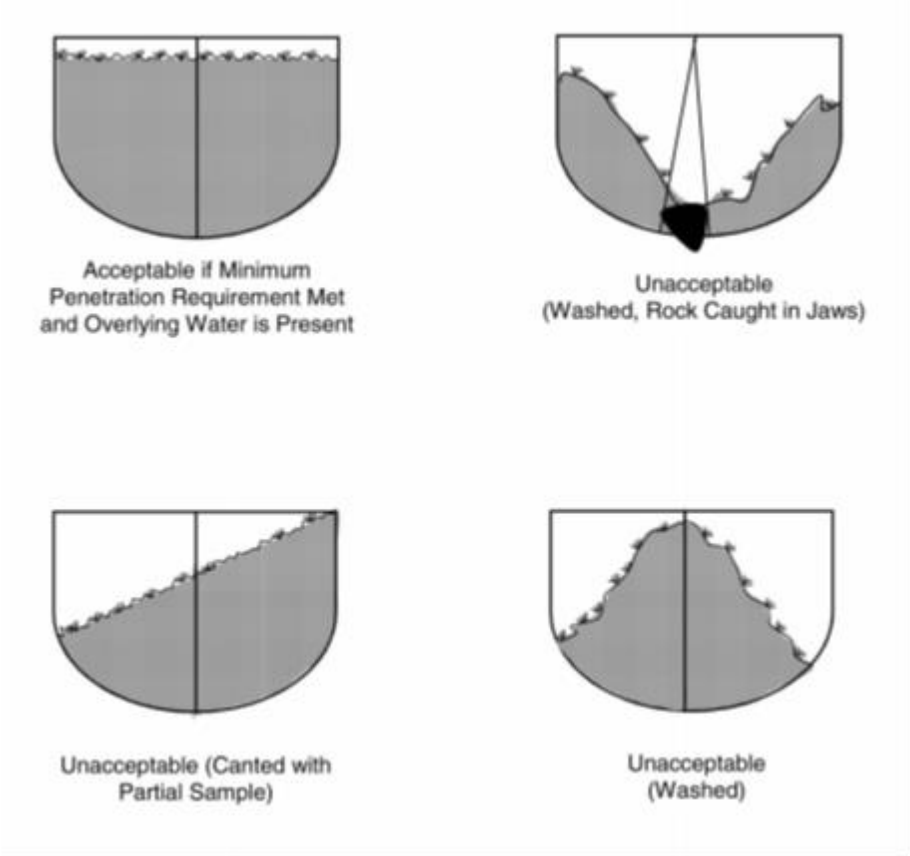


Figure 5. Illustrations of acceptable and unacceptable grab samples (USEPA 2001)

5.2 Sediment Core Collection

Sediment sampling may be conducted with the use of self-contained core tubes, collected by SCUBA diver or ROV in accordance with the following methodology and quality criteria:

- Core samples will be collected using transparent core tubes, allowing a clear view of the entire sample surface;
- Core tubes must be equipped with a means of either directly analyzing the top 2 cm of sediment for required geochemical parameters or extracting a suitable subsample while maintaining an undisturbed sample surface;
- Cores are to be inserted vertically, collecting a sample at least 5 cm depth;
- If the sample area is disturbed or contaminated, the dive crew must select a new sample area as close as possible to the original station without sampling previously disturbed substrate;
- Core samples are to be sealed as soon as possible following sample collection;
- Cores must remain vertically oriented and maintain a relatively flat and undisturbed sediment-water interface until analysis is performed or a subsample is extracted;
- Retrieved cores are to be photographed and evaluated for disturbance level (i.e. very clear with no disturbance, clear with minimal disturbance, cloudy with moderate disturbance or not clear and disturbed) after surfacing;
- Any subsamples collected must contain a minimum of 5 mL of sediment and be clearly labeled with a station and sample ID;
- If it is determined that an acceptable core sample cannot be collected within approximately 10 m of the monitoring location due to sediment composition or consolidation, the alternate station should be sampled;
- If an alternate station can not be established which meets the above criteria, visual monitoring, as described in Section 4.2.2, will be conducted in lieu of sediment sampling at the initially selected location; and
- If it is determined that an acceptable core sample cannot be collected within approximately 10 m of the monitoring location due to sediment composition or consolidation at the alternate location, the station will be considered a ‘hard bottom station’ for that monitoring event. Visual monitoring, as described in Section 4.2.2, will be conducted in lieu of sediment collection.

5.3 Sediment Storage and Transportation

Samples should be analyzed as quickly as possible following retrieval. If samples are to be stored and transported for analysis at a later time, the following guidelines must be followed:

- Sample storage containers used must not be made of any material or used in such a way that may negatively impact subsequent laboratory analysis;
- Samples must be sealed against the intrusion of air and contain no apparent air bubbles throughout the sample;
- A flexible, impermeable barrier, such as Parafilm® or Saran Wrap® should be used in addition to a tight-fitting cap in order to ensure an air-tight seal;
- If headspace is unavoidable in a sample or subsample vessel, inert gas (e.g. nitrogen or argon gas) may be used to cover the sample prior to closure of container;

- As soon as possible following sample or subsample collection, sediments must be stored in the dark at 2-5 ºC until they can be analyzed;
- A thermometer for immediate reference and a continuous temperature logger, recording at a minimum 30-minute interval, must accompany samples until laboratory analysis is conducted; and
- Sample temperature data must be maintained by the monitoring party and may be requested by NSDFA to assess quality assurance and quality control (QA/QC) of sediment storage and transport.

5.4 Sediment Collection Observation Requirements

For each successful sediment retrieval attempt which meets the appropriate methodology and quality criteria outlined above, detailed observations are to be made, recorded and submitted to NSDFA. Observations recorded for each sample retrieval should include, but are not limited to:

- Sampling Observations:
    - Waterbody
    - Aquaculture lease number
    - Station ID and replicate number
    - Distance along established transect (where applicable)
    - Date and time of sample collection
    - Water depth
    - Latitude and longitude of monitoring location
    - Type of sampling equipment utilized
    - Name of personnel collecting samples
    - Number of collection attempts required
    - Any deviations from prescribed standard operating procedures

- Sample Observations:
    - Sediment colour at surface and subsurface
    - Sample composition (e.g. mud, sand, cobble etc.)
    - Sample odor
    - Total sample depth
    - Macrofauna observed
    - Macroflora observed
    - Presence and relative abundance of uneaten finfish feed
    - Presence and relative abundance of finfish faeces
    - Presence and relative abundance of other organic detritus
    - Presence of gas bubbles released from sediment
    - Presence and relative abundance of *Beggiatoa*-like bacterial mats
    - Presence and relative abundance of polychaete complexes
    - Presence and description of anthropogenic debris

6 ANALYSIS OF SEDIMENT SAMPLES

Information contained within this section provides guidance for the analysis of sediment samples for the Nova Scotia EMP. The procedures outlined below are based on information found in Wildish et al. (1999) and Wildish et al. (2004). Revisions have been made according to discussions and feedback from the April 2014 Nova Scotia Aquaculture Environmental Coordinating Committee (AECC) meetings.

The NSDFA has approved the Accumet AP63 and AP125 Portable pH/Ion Meter, Orion Silver/Sulfide Ionplus® Sure-Flow® Solid State Combination Ion Selective Electrode (Cat. No. 9616BNWP), and Orion Epoxy Sure-Flow Combination Redox/ORP Electrode (Cat. No. 9678BNWP) for measurement of sulfide and redox. Once per year, prior to the initiation of EMP sediment analyses, the analytical party must submit to NSDFA, for approval, a list of chemicals (name, CAS#, and expiry date) and analytical equipment (name and model #) intended for EMP sediment analysis. Each instrument must be associated with a unique identifier and recorded. Laboratory records (e.g., logbooks, original records) may be requested by NSDFA for QA/QC laboratory audits. A sample of the data recording sheet can be found in **Appendix A5** respectively. Original records of sampling data must be retained for a minimum of seven (7) years.

6.1 Redox Analysis (Eh)

Oxidation-reduction potential (redox), measured in millivolts (mV), is a measure of oxidation-reduction potential in sediments and is an indirect indicator of aerobic versus anaerobic conditions.

6.1.1 Materials

- Accumet AP63 or AP125 Portable pH/Ion Meter (Cat. No. 13-636-AP63 or 13-636-AP125)
- Orion Epoxy Sure-Flow Combination Redox/ORP Electrode (Cat. No. 9678BNWP)
- Accumet ATC probe (Cat. No. 13-620-19)
- 4 M KCL saturated with Ag/AgCl (Cat. No. 900011)
- ORP standard (Cat. No. 967901 or 967961)
- Sampling receptacles (labelled)
- Timer
- **Appendix A5** data record sheet or an additional record sheet containing the exact fields

6.1.2 ORP Electrode Accuracy Check

An accuracy check is to be performed **before and after** analysis using the commercially available ORP standard solution. The redox electrode must be filled with 4 M KCl saturated with Ag/AgCl at least 24 hours before use (Wildish et al., 1999). Place the electrode in a sample of 25 °C ORP standard solution and record the mV reading. At 25 °C, absolute mV values should equal 220 ± 3 mV. Accuracy check readings are to be recorded on the data recording sheet. Include notes regarding any errors or irregularities on data sheets. See **Appendix A6** for a suggested procedure to detect coatings on the electrode platinum surface. The electrode manual should be read before usage (Thermo Scientific, 2007).

6.1.3 Redox Measurements

Triplicate subsamples taken from each monitoring station will be analyzed for redox in accordance with the protocol outlined below.

- Measurements will be completed within 72 hours of sample collection. AAR requirements dictate that measurements must be completed within 36 hours. If storage is required, samples must be stored in the dark, on ice (chilled, not frozen) in the field and transferred to a refrigerator held at 2 – 5 °C (a temperature logger must be used to measure storage temperatures (see Section 5.0);
- From the cut-off 5 mL or 10 mL syringe, the first 2 mL (or 5 mL if a 10 mL syringe is utilized) are isolated from the upper 3 mL (5 mL) by first extruding 2 mL (5 mL) into a labelled, decontaminated, pre-weighed (g) receptacle for sediment porosity and percent organic matter analysis. The upper 3 mL (5 mL) are extruded into a separate labelled, receptacle for redox and sulfide analysis;
- Receptacles used for redox and sulfide analysis should have a volume capacity that minimizes headspace;
- Measurements will be taken with Accumet AP63 or AP125 Portable pH/Ion Meter, Orion Epoxy Sure-Flow Redox/ORP Electrode and Accumet ATC probe;
- The redox probe should be held stationary during analysis. Hold the probe firmly in place below the sediment surface (Hargrave, personal communication);
- Redox measurements will be recorded as millivolts relative to the normal hydrogen electrode (mV_{NHE}) using the equation $mV_{NHE} = Eo + (224 - T)$, where Eo = mV of unknown and T = temperature of unknown (°C). Record the mV and temperature readings once the mV value has stabilized (stable reading displayed on meter or mV drift is < 10 mV/minute). If stabilization is not achieved, record the mV and temperature values when two (2) minutes has elapsed (use a timer to achieve consistency among samples). Note on **Appendix A5** data sheet which readings were taken at 2-minutes;
- The redox electrode will be rinsed with distilled water and dried between measurements (gently blot dry with Kimwipe);
- Redox and sulfide measurements must occur sequentially on one subsample before commencing redox analysis on the next subsample;
- All replicate 1's from each monitoring location must be analyzed first, followed by all replicate 2's and then 3's to disperse evenly across all samples any potential influence that probe drift may have on measurements throughout the period of analysis; and
- The order of subsample analysis, based on station ID, should be the same when each replicate group is analyzed.

6.2 Sulfide Analysis

Total dissolved sulfide, measured in micromolar (μM), quantifies the accumulation of soluble sulfides, a major product of sulfate reduction that occurs under anaerobic conditions. This is a sensitive indicator of habitat degradation due to organic loading and currently the main indicator used to determine direct impact of an aquaculture operation.

As an accuracy check for the internal meter calculation, record the associated millivolt (mV) value for both the calibration and sulfide analysis. This allows calculation of sulfide concentrations directly from the calibration curve.

6.2.1 Materials

- Accumet AP63 or AP125 Portable pH/Ion Meter (Cat. No. 13-636-AP63 or 13-636-AP125)
- Orion Silver/Sulfide ionplus® Sure-Flow® Solid State Combination Ion Selective Electrode (Cat. No. 9616BNWP)
- Accumet ATC probe (Cat. No. 13-620-19)
- Orion Optimum Results B filling solution (Cat. No. 900062)
- Sodium sulfide (Na_2S) standards (100, 500, 1000, 5000, 10000 µM)
- Sulfide antioxidant buffer (SAOB) + L-ascorbic acid
- **Appendix A5** data record sheet or similar recording sheet

6.2.2 Sulfide Electrode Calibration

Five sodium sulfide standards will be used to calibrate the sulfide electrode prior to sample analysis (100, 500, 1,000, 5,000 and 10,000 µM). Sodium sulfide standards are unstable and oxidize readily in aerobic conditions and should be prepared fresh with deaerated water (distilled or deionized). SAOB + L-ascorbic acid are combined and added to standards just prior to calibrating. See Wildish et al. (1999) for preparation of sodium sulfide standards and SAOB + L-ascorbic acid solution. An exothermic reaction is initiated during the preparation of SAOB therefore, this solution must be cooled to 2 – 5 °C prior to use. See the electrode and meter manuals for calibration steps (Thermo Scientific, 2009 and Fisher Scientific, 2009).

The sulfide electrode will be filled with Orion Optimum Results B filling solution at least 24 hours before use (Wildish et al., 1999);

- SAOB may be stable for a maximum of 3 hours following the addition of L-ascorbic acid (Wildish et al., 1999). If the SAOB + L-ascorbic acid solution exhibits a colour change prior to the 3-hour expiration, it is recommended to prepare a fresh solution. Record time that L-ascorbic acid is added to SAOB and time solution expires or colour change is observed on **Appendix A5** data sheet;
- Always dilute standards using a 1:1 ratio with SAOB + L-ascorbic. Do not add SAOB + L-ascorbic acid to standards until just prior to calibration;
- Standards should not be shaken, rather gently swirled or stirred to adequately mix the SAOB + L-ascorbic acid and standard;
- Each standard and SAOB + L-ascorbic acid solution must reach the same target temperature (between 20-25 °C) before calibrating the electrode;
- Follow the meter calibration steps (Fisher Scientific, 2009). Record both µM and mV readings once the target temperature is reached for each standard. Also, record the displayed slope value provided after 500 µM (**must be between -27 to -33**) standard on the **Appendix A5** data sheet.
- Calculate the 10-fold mV change (slope). This value provides the best means for checking electrode operation (see Thermo Scientific, 2009).
    - mV (5,000 µM) – mV (500 µM) = 10-fold mV change.
    - mV (10,000 µM) – mV (1,000 µM) = 10-fold mV change.
    - The acceptable value range is **-25 to -30 mV.**
- The Accumet AP63 and AP125 Portable pH/Ion meter's default calibration values are a factor of 10 times less than the actual standard concentrations; therefore, the displayed

calibration value must be multiplied by 10 to obtain the correct concentrations;
- Include notes regarding any calibration problems on **Appendix A5** data sheet; and
- Calibration of the sulfide electrode is stable for a maximum of three hours. Record time calibration completed and time of expiry on the **Appendix A5** data sheet.

6.2.3 Sulfide Measurements

Triplicate subsamples taken from each monitoring station will be analyzed for sulfide in accordance with the protocol outlined below.

- Measurements will be completed within 72 hours of sample collection (Wildish et al., 1999). AAR requirements dictate that measurements must be completed within 36 hours.
- Measurements will be taken with Accumet AP63 or AP125 Portable pH/Ion Meter, Orion Silver/Sulfide ionplus® Sure-Flow® Solid State Combination Ion Selective Electrode and Accumet ATC probe.
- Receptacles used during analysis should have a volume capacity that minimizes headspace.
- Always dilute samples using a 1:1 ratio with SAOB + L-ascorbic. (i.e., each 3 mL sediment subsample will be mixed with 3 mL of SAOB + L-ascorbic acid).
- Samples should not be shaken, rather gently swirled or stirred to adequately mix the SAOB + L-ascorbic acid and sample.
- Sulfide readings will be taken once the SAOB + L-ascorbic acid and sample mixture reaches the same temperature at which the electrode was calibrated, and stabilization is achieved ('stable' displayed on meter). Note samples that are up to temperature but have not stabilized within 2 minutes. Record µM and mV values. Multiply µM values by a factor of 10 and record as 'adjusted'.
- The sulfide electrode is to be rinsed with distilled water and dried between sample measurements (gently blot dry with Kimwipe).

6.3 Sediment Porosity

Porosity is the percentage (%) of pore volume or void space, or the volume within any material (e.g., bottom sediment) that can contain fluids. Porosity is an indirect measure of grain size and is used to detect changes in sediment consistency which may result from sedimentation of faeces and excess feed.

The method described below is to be performed using a gravity convection drying oven (e.g., Lindberg/Blue M 260) and an analytical balance (e.g., Denver Instrument Summit Series, SI 234); Other makes and models are acceptable:

6.3.1 Materials

- Gravity convection drying oven
- Analytical balance with four (4) decimal places
- Labelled, pretreated, pre-weighed (g) receptacles
    - If receptacles are being reused, they must be acid washed between analyses to avoid cross contamination.
- Vacuum desiccator
- Worksheet

6.3.2 Porosity Measurements

- Pre-heat drying oven to 60 °C;
- Record wet weight (g) of pre-weighed receptacle and sediment sample;
- Place weighed receptacles and sediment in the drying oven for 24 hours at 60 °C;
- Following 24 hours, place dried samples in a vacuum desiccator to bring to room temperature prior to weighing; and
- Record dry weight (g) of receptacle and sediment sample. Weight recordings (g) should be recorded to at least 4 decimal places. The porosity value can be calculated as a percentage of the total volume of material:

(Wet sediment and receptacle weight) – (receptacle weight) = Wet sediment weight (g)
(Dry sediment and receptacle weight) – (receptacle weight) = Dry sediment weight (g)

[(Wet sediment weight – Dry sediment weight) / Wet sediment weight] x 100 = porosity (%)

6.4 Sediment Percent Organic Matter (POM)

Organic matter is observed to determine the portion (%) of sediment that is of plant or animal origin (combined). This variable is a good measure of organic loading.

The method described below is to be performed on the pre-dried samples from porosity analysis (section 6.3) using a muffle furnace (e.g., Barnstead/Thermolyne, Type 48000). Other make/models are acceptable:

6.4.1 Materials

- Receptacles used for organic matter analysis must be pre-ashed, and pre-weighed (g), before sediment is introduced
- Tweezers
- Ceramic tray (not required but helpful for keeping track of samples)
- Muffle furnace
- Analytical balance with four (4) decimal places
- Vacuum desiccator
- Worksheet

6.4.2 Percent Organic Matter Measurements

- Handling the labelled, pre-weighed (g), pre-ashed receptacle with tweezers, add approximately 0.5 g of ground, homogenized, dried sediment from the porosity analysis to the muffle furnace-safe receptacle. Record the weight. Weight recordings (g) should be recorded to at least 4 decimal places.
    - Sample homogenization is only required if the dried sediment is subsampled for POM measurements. Take care to avoid cross contamination between samples;
- Place samples in a cold muffle furnace. Set muffle furnace to 490 °C for a minimum of 8 hours;
- Allow furnace to cool down before handling samples. Place ashed samples in a vacuum desiccator to bring to room temperature prior to weighing;
- Record weight of receptacle and ashed sediment sample;
- Percent organic matter can be calculated as follows:

Dried sediment – ashed weigh boat = Dried sediment (g)
Ashed sediment – ashed weigh boat = Ashed sediment (g)
Dried sediment – ashed sediment = Sediment organic content (g)

[Sediment organic content (g) / Dried sediment (g)] x 100% = organic matter (%)

7 RECORD KEEPING

NSDFA will review all environmental monitoring performed as part of this program. Pre-monitoring submissions are required to be submitted to NSDFA a minimum of two weeks prior to monitoring. Data submissions are required to be submitted to NSDFA 14 days following sample collection. In summary, the final submission must include:

- **Pre-monitoring**
  - Once a year: A list of chemicals (name, CAS#, and expiry date) and equipment (model name and #) intended for use for the EMP season.
  - 2 weeks prior to monitoring: Pre-monitoring submission (Section 3.1)

- **Within 14 days of sediment collection:**
  - **Appendix A2** – Coordinate and Lab Results Table
    - All data fields completed
  - **Appendix A3** – Video and Sediment Sample Log Sheet (1 per station)
  - **Appendix A4** – Video Transect Log Sheet (1 per station)
  - Sediment sample photos
  - Video recordings
  - **Appendix A5** – Analytical Data Record Sheet
    - Site name/# date of monitoring and analysis etc., redox probe accuracy check and sulfide calibration results (redox and sulfide sediment results will be included in A2)

APPENDIX A: ASSOCIATED FIELD AND ANALYTICAL SHEETS

The following appendices include templates and guidance documents to be used as part of the standard operating procedures.

Appendix A1 is a monitoring equipment decision tree.

Appendix A2 includes a coordinate table to record and submit all coordinates used to determine precise monitoring station locations. This template also includes columns to input summary laboratory results.

Appendix A3 is a log sheet to record field notes.

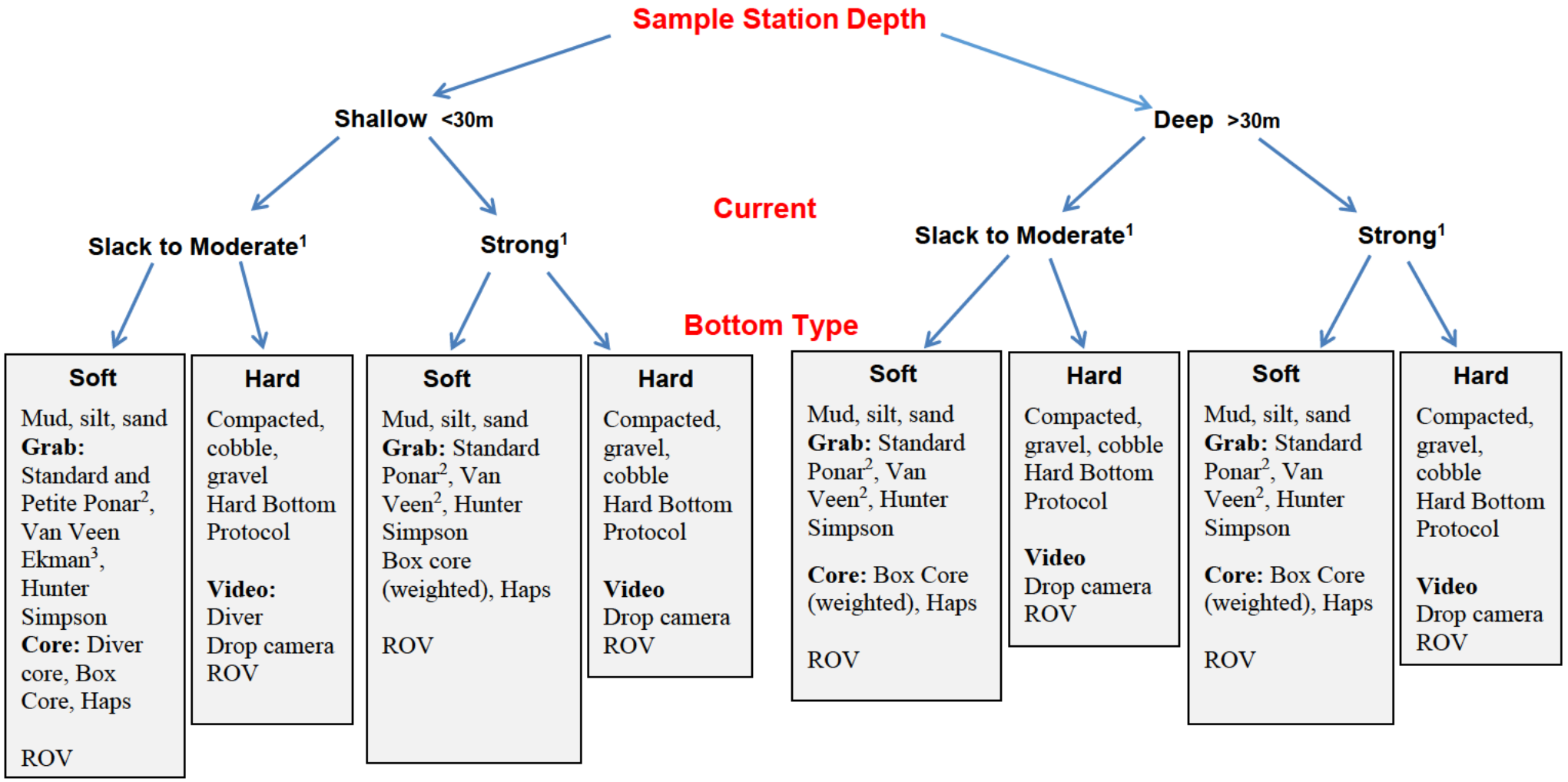
Appendix A4 is a log sheet to record information recorded by video transects at hard bottom stations

Appendix A5 is a data worksheet to record the redox accuracy check, sulfide calibration and measured values of redox potential and sulfide in sediment samples.

Appendix A6 is a suggested procedure for pre-season preparation and on-going use of ORP electrodes.

Appendix A7 is a checklist which outlines all information pieces required for submission.

APPENDIX A1: Decision Guidelines for Selecting Monitoring Equipment



[1] As a guide slack to moderate is considered to be 0-1 knot (0-0.5 m/s) while strong is greater than 1 knot (0.5m/s).
[2] To stiffen the flaps, modify the Ponar and Van Veen grab samplers with thicker rubber flaps.
[3] Ekman not appropriate for use in moderate current.

APPENDIX A2: Coordinate and Lab Results Template

This template should be submitted in editable electronic spreadsheet format (i.e., excel) for all sampling events including baseline and Level I to III monitoring events. The coordinates should be submitted in NAD83 CSRS (decimal degrees). This template also includes columns to input summary laboratory results. Please submit this table with completed laboratory analysis of sample temperature, redox potential, total dissolved sulfide, porosity and percent organic matter. Data pertaining to individual replicates must be provided.

Monitoring Date	Sample ID		Longitude	Latitude	Location	Lease #	Sample temp. (°C)	Redox (mV)	Redox (mV_{NHE})	Sulfide (µM) adjusted	Sulfide (mV)	Porosity (%)	Organic Matter (%)
	Station ID	Replicate											
8-Aug-13	NSH01	1	43.33333	65.55555	Scotia Bay	0001							
8-Aug-13	NSH01	2	43.33333	65.55555	Scotia Bay	0001							
8-Aug-13	NSH01	3	43.33333	65.55555	Scotia Bay	0001							
8-Aug-13	NSH02	1	43.44444	65.66666	Scotia Bay	0001/Ref							
8-Aug-13	NSH02	2	43.44444	65.66666	Scotia Bay	0001/Ref							
8-Aug-13	NSH02	3	43.44444	65.66666	Scotia Bay	0001/Ref							

APPENDIX A3: Video and Sediment Sampler Log Sheet

Date:			**Wind direction and speed:**	
Water body:			**Wave action:**	
Lease name and #:			**Direction and speed of current:**	
Monitoring Station ID:			**Tide schedule:**	
Latitude (decimal degrees):			**Video Notes:**	
Longitude (decimal degrees):				
Dist. and dir. from WP:				
Time:			**Comments:**	
Recorder name:				
Sample collector:				
Type of sediment sampler:			**Benthic Descriptor Key:**	
Station Depth (m):			1. Oxic layer thickness, gas bubbles, feed, faeces, sediment: colour, type and consistency	
Gear Present on Bottom (Description)			2. Degree of odour (strong, slight, none)	
Video (Y/N):			3. Flora/Fauna (e.g., eel grass, kelp, lobster, crab, starfish, *Beggiatoa*-like, polychaetes etc.)	
# sediment collection attempts:				

Sediment Samples	Sample (y/n)	Sample ID	Sediment Sampler Retrieval Speed (cm/s)	Sediment Description[1]	Sediment Sample Depth (cm)	Odour[2]	Flora / Fauna[3]
Benthic Replicate A							
Benthic Replicate B							
Benthic Replicate C							

Key Terms of Video and Sediment Sampler Log Sheet

Date – Date sample was collected.

Water body – Bay or Harbour name.

Lease name/# - Lease name/NSDFA lease number

Monitoring station ID - Indicate the predetermined station identification code (e.g. SBH03)

Latitude –Monitoring station coordinate in decimal degrees (hddd.ddddd°)

Longitude – Monitoring station coordinate in decimal degrees (-hddd.ddddd°)

Dist. and dir. from WP – Indicate the distance (m) and direction from the intended waypoint.

Time – Time sample was collected.

Recorder Name - Name of person taking notes.

Sample Collector/Diver(s) Name – Name of person who collected the sample using surface deployed sampler or diver who collected the core.

Type of sediment sampler – Sediment sampler type (i.e., core tube or grab type).

Station depth – Station water depth (m) at time of monitoring.

Gear present on bottom- Note any visible gear that is related to the aquaculture operation on the bottom (i.e. nets)

Video (Y/N) – Indicate if video was successfully collected. If no video collected, note the reason.

 # sediment collection attempts – State the number of sediment sampler deployments, in total, per replicate sampling.

Wind direction and speed – Describe the relative wind direction (e.g., N, SE, etc.) and relative speed (e.g., 10 knots).

Wave action – Describe the relative water conditions (e.g., flat, chop, swell, etc.).

Direction and speed of current – Describe the relative direction path (e.g., N-S, SW-NE, etc.) and relative speed (e.g., 10 knots) of the predominant current.

Tide schedule – State the times of high and low tide.

Video notes – Sediment type, consistency and colour. Presence of biota (flora and fauna), presence of gas bubbles, presence of fish feed and/or faeces.

Comments – Include any notes pertaining to site changes, sampling difficulties, anchoring/mooring, differences between observed seafloor conditions and collected sediment sample, notes regarding sampling difficulties, weather issues, deviations from the SOP, etc.

Sample (Y/N) – Indicate if a replicate sample was collected.

Sample ID - List identification number listed on replicate core.

Sediment description – Describe sediment characteristics of sediment sample. See Benthic Descriptor Key'.

Sediment sample depth – The measurement of the depth (cm) of the sediment within the sampler.

Odour – Indicate degree of odour from the sediment (strong, slight, none). See 'Benthic Descriptor Key'.

Flora/Fauna – Describe flora/fauna characteristics collected along with sediment sample. See 'Benthic Descriptor Key'.

APPENDIX A4: Video Monitoring Transect - Summary of Observations for Station

	0 M	10 M	20 M	30 M	40 M	50 M
GPS coordinates NAD83 CSRS						
Sediment colour (brown, black, grey)						
Sediment consistency (mud; clay; rock; cobble; sand/silt)						
Sediment surface consolidation (firm packed; consolidated but easily disturbed; unconsolidated but very easily disturbed)						
Gas bubbles (none; rare; some; prevalent)						
Beggiatoa-like bacteria presence/absence and % coverage						
Opportunistic Polychaete worm Complexes (OPC) presence/absence						
Presence of feed (none; rare; some; prevalent)						
Presence of faeces (none; rare; some; prevalent)						
Macro fauna/flora (none; relative abundance of polychaetes, molluscs; echinoderms and crustaceans; note which species are in relative abundance)						
Presence of gear on bottom						

APPENDIX A5: Analytical data record sheet

Lease # / name: _____

Date samples collected: _____ Time samples collected (x – x): _____

Date of analysis: _____ Analysis start time: _____ Analysis end time: _____

Analysis performed by: _____

Redox Probe Accuracy Check (25°C)

ORP standard (start): _____ ORP standard (finish): _____

Sulfide Calibration

Temperature target (20-25°C): _____

Na_2S standard concentration (µM)	mV	Meter calculated slope (mV) Acceptable range: -27 to -33 mV after 500	Calculated 10-fold change (mV)	Verification check	
				µM	mV
100			1. 5,000 mV – 500 mV 2. 10,000 mV – 1,000 mV Acceptable range : -25 to -30 mV		
500					
1,000					
5,000			1.		
10,000			2.		

Time calibration completed: _____ Time L-ascorbic acid added to SAOB: _____
Time calibration expires: _____ Time SAOB + L-ascorbic acid expires: _____

Sample ID	Replicate #	Temp. (°C)	Redox (mV)	Sulfide (µM) unadjusted	Sulfide (mV)	NOTES:

APPENDIX A6: Suggested procedure for pre-season preparation and on-going use of ORP electrodes

1. Use only a refillable combination ORP electrode for Eh potential measurements in sediments. Gel-filled electrodes are not suitable.
2. Fill the electrode filling chamber with 4 M KCL saturated with Ag/AgCl and let stand for at least 24 hours prior to use.
3. Use the electrode to determine potential values of the ORP standard solution. Rinse (distilled water) and dry electrode on transfer between solutions and after use.
4. Place the electrode in aerated seawater and check readings every min for 5 min. Potentials should stabilize with minimum variability (± 10 mV) within 5 min. If potentials do not stabilize repeat step 2. Rinse and dry the electrode and it is ready to use.
5. Record the Eh potential in aerated seawater at the beginning and end of each day of use and enter the values on sample data sheets.
6. Check the level of the reference 4 M KCL filling solution in the electrode daily. If it falls below the filling hole add more solution to bring the level up to the hole.
7. If the electrode is to be unused for an extended period of time empty the filling chamber, rinse with distilled, deionized water and store the electrode dry.

Comments

A brand new, accurately performing ORP electrode should have NHE-corrected Eh potentials in aerated seawater between 400 and 500 mV. Used probes generally have a lower range (300-400 mV). The raw potential on the meter before applying the NHE correction should be approximately + 250 (± 50) mV for new probes and + 150 (± 50) mV for used probes. The potential will be variable and differ between electrodes reflecting the absence of strong redox reactions in aerated seawater and differences in surface properties of the Pt tip of each electrode.

It is especially important to perform this procedure prior to using a new electrode in order to determine baseline potential values under oxic conditions. This check should also be applied routinely (at least daily) to determine if the electrode has been poisoned during use (Wildish et al., 2004). The Pt tip of an ORP electrode can be polished to remove oxic coatings. The electrode's response should be compared to the initial baseline value on a regular basis to ensure that the surface of the Pt tip has not been altered or damaged during use.

If Eh potentials fall below expected values in aerated seawater and polishing does not correct the electrode response to expected potentials the orifice between the filling solution and Pt tip may have become blocked with sediment. Wildish et al. (2004) described cleaning procedures to ensure that the orifice is open. If the orifice is not blocked the Pt tip has become damaged and the electrode should be replaced (Wildish et al., 2004).

APPENDIX A7: Checklist

Pre-monitoring

- Once/year: Submit to NSDFA a list of chemicals (name, CAS#, and expiry date) and equipment (model name and #) intended for use for the upcoming EMP season. **(*Note: due to the time to acquire/order materials, submit this list a minimum of 30 days prior to the commencement of analyses).***
- Two weeks prior to monitoring: Pre-monitoring submission (Section 3.1)

Monitoring

- Underwater video recordings of the seafloor at each station with GPS overlay
- 3 deployments of sediment samplers per station, 1 syringe subsample/sediment sampler (3 sediment subsamples per station)
- Photographs of each sediment sample
- **Appendix A3** – Video and Sediment Sample log sheet completed (1 per station)

Sediment analysis

- Redox
- Sulfide
- Porosity
- Organic matter
- **Appendix A2** – Coordinate and Lab Results Table completed (Excel)
- **Appendix A5** – Analytical Data Record Sheet completed

Submissions and timelines

- Within 14 days of sediment collection:
    - **Appendix A2** – Coordinate and Lab Results Table
        - All data fields completed
    - **Appendix A3** – Video and Sediment Sample Log Sheet (1 per station)
    - **Appendix A4** – Video Transect Log Sheet (1 per station)
    - Sediment sample photos
    - Video recordings
    - **Appendix A5** – Analytical Data Record Sheet
        - Site name/# date of sampling and analysis etc., redox probe accuracy check and sulfide calibration results (redox and sulfide sediment results will be included in A2)

For further information on timelines for monitoring events, submissions and necessary mitigation please refer to Section 5.0 Annual Schedules of the EMP Framework (PNS 2021A).

LIST OF REFERENCES

Environment Canada 1994. Guidance document on collection and preparation of sediments for physiochemical characterization and biological testing. ISBN 0-660-16297-0; Cat. No. En49-24/1-29E.

Environment Canada. 2012. Metal mining technical guidance for environmental effects monitoring. ISBN 978-1-100-20496-3. Cat. no.: En14-61/2012E-PDF.

Fisher Scientific, 2009. Accumet AP 100, AP 115 and AP 125 Instruction Manual. http://static.fishersci.com/cmsassets/downloads/segment/Scientific/pdf/accumet/AP110_AP115_AP125_usermanl.pdf.

Government of Canada 2021. Aquaculture Activities Regulations (AAR)

Hargrave, B.T. [ed.]. 1994. Modelling benthic impacts of organic enrichment from marine aquaculture. Can. Tech. Rep. Fish. Aquat. Sci. 1949: xi + 125p.

Hargrave, B.T. 2009. General Services Contract for Nova Scotia Department of Fisheries and Aquaculture*:* Sampling protocols when using diver and core tubes. Owen Sound, Ontario.

Hargrave, B.T. 2010. Empirical relationships describing benthic impacts of salmon aquaculture. Aquacult. Enivron. Interact. 1: 33-45.

Province of Nova Scotia (PNS). 2021A. Environmental Monitoring Program Framework for Marine Aquaculture in Nova Scotia.

Province of Nova Scotia (PNS). 2021B. Standard Operating Procedures for Baseline Monitoring of Marine Shellfish Aquaculture in Nova Scotia.

Smith, J., Grant, J., and Stuart, R. 2002. *Design of the Environmental Monitoring Program for the Marine Aquaculture Industry in Nova Scotia.*

Thermo Scientific, 2007. User Guide – Redox/ORP Electrodes.

Thermo Scientific, 2009. User Guide – Silver/Sulfide Ion Selective Electrode. http://www.thermoscientific.fr/eThermo/CMA/PDFs/Product/productPDF_4690.pdf.

U.S. EPA 1986. Test methods for evaluating solid waste (SW-846): physical/chemical methods. U.S. Environmental Protection Agency, Office of Solid Waste, Washington, DC.

U.S. EPA 2001. Methods for Collection, Storage and Manipulation of Sediments for Chemical and Toxicological Analyses: Technical Manual. EPA 823-B-01-002. U.S. Environmental Protection Agency, Office of Water, Washington, DC.

Wildish, D.J., Akagi, H.M., Hamilton, N. and Hargrave, B.T. 1999. A recommended method for monitoring sediments to detect organic enrichment from mariculture in the Bay of Fundy. Can. Tech. Rep. Fish. Aquat. Sci. 2286: iii + 31 p.

Wildish, D.J., Akagi, H.M., Hargrave, B.T. and Strain, P.M. 2004. Inter-laboratory calibration of redox potential and total sulfide measurements in interfacial marine sediments and the implications for organic enrichment assessment. Can. Tech. Rep. Fish. Aquat. Sci. 2546: iii + 25 p.