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

Fisheries and Aquaculture

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

1. INTRODUCTION

The following information describes the sampling methodologies for the environmental monitoring of finfish farms in Nova Scotia (NS). This document will also serve as the basis for Nova Scotia shellfish monitoring. Provided in this document are a series of sampling instructions, laboratory guides, and field templates that are designed to assist farm operators. This document in intended to be a template for Nova Scotia marine monitoring that will be reviewed yearly to include changes and innovations to field methods, technologies and regulatory approaches.

This protocol originated in 2002 as part of the document *Design of the Environmental Monitoring Program for the Marine Aquaculture Industry in Nova Scotia* (Smith et al., 2002) and has evolved with recent advancements in science and technology. Starting field work in 2003 as a pilot project, Nova Scotia Department of Fisheries and Aquaculture (NSDFA) has regularly monitored each marine finfish aquaculture operation throughout the province as part of the Environmental Monitoring Program (EMP). A number of revisions were incorporated to keep the EMP up-to-date, relevant and effective. Recognizing that the EMP is a mandatory requirement and integral part of the lease and license process, the eventual goal of this pilot study was to task individual farms with the responsibility to conduct their own EMP and to provide results to NSDFA as required. All farm operators must now adhere to this program and follow these instructions below as standard operating practices for environmental monitoring.

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2. LOCATION AND NUMBER OF SEDIMENT SAMPLING STATIONS

Through the EMP, monitoring has been conducted in and around aquaculture sites throughout the province. Using findings from historical data collected since 2003, sampling locations are determined based on environmental performance, species type (finfish/shellfish) and level of production. This is detailed in the companion paper *Environmental Monitoring Program Framework for Marine Aquaculture in Nova Scotia* (EMP Framework; PNS, 2010).

2.1 Finfish Monitoring Stations

There will be a minimum of three monitoring stations within a lease. Stations will be evenly spaced on either side of centre along the longitudinal axis beginning with a station at either end of the axis (see Figure 1). If production is greater than 450,000 fish or rotated within the site, then additional stations will be assigned. If the grid is more than 2 rows, stations will be further offset to occur in each row.

Table 1 details the level of monitoring required based on production levels at each site.

Table 1: Number of Sampling Stations Required

Maximum # of fish during production cycle	Number of sampling stations required (not including reference stations)
0 to 450,000	3
450,001 to 600,000	4
600,001 to 750,000	5
750,001 to 900,000	6
900,001 to 1,050,000	7
1,050,001 to 1,200,000	8

The location and number of sampling stations varies with stocking and configuration. An example of Level 1 monitoring based on the above table, for a farm with 750,001 to 900,000 fish, is shown in Figure 1.

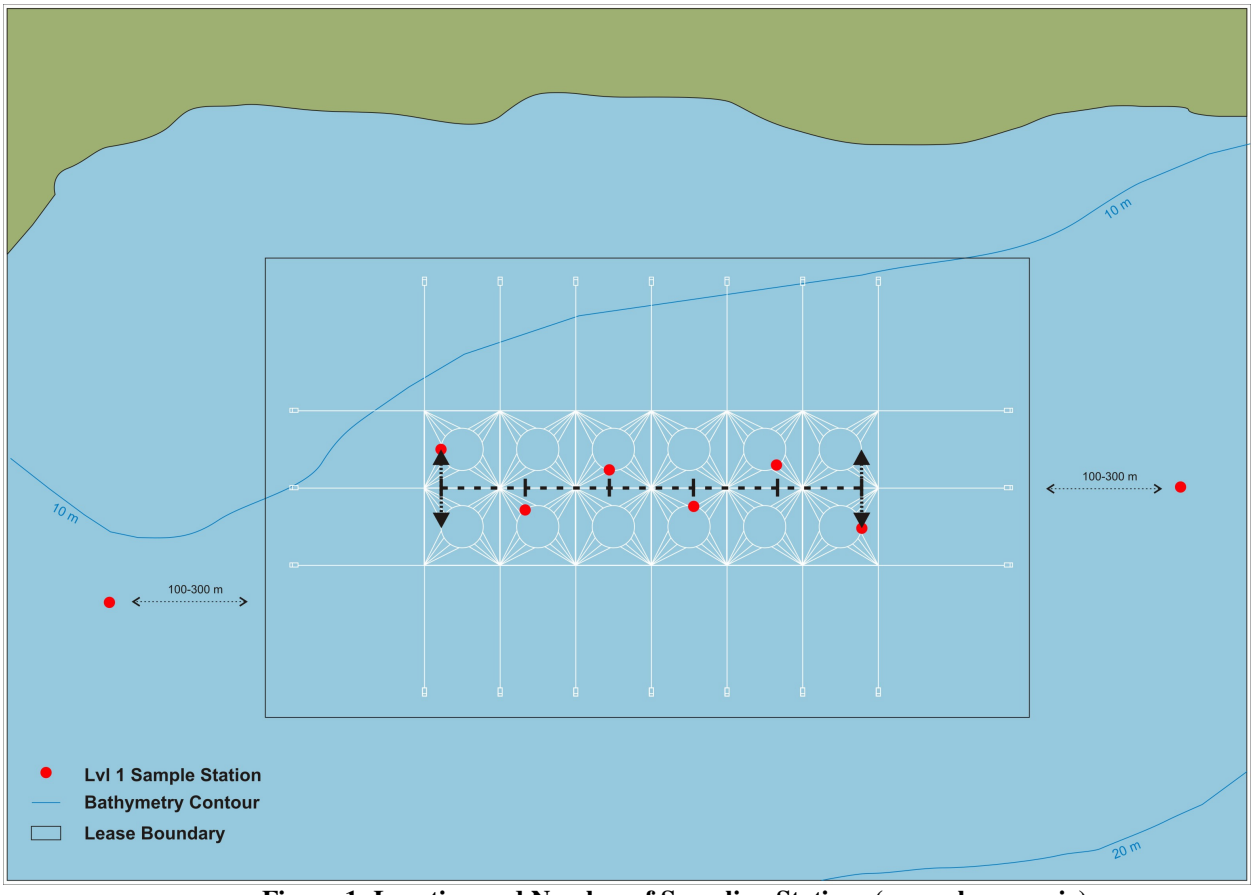

Figure 1: Location and Number of Sampling Stations (example scenario)

Accuracy of sampling stations is critical to program efficacy with the goal of achieving consistency and repeatability. For this reason, sampling vessels must be moored during

collection of replicates. Samples will be collected on the cage edge (between boat and cage if using grab) and will target the downstream side of the highest production cages in that region of the site. Using a GPS device, a waypoint will also be logged at every sampling location (NAD83 in decimal degrees or UTM metres) **and submitted in editable electronic format to NSDFA, preferably in Excel**.

In addition to stations for Level I, the sampling stations with historically high values (ie. >3000 µM) must be re-sampled every Level I EMP trip until stations return to oxic conditions. All repeat sampling should occur within 10 m of the original coordinates. Any station further than 10 m shall be considered a new station. In cases with multiple stations within relative proximity, NSDFA may reduce number of stations required for re-sampling. Historic high stations are not required as part of Level II.

If necessary, revised sampling station locations may be determined once on-water field work begins. Where cage gear prevents access to the pre-assigned monitoring stations, or no high-density stocked cages are within 10 m of the coordinates provided, sampling will take place as close as possible to the station without risking entanglement of equipment. As with any other sampling station, another waypoint must be logged at the new location. Record the distance and direction from the proposed waypoint. Those coordinates, with explanation of spatial variation, must be provided with the submission of the final environmental summary in editable electronic format. A template is provided in Appendix A1.

2.2 Shellfish Monitoring Stations

Shellfish leases may require fewer sampling stations per site than finfish leases. For active shellfish farms, sampling will be consistent with the stations required for finfish sites. However, 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

There will be at least 2 reference stations per lease. Reference stations will be 100 – 300 m from the site 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 water depths similar to that of the lease.

2.4 Monitoring Levels

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

Level II – Additional samples required are based on Level I outcomes (i.e. if site is classified as Hypoxic B or Anoxic). See Figure 2 below. Porosity and organic matter data are not required for Level II sampling. A consistent rationale for re-sampling will be applied based on the following sampling objectives:

a) Better delineating the affected area. This will include sampling (cage edge) on all adjacent cages immediately around the Level I sample station(s) that had recorded elevated sulfide numbers > 3000 µM.

b) Better delineating the zone of influence. This will involve collecting sediment samples from the 4 corner compensator buoys and additional compensator buoys at no more than 200 m spacing along the outer edge of the cage configuration.

The Level I and II stations will be combined to determine state of the benthic environment within the lease. However, Level I results will be the sole determinate for site classification purposes.

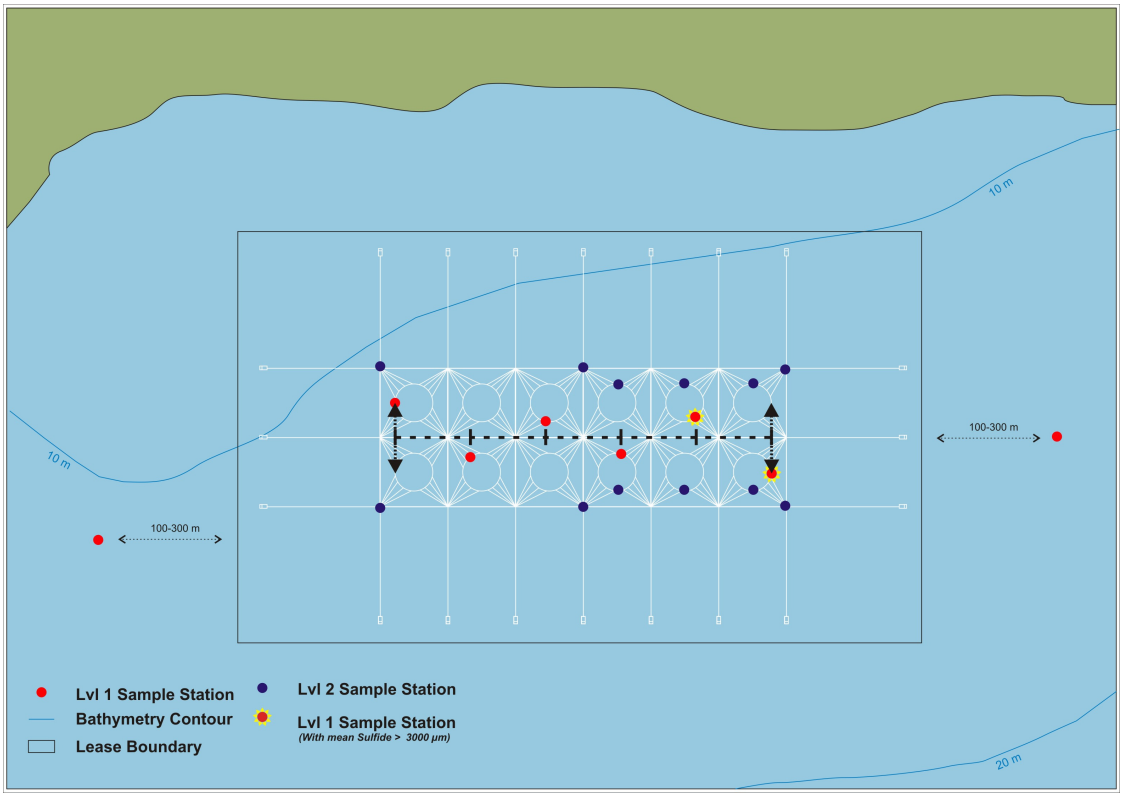

Figure 2: Typical Location of Level II Sampling Stations (example scenario)

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 to be determined by NSDFA in discussion with site operator.

3. VIDEO RECORDING METHODOLOGY

This is the only method of data collection that can be consistently and repeatedly collected at all marine sites. It provides the best qualitative (and possibly quantitative) method of assessing and comparing benthic conditions around the province and serves as a basis for further evaluation of benthic conditions.

Video will be collected at every sampling station for all levels of monitoring. Video will be obtained before grab samples to show undisturbed sediment. 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 until impact with sediment on the seafloor. Once at the bottom, the camera will hover just off bottom and bounce several times (ie. frame or diver hand contacts sediment to show sediment consistency). Each station requires a minimum of 2 minutes of benthic footage covering a minimum of 5 m^2 of bottom with drift of vessel, movement along the vessel deck, or diver swim.

Video imagery of the sediment surface will be obtained at all designated stations using a submersible video camera (drop camera or diver remote) and recorded continuously using an acceptable high-resolution format (eg. AVI). The field of view will include a visible frame measuring between 0.5 and 1.0 m on either side (i.e. 0.25 to 1.0 m^2) with a scale bar. Each station will be clearly labeled on the video by using a placard (date, sample station number) prior to submersion. The drop camera video shall be equipped with a digital overlay detailing real time latitude and longitude of the sampling location. Diver video will be accompanied by coordinates of swim.

Video image quality must be sufficient for a trained biologist to recognize and identify sediment type, condition, and any benthic macrofauna/flora present. Backup lighting may be required in reduced visibility in order to obtain acceptable image quality.

The video, with chapters sorted by sampling station ID, shall be submitted according to the timelines presented Section 5 of the EMP Framework.

4. SEDIMENT SAMPLING METHODOLOGY

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

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, 2004). Additional comments on diver coring methodologies were also submitted to NSDFA by Dr. Barry Hargrave (2009) and summarized below. This submission can be made available if requested. The main goal is to use an appropriate sampling method, or device, which maintains an intact sediment-water interface.

4.1 Remote Grab Collection

NSDFA has approved the Eckman grab for sample collection. This method has proved effective for most sediment conditions specific to the Nova Scotia aquaculture industry. With other grab types there is a risk of disturbing the seafloor or collecting a compromised sample. To ensure acceptable results, NSDFA approval must be obtained prior to the use of other methods for sediment sample collection.

If using a grab, 1 plastic syringe core, e.g. similar to Becton-Dickson 5 cc (Fisher # 14-823-35), will be used to remove surface sediment from 3 points on the sediment surface. This is done by pushing the cut-off syringe into the sediment then gently withdrawing 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. The first point will remove 2 cm, the second point will remove 2 cm and the third point will remove 1 cm. Ensure no head space or air cavities are present in the syringe. This syringe, containing 5 cm of sediment from 3 points within the grab, will be kept dark and chilled (not frozen) until processed. Each plastic core syringe shall be labeled with a sample ID sticker.

This process will be repeated twice more for a total of 3 syringes from 3 different grab replicate extractions at each sampling station (3 total) to achieve a desirable sampling resolution over the 5 m^2 area.

4.2 Diver Core Collection (comments by Hargrave, 2009)

When locations and sampling conditions allow cores to be collected by divers, cores should be inserted into the bottom to minimize disturbance of the sediment surface. As mentioned earlier, NS geochemical measurements are to be made on the 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 various depths to allow lateral sampling of the surface layer closest to the sediment-water interface using 5-mL cut-off plastic syringes. The length of sediment 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. Transfer of sediment-filled cores between small boats and shore or into vehicles should be done gently to minimize disturbance of the sediment-water interface. 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 or placed in a refrigerator (5 ºC) until analysis.

In addition, the following recommended practices should be applied to all sample collection:

- Rinse all sampling equipment with saltwater between deployments to remove all debris and sediment.
- Siphon (not pour) the overlying water from the sample. It is important to maintain an

undisturbed sediment sample and avoid getting surface water in syringe.
- If sample is spoiled at any point during the collection (e.g. equipment malfunction, human error), repeat steps from beginning to collect undisturbed sample.
- If bottom type does not allow for sample collection after 5 attempts the sampling team should move to another station location within the lease. Collect new waypoints and make note to indicate non-standard sampling.
- When moored to static structure take care not to repeat exact sampling position of initial grab.

5. FIELD OBSERVATIONS

Field reporting presents an overview of the site and benthic conditions on and around 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 quality assurance during NSDFA review.

Field observations will include details such as locations, date, names of people involved with sample collection. Water depth and temperature, at the time of sampling, are to be recorded as well.

A description of the site and any other notes of interest should also be recorded. These notes could include, but not be limited to, weather issues, whether vessel is anchored or tied to cage, sampling difficulties, etc.

Notes as to sediment type/description, flora/fauna and depth of the sediment sample within grab (e.g. 0-30 cm) should be written on log sheet for every grab sample. Diver cores should be photographed 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 were made according to discussions and feedback from the March 2010 NS EMP Technical Review and Laboratory Workshop held in Halifax, NS (Grant, 2010).

The NSDFA EMP lab uses the Accumet AP63 Portable pH/Ion Meter, Orion 9616BNWP Sure-Flow™ Combination Silver/Sulfide Electrode 9616BNWP and Orion Epoxy Redox/ORP electrode 9678BNW for measurement of redox and sulfide. *Use of comparable probes and meters is acceptable. Consult the manufacturers instructions for proper details on set up and use of probes and meter.*

If any of the chemicals or electrodes/instrumentation used for these analyses are other than those outlined in the above references (i.e purchased from a supplier), provide supplier name and product number.

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 an indirect indicator of aerobic versus anaerobic conditions. This measure is less reliable than sulfides, but used to support the findings.

Electrode accuracy check

Standardization of the redox electrode is to be performed at the beginning AND end of each set of analyses and to be recorded on the data recording sheet.

Accuracy check to be performed using Zobell's A and B standard solution (see Wildish *et al*., 1999 for preparation of standard solutions). Include notes regarding any calibration problems on data sheets.

1. Place electrode in a sample of Zobell's A standard (should give a reading of +234 (±9) mV)

2. Place electrode in a sample of Zobell's B solution (should give a reading of +300 (±9) mV).

There should be a 64 ± 9 mV difference between the readings.

Redox measurements

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

The redox electrode will be filled with 4M KCl at least 24 hours before use;

- Measurements will be taken within 72 hours of sample collection. If storage is required, samples must be stored in the dark and on ice.
- From the cut-off 5 cm syringe, the first two cms are analyzed for sediment porosity and percent organic matter. The upper 3 cm are analyzed for redox and sulfides
- Measurements will be taken with Accumet AP63 Portable pH/Ion Meter and Orion Epoxy Redox/ORP electrode 9678BNW.
- Redox measurements will be recorded as millivolts relative to the normal hydrogen electrode (mV NHE) using the equation mVNHE=Eo+(224-T), where Eo=mV of unknown and T=temperature of unknown (oC) or as millivolts (mV), once the value has stabilized (drift < 10 mV/minute) or 2 minutes after commencement of measurement. Note samples that require 2 minutes.
- The redox electrode will be rinsed with distilled water and dried between measurements (gently blot dry with Kimwipe).

6.2. Sulfide analysis

Total dissolved sulfides, measured in micromolar (μM), are 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 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 curve and sulfide samples. This allows calculation of the sulfide value directly from the calibration curve.

Electrode accuracy check

Five calibration standards will be used to check the accuracy of the sulfide electrode prior to sample analysis (100 μM, 500 μM 1000 μM, 5000 μM and 10000 μM); *record both μM and mV readings*. Include notes regarding any calibration problems on data sheets.

- Calibration of the sulfide electrode is stable for up to 3 hours.
- The Accumet AP63 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;

Sulfide measurements

The sulfide electrode will be filled with Orion Optimum Results B (cat. No. 900062) at least 24 hours before use;

- Measurements will be taken within 72 hours of sample collection.
- Measurements will be taken with Accumet AP63 Portable pH/Ion Meter and Orion 9616BNWP Sure-Flow™ Combination Silver/Sulfide Electrode 9616BNWP
- Each 3 mL sub-sample will be mixed with 3 mL of sulfide antioxidant buffer (SAOB) + L-ascorbic acid (SAOB + L-ascorbic acid is stable for a maximum of 3 hours).
- Sulfide readings will be taken once the SAOB + L-ascorbic acid/sample mixture reaches the same temperature at which the electrode was calibrated.
- Sulfide reading will be recorded once the value has stabilized (usually within 2 minutes). Note samples that require 2 minutes. Record μM and mV values.
- 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 that volume within any material (e.g. bottom sediment) that can contain fluids.

The method described below is to be performed using a gravity convection drying oven (Lindberg/Blue M 260) and Denver Instrument Summit Series Analytical Balance, SI 234 *however this method will apply to other make/models*:

- Pre-heat drying oven to 60°C
- Extrude ~ 2 mL (from 5 cc syringe) of sediment sample into corresponding pre-weighed (g) scintillation vial.
- Record wet weight (g) of pre-weighed scintillation vial and sediment sample and place in the drying oven for 24-48 hours (at 60°C).
- At the end of drying period, re-weigh scintillation vial and sediment sample.
- The porosity value can be calculated as a percentage of the total volume of material:

(Final vial + dried sediment weight (g)) – vial weight (g) = dried sediment weight (g)

$$100 - \frac{\text{Dried sediment weight (g)}}{\text{Wet sediment weight (g)}} \times 100\% = \%\ \text{Porosity value}$$

6.4. Sediment Percent Organic Matter (POM)

Organic content 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 in on the pre-dried samples from porosity analysis (section 5.3) using a muffle furnace (Barnstead/Thermolyne, Type 48000) *however this method will apply to other make/models*:

- Handling the weigh boat with tweezers, add approximately 0.5 g of ground dried sediment from the porosity analysis to a pre-weighed, pre-ashed muffle furnace safe weigh boat.
- Place in muffle furnace at 490°C for 8 hours.
- Percent organic matter can be calculated as follows:

Dried sediment weight – (Weight @ 490°C – weigh boat (g)) = sediment organic content (g)

$$\frac{\text{Sediment organic content (g)}}{\text{Sediment dry weight (g)}} \times 100\% = \%\ \text{percent organic matter}$$

7. RECORD KEEPING

NSDFA will review all environmental monitoring performed as part of this program. Data will be submitted to NSDFA within 14 days of collection (except for porosity and percent organic matter which can be submitted up to 21 days after collection). In summary, the final submission will include:

- Spreadsheet # 1: Complete results of laboratory analysis of total dissolved sulfide, redox potential and porosity and percent organic matter in editable electronic format.
- Spreadsheet # 2: Coordinates for all locations where any sampling took place, in editable electronic format, with associated summary results of laboratory analysis (Appendix A1).

- Copies of completed log sheets for every sampling station. This would also contain description of, and reasoning for, any discrepancies between NSDFA sample plan and actual samples taken (Appendix A2).
- High quality indexed (chaptered) DVD with video from every sampling station.

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.

SOP APPENDIX A: Associated Field Sheets

The following appendices are 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 sheet for redox/sulphide calibration records.

APPENDIX A1: COORDINATE & LAB RESULTS TEMPLATE

This template should be provided in editable electronic spreadsheet format (e.g. 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 in addition to completed laboratory analysis of total dissolved sulfide, redox potential and porosity and percent organic matter.

Sample ID		Location	Redox (mV)	Sulfide (µM)	Porosity (%)	Organic Content (%)	Actual Latitude	Actual Longitude
Station	ID #							
NSH01	1							
	2							
	3							
NSH02	1							
	2							
	3							

APPENDIX A2: LOG SHEET

Date:		**Site Description:**
Location:		**(e.g. notes regarding sampling difficulties, weather issues, etc.)**
Time:		
Recorder Name(s):		
Sample Collected By:		
Lease # or Reference Site:		
Sampling Station #:		
Distance and Direction from WP:		
		Benthic Descriptor Key:
Station Depth (m):		1. Oxic layer thickness, sediment colour, gradient type, sediment type
Water Temperature (ºC):		2. Degree of odour (strong, slight, none)
Video (y/n):		

Grab/Core Sample	Sample (✔)	Sample label #	Sediment Description [1]	Grab Depth (cm)	Odour [2]	Flora / Fauna
Benthic Replicate A						
Benthic Replicate B						
Benthic Replicate C						

KEY TERMS OF LOG SHEET

Date – Date sample was collected.

Location – Bay or Harbour name.

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.

Lease # or Reference Site – Indicate the actual lease number if on site. If not on site, list as reference station.

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

Distance and Direction from WP – Always provide coordinates for each sampling station. Indicate the distance and direction from the intended waypoint.

Station Depth – Water depth at time of sampling.

Water Temperature – Surface water temperature at time of sampling

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

Site Description – Include any additional notes pertaining to site changes, sampling difficulties, anchoring/mooring, weather, observations of interest, etc).

Sample (✓) – Indicate if replicate core was collected.

Sample Label # - List identification number listed on replicate core.

Sediment Description – Describe sediment characteristics from grab sample.

Grab Depth – The measurement, in centimeters, of the depth of the sediment within the grab.

Odour – Indicate degree of odour from the sediment (strong, slight, none).

Flora/Fauna – Describe flora/fauna characteristics from grab sample.

APPENDIX A3: REDOX-SULFIDE CALIBRATION & DATA RECORD

Date of analysis: _____ Start time: _____

Location: _____ End time: _____

Analysis performed by: _____

Sulphide calibration:

Calibration value	µM	mV
STD 100		
STD 500		
STD 1000		
STD 5000		
STD 10000		

Redox Calibration

START: Zobell's A: _____ Zobell's B: _____ Diff: _____ Other std: _____

FINISH: Zobell's A: _____ Zobell's B: _____ Diff: _____ Other std: _____

Samples:

Sample ID	Station	Redox (mV)	Sulphide (µM)	Sulphide (mV)	NOTES:

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), Habitat Protection and Sustainable Development (HPSD) Division. 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
PO Box 2223
Halifax, NS
B3J 3C4

Location of Stations
The following outlines the required stations for the baseline sampling program. DFO-HPSD 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 three leases. 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 - HPSD 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. 3 syringes from 3 separate grab or cores 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 two centimeters of sediment only.**

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.**

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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