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National Capital Region

Canadian Science Advisory Secretariat
Science Advisory Report 2022/049

EVALUATION OF FACTORS AFFECTING THE ION-SELECTIVE ELECTRODE (ISE) ELECTROCHEMICAL MEASUREMENT OF TOTAL FREE SULFIDE IN MARINE SEDIMENTS

An aerial image of a finfish aquaculture facility in Eastern Canada (Photo: Émilie Simard, DFO)

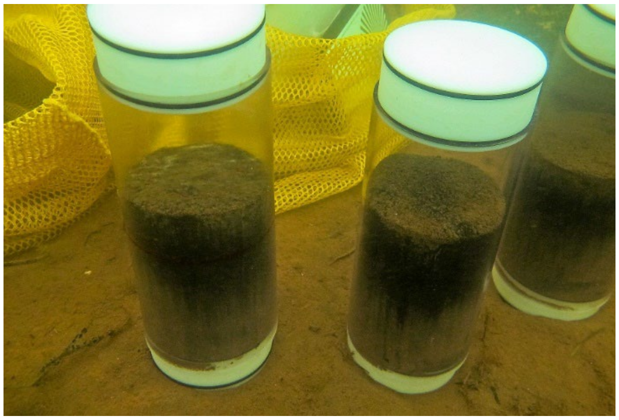

Figure 1. Diver-collected sediment cores used to measure total free sulfide concentrations using the ISE Method (Photo: Chris Mckindsey, DFO)

Context:

Under s.36 of the Fisheries Act *and associated* Aquaculture Activities Regulations*, Fisheries and Oceans Canada (DFO) regulates the conditions under which an aquaculture operator may deposit deleterious substances. The aquaculture industry is required to conduct seafloor monitoring of finfish aquaculture sites. The measurement of sulfide from sediment samples is used as a proxy for oxic state and biodiversity and is an accepted standard practice for monitoring the status of soft-bottom seabeds.*

The currently applied DFO monitoring protocols used at marine finfish sites may result in differences in the time between sample collection and the measurement of sulfide in the samples, as well as differences in the temperature at which samples are held prior to processing. DFO Aquaculture Management requested information on the effects of several factors on the measurement of sulfides in sediment samples and have requested Science advice to support the development of a nationally harmonized approach to the collection and testing of sediment sulfide.

This Science Advisory Report is from the May 10-12, 2022 National Peer Review on the Evaluation of factors affecting the ion-selective electrode (ISE) electrochemical measurement of total free sulfide in marine sediments. Additional publications from this meeting will be posted on the Fisheries and Oceans Canada (DFO) Science Advisory Schedule as they become available.

SUMMARY

- Sediment sulfide concentrations are used as an indicator of oxic state and biodiversity in soft sediments. Under the DFO *Aquaculture Activities Regulations* (AAR) and provincial regulations, aquaculture industry operators are required to conduct monitoring of marine sediment sulfide near finfish aquaculture sites. Sediment sulfide concentrations are used to

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assess the potential impact of organic matter on the benthic environment. Should regulatory thresholds be exceeded, management actions are required.

- The ion-selective electrode (ISE) method is the prescribed method for the measurement of sulfide concentrations from sediment samples in the monitoring standard associated with the AAR, as well as provincial aquaculture regulatory documents specific to New Brunswick and Nova Scotia.
- Sulfide concentrations in marine sediment are difficult to measure because they can change when exposed to oxygen, which is likely to occur during sampling and/or analysis. Multiple factors increase the error and variability of results generated using the ISE method. These include differences in procedures (e.g., sampling, sample storage, and analysis protocols), as well as potential lack of consistency in the implementation by analysts and laboratories across the country, resulting in varying levels of differences in measured sulfide concentrations.
- As compared to the total free sulfide measured immediately after sample collection, the effect of storing sediment samples on the measurement of total free sulfide by ISE is unpredictable and inconsistent. Within four hours, the magnitude of the differences can exceed either a decrease or increase of 15% relative to the initial measurement and, in some cases, can be much greater than the initial measurement. Sulfide in different sediment compositions responds differently to the same storage conditions and duration.
- To minimize uncertainties associated with sediment storage, ISE measurements on sediment samples ideally should be conducted as soon as the sample is collected. Where this is not feasible, the interpretation of the results should take into account the uncertainty associated with storage. At this time, it has not been determined which storage conditions and/or durations result in expected total free sulfide measurements within ± 5%, 10% or 15% of the value obtained from measuring total free sulfide immediately following sample collection.
- The refinement and nation-wide adoption of an updated standard operating procedure (SOP) for ISE measurement of total free sulfide from marine sediment will reduce uncertainty.
- When equipment is properly and newly calibrated and samples are not stored, the precision associated with the ISE analytical methodology is typically about an order of magnitude less than the spatial variability among sediment samples collected at various locations around a marine finfish aquaculture site or reference site.
- Despite the uncertainties, the ISE method has the ability to resolve differences between low (e.g., hundreds µM, i.e., oxic) and high (e.g., thousands µM, i.e., anoxic) sulfide concentrations characteristic of enriched sediments. However, in the middle ranges there is increased variability due to the patchiness of organic deposition and other sources of variability. In this case, sampling design may be modified to account for this variability.
- It is recommended that the ISE method be formally validated for its use in regulatory monitoring and decision-making to ensure increased robustness and confidence in results with respect to regulatory thresholds.
- The strengths and weaknesses of ISE-based measurements of sulfide in the context of potential alternative measurement methods and regulatory thresholds could also be examined to determine if more suitable or additional alternatives exist.

INTRODUCTION

During aquaculture operations, organic material such as unconsumed feed, fecal matter, shellfish drop-off, and other organisms and materials are released into the surrounding waters and can sink to the seafloor. This organic matter is, in turn, used by benthic organisms; however, if it accumulates, its decomposition process alters the chemistry of the underlying sediment by depleting available dissolved oxygen and increasing ‘free’ sediment sulfide concentrations. Because benthic species rely on oxygen for survival, an accumulation of organic matter from aquaculture operations can harmfully alter benthic habitat and decrease levels of benthic biodiversity.

Under both the Federal *Aquaculture Activities Regulations* (*AAR*) and provincial regulations, the aquaculture industry is required to conduct seafloor monitoring of finfish aquaculture sites. In areas where the seafloor has soft sediment (i.e., it consists of loose particles such as clay, mud, marl, sand, pebbles, gravel, shells or small stones), total free sulfide concentrations (i.e., dissolved sulfides) in marine sediment samples are measured and used as a proxy for oxic state (i.e., the oxygen content of the sediment). Recognizing that generally the higher the oxygen content in marine sediment the greater the benthic biodiversity, the *AAR* have set regulatory sulfide thresholds (i.e., concentration limits) and should they be exceeded, management actions are required.

The protocols for conducting sampling and for the measurement of sulfide from sediment samples are outlined in the monitoring standard under the *AAR* and provincial aquaculture regulatory documents specific to New Brunswick (NB DELG, 2018) and Nova Scotia (NS DFA, 2021). According to these monitoring standards, the method prescribed for determining the free sulfide in sediments is the ion-selective electrode (ISE) method.

This Science Advisory Report presents consensus advice on factors affecting the measurement of sulfides in marine sediment samples using the ISE method. This was developed in response to the following objectives and questions asked by DFO’s Aquaculture Management Directorate:

- What are the effects of sediment sample storage time and conditions (e.g., temperature, vacuum-sealed) on the measurement of total free sulfide as compared to total free sulfide measured immediately upon sample collection?
- Are these relationships consistent across sediment types and/or total free sulfide concentrations?
- Is there a combination of storage conditions and storage time post collection that would result in expected total free sulfide measurements within +/- 5%, 10%, and 15% of the value obtained from measuring total free sulfide immediately following sediment sample collection?
- Are there steps in the ion-selective electrode (ISE) total free sulfide measurement protocol that are open to interpretation by the analyst and to which differences will result in different measured concentrations of total free sulfides?
- Review ISE total free sulfide measurement methodologies and develop standard procedures for sample storage time, storage conditions, and analyses.
- In the consideration of the above questions, characterize the method variability in the context of natural, in situ, spatial variability of sediment sulfide levels.

This science advice was requested to support the development of a nationally harmonized approach to the collection and testing of sediment sulfide, which is linked to management biodiversity protection goals.

ANALYSIS

ISE Method and Variability in Description

The ISE method requires the use of an ion-selective electrode, selective to the sulfide ion (S^{2-}), that is attached to a meter that records millivolts. ISEs are essentially electrochemical half-cells in which a potential difference, which is dependent on the concentration (activity) of a particular ion in solution, arises across the electrode/electrolyte interface. ISEs have their limitations, but with careful use, frequent calibration, and an awareness of the limitations, ISEs can compare favourably with analytical techniques that require far more complex and expensive instrumentation (Rundle 2000; Cranford et al. 2020).

All analytical measurements are prone to error, i.e., the difference between the observed value and the true value of the sample being analyzed. These errors can originate throughout the steps of the sample analysis process and contribute to variability of the generated results, i.e., a difference in measurements produced from the same sample. Multiple factors increase the error and variability of results generated using the ISE method and collectively contribute to measurement uncertainty. These factors include differences in procedures (e.g., sampling, sample storage, and analysis protocols), as well as potential lack of consistency in the implementation by analysts and laboratories across the country. Figure 2 outlines some of the possible sources of variability associated with measuring sulfide using ISE.

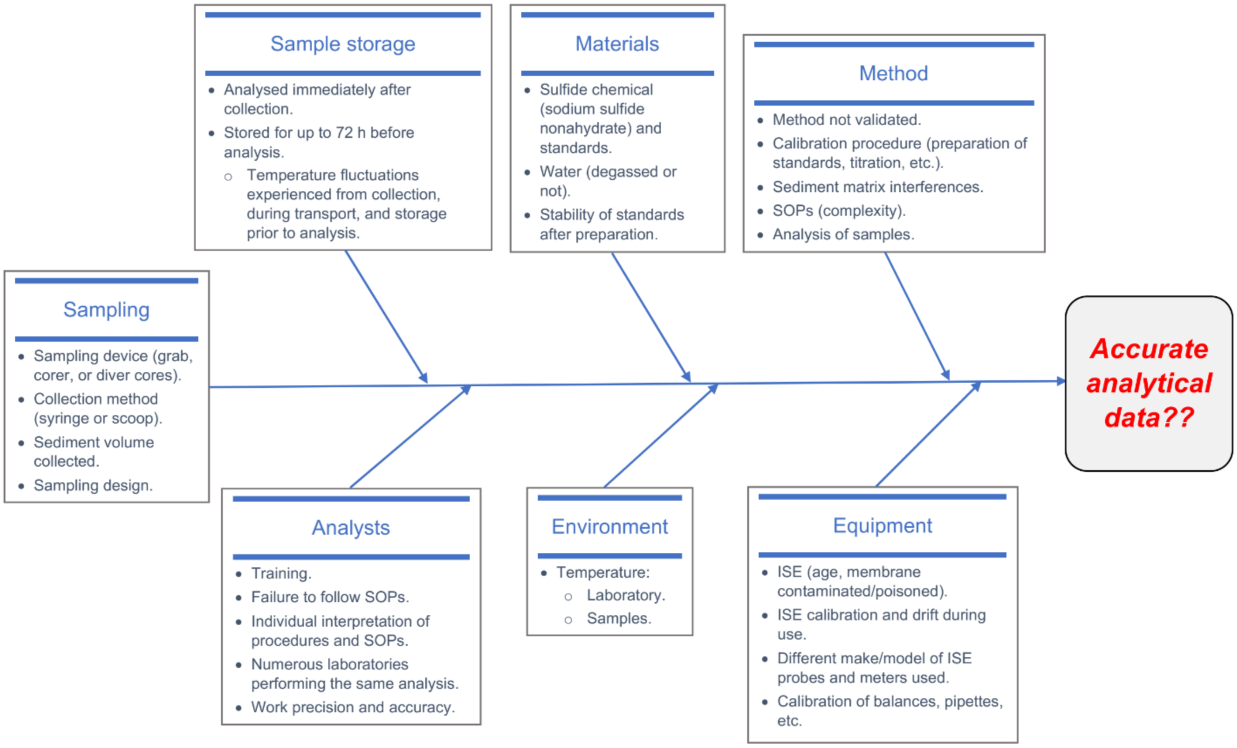

Figure 2. Sources of possible variability associated with the sulfide ISE method.

The protocols for conducting sampling and for the measurement of sulfide from sediment samples are outlined in the monitoring standard under the Federal *AAR* and provincial aquaculture regulatory documents specific to New Brunswick (NB DELG, 2018) and Nova Scotia (NS DFA, 2021). ISE method protocols outlined in Canadian federal and provincial regulations were compared. Results showed that there are inconsistencies between regulators, including:

- Number of calibration points.
- Filling solution used for the make/model of ISE.
- Sampling design (farm and reference sites), sediment sample collection method (grab, core), and volume of sediment sample analyzed.
- Time from sample collection to analysis (from within five minutes and up to 72 hours).

To reduce method-induced variation in sulfide measurements using the ISE method, it is recommended that the following changes be adopted:

- Titration of prepared ck solutions should be performed to confirm actual sulfide concentration prior to preparation of calibration curves.
- Water (distilled/deionized), used for preparing calibration standards, should be degassed by sparging with an inert gas.
- Calibration standards should be used as soon as possible after preparation for accurate calibration of the ISE.
- The use of a 3-point calibration (100, 1 000 and 10 000 µM), to operate within a one order of magnitude difference for the calibration standards, should be used. Alternatively, and preferably, a regression approach, using three or more calibration points covering the expected range of sulfide concentrations, could be used. This approach enables an uncertainty level to be associated with the calibration parameters.
- The ISE should be recalibrated after each 2 hours of use to minimize loss of accuracy.
- The performance of the ISE (i.e., calibration) should be checked at the end of the analyses to determine the loss in accuracy over the analysis period.

A flow chart highlighting important methodological steps required for the determination of sulfide in sediment samples is presented in Figure 3.

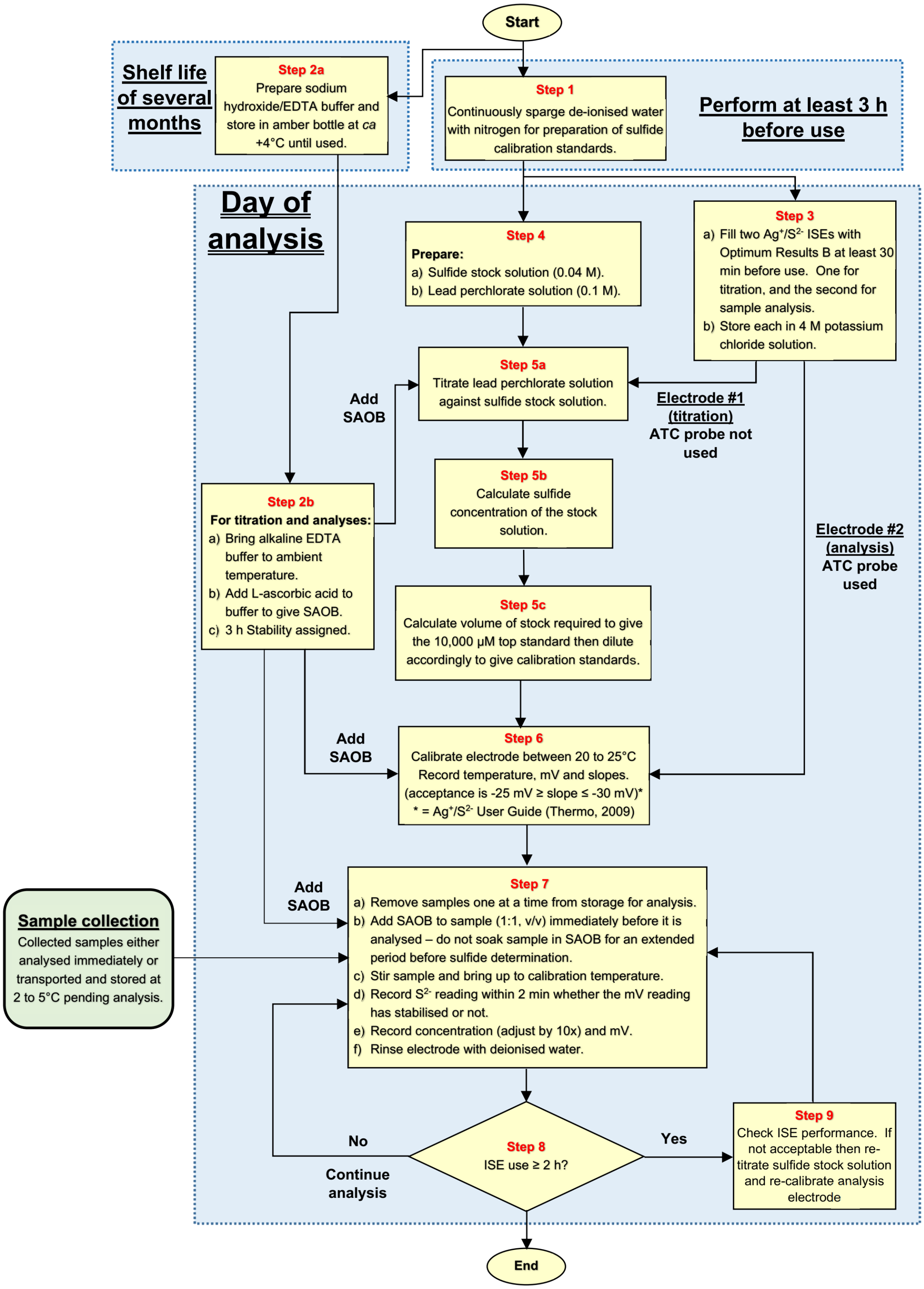

Figure 3. Flowchart of important analytical steps required for the determination of sulfide in sediment samples. EDTA = ethylenediaminetetraacetic acid; ISE = ion-selective electrode; SAOB = sulfide antioxidant buffer. The flowchart applies to the use of Orion 9616BNWP probes attached to an Accumet AP25 meter.

A lack of standardization of the ISE method has led to steps in the method being performed inconsistently between analysts and laboratories. To minimise method-induced variability in sediment sulfide measurements using the ISE method, an updated standard operating procedure (SOP) should be refined and adopted nationwide. Though extensive research has been conducted to evaluate the ISE method for measuring sulfide in marine sediment, a formal evaluation and validation process has not been conducted. Method validation, which uses laboratory studies to determine the suitability of a method for an intended purpose, would ensure increased robustness and confidence in results with respect to regulatory thresholds.

Storage Stability

Based on the *AAR* and provincial monitoring standards, the time from collection of sediment samples to free sulfide analysis by ISE method differs between jurisdictions. The AAR stipulate that sediment samples must be analyzed within 36 hours of collection, except in BC where sediment samples must be analyzed within five minutes. New Brunswick (NB DELG 2018) and Nova Scotia (NS DFA 2021) allow analysis of sediment samples within 72 hours.

Experiments have shown that the oxidation of sulfide in seawater is not constant but varies due to many factors, including dissolved oxygen concentration, pH, and temperature. Sulfide degradation in sediment is further complicated by other factors such as benthic microorganisms, organic matter, trace metals, etc.

To explore the effect of adding sulfide antioxidant buffer (SAOB) to sediment samples prior to storage, an experiment was conducted using sediment collected from sites in Oak Bay, NB and Shelburne, NS. ISE was used to measure sulfide concentration at two-minute intervals starting one minute after the addition of SAOB, for a total of 31 minutes. Results showed that sulfide concentration increased in the sediment sample from NS but decreased in the sediment sample from NB. Based on the change in sulfide concentration over time after the addition of SAOB, these results support previous recommendations that SAOB should not be added prior to sediment storage. Results also highlight the importance of taking ISE measurements immediately after SAOB addition.

Due to the volatility and ease of oxidation of sulfide, the viability of vacuum sealing sediment in capped syringes to limit oxidation of sulfide in the samples was examined through a series of experiments. These experiments included measurements of sediment samples collected from Shelburne, NS and Oak Bay, NB and stored for six days at different temperatures (ambient, 10°C, and 0°C) with and without vacuum sealing. Results were variable and no single storage condition produced results that consistently fell within the +/-5%, 10%, or 15% of the value obtained from measuring total free sulfide immediately following sample collection.

To assess sulfide stability over time (i.e., up to six days), a series of controlled experiments was conducted in which bulk samples of sediment were collected from two southwestern NB locations (Oak Bay and Lime Kiln Bay, including at an aquaculture site actively growing salmon at the time of sampling). SAOB was added immediately prior to sample analysis by ISE. The sediments examined in these experiments ranged from oxic to anoxic. Experiments showed that sediment samples produced very different degradation profiles to each other over the six days of storage. These results suggest that the rate and pattern of sulfide degradation in sediments is not readily predictable and predominantly dependent on sampling locations/sediment type. Therefore, it may not be practically possible to predict if sulfide measurement for a particular sediment sample will fall within +/-5%, 10%, or 15% of the value obtained from measuring total free sulfide immediately following sample collection.

Uncertainty of ISE Measurements

All estimates and measurements have uncertainty associated with them. When possible, measurements should be reported as the measured value plus or minus an uncertainty (i.e., an interval of values within which the true value is believed to lie with a stated probability). As with any measurement or index, an understanding of the associated uncertainty is essential to making meaningful interpretations of the values. The uncertainty and variability of ISE measurements was examined, including bias and precision. Bias, or systemic error, is a consistent difference between the true value and the measured or estimated value. Precision refers to the random variation associated with a measurement.

Like all measurements, ISE determinations of sediment sulfide concentration have errors and uncertainties associated with them. These uncertainties may be due to multiple factors including those associated with laboratory procedures, such as probe calibration, standard solution preparation, probe storage, and sample storage; however, they may also be attributed to factors related to sample collection procedures, such as grabs or cores, and temporal and spatial heterogeneities in the environment (Cranford et al. 2011).

Under ideal homogeneous conditions (i.e., measurements on freshly prepared standard solutions), uncertainty in ISE measurement of total free sulfide concentration can be less than 10% (Chang et al. 2014). The examination of the methodological-related uncertainties indicates that storage of electrodes, standard solutions, and field sediment samples has an influence on, and usually increase the uncertainty associated with, ISE measurements and introduce a bias to the measurements, usually a negative bias. The increase in uncertainty, either bias or precision, can routinely exceed 10% and sometimes several tens of percent. The magnitude of the bias is not constant and hence measurements cannot easily be adjusted to account for the bias. Therefore, storage of electrodes, standards, and samples should be minimized to reduce uncertainty.

Indices of variability in ISE measurement of total sulfide concentration are not independent of the mean sulfide concentration. Variability in unhomogenized sediment sulfide concentration from field samples increases with the mean sulfide concentration. Logarithmic transformation of the ISE measurements reduces this dependence.

The variation in ISE sulfide measurements in relation to the separation distance between samples was examined, including within sample collection units, between multiple sediment samples taken at ostensibly the same geographic coordinates but a few meters apart, and samples collected from a reference area or different locations within a farm site (Table 1 and Figure 4). Results suggest that there is more variability related to site heterogeneity than the ISE method and that variability due to site heterogeneity typically increases with the distance between samples. The variability in ISE derived sulfide measurements at the farm and reference location scale is large with a coefficient of variation (CV) central tendency of greater than 60% and a variation from less than 20% to greater than 100%. The variability in baseline ISE measurement uncertainty is typically less than 10% (i.e., considerably less than the in-situ variability).

Table 1. Description of data sources and sample groupings used for estimation of ISE sulfide variation shown in Figure 4.

Scale	Description	Source(s)
ISE Standard	Measurements made on sulfide standard aqueous solutions that have not been stored and measurements have been made with freshly calibrated electrodes using titrated stock solutions. The solutions have no sediment. Measurements are separated by distances of effectively ~0.0 m.	(Chang, et al., 2014)
Mixed Sediment	Measurements made on homogenized sediment. Measurements are separated by distances of effectively ~0.0 m.	(Wildish D. M., Akagi, Hamilton, & Hargrave, 1999); Data presented at this meeting
Within Samples	Measurements made within the confines of a sampling unit such as a grab. Measurements are separated by distances of ~0.1 m.	(Chang, Cooper, Page, & Losier, 2017); (Wildish, Akagi, & Martin, 2002); (Wildish D. W., Akagi, Hargrave, & Strain, 2004); (Chang, Page, Losier, McCurdy, & MacKeigan, 2011); (Chang, et al., 2013)
Within Stations	Measurements made at the sampling station. Multiple grabs or cores were collected from the same surface location. Measurements are separated by distances of ~10 m.	(Chang, Cooper, Page, & Losier, 2017); (Chang, Page, Losier, McCurdy, & MacKeigan, 2011); (Chang, et al., 2013)
Within Reference Areas	Measurements made at multiple stations within are separated by distances similar to the length scales of fish farms, Measurements are separated by distances of ~100 m.	(Chang, Cooper, Page, & Losier, 2017); (Cranford, et al., 2011); (Bugden, Hargrave, Strain, & Stewart, 2001)
Within Farm Sites	Measurements made at multiple stations within and around a fish farm. Measurements are separated by distances of ~100 m.	(Chang, Cooper, Page, & Losier, 2017); (Cranford, et al., 2011); (Hargrave, Doucette, Phillips, Milligan, & Wildish., 1998)

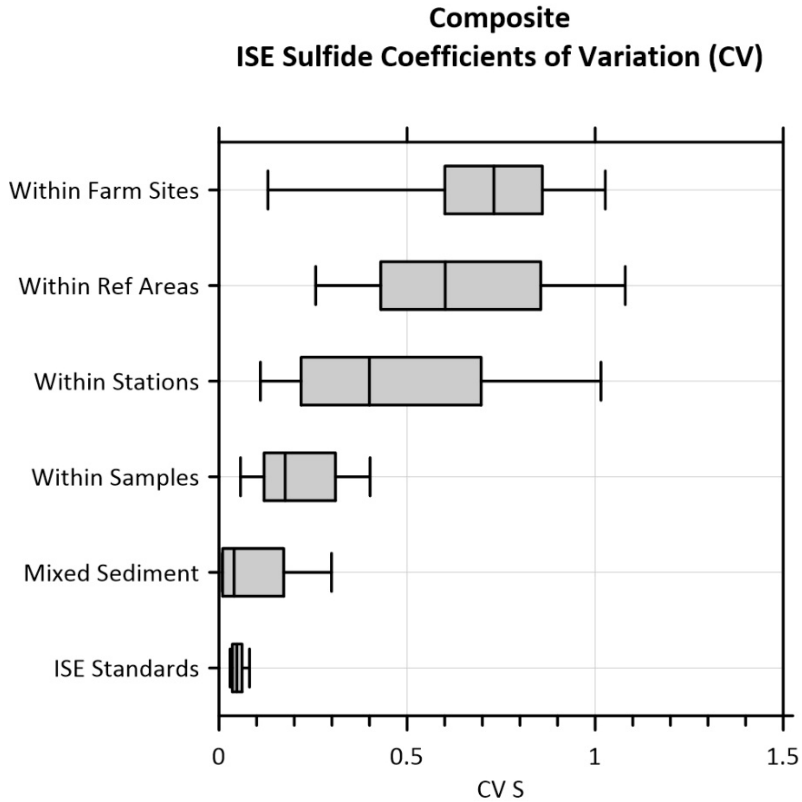

Figure 4. The variation in ISE sulfide measurements in relation to the separation distance between samples. The whisker extremes represent the 10% and 90% values, the shaded box limits represent the 25th and 75th percentiles of the values and the central line represents the median value. The data sources are listed in Table 1.

In summary, when equipment is properly and newly calibrated and samples are not stored, the precision associated with the ISE analytical methodology is typically about an order of magnitude less than the spatial variability among sediment samples collected at various locations around a marine finfish aquaculture site or reference site.

Despite the uncertainties, the ISE method has the ability to resolve differences between low (e.g., hundreds μM, i.e., oxic) and high (e.g., thousands μM, i.e., anoxic) sulfide concentrations characteristic of enriched sediments. However, the uncertainties associated with the measurements can make it difficult to distinguish between intermediate levels of sulfide concentration.

Sources of Uncertainty

Additional uncertainties in the ISE method for measuring total free sulfide concentration in marine sediment samples exist and their individual and specific effects on the measurement of sulfides is not completely known. For example, it is unclear what the effects of the sediment sample's matrix are on dissolved sulfide in the porewater. Water content in sediment samples varies and this has not been accounted for in the storage stability experiments.

Calibration of the ISE is performed using deionized water standards (i.e., freshwater) whereas the analyzed samples are in seawater and sediment; therefore, it is unknown what effect this salinity difference and sediment matrix effect have on accurate quantification.

Storage stability experiments were based on the results of a single analyst and the variability in sulfide measurements may be greater if the experiment included analyses conducted by a range of analysts and instruments.

Storage stability experiments were not conducted in large grain size (sandy) sediments; therefore, it is unclear how sulfide measurements may vary in these sediment types.

While sediment samples are typically taken from the top 2 cm of sediment, the sulfide content may not be homogeneous throughout the sample. The impact of this on variability was not assessed.

In most cases, the measurement of variability among samples collected at various locations also includes methodological variability and values have not been corrected for this (i.e., they may be over- or under-estimated).

CONCLUSIONS AND ADVICE

Multiple factors increase the uncertainty of total free sulfide concentration measurements from marine sediment samples determined using the ISE method. Some of these factors include differences in methods that are absent from, or not clearly described in, the standard operating procedures (e.g., titration of the stock solution, oxygen removal from water, preparation and storage of standard solutions, calibration, sample preparation and analysis). Therefore, these steps may not be performed consistently between analysts and laboratories which may result in differences in measured concentrations of sulfides.

Storage of sediment samples impacts sulfide measurements. The degree of change is inconsistent, unpredictable, and can exceed ±15% of the initial ISE measurement (i.e., the measurement soon after sample collection and without storage). Based on data derived from storage stability experiments, the response of sulfide to short-term storage appears to be dependent on the sampling location since sediment from different spatial locations produced different sulfide degradation profiles. The reason for this effect is unknown at this time. Therefore, it is not presently feasible to predict how sediments collected from spatially different locations and with different matrix characteristics will respond to storage. Likewise, it is not possible to determine the ideal storage conditions that will result in sulfide concentrations within ±5%, 10%, or 15% of the value obtained from measuring total free sulfide immediately following sample collection. Alternative preservation methods tested, including storing sediment with SAOB and vacuum sealing sediment samples, do not appear to be effective at stabilizing sulfide in stored samples.

To minimize uncertainties associated with sediment storage, ISE measurements on sediment samples should be conducted as soon as the sample is collected. Where this is not feasible, the interpretation of the results should take into account the uncertainty associated with storage. The refinement and nation-wide adoption of an updated SOP for ISE measurement of total free sulfide from marine sediment will reduce methodological uncertainty.

Under ideal homogeneous conditions (i.e., measurements on freshly prepared standard solutions), the uncertainty in ISE sulfide measurements can be ≤10%. When equipment is properly and freshly calibrated and samples are not stored, the precision associated with the

ISE analytical methodology is typically about an order of magnitude less than the variability among sediment samples collected at various locations within a farm or reference site.

Despite the uncertainties, the ISE method has the ability to resolve differences between low (e.g., hundreds µM, i.e., oxic) and high (e.g., thousands µM, i.e., anoxic) sulfide concentrations characteristic of enriched sediments. the uncertainties associated with the measurements can make it difficult to distinguish between intermediate levels of sulfide concentration.

It is recommended that the ISE method be formally validated for its use in regulatory monitoring and decision-making to ensure greater robustness and confidence in results with respect to regulatory thresholds. The process of validation should include an analysis of linearity, accuracy, precision, robustness, and reproducibility. The strengths and weaknesses of ISE-based measurements of sulfide in the context of potential alternative measurement methods and regulatory thresholds could also be examined to determine if more suitable or additional alternatives exist.

OTHER CONSIDERATIONS

The method for sediment sample collection (e.g., grab, core, distance of sampling from farm, timing of sampling in relation to production cycle) and how this may affect the measurement of total free sulfide in marine sediments was not examined. This should be taken into consideration when comparing values collected from different places and times.

Spatial heterogeneity of the seafloor has not been well characterized. It is not known to what degree any given sediment sample is representative of the seafloor. Spatial heterogeneity is expected to be a significant source of variability in the measurement of sulfides using the ISE method that increases with spatial scale. As such, sampling design should be carefully considered and clearly defined.

The feasibility of applying the recommendations provided in this document to the ISE method for measuring total free sulfide concentration from marine sediment samples was not examined. If collected samples are to be analyzed immediately after collection, as per recommendations in this document, the practicality of titrating and analyzing in the field, whether in a land-based mobile lab or on a vessel, should be investigated. Other options could be assessed to reduce error and time needed to prepare calibration curves, e.g., using certified standard sulfide reference material.

Use of a standardized matrix (i.e., using solely porewater rather than a sediment slurry) may reduce any matrix effects associated with sediment type. Methods are available to collect porewater which could be evaluated. Further studies are required to compare sulfide measurements in extracted porewater with measurements in sediment-porewater slurries to better understand matrix effects (Brown et al. 2011). A matrix change would also require the re-evaluation of the relationship between biodiversity and sulfide concentration.

Other methods for measuring sulfides in porewater (e.g., ultraviolet spectrophotometric, methylene blue) and other indicators of biodiversity are available (Cranford et al. 2020). Further studies are needed to compare results between methods. While outside the scope of this report, other methods for measuring sulfides and other indicators of biodiversity could also be considered in the context of potential alternative or additional measurement methods and regulatory thresholds.

LIST OF MEETING PARTICIPANTS

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*NCR = National Capital Region
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SOURCES OF INFORMATION

This Science Advisory Report is from the May 10-12, 2022 National Peer Review on the Evaluation of factors affecting the ion-selective electrode (ISE) electrochemical measurement of total free sulfide in marine sediment. 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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