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Sciences des écosystèmes  
et des océans

## **Canadian Science Advisory Secretariat (CSAS)**

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**Proceedings Series 2022/039**

**National Capital Region**

### **Proceedings of the National Peer Review Meeting on Evaluation of factors affecting the ion-selective electrode (ISE) electrochemical measurement of total free sulfide in marine sediments**

**May 10–12, 2022**  
**Virtual Meeting**

**Chairpersons: Jay Parsons and Brittany Beauchamp**

**Editors: Alex Tuen, Fred H. Page, David K. Wong, Brittany Beauchamp, Emily Ryall, and Jay Parsons**

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## Foreword

The purpose of these Proceedings is to document the activities and key discussions of the meeting. The Proceedings may include research recommendations, uncertainties, and the rationale for decisions made during the meeting. Proceedings may also document when data, analyses or interpretations were reviewed and rejected on scientific grounds, including the reason(s) for rejection. As such, interpretations and opinions presented in this report individually may be factually incorrect or misleading, but are included to record as faithfully as possible what was considered at the meeting. No statements are to be taken as reflecting the conclusions of the meeting unless they are clearly identified as such. Moreover, further review may result in a change of conclusions where additional information was identified as relevant to the topics being considered, but not available in the timeframe of the meeting. In the rare case when there are formal dissenting views, these are also archived as Annexes to the Proceedings.

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### ***Aussi disponible en français :***

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## **SUMMARY**

These proceedings summarize the relevant discussions and key conclusions that resulted from the Fisheries and Oceans Canada (DFO) Canadian Science Advisory Secretariat (CSAS) National Peer Review Meeting to evaluate the factors affecting the ion-selective electrode (ISE) electrochemical measurement of total free sulfide in marine sediments. This meeting was held virtually May 10-12, 2022. The science advice will support the development of a nationally harmonized approach to the collection and testing of sediment sulfide, which is used as a proxy for oxic (presence of oxygen) state and biodiversity, which is linked to management protection goals.

The conclusions and advice resulting from this meeting are provided in the form of a Science Advisory Report which is available on the CSAS website. Supporting Research Documents reviewed and discussed at the meeting will also be made available on the CSAS website.

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## INTRODUCTION AND CONTEXT

A Canadian Science Advisory Secretariat (CSAS) National Peer Review Meeting was virtually held May 10–12, 2022 to conduct an evaluation of factors affecting the ion-selective electrode (ISE) electrochemical measurement of total free sulfide in marine sediments.

Participants introduced themselves (Appendix 3). The Chairs provided an overview of the CSAS policies, reviewed the Terms of Reference (Appendix 1) that served as the foundation for this CSAS process, and reviewed the Agenda (Appendix 2).

Three Working Papers were drafted to address the six Objectives of the Terms of Reference as follows.

- Working Paper 1, *ISE Method Overview and Variations in Implementation Descriptions*, addresses:
  - Objective 4: Are there steps in the 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?
  - Objective 5: Review ISE total free sulfide measurement methodologies and develop standard procedures for sample storage time, storage conditions, and analyses.
- Working Paper 2, *Storage Stability: Effect of Storage Time, Temperature and Preservation Method on Sulfide Measurements*, addresses:
  - Objective 1: 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?
  - Objective 2: Are these relationships consistent across sediment types and/or total free sulfide concentrations?
  - Objective 3: 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?
- Working Paper 3, *Considerations of Bias and Precision associated with ISE Based Measurements of Sediment 'Free' Sulfide*, covers uncertainty analyses. It contributes to the first three Objectives but primarily focuses on:
  - Objective 6: In the consideration of the above questions, characterize the method variability in the context of natural, in situ, spatial variability of sediment sulfide levels.

The *Aquaculture Activities Regulations* (AAR) clarify conditions under which aquaculture operators can install, operate, or remove facilities, or undertake measures to treat their fish for disease and parasites, as well as deposit organic matter. This is to avoid, minimize and mitigate any potential detriments to fish and fish habitat. Under both the AAR and provincial regulations, 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 soft-bottom seabeds.

When measuring sulfide, it is best to analyze the results immediately after sediment sample collection to minimize uncertainties. However, due to logistical considerations such as the impracticality of performing analyses on a vessel, samples are frequently stored and brought to the laboratory for analysis.

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DFO (Fisheries and Oceans Canada) Aquaculture Management requested information on the effects of several factors on the measurement of sulfides in sediment samples, and asked DFO Science to provide advice to support the development of a nationally harmonized approach to the collection and testing of sediment sulfide.

## **WORKING PAPER 1: ISE METHOD OVERVIEW AND VARIATIONS IN IMPLEMENTATION DESCRIPTIONS**

### **PRESENTATION**

Presenter: David K Wong

The variability in the measurement of sulfide using ISE can be attributed to a wide range of factors, including:

- Storage
  - Samples are prone to degrade soon after collection.
  - Storage conditions are not consistent between laboratories.
  - The recommendation is to minimize or eliminate storage time, and test immediately on-site if possible.
- Human factors
  - Preparation and testing of samples may differ between analysts or laboratories.
  - Analytical biases, precisions, and interpretation of results may differ between analysts or laboratories.
- Quality of chemicals used
  - The purity begins to degrade once a fresh container is opened, as the hygroscopic crystals draw atmospheric moisture.
  - The recommendation is to prepare stock and calibration solutions, and to use them immediately to calibrate the electrode.

One method discussed was titration. Titration should be used to determine the concentration of sulfide in the stock solution.

The ISE method for measuring sediment sulfide has not been formally validated to demonstrate its suitability for this intended purpose. The validation should address repeatability and reproducibility. Repeatability is the variability in measurement value associated with the same analyst repeating the measurements multiple times on a single sample. Also, since the method is utilized by numerous different laboratories, its reproducibility (or between-lab reproducibility) has not been assessed. This is an important factor for method standardization and expresses the precision between laboratories.

### **REVIEWER PRESENTATIONS AND DISCUSSION**

Reviewers: Andr ea Weise (for Chris Mckindsey) and Leah Lewis-McCrea

The reviewers provided several comments about uncertainties, the inconsistencies in the existing methods, and the lack of details in the existing methods. They agreed that the method should be formally validated, and a Standard Operating Procedure should be developed to

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combat variability in method execution. The validity of the ISE method was discussed amongst all participants:

- At some point, a decision should be made about whether to try to improve and continue to use the ISE method, or to abandon it (while acknowledging no real alternative currently exists). This could be a future CSAS process or future work.
- The ISE method was acknowledged for its lack of robustness, but there is also no real alternative currently available. Due to so many variables, having a Standard Operating Procedure may not address all the limitations.
- It would be useful to update or standardize the ISE method because historical data are based on ISE. This would allow a like-for-like comparison. This could be a research recommendation.
- The current method measures sulfide in a sediment slurry, rather than directly in the sediment porewater, and this can increase variability due to potential matrix effects.
- Accuracy, precision, and reproducibility are all important, but of the three, reproducibility between analysts and laboratories is most important.

The Science Advisory Report, “Other Considerations” section, could include the following additions that may be outside the scope of this CSAS process:

- The recommendation to use certified reference solutions to eliminate the need to weigh chemicals which may make preparation of calibration solutions easier.
- The need to determine what the regulatory objective is. Either the regulations have to be consistent with practicality, or the method is not appropriate to meet the desired regulatory objective.
- Feasibility and implications, which are important for decision-makers to consider.

## **WORKING PAPER 2: STORAGE STABILITY: EFFECT OF STORAGE TIME, TEMPERATURE AND PRESERVATION METHOD ON SULFIDE MEASUREMENTS**

### **PRESENTATION**

Presenter: David K Wong

Time from sample collection to analysis differs by jurisdiction. Storage of samples can impact the measurements and increase uncertainty in the results.

Alternate storage methods and possible ways of preserving sulfide include:

- Chemical
  - Addition of SAOB (Sulfide Antioxidant Buffer) to prevent oxidation of sulfide.
  - Reaction with aqueous metal salt (for example, zinc (II) acetate) to form an insoluble metal sulfide.
- Physical
  - Occlude oxygen to prevent oxidation, trialed here.

It was observed that degradation happens even in deoxygenated Milli-Q water.

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The results were presented from different experiments to test the storage stability by manipulating the variables of storage time, temperature, and preservation method.

- There is no single perfect solution. The results were highly unpredictable and variable.

Participants questioned the comparability of the two sampling sites chosen:

- Oak Bay, New Brunswick. This is a mud flat exposed to air for 10–12 hours per day.
- Shelburne, Nova Scotia. This is covered by seawater all day.

To combat the highly variable situation, a very large number of samples should be taken to ensure bias is minimized or not introduced. However, the biggest recommendation is to simply not store sediment samples.

Changes in sulfide concentration are likely, in part, a reflection of organic material in the sediment. The organic matter amount will differ and will not be stable between intertidal, surface, and subsurface zones.

## **REVIEWER PRESENTATIONS AND DISCUSSION**

Reviewers: Terri Sutherland and Gretchen Wagner

For consistency and standardizing a Standard Operating Procedure, there should be a distinction made between “SAOB” and “SAOB + L-ascorbic acid”. SAOB alone is stable for months but is only stable for three hours after adding L-ascorbic acid. The authors agreed to clearly distinguish between:

- “Alkaline EDTA (ethylenediaminetetraacetic acid) solution” (before L-ascorbic acid is added), and
- “SAOB solution” (after L-ascorbic acid is added).
- The authors recommend mixing fresh SAOB solution as required to keep the practice standard, rather than relying on manufacturer-produced “store-bought” SAOB.

The Working Paper did not include information on sediments such as sandy bottoms where sulfide concentrations and measurements may vary. The reviewer’s recommendation was to include the rationale behind why sandy bottoms were not considered, or to include some information about sandy bottoms.

- The authors stated they did not investigate sandy sediment since past experience showed virtually no sulfide presence in this sediment type in New Brunswick.
- Participants indicated that sandy sediments can sometimes have enough mud content to have high sulfides.
- Storage stability may or may not be different in sandy sediments. It depends on how much organic matter is in the samples and the rate of sulfate reduction. The more oxygen, the more the sulfide will be oxidized.
- The higher porosity in sandy type environments lends itself to greater exchange or loss of porewater from sediments.
- These points about sediment types, “muddy” versus “muddy sand” versus “sandy”, and porosity can be included in the Science Advisory Report, “Uncertainties” or “Other Considerations” section.

The reviewers assessed the ability of the authors to address Terms of Reference Objectives 1, 2, and 3.



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- Objective 1: Highly variable results.
  - Objective 2: Unknown.
  - Objective 3: Not possible to determine since each sediment responds differently to the same storage conditions.

The Chairs forwarded the reviewers' written comments to the authors to address in their revisions to the Working Paper.

The authors invited participants to share specific literature for any of the above points, along with any sources for "store-bought" SAOB, with the potential to recommend which brands of SAOB were acceptable.

## **WORKING PAPER 3: UNCERTAINTY ANALYSES**

### **PRESENTATION**

Presenter: Fred H Page

Definitions were provided for the terminology used in this presentation. These included "accuracy", "precision", "repeatability", "reproducibility", "bias", and "trueness".

Influencing factors include:

- The storage time of standard solutions, electrodes, and samples.
- Response time of meter.
- Time at which meter is read.
  - A participant expressed mistrust in the millivolt reading on Orion meters.
  - The authors indicated that in the measurements they had taken, both millivolt and micromolar readings were recorded.
- Matrix effects (water content and grain size).
- Collection method ("grab" versus "core" versus "Rhizon samplers").

As expected, a longer storage time will increase bias and variability, and will decrease precision. Therefore, the recommendation is to analyze the sample immediately ( $T_0$ ) after collection for best results.

Sediment water content can have a large influence on ISE sulfide measurements. It is possible to correct for water content in sediment, but the uncertainty associated with those corrections is not well known and a sediment porosity determination would need to be made for each sample. This has not yet been investigated thoroughly.

The Working Paper includes a box-and-whisker figure entitled, "Composite ISE Sulfide Coefficients of Variation (CV)", which was a useful visual demonstration of how variability increases when deviating away from the ISE Standards.

Ideally, there should be no bias. If a bias is known and is stable, methods or interpretations can account for that bias.

- Bias of field samples is generally not known.
- Laboratory bias error can potentially be as large as precision errors.

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Because the method is not rigorous, results can potentially be skewed by the combined effects of:

- Precision and bias
  - The method used is an order of magnitude method. Very careful implementation and caution should be exercised when it is used to try to detect differences smaller than an order of magnitude.
  - A sufficiently robust method and sample design are needed to be able to properly detect signals that are considerably less than an order of magnitude.
- Human factors
  - Sample collection, preparation, and testing.
  - Analyst biases and interpretation of results.

Averaging affects precision. Site classification is often based on a mean (average) of the sulfide. Bias cannot be removed by averaging. Biasing the average can lead to the wrong conclusion.

- Authors agreed with the participant who suggested using a median (middle) value instead of a mean. The mean forces symmetry, but the true scenario is asymmetrical. Log transformation should also be considered.

## **REVIEWER PRESENTATIONS AND DISCUSSION**

Reviewers: Barry Hargrave and Dounia Hamoutene

Reviewers found Working Paper 3 contained repetition from, and overlap with, Working Papers 1 and 2.

Reviewers offered suggestions on how to combine three Working Papers into one, how to combine the six Terms of Reference Objectives to two, and how to present results and conclusions in tables that would be simple for practitioners to follow.

The box-and-whisker figure entitled, “Composite ISE Sulfide Coefficients of Variation (CV)”, was well-received, highlighting that the heterogeneity in the sediment is natural and will never be homogenous.

Summary and conclusions:

- Non-systematic error associated with ISE methodology is typically small compared to environmentally induced variability.
- The smallest biases and highest precision occur when analyses are conducted very soon after sample preparation (no storage time).
- Increased sample numbers (minimum of 5) are required to determine if observations exceed thresholds at  $p < 0.05$ .

Recommendations:

- Revise Standard Operating Procedure (calibration procedures, water content correction) to achieve as high an accuracy as possible. Minimize sample storage time.
- Compare measured total free sulfide (sediment + porewater) using the revised Standard Operating Procedure with parallel measurements (porewater extracted) by ultraviolet and methylene blue in different sediment types and locations.

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- Determine if the oxic-anoxic classification system for measuring enrichment gradients based on ISE can be adjusted for total free sulfide dissolved in porewater for regulatory purposes.
  - Consider presenting the steps of the method in table format for practitioners to easily follow.
  - Consider presenting the conclusions in table format.

The Science Advisory Report, “Other Considerations” section, could include the following additions that may be outside the scope of this CSAS process:

- Characterization of spatial heterogeneity.
- Weight of water translates to volume. Evaporate the water and account for its removal from the sediment sample.
- Sampling the top two centimeters of sediment aims to obtain a uniform measurement. However, concentrations vary with depth into the sediment. For example, the first 1.5 centimeters might have zero sulfide, and then the final 0.5 centimeters might have high levels of sulfide. When averaged together, it could be equivalent to an intermediate sulfide level.
- Collection of diver cores is impractical in British Columbia since the shallowest depth around aquaculture sites is approximately 32 meters. This is the same situation in Newfoundland. This would make it difficult to establish a standardized protocol for collecting samples using this method. DFO should consider regional differences in sites should a national standard protocol for sampling marine sediments be developed.
- Results indicate that ideally sediment samples should not be stored. Storage of samples increases uncertainties. When designing regulatory thresholds, it is management’s decision on how much error to accept.
- The sampling distance from cages is already hugely different between West and East Coasts.
- A major knowledge gap is the degree to which a small sample volume (5 mL) collected for analysis is representative of the oxic condition of the seafloor.

## **EXPECTED PUBLICATIONS AND THEIR NEXT STEPS**

Participants collaborated in real time to draft the Summary Bullets for the Science Advisory Report. Terminology in the Bullets should be made clear in the Report. This includes “uncertainty” and “variability”. “Uncertainty” was chosen because everything else has a specific meaning. Consensus was achieved on the Bullets.

Participants agreed to upgrade the three Working Papers to three Research Documents. The three Working Papers will not be combined into a single Research Document. The third Research Document will include tables to outline the methods and conclusions.

The revised draft Science Advisory Report, Research Documents, and Proceedings will be reviewed against the agreed-to changes discussed during the meeting prior to their final publication.

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## APPENDIX 1: TERMS OF REFERENCE

### Evaluation of factors affecting the ion-selective electrode (ISE) electrochemical measurement of total free sulfide in marine sediments

#### National Peer Review – National Capital Region

May 10-12, 2022  
Virtual Meeting

Chairpersons: Jay Parsons and Brittany Beauchamp

#### 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. DFO recognizes that there are interactions between aquaculture operations and the natural environment. The risks associated with these interactions are considered and addressed through a suite of regulatory tools, at both the federal and provincial levels. Examples of such tools include requiring that aquaculture operators monitor the oxic state of the seafloor beneath farms and having defined thresholds and associated management actions that are required should the regulatory thresholds be exceeded.

Under both the *Aquaculture Activities Regulations* and provincial regulations, the aquaculture industry is required to conduct seafloor monitoring of finfish aquaculture sites. The measurement of sulfide from sediment samples is an accepted standard practice for soft-bottom seabeds. The protocols for conducting sampling, and for the measurement of sulfide from sediment samples, are outlined in the monitoring standard associated with the *Aquaculture Activities Regulations*.

The currently applied DFO monitoring protocols used at marine finfish sites were adopted by DFO during the development of the *Aquaculture Activities Regulations* and incorporate some regional differences in Provincial program design. Specifically, these protocols 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 have asked for 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, which is used as a proxy for oxic state and biodiversity, which is linked to management protection goals.

#### Objectives

The objectives of this meeting are to determine:

1. 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?
2. Are these relationships consistent across sediment types and/or total free sulfide concentrations?
3. 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?

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4. 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?
  5. Review ISE total free sulfide measurement methodologies and develop standard procedures for sample storage time, storage conditions, and analyses.
  6. In the consideration of the above questions, characterize the method variability in the context of natural, in situ, spatial variability of sediment sulfide levels.

#### **Expected Publications**

- Science Advisory Report(s)
- Proceedings
- Research Document(s)

#### **Expected Participation**

- Fisheries and Oceans Canada (DFO) (Ecosystems and Oceans Science and Aquatic Ecosystems sectors)
- Provinces
- Aboriginal communities/organizations
- Academia
- Aquaculture Industry
- Other invited experts (e.g., environmental non-government organizations)

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## APPENDIX 2: AGENDA

### Agenda of the National CSAS Process

#### Evaluation of Factors Affecting the Ion-Selective Electrode (ISE) Electrochemical Measurement of Total Free Sulfide in Marine Sediments

May 10–12, 2022

11:00 am – 3:00 pm (Eastern Time)

Co-Chairs: Jay Parsons and Brittany Beauchamp, DFO Science

MS Teams Virtual Meeting

DAY 1 – May 10, 2022

Time	Subject	Presenter
11:00-11:20	Welcome, Introductions, Housekeeping and Review of Agenda and Terms of Reference	Jay Parsons and Brittany Beauchamp (Co-Chairs)
11:20-11:25	Overview of considerations for an evaluation of factors affecting the electrochemical measurement of total free sulfides in marine sediments	Michael Ott
11:25-11:55	Presentation #1: ISE Method Overview and Variations in Implementation Descriptions (20 minutes) Questions and discussion (10 minutes)	David Wong
11:55-12:25	Reviewer Presentations (15 minutes each)	Chris Mckindsey and Leah Lewis-McCrea
BREAK (30 minutes)		
12:55-1:25	Open Discussion and Preparation of Summary Bullets	Everyone
1:25-1:55	Presentation #2: Storage Stability: Effect of Storage Time, Temperature and Preservation Method on Sulfide Measurements (20 minutes) Questions and discussion (10 minutes)	David Wong
1:55-2:25	Reviewer Presentations (15 minutes each)	Terri Sutherland and Gretchen Wagner
2:25-2:55	Open Discussion and Preparation of Summary Bullets	Everyone

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<b>Time</b>	<b>Subject</b>	<b>Presenter</b>
2:55-3:00	Summary and Adjournment	Co-Chairs

DAY 2 – May 11, 2022

<b>Time</b>	<b>Subject</b>	<b>Presenter</b>
11:00-11:10	Welcome and Review of Day 1	Co-Chairs
11:10-11:40	Presentation #3: Uncertainty Analyses (20 minutes) Questions and discussion (10 minutes)	Fred Page
11:40-12:10	Reviewer Presentations (15 minutes each)	Barry Hargrave and Dounia Hamoutene
12:10-12:40	Open Discussion and Preparation of Summary Bullets	Everyone
BREAK (30 minutes)		
1:10-2:55	Science Advisory Report Development	Everyone
2:55-3:00	Summary and Adjournment	Co-Chairs

DAY 3 – May 12, 2022

<b>Time</b>	<b>Subject</b>	<b>Presenter</b>
11:00-11:10	Welcome and Review of Day 2	Co-Chairs
11:10-1:00	Science Advisory Report Development	Everyone
BREAK (30 minutes)		
1:30-2:50	Science Advisory Report Development	Everyone
2:50-3:00	Conclusions and Next Steps	Co-Chairs

### APPENDIX 3: LIST OF PARTICIPANTS

Name	Affiliation
Barrell, Jeff	Fisheries and Oceans Canada Science - Gulf
Beauchamp, Brittany	Fisheries and Oceans Canada Science – NCR*
Bennett, Aaron	Mowi Canada East
Blasco, Nathan	Fisheries and Oceans Canada Science - Maritimes
Gibb, Olivia	Fisheries and Oceans Canada Science - Newfoundland
Grant, Jon	Dalhousie University (Nova Scotia)
Hamoutene, Dounia	Fisheries and Oceans Canada Science - Maritimes
Hargrave, Barry	Independent consultant (Ontario)
Kraska, Peter	Fisheries and Oceans Canada Science - Maritimes
Lewis-McCrea, Leah	Centre for Marine Applied Research (Nova Scotia)
Marshall, Kimberly	Fisheries and Oceans Canada Science - Newfoundland
Ott, Michael	Fisheries and Oceans Canada Aquaculture Management - NCR
Page, Fred	Fisheries and Oceans Canada Science - Maritimes
Parsons, Jay	Fisheries and Oceans Canada Science - NCR
Ryall, Emily	Fisheries and Oceans Canada Science - NCR
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Sweeney, Bob	SIMCorp Environmental Sciences Lab (New Brunswick)
Tuen, Alex	Fisheries and Oceans Canada Science - NCR CSAS
Wagner, Gretchen	Government of Nova Scotia
Weise, Andréa	Fisheries and Oceans Canada Science - Québec
Wong, David	Fisheries and Oceans Canada Science - Maritimes

\*NCR = National Capital Region

\*\*CSAS = Canadian Science Advisory Secretariat