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

July 2014

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

1. INTRODUCTION

This document titled, *Standard Operating Procedures for Environmental Monitoring of Marine Aquaculture Sites in Nova Scotia*, describes the sampling and laboratory methodologies for the Nova Scotia (NS) Environmental Monitoring Program (EMP). Marine finfish and shellfish farms in NS are required by the Nova Scotia Department of Fisheries and Aquaculture (NSDFA) to comply with the EMP as a condition of leases and licenses issued under authority of the *Fisheries and Coastal Resources Act*. Provided in this document are sampling instructions, laboratory guides, field templates and reporting requirements designed to assist those conducting the environmental monitoring. 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). A pilot project was initiated in 2003 by NSDFA who regularly monitored each marine finfish aquaculture operation throughout the province as part of the EMP. A number of revisions have since been incorporated, considering advancements in science and technology, to ensure the EMP is up-to-date, relevant and effective. Recognizing that 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 below and provide results to NSDFA as required.

Should readers of this document have any questions, please contact Jessica Whitehead, EMP Supervisor, at (902) 875-7436 or whitehja@gov.ns.ca.

2. LOCATION AND NUMBER OF SEDIMENT SAMPLING STATIONS

Using historical EMP monitoring results dating as far back as 2003, sampling station locations for each individual aquaculture site are based on past environmental performance, current species cultured, level of production, and configuration and type of cages. Further explanation is provided in the companion paper *Environmental Monitoring Program Framework for Marine Aquaculture in Nova Scotia* (PNS, 2014).

2.1 Finfish Monitoring Stations

For each finfish aquaculture site, the minimum number of sampling stations within each lease is based on the maximum number of fish onsite during the current production cycle as shown in Table 1. Biomass/cage must be used to determine station locations; therefore biomass/cage must be determined, prior to sampling, to justify station locations. A detailed site diagram, indicating kg of fish/cage and the number and location of proposed sampling stations, must be submitted electronically to NSDFA for review, at least one week prior to sampling. Stations must be located on stocked cages only. However, if fish are not present on site, at the time of monitoring (e.g., fish are harvested) or the number of stocked cages is less than the minimum required

number of sampling stations, highest biomass/cage must be determined from the most recent date when the site biomass was highest and stations positioned accordingly. One station must be located at each extreme end of the site (Stations 1 and 2 in Figures 1 - 3) on the outside edge of the highest biomass cage and positioned to align with the longest row (single arrangement of cages). The third station, Station 3 in Figures 1 - 3, must be located on the cage with the highest biomass within the cage array. Additional stations, if required according to Table 1, will be located on cages with the next highest biomass relative to the previously assigned highest biomass cage, as depicted by Stations 4, 5, 6, 7, 8 and 9 in Figures 1 - 3. For example, Station 3 must be located on the highest biomass cage whereas Station 9 must be located on the cage with the lowest ranked highest biomass. If one of the end cages is already flagged as Station 1 or 2 and its biomass is such to indicate that an additional station is required, the station must be located on another cage, which has the next highest biomass. Stations 3 – 9 must be located between cages and must not be located on the outer perimeter of the cage array, where possible. For sites with one row of cages, stations 3 - 9 must be located on the side of the cage where the adjacent cage has the higher biomass (Fig. 1). When 2 or more rows of cages are present, stations 3 - 9 must be located between cages in opposite rows, where possible (Fig. 2). Steel cage configurations will likely require that stations 3 - 9 be positioned around the perimeter of the site (Fig. 3). The EMP Supervisor must be consulted prior to conducting EMP sampling to obtain approval of proposed station positioning.

Table 1. Minimum Number of Sampling Stations Required

Maximum # of fish onsite during production cycle	Minimum Number of sampling stations required (not including reference stations)
0 to 300,000	3
300,001 to 600,000	5
600,001 to 900,000	7
900,001 to 1,200,000	9

Figure 1. Location and Number of Sampling Stations (one row scenario)

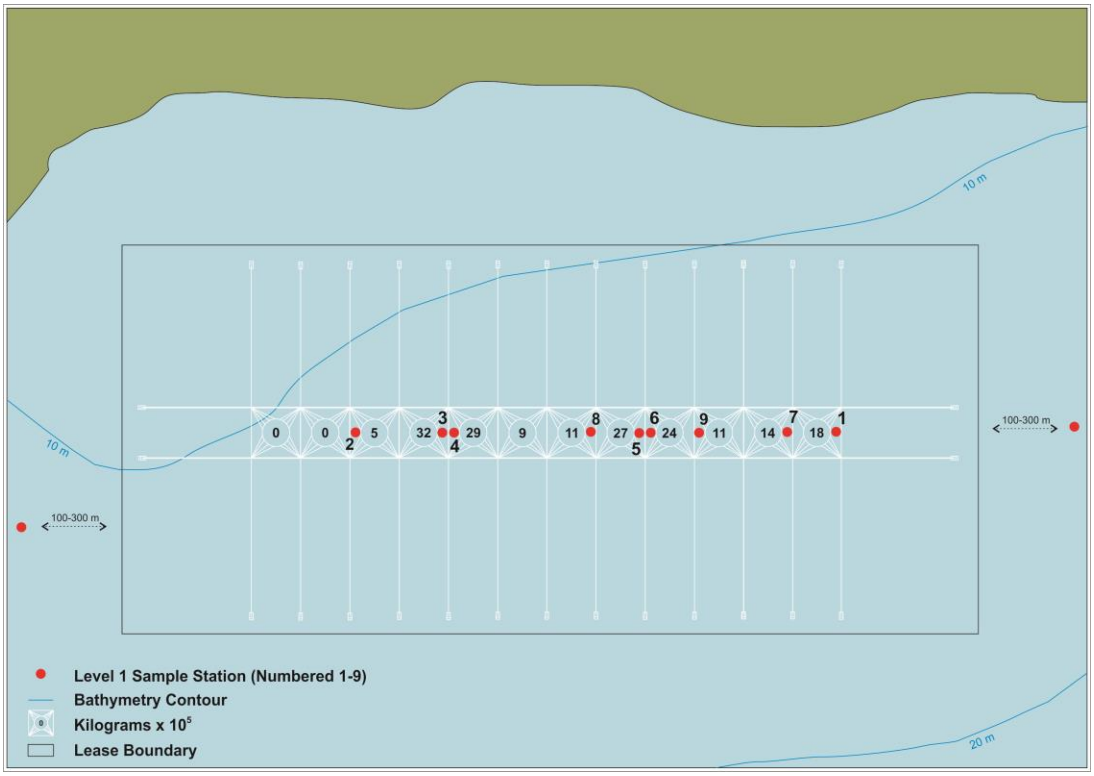


Figure 2. Location and number of sampling stations (two row scenario)

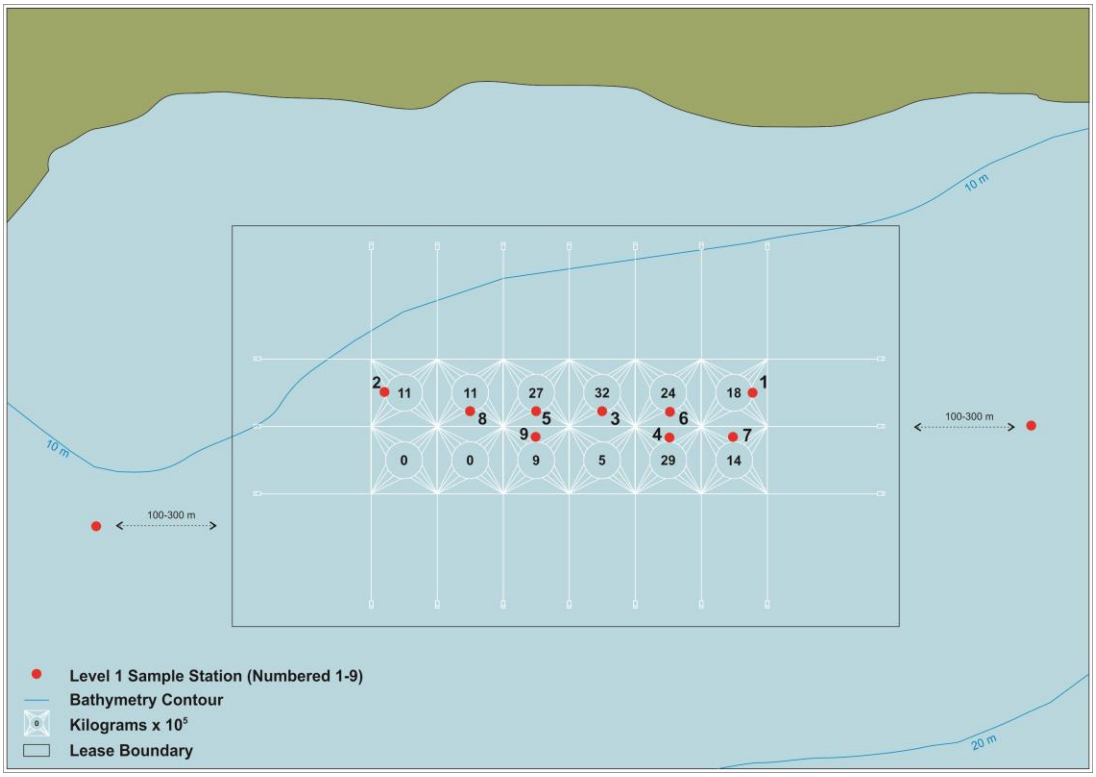
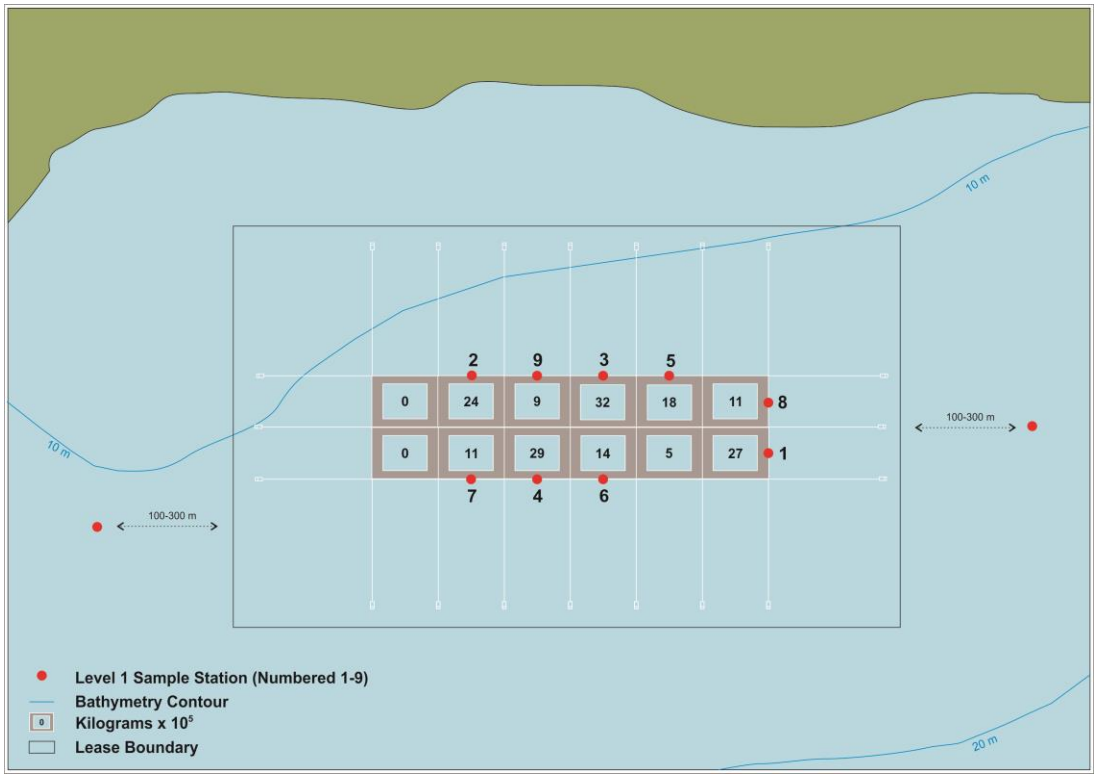


Figure 3. Location and number of sampling stations (steel cage scenario)



Accuracy of sampling station location is very important for the efficacy of the EMP and the goal of achieving consistency and repeatability. For these reasons, sampling vessels must be moored to cages during sample collection. Mooring is not required for stations that are not located on cages (see historic high and reference stations below); however, an appropriate method to remain on station must be employed. For on-cage stations, samples must be collected at cage edge, between the boat and cage if using a grab sampling device. Using a GPS device, a DGPS waypoint must be recorded at every sampling location during each sampling event (NAD83 in decimal degrees or UTM meters)**.**

In addition to the stations required as a minimum within the lease according to Table 1, historical sampling station means that have exceeded 3000 µM must be re-sampled if the most recent monitoring indicates the station mean sulfide concentration has not since decreased to < 1500 µM. These sampling stations are referred to as historic high stations. Historic high stations must be located within 10 m of the original coordinates, if site infrastructure allows, or else the sampling will be considered to have taken place at a another station. In cases with multiple historic high stations within close proximity, NSDFA may consider reducing the number of stations required for re-sampling upon request.

If necessary, revised sampling station locations may be determined once on-water field work begins. Where site infrastructure prevents access to the sampling stations, or no cages with the highest biomass are within 10 m of the proposed station locations, sampling will take place as close as possible to the station, at the closest cage with the highest biomass, without risking entanglement of equipment (this does not apply to historic high stations). As with any other

sampling station, another waypoint must be logged at the new location. If target stations (reference and historic high stations) cannot be sampled, record the distance and direction from the target waypoint. When sampling station locations are revised, coordinates and an explanation of spatial variation must be provided with the submission of the final environmental summary. A template is provided in Appendix A1.

2.2 Shellfish Monitoring Stations

For active shellfish farms, fewer sampling stations may be required per site compared to finfish leases. Monitoring will be scaled to level of risk (considering production levels, percent of bay volume and historical environmental performance). For inactive shellfish farms with no production, no sampling stations will be required. Refer to Figure 2: Risk Based Decision Making Matrix of *Environmental Monitoring Program Framework for Marine Aquaculture in Nova Scotia* for elaboration on appropriate monitoring actions.

Alternative levels of sampling are proposed for shellfish aquaculture sites that have repeatedly shown no or limited potential for impact. These include a reduced sampling requirement to video monitoring only and/or sampling repeated at extended spatial and temporal intervals (fewer stations, every 5 years, etc.).

2.3 Reference Stations

For each marine finfish and shellfish aquaculture site that requires monitoring, there will be at least 2 reference stations. Reference stations will be 100 – 300 m from the lease boundary in an alongshore axis, both upstream and downstream of the site. Reference samples must be collected from a similar depth and sediment type to lease stations.

In the event that the off-site distance criterion cannot be achieved, reference samples should be collected from a new sampling station with similar characteristics to the lease stations (water depths and sediment type, etc.).

2.4 Monitoring Levels

Level I – As outlined in Figures 1 - 3 and described in Section 2.1 - 2.3.

Level II – Additional monitoring is required based on Level I outcomes (i.e., if site is classified as Hypoxic B or Anoxic). See Table 2 and Figure 4 below. Sampling historic high stations and analysis of porosity and organic matter are not required for Level II sampling. A consistent rationale for additional monitoring will be applied based on the following sampling objectives:

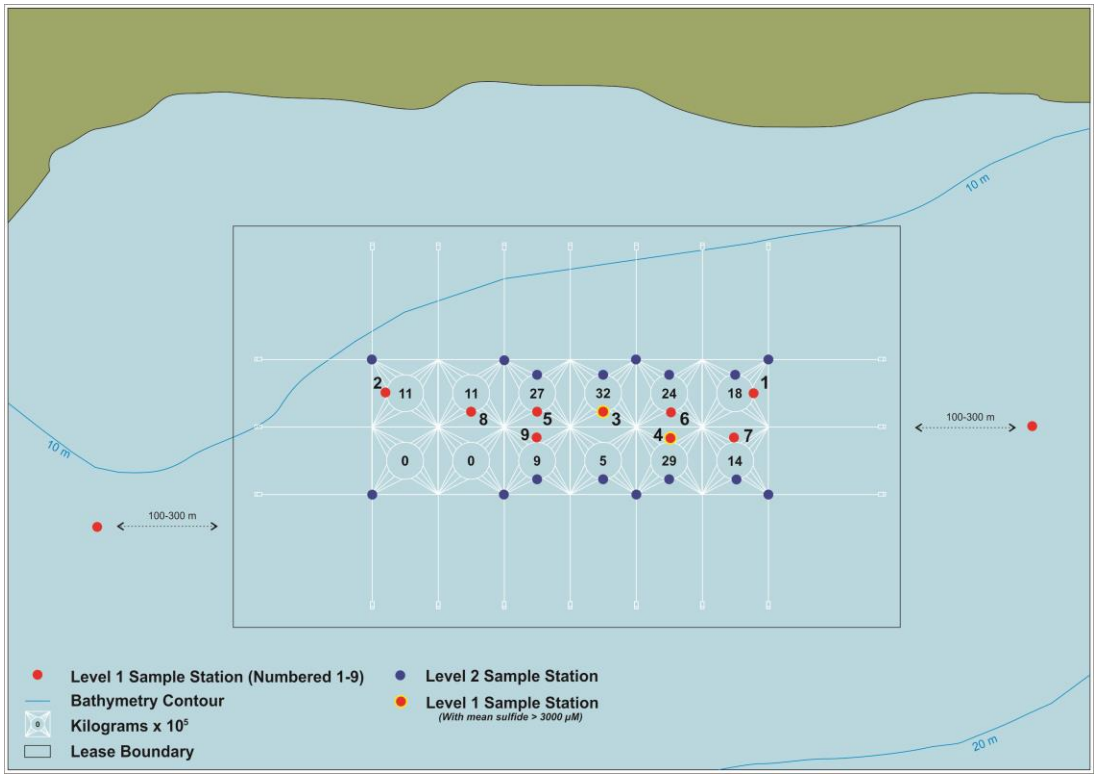
a) Improved spatial delineation of the impacted area. This will include sampling at cage edge of all cages immediately adjacent to the Level I sampling station(s) with mean sulfide concentrations > 3000 µM.

b) Improved spatial delineation of the zone of influence. This will involve sampling at the 4 corner compensator buoys and additional compensator buoys at no more than 200 m spacing along the outer edge of the cage configuration.

Level I results will be the sole determinate for site classification purposes. Site classifications dictate appropriate site management responses, including follow-up monitoring and mitigation

requirements (see section 3.0: PNS, 2014). If Level II is required, monitoring results from Level I and II will be combined to determine state of the benthic environment within the lease. This information will be used by regulators to provide site-specific recommendations for any remedial action required.

Figure 4. Typical Location of Level II Sampling Stations (example scenario)



Level III – Variation of Level II repeated to capture seasonal variation (likely in winter or early spring) and to more closely monitor affected areas. Requirements for Level III follow-up will be determined by NSDFA in discussion with the site operator.

Table 2. Sediment classification thresholds and determination guide

Sediment classification		Minimum proportion of station means within each sulfide threshold range to determine sediment classification	Sulfide concentration thresholds (µM)	Monitoring prompted
Oxic	A	≥ 0.50	< 750	
	B	≥ 0.50	750 -1500	
Hypoxic	A	≥ 0.50	1500 - 3000	
	B	≥ 0.50	3000 - 6000	Level II
Anoxic		≥ 0.70	> 6000	Level II, Level III

*Note: The classification indicating the most adverse sediment condition will be assigned to a site when sulfide data suggests even distribution between two classifications.

3. VIDEO RECORDING METHODOLOGY

Video must be collected at every sampling station for all levels of monitoring using a submersible video camera (drop camera or diver remote) using an acceptable high-resolution format (i.e., AVI). Video must be obtained before grab samples to show undisturbed sediment. 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 with the video submissions. Each station must be clearly labeled on the video by using a placard (lease name/#, date, sample station ID) prior to submersion. The drop camera video must be equipped with a digital overlay detailing real time latitude and longitude (NAD83, decimal degrees) of the sampling location. Diver video must be accompanied by coordinates of swim. For each station, each video must include a 360º panorama (or as close as possible) of the water surface view plane prior to submersion. Video requirements include continuous footage of initial descent, impact with the seafloor, camera ascent and retrieval on deck. Once at the bottom, the camera will hover just off bottom and bounce several times (i.e., frame or diver hand contacts sediment to show sediment consistency). Each station requires a minimum of 2 minutes of seafloor footage, covering a minimum area of 5 m^2, achieved either by drift of vessel, movement of the handler along the vessel deck, or diver swim.

Video image quality must be sufficient to recognize and identify sediment type, condition, and any benthic macrofauna/flora present. Sufficient lighting must be used in locations where poor visibility due to benthic conditions is anticipated. Lighting must illuminate the field of view surrounding the reference scale. The lighting angle must be such to reduce backscatter of suspended particles. The video, with chapters sorted by sampling station ID, must be submitted according to the timelines presented in Section 5 of the EMP Framework.

4. SEDIMENT SAMPLING METHODOLOGY

Sampling of benthic sediments will be conducted to determine: redox potential, total dissolved sulfide, porosity and sediment organic matter. Although sulfide is the main regulatory determinant, the other 3 variables are used to validate and confirm accuracy of sulfide results via

empirical relationships of measured variables (Hargrave, 2010) and the Benthic Enrichment Index (BEI) (Hargrave, 1994). Refer to section 2.3 of the EMP Framework for more information.

Samples can be collected using either diver or grab. The sampling methodology for using a diver to collect sediment samples is outlined in Wildish *et al*., (1999 and 2004). Additional comments on diver coring methodologies were also submitted to NSDFA by Dr. Barry Hargrave (2009) (see section 4.2). This submission can be made available if requested. The main goal is to use an appropriate sampling method and device, which maintains an intact sediment-water interface. To ensure acceptable results, NSDFA approval must be obtained prior to the use of non-approved equipment and methodologies for sediment sample collection and sub-sampling not described herein. Any deviations from the approved equipment and methodologies must be justified and described in the final submission.

The following practices MUST be applied to ALL sample collection (sections 4.1 and 4.2, where applicable):

- A temperature logger (e.g., Tidbit v2 Water Temperature Data Logger – UTBI-001) must be used to record, at least every 30 minutes, the temperature of the environment where sediment samples are stored (i.e., cooler and refrigerator). The same temperature data logger must measure the storage temperature throughout the lifecycle of each set of sediment samples (i.e., from the onset of sediment collection to the completion of analysis). 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 temperatures.
- Rinse all sampling equipment with saltwater between deployments to remove all debris and sediment.
- Siphon (do not pour) the overlying water from the sample. It is important to maintain an undisturbed sediment sample and avoid getting surface water in the syringe.
- If the sample is spoiled at any point during the collection (e.g., leakage from sampling device, sediment surface layer not intact, equipment malfunction, human error etc.), repeat steps from beginning to collect undisturbed sample.
- If an appropriate grab sampler for the site-specific benthic conditions is used and is unsuccessful (lack of sufficient quantity of sediment) after the 3rd sediment collection attempt and free-falling deployments are unsuccessful for the 4th and 5th sediment collection attempts (see section 4.1) the sampling team must move to another station location, within the lease for farm stations and to an appropriate location for reference stations (see. 2.1-2.3), to collect sediment in place of the unsuccessful attempts. Collect new waypoints, make note to indicate non-standard sampling and provide a justification for the appointed sampling station.
- When moored to static structure take care not to repeat exact sampling position of initial grab.
- Excess sediment from grabs must be discarded downstream, away from the sampling location.

4.1 Remote Grab Collection

NSDFA has approved certain sediment grabs (i.e., Ponar and Ekman) for sample collection. These grabs have proved effective for the sediment conditions specific to the Nova Scotia

aquaculture industry. The condition of grabs, as well as the method of deployment and retrieval must ensure the sediment-water interface is maintained (e.g., water should not drain through the sample and out the bottom of the grab). The speed at which the grab descends in the water column must minimize the dissipation of the benthic surface sediment due to the force of water being displaced by the falling grab. Free-falling grab deployments are unapproved. In the rare case that an appropriate grab sampler (for the benthic conditions) has unsuccessfully collected a sufficient quantity of sediment, after 3 attempts at the same station, due to the site specific benthic conditions (i.e., sediment type and current) sediment can be collected by allowing the grab to free-fall. Free-falling is a deviation; therefore this technique must only be utilized in the aforementioned described situation and must be noted in the final submission. The speed at which the grab is retrieved must be consistent and such to avoid mixing the sediment sample and to prevent water from washing through the sample and out the bottom of the grab. A target speed for grab deployment and retrieval is suggested to be no greater than 30 cm/s (Environment Canada 2012). The grab must descend and ascend vertically to ensure the sampler connects evenly with the seafloor and that the collected sample is not shifted during retrieval. Grabs that ascend or descend diagonally are inappropriate for sample collection and should either be fixed with additional weight to achieve verticality or exchanged with an appropriate grab more suited to the conditions.

If using a grab, 1 plastic syringe core with a rubber-tipped plunger (similar to Becton-Dickson 5 mL, Fisher # 14-823-35) and mL increments displayed, must be used to remove surface sediment from 3 points on the sediment surface. This is done by pushing the cut-off syringe (tip removed to widen the syringe opening) into the sediment then gently withdrawing the sample while taking care to avoid collecting air spaces in the plastic tube. It is **critical** that sediment samples are obtained from the top 2 cm of sediment only.
A minimum sediment volume equaling 5 mL is required to be subsampled per grab. If using a 5 mL syringe, the first point will remove 2 mL, the second point 2 mL and the third point 1 mL for a minimum total of 5 mL of sediment. If a 10 mL syringe is used, collect 2 mL of sediment from 5 points within the grab, totaling 10 mL. Neither head space nor air cavities can be present in the syringe. The open syringe end must be immediately capped and stored in a dark environment held at 2 – 5°C until processed. Each syringe core must be labeled with a sample ID.

To achieve a desirable sampling resolution over the 5 m^2 area, 3 separate grab collections must be collected per station, with a minimum of 1, 5 mL subsample extracted from each grab, for a total of 3 grabs and 3 subsamples, totalling a minimum of 15 mL of sediment analyzed per station.

4.2 Diver Core Collection

When locations and sampling conditions allow cores to be collected by divers, cores must be inserted into the sediment, while minimizing disturbance of the sediment surface. As mentioned earlier, geochemical measurements are to be made on the sediment surface layer (0-2 cm) of undisturbed sediment. Open-ended cores should be slowly inserted into the bottom with a gentle twisting action to minimize sediment compression. Cores should have drilled holes at least every 2 cm's to allow lateral sub-sampling of the surface layer closest to the sediment-water interface using a minimum 5-mL cut-off plastic syringe (see section 4.1 for syringe information). The length of sediment collected in cores obtained will be determined by the grain size and water

content of the deposits being sampled. For example, if 30-cm long acrylic core tubes are used in soft, mud-rich sediments these should be ~50% full with a 15 cm sediment column and 15 cm of overlying water. Once sediment is in the core, the diver seals the upper end with a cap to maintain overlying water above the undisturbed sediment surface. Vertically intact cores must be brought to the surface in an upright position. Clarity of overlying water can be used to visually confirm that the sediment surface is as undisturbed as possible. Intact sediment cores should be stored upright in an ice-filled cooler (2 - 5 ºC) upon collection. Transfer of sediment-filled cores between small boats and shore must minimize disturbance of the sediment-water interface. Sediment sub-sampling from cores must occur between vessel and automobile transportation. Subsamples must be immediately stored at 2 – 5°C. The required subsample volume must equal a minimum of 5 mL per core. Each core and corresponding syringe must be labeled with a sample ID.

5. FIELD OBSERVATIONS

Field reporting presents an overview of the conditions on, around and beneath a farm site. There is a requirement to collect and submit field observations. A sample log sheet is provided in Appendix A2. Often, these log sheets will be used for QA/QC during NSDFA review.

Field observations must be recorded and will include (Environment Canada, 2012):

- Sampling water body, site name and lease number
- Relative descriptions/estimates of ambient weather conditions, including wind speed and direction[1], wave action (e.g., chop, swell, etc.), direction[1] and strength of the predominant current and tide schedule
- Sampling station coordinates
- Station ID and replicate number
- Time and date of each sample collection
- Type of vessel used for sampling
- Type of sampling equipment and any modifications
- Water depth at each sampling station (m) and the depth of collected sediment (cm)
- Water temperature (°C)
- Name of personnel collecting the samples
- Number of sediment collection attempts at each station
- Details pertaining to unusual or unpredicted events that might have occurred during the operation of the grab sampler (e.g., equipment failure, unusual appearance of sediment integrity and control of vertical descent and ascent of the sampler)
- Deviations from standard operating procedures

Benthic descriptions (applies to the area covered for video footage and all grab collections)

- Description of the sediment type, consistency, colour and odour
- Presence of biota (flora and fauna)
- Presence of gas bubbles
- Presence of fish feed and faeces

[1] Relative to true north

Provide a description of unusual or unpredicted events that may have occurred during the operation of the grab sampler, weather issues, whether the vessel is moored and sampling difficulties, etc.

Grabs and diver cores containing collected sediment should be photographed upon retrieval with image copies included in the submission to NSDFA.

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). Recent 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. 9678BNW) 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 and CAS#) 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., log books, original records etc.) may be requested by NSDFA for QA/QC laboratory audits. A sample of the data recording sheet can be found in Appendix A3, respectively. Please retain original record of sampling data.

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. 9678BNW)
- 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, decontaminated and pre-weighed (g))
- Timer
- A3 data record sheet

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 A4 for a suggested procedure to detect coatings on the electrode platinum surface (this is not mandatory).

6.1.3 Redox measurements

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

- Measurements will be completed within 72 hours of sample collection. 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 4.0).
- From the cut-off 5 mL (or 10 mL) syringe, the first 2 mL (5 mL) 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, decontaminated, pre-weighed (g) 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 2 minutes has elapsed (use a timer to achieve consistency among samples). Note on A3 data sheet which readings were taken at the 2 minute mark.
- 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 sampling 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.
- 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), is a measure of 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 currently 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
- A3 data record 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, 1000, 5000 and 10000 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, 2007b 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 is 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 A3 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. Also, record the displayed slope value for the 10,000 µM standard on the A3 data sheet (the acceptable range -27 to -33 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.
- Calculate the 10-fold mV change (slope). This value provides the best means for checking electrode operation (see Thermo Scientific, 2007b).

  - mV (5000 µM) – mV (500 µM) = 10-fold mV change.
  - mV (10000 µM) – mV (1000 µM) = 10-fold mV change.
  - The acceptable value range is -25 to -30 mV.
- Include notes regarding any calibration problems on A3 data sheet.
- Calibration of the sulfide electrode is stable for a maximum of 3 hours. Record time calibration completed and time of expiry on the A3 data sheet.

6.2.3 Sulfide measurements

Triplicate subsamples taken from each sampling 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).
- 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 make/models are acceptable*:

6.3.1 Materials

- Gravity convection drying oven
- Analytical balance
- Labelled, pretreated, pre-weighed (g) receptacles
  - Glass receptacles must be acid washed between analyses to avoid cross contamination.

    - Receptacles used for both porosity and organic matter analysis must be pre-ashed before sediment is introduced.
- 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.
- 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

- Pre-ashed receptacles (labelled and pre-weighed (g))
- Tweezers
- Ceramic tray
- Muffle furnace
- Analytical balance
- Vacuum desiccator
- Worksheet

6.4.2 *POM 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 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-sampling submissions are required to be submitted to NSDFA a minimum of one week prior to sampling. Data submissions are required to be submitted to NSDFA 14 and 21 days following sample collection. In summary, the final submission must include:

- **Pre-sampling**
    - Once/year: A list of chemicals (name and CAS#) and equipment (model name and #) intended for use for the EMP season.
    - One/site: Electronic site diagram (kg fish/cage and location of proposed sampling stations)
- **Within 14 days of sediment collection:**
    - A1 – Coordinate and Lab Results Table
        - All data fields completed except for porosity and organic matter
    - A3 – 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 A1)
- **Within 21 days of sediment collection:**
    - A1 – Coordinate and Lab Results Table (completed)
        - All data fields completed
    - A2 – Video and Grab Log Sheet (1 per station)
    - Grab photos
    - Video recordings

8. BASELINE REQUIREMENTS

New sites and site expansions are subject to baseline environmental reporting. This would include environmental monitoring specific to each application. Appendix B includes typical baseline requirements for marine finfish aquaculture. Shellfish applications are required to complete similar studies; however, the level of monitoring is based on the level and type of proposed production. See Appendix B for details.

SOP APPENDIX A: ASSOCIATED FIELD SHEETS

The following appendices are record templates and field sheets that are to be used as part of the standard operating procedures.

Appendix A1 includes a coordinate table to record and submit all coordinates used to determine precise sampling station locations. This template also includes columns to input summary laboratory results.

Appendix A2 is a log sheet to record field notes.

Appendix A3 is a data worksheet to record the redox accuracy check, sulfide calibration and measured values of redox potential and sulfide in sediment samples.

APPENDIX A1: Coordinate and Lab Results Template

This template should be submitted in editable electronic spreadsheet format (i.e., excel). The coordinates should be submitted in NAD83 (decimal degrees or UTM meters). 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.

Sampling 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 A2: Video and Grab Log Sheet

Date:		**Wind direction and speed:**	
Water body:		**Wave action:**	
Lease name and #:		**Direction and speed of current:**	
Sampling Station ID:		**Tide schedule:**	
Latitude (decimal degrees):		**Comments:** (e.g., differences between observed seafloor conditions and grab sample, notes regarding sampling difficulties, weather issues, deviations from the SOP, etc.)	
Longitude (decimal degrees):			
Dist. and dir. from WP:			
Time:			
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	
Water Temperature (ºC):		2. Degree of odour (strong, slight, none)	
Video (y/n):		3. Flora/Fauna (e.g., eel grass, kelp, lobster, crab, starfish, beggiatoa, polychaetes etc.)	
Number of grab attempts:			

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

Key Terms of Video and Grab Log Sheet

Date – Date sample was collected.

Water body – Bay or Harbour name.

Lease name/# - Lease name/NSDFA lease number

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

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

Longitude – Sampling 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 remote grab 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 sampling.

Water Temperature – Surface water temperature (°C) at time of sampling.

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

Number of grab attempts – State the number of grab 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.

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

Sample (yes or no) – Indicate if a replicate sample was collected.

Sample ID - List identification number listed on replicate core.

Sediment Description – Describe sediment characteristics from grab sample. See Benthic Descriptor Key'.

Grab Depth – The measurement of the depth (cm) of the sediment within the grab.

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

Flora/Fauna – Describe flora/fauna characteristics from grab sample. See 'Benthic Descriptor Key'.

APPENDIX A3: 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)	µM	mV*	Meter calculated slope (mV)	Calculated 10-fold change (mV)
100			1. Record the displayed slope value for the 10,000 µM standard. Acceptable range: -27 to -33 mV	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			1.	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 A4: ***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 (±10mV) within 5 min. If potentials do not stabilize repeat step 2. Rinse and dry the electrode.
5. The electrode is ready to use.
6. 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.
7. 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.
8. If the electrode is to be unused for an extended period of time empty the filling chamber 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 A5: Checklist

Pre-sampling

- ☐ Once/year: Submit to NSDFA a list of chemicals (name and CAS#) 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).***
- ☐ One week prior to sampling: Submit to NSDFA for review, an electronic site diagram with kg fish/cage and location of proposed sampling stations displayed (see section 2.1)
- ☐ Communicate tentative sampling date(s) to NSDFA at least one week prior to sampling (site-specific, sampling timeframes are provided to industry, by NSDFA, 1 month prior to the EMP season)

Sampling

- ☐ Underwater video recordings of the seafloor at each station with GPS overlay
- ☐ 3 grabs per station, 1 syringe subsample/grab (3 sediment subsamples per station)
- ☐ Photographs of each grab sample
- ☐ A2 – Video and grab log sheet completed (1 per station)

Sediment analysis

- ☐ Redox
- ☐ Sulfide
- ☐ Porosity
- ☐ Organic matter
- ☐ A1 – Coordinate and Lab Results Table completed (excel)
- ☐ A3 – Analytical Data Record Sheet completed

Submissions and timelines

- Within 14 days of sediment collection:
  - ☐ A1 – Coordinate and Lab Results Table
    - All data fields completed except for porosity and organic matter
  - ☐ A3 – 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 A1)
- Within 21 days of sediment collection:
  - ☐ A1 – Coordinate and Lab Results Table (completed)
  - ☐ A2 – Video and Grab Log Sheet (1 per station)
  - ☐ Grab photos
  - ☐ Video recordings

For further information on timelines for monitoring events, submissions and necessary mitigation please refer to section 5.0 Annual Schedules of the EMP Framework document (PNS, 2014).

APPENDIX B: BASELINE REQUIREMENTS

With any new site developed in Nova Scotia, or any significant site amendment or re-activation, it is important that appropriate baseline data be collected and that ongoing monitoring reflects the original data requirements. A typical application for a finfish operation would require complete sediment analysis and video collected from lease and reference locations, plus video monitoring of all lease corners, current measurements and additional site characteristics, as determined by NSDFA after reviewing site application.

There are many shellfish sites in NS that pose little environmental risk, and therefore warrant a different degree of baseline monitoring within the EMP. Sampling of shellfish sites to date by the EMP has shown low risk interactions with the marine environment compared to larger aquaculture operations. However, some areas with a high level of shellfish culture may justify complete baseline and routine monitoring, based on a bay-specific risk-assessment process.

Typical Baseline Monitoring Requirements for Proposed Finfish Aquaculture in Nova Scotia

In order to evaluate benthic habitat conditions within the lease area information concerning currents, sediment grain size, percent organic matter, porosity, redox potential and sulfide concentration must be provided. The following information describes the sampling locations and the methodologies for data acquisition required by the Nova Scotia Department of Fisheries and Aquaculture (NSDFA), Aquaculture Division and Fisheries and Oceans Canada (DFO), Fisheries Protection Program (FPP). Two hard copies, and an electronic copy, of the required information and video must be sent to:

Attention: Manager, Aquaculture Development
Nova Scotia Department of Fisheries and Aquaculture, Aquaculture Division
1575 Lake Road
Shelburne, NS
B0T1W0

Location of Stations

The following outlines the required stations for the baseline sampling program. DFO-FPP in conjunction with the NSDFA determined the locations for this sampling program. Please see the diagrams located in SOP Section 2 for the number and location of baseline monitoring stations.

Sampling stations are located at the corners of the lease and within the proposed lease tenure. Reference stations are located outside of the lease area.

The choice of reference sites for benthic comparison includes a consideration of general benthic conditions in the area and within the lease area. The location of the reference station will have similar depth contour and bottom characteristics as those contained within the lease site. These reference sites should be 100 – 300 m from the site in an alongshore axis both upstream and downstream of the site.

Video Monitoring
Video monitoring will be conducted at all stations for all leases conducting baseline. The Observation Key located in Appendix B1 should be used to complete the Summary of Observations located in Appendix B2, based on the video collected. High quality copies of the original, unedited footage should be provided to DFO - FPP and NSDFA - Aquaculture Division.

A detailed process for the collection of video is described in SOP Section 3: Video Recording Methodology.

Current Meter
A current meter will be deployed in the center of the proposed lease tenure. Measurements of current speed and direction must be recorded at the center of the site, at least every 15 minutes, over a minimum duration of 30 days, using an ADCP current meter set at 1 m sampling bins. Each observation of speed and direction must be made over at least a 5-minute averaging period, and expressed as that average. The current meter must be correctly calibrated and dated calibration sheets must be submitted along with the entire current meter record.

NOTE: Current data may not be required for some finfish expansions or shellfish applications and/or may not require an ADCP for a full 30 day deployment (contact NSDFA to confirm).

Sulfide, Redox, Organic Content, Porosity and Grain Size
All stations listed above, with the exception of the corner stations have been designated for benthic sampling. Samples will be collected in triplicate (i.e. 1 syringe/grab, 3 separate grabs in total per station) and analyzed for oxidation-reduction potential (redox), sulfide ion content, percent organic matter and porosity (both expressed as a percentage), and sediment grain size. Sulfide levels, redox potential, percent organic matter, and porosity represent four fundamental sediment conditions that together provide information on fish habitat in the benthic environment.

The methods to be utilized to determine the levels of sulfide and redox in the sediment samples are contained in the document titled "*A Recommended Method for Monitoring Sediments to Detect Organic Enrichment from Mariculture in the Bay of Fundy*", Wildish et al. (1999). **Critically, measurements should be obtained using the top 2 cm's of sediment only.** See section 4.0 Sediment Sampling Methodology for guidelines.

All samples will be analyzed for redox and sulfide in accordance with the standard operating procedures employed by DFO and modified from those in Wildish et al. (2004). **The modification is that redox and sulfides will be measured in a vial of extracted sediments to reduce the small-scale spatial variation that occurs when the redox probe is inserted into cores.** See section 6.0 Analysis of Sediment Samples for guidelines.

Other parameters discussed (e.g. video) will serve as confirmation mechanisms for the geochemical analysis. An important consideration used in this process is that coastal habitats in Nova Scotia can be organically rich due to natural processes and therefore may naturally exhibit variable oxygen conditions. This is a condition that may be reflected in reference samples and where it occurs, it will be a consideration of management decisions.

APPENDIX B1: Observation Key

- ✓ *Depth:* Provide the depth of water.
- ✓ *Time:* Provide the time the video footage was shot.
- ✓ *Sediment Type:* Give the sediment type as well as its relative proportion (e.g., 33% silt, 66% coarse sand).
- ✓ *Sediment Colour:* Give the approximate colour.
- ✓ *Macrofauna/flora Qualitative Presence/Absence*: List the common names of organisms that are present.
- ✓ *Comments:* Provide any additional information that may be of interest to this station.

Descriptive name		**Diameter (mm)**
Gravel	Boulder	>256
	Cobble	64-256
	Pebble	4-64
	Granule	2-4
Sand	Very coarse	1-2
	Coarse	0.5-1
	Medium	0.25-0.5
	Fine	0.125-0.25
	Very fine	0.063-0.125
Mud	Silt	0.004-0.063
	Clay	<0.004

APPENDIX B2: Video Monitoring Summary of Observations for Sample Site

Stn. #	Depth (m)	Time	Observation			
			Sediment Type and Proportion	Sediment Colour	Macrofauna/flora Qualitative Presence/Absence	Comments

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