MINISTRY OF WATER, LAND AND AIR PROTECTION

Protocols for Marine Environmental Monitoring

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Introduction

To support the *Finfish Aquaculture Waste Control Regulation*, the Ministry of Water, Land and Air Protection (WLAP) has developed *Protocols for Marine Environment Monitoring.* These protocols will ensure that high quality data are collected, thereby leading to sound decisions as to whether environmental standards are being met. WLAP developed the protocols with assistance from various government agencies, consultants, literature reviews, and equipment manufacturers.

The protocols were developed with regard to the aquaculture industry. However, these protocols may also be relevant to monitoring or assessing impacts of other anthropogenic activities.

The protocols comprise 7 sections:

Section 1 lists acceptable types of current meters for generating data on currents at BC aquaculture operations. It also specifies the supporting information that monitoring agencies must submit.

Section 2 specifies the video equipment for completing video surveys (typically of hard-bottom sites) and outlines procedures for deploying the camera and generating acceptable quality video.

Section 3 describes materials and methods for soft-bottom sampling, including procedures for obtaining specific types of data.

Section 4 outlines the quality assurance/ quality control requirements for physical and chemical parameters and biological samples.

Sections 5 and 6 describe procedures for standardizing and calibrating field meters for sulphide and Eh measurements. The procedures are specific to ThermoOrion meters and probes, the most widely used brand. Other companies' meters and probes are acceptable, provided the standardization and calibration procedures provided by the manufacturer are followed.

Section 7 describes statistical tools for analysing sampling data from soft-bottom sites and the video from hard-bottom sites.

Appendix A summarizes the video survey requirements for baseline inventory monitoring.

Appendix B summarizes the sediment sampling requirements for both baseline inventory and operational monitoring.

Appendix C describes statistical procedures to be used for existing facilities and new facilities respectively.

Acronyms, Abbreviations, & Definitions

ANOSIM: analysis of similarities

ANOVA: analysis of variance

BACI: before-after-control-impact (study design)

Baseline monitoring: sampling conducted before operation of a finfish aquaculture facility

BCGS: British Columbia Geographic System

***Beggiatoa*:** a genus of bacteria that forms white mats on the sediment surface in areas of intense organic enrichment

***Capitella*:** a genus of polychaetes that thrives in areas of intense organic enrichment

CEAA: *Canadian Environmental Assessment Act*

Cu: copper concentration (expressed in µg/ g dry sediment)

DGPS: Differential Global Positioning System

DI: de-ionized

EDTA: ethylenediaminetetraacetic acid

E_h: redox potential (expressed in millivolts, mV)

Epifauna: animals that live on top of the substratum

ES_{crit}: critical effect size

H_A: alternate hypothesis

H_O: null hypothesis

Infauna: animals that live within the substratum

LWBC: Land & Water British Columbia Inc.

M: median

Macrofauna: animals with body sizes on the scale of millimetres

MAFF: BC Ministry of Agriculture, Fisheries and Food

MCI: multiple control/ impact (study design)

MDS: multi-dimensional scaling

Megafauna: animals with body sizes on the scale of centimetres

MLR: multiple linear regression

N: sample size

NAD: North American datum

NLR: non-linear regression

Operational monitoring: sampling conducted during operation of a finfish aquaculture facility and as outlined in Schedule B of the Finfish Aquaculture Waste Control Regulation

QA/ QC: quality assurance/ quality control

ROV: remotely operated vehicle

$S^{=}$: free sulfide concentration (expressed in micromolar, µM)

SAOB: sulphide anti-oxidant buffer

SD: standard deviation

SGS: sediment grain size

SLR: simple linear regression

TOC: total organic carbon (expressed in µg/ g dry sediment)

TVS: total volatile solids (expressed as a percentage)

WLAP: BC Ministry of Water, Land and Air Protection

$\bar{x}$ **:** sample mean

Zn: zinc concentration (expressed in µg/ g dry sediment)

1 – ß: power (of statistical test)

a: Type I error rate (significance level)

ß: Type II error rate

$ß_1$: rate of increase (non-linear) or slope (linear) of population regression line

µ: population mean

1. Currents Metering

Equipment

Electronic current meters capable of determining both speed and direction are available from several manufacturers (Aanderaa, Sontek, Nortek, RD Instruments, Applied Microsystems, InterOcean Systems, etc). We recommend a meter with an internal data-logger, which can be pre-set for the correct interval and record the results automatically. Both vector-averaging and instantaneous type meters are acceptable. Only experts should attempt to program and deploy these devices, or extract and process the collected data.

Procedures

1. Measure currents at 2 depths: approximately 15 m below the surface and approximately 5 m above the bottom.

2. Report current direction in degrees True (include magnetic north reading and correction factor) and speed in cm/ s.

3. Record current speed and direction at least once every 30 min over a period of at least 30 days.

4. At sites with infrastructure in place, locate the meter away from attenuation effects of any infrastructure and in line with the prevailing current direction. Moor the current meter approximately 30 m from the offshore side of the containment structure unless circumstances do not allow it.

5. At sites where infrastructure has not yet been installed, metering locations should represent currents within the tenure, especially near containment structures.

Reporting

This information must be included with both the raw data and data summaries:

1. Current meter moorings and deployment locations

Supply a diagram showing how the current meters were deployed within the tenure area. Include in the diagram:

- the type and position (surface or sub-surface) of the flotation devices used to support the current meters during deployment
- the distances between the current meter and the flotation device
- the type and weights of anchors used.

Also, show and describe any other components or instruments attached to the mooring apparatus (e.g. mechanical or acoustic releases).

Supply DGPS co-ordinates for the deployment locations and a written description of the locations (e.g. 30 m at 270º from the southwest corner of the containment structures), indicating the locations on a map. A 1:20,000 scale BCGS map is recommended, but equivalents are acceptable. Indicate whether the DGPS co-ordinates and maps are based on the NAD 27 or NAD 83 co-ordinate system.

2. Start date and time

Record the date and time that the current monitoring commenced (i.e. the individual date and time that each meter began to collect and record good-quality data of local currents). Indicate whether time is recorded as Pacific Standard Time (UTC-8) or Pacific Daylight Saving Time (UTC-7).

3. End date and time

Record the date and time that the current monitoring was terminated for each meter (i.e. the date and time the current meter collected its last good record of the currents before it was recovered). Clearly indicate whether time is recorded as above.

4. Instrument

Provide the make and model of the current meters used, including a copy of the manufacturer's specifications, and date of last calibration and servicing.

5. Number of data points

Report the actual number of instantaneous or average measurements recorded by the meter. If measurements are taken every 30 min, there will be approximately 1400 measurements in the monitoring period and therefore 1400 data points. This number assists in calculating averages.

6. Sample interval

Report the sample interval (min) between consecutive measurements made by the meter. A sample interval must be 30 min or less.

7. Data processing and reporting

Describe the data-processing methods and software used to correct and process the current meter data. Indicate whether the current direction is in degrees *True* (recommended) or degrees *magnetic*.

Indicate whether the current meter records average or instantaneous measurements, and describe the instrument's set-up or configuration. If the meter records average measurements, indicate the averaging interval.

Details should be provided in distinct sections of the report under the appropriate section headings or titles.

8. Depth of meter

Report the depth of the meter below the water surface or the distance of the meter from the bottom. The meter should be 15 m below the surface for surface-currents measurements and approximately 5 m above bottom for bottom-currents measurements.

9. Water depth

Report the water depth at the location of deployment.

10. Average current speed

Calculate the average current speed for the entire data-collection period (30 d). This should be calculated from the entire dataset, not from the summary data.

11. Contact names

Provide the name and contact information of the staff person or consulting company responsible for collecting and reporting the current measurements.

2. Video Surveys

Equipment

Acceptable vehicles for carrying video equipment include:

- An ROV
- A cable camera apparatus
- Scuba divers

Video equipment must meet these criteria:

- be capable of producing broadcast-quality images
- have supplemental light to increase clarity and maintain good colour balance
- have a reference object or superimposed image to show scale on the viewing screen in metres
- have an in-built DGPS unit or similar tracking device to define the transect or station being videotaped
- original video must be transferable to digital-format storage media.

Acceptable quadrat types include:

- A wire frame (1 × 1 m, with nine 33 × 33 cm sections) placed on the seabed
- A wire frame mounted on the cable camera or ROV
- A laser-delineated frame

Acceptable transect lines include:

- Brightly-coloured polypropylene ropes, weighted, and with flagging tape placed at regular intervals
- Brightly-coloured measuring tapes, weighted

Procedures

See Appendix A for summary of design information.

A. Baseline Monitoring

1. Survey several transects across the entire tenure, capable of mapping biophysical characteristics to a 50 m resolution.
2. Within the tenure, survey a minimum of one transect perpendicular to shore, starting at the shore and terminating at the opposite perimeter of the tenure to describe depth

variation. Surveys should encompass all area(s) of probable footprint(s) expected for future containment structures.

3. Survey a minimum of 2 transects at each of 2 reference stations, each 100 m long with one oriented perpendicular to shore.
4. Use a sufficient number of macrofauna quadrats to represent each substratum type.
5. Quadrats must measure 1 × 1 m, with nine 33 × 33 cm sections. Note that quadrat size must be the same at all stations.
6. Place at least 5 quadrats at each station.

B. Selection of Reference Stations

1. Locate stations within a range of 0.5 – 2.0 km from facility.
2. Locate stations at least 0.5 km apart.
3. Ensure that the mean depth is within 20% of the mean depth of the facility tenure.
4. Ensure that characteristics such as topography, angle of repose, current and tidal regimes, amount of freshwater run-off, etc. are similar to those at facility stations;
5. If facility stations are potentially influenced by other human activities (e.g. log dumping), seek reference stations that may be similarly influenced.

C. Deployment

1. Place transect line on bottom for camera or ROV to follow on a selected bearing.
2. Attach a small boat anchor and vertical line to one end of the transect line. The camera or ROV will use the vertical line as a guide to the transect line.
3. A DGPS reading must be taken at the beginning and end of each transect and at each quadrat. Readings associated with transects are to be taken when the transect line has been pulled taut.
4. If possible, deploy the camera during slack tide to minimize drifting.
5. Deploy the camera during daylight, when there is plenty of well-diffused light. Avoid taking videos at night or in extreme overcast conditions.
6. Keep the camera or ROV close enough to the bottom to provide optimum resolution of the bottom and never more than 1.5 m above the substratum.
7. Manoeuvre the camera or ROV at a maximum speed of 0.25 m/ s.
8. Position the transect line at the edge of the camera's field of view so that it focuses on substratum and not on the line.
9. In areas of extreme slope and or boulder complexes, move the camera from deeper to shallower water to ensure that the field of view includes the substratum.

D. Reporting

Submit data after filling out templates provided by WLAP. Provide either audio (i.e. voice dubbing) or text narration of the video. For each quadrat, describe the angle of repose as either horizontal, vertical, or oblique.

3. Sediment Sampling

Equipment

1. Acceptable sampling devices for chemical/ physical sampling include Petite Ponar, Ponar, Smith-MacIntyre, VanVeen or other appropriate equipment.

2. For biological sampling, use a Smith-MacIntyre, VanVeen, or other appropriate large-volume sediment sampling device with a 0.1 m^2 footprint.

3. Various probes and chemicals, described fully in Sections 4 & 5, are to be used.

Procedures

For a summary of sampling design, see Appendix B.

A. Baseline Monitoring

- Within each of the probable footprints, at least 3 grab samples must be taken for each sediment type. If only one sediment type predominates, at least 5 grab samples must be taken.

- 2 reference stations must be selected as described above for video surveys; at least 3 grabs must be taken at each reference station.

B. Operational Monitoring

- Ensure the transect is parallel to the predominant current direction.
- Use at least one transect for each dominant current direction or alternate design, provided extent and magnitude of effects is represented.
- Sample at least 3 stations on each transect: at perimeter of the containment structure, at 30 m from zero metre station, and at perimeter of tenure.

C. Selection of Reference Stations

- Sample at least 2 reference stations for each facility.
- Ensure the stations are within 0.5 – 2.0 km of the facility tenure, if possible.
- Ensure reference stations are at least 0.5 km apart, if possible.
- Ensure the mean depth is within 20% of the mean depth of facility stations.
- Ensure the SGS fractions are within 15% of the facility stations' SGS fractions.
- Ensure that characteristics such as topography, current and tidal regimes, amount of freshwater run-off, etc., are similar to that of the facility stations.
- If the facility stations appear to have been influenced by anthropogenic activity ensure that the reference stations have similar characteristics to that of the facility stations (e.g. log dumps).

D. Sampling Preparation

1. Prepare a sulphide stock solution and EDTA/ NaOH solution in advance. Note that:

- 10,000 µM sulphide stock solution is stable for up to 5 d, if it is kept cool with limited head space.
- EDTA/ NAOH solution is stable for up to 7 d, if kept cool.

2. Check tidal conditions. If possible, do not sample during maximum flood and ebb tides or strong wind conditions.

4. Obtain latitude/ longitude using DGPS, with a minimum accuracy of ± 5 m at each station.

5. When sampling on a transect, use polypropylene rope pre-marked in metre increments to ensure accurate measurements.

6. When sampling on a transect, note bearing. Report the true-north bearing as well as the magnetic north reading and correction factor.

7. Report water depths in metres.

8. Check the Eh electrode against standard (re-check every 4 hr and when recalibrating the sulphide meter).

9. Calibrate the sulphide electrode, and recalibrate it at least every 3 - 4 hr.

10. Drift: before recalibration, and hourly during sampling or at a minimum at the completion of each sampling station, check and record the drift by measuring the sulphide concentration against each of the standard concentrations originally used to calibrate the electrode. Always use fresh standards (1000, 100, 10 µM) by serial dilution from the stock solution when recalibrating or checking drift. Do not attempt to correct the data for any observed drift. A drift of up to 20% is acceptable.

E. Collect and describe samples

1. Deploy and retrieve sampling device at a maximum rate of 0.3 m/ sec. Rinse all equipment with ambient seawater between grab deployments. Take care that 2nd and 3rd grab samples are not taken from the crater formed by the first grab sample. Typically, this is only of concern at the when the sampling vessel is moored at the edge of the containment structure.

2. Check for these indicators of an acceptable sample:

   - overlying water present – indicating minimal leakage
   - overlying water not excessively turbid – indicating minimal sample disturbance
   - sediment surface relatively flat – indicating minimal sample disturbance or washing
   - desired penetration depth achieved – at least 4 to 5 cm for characterizing surficial sediments
   - overfilled sampling device – if occurring routinely some or all of the detachable weight might have to be removed

3. Do not make more than 4 deployments of the grab to obtain a suitable sample. If unsuccessful, provide a video of the station as an alternative (see Section 2)

4. Siphon the overlying water from the sample. Retain it for sieving if biological samples required.

5. Examine the sediment sample and record the following:

- Sediment texture, colour, odour, presence/absence of gas bubbles, *Beggiatoa*, fish feed, fish feces, flocculent organic material, macrophytes, terrigenous material, and farm litter

6. Take a colour photo of the sample or score sediment colour by comparing with colour charts

7. Record the penetration depth of the sampler in centimetres.

F. Measure $S^{=}$ and E_h levels

1. Extract 2 sub-samples by removing the top 2 centimetres of sediment from the centre of each side of the sampling device. Limit the volume of each sub-sample to what is needed for the required tests as summarised in Appendix B (i.e. 50 mL required for Eh potential and sulphide concentration). Place the 2 sub-samples in a suitable container and homogenize by gently stirring with a flat tipped steel spatula. Sulphide and Eh analyses must done within 60 min of sampling to avoid sample degradation. Wear gloves if you will be touching the sediment.

2. Measure sulphide:

   a. Rinse the electrode with distilled water and blot it dry. Then insert it into sample.

   b. When the initial sample is obtained and accepted, add 8.75 mg L ascorbic acid to 250 mL of previously prepared EDTA/ NaOH buffer and thoroughly mix to create SAOB buffer. (Various amounts of SAOB can be made, provided the 8.75 g L ascorbic acid: 250 mL EDTA/ NaOH ratio is maintained).

   c. Combine equal volumes of sediment and SAOB in a suitable container (5 mL of each is typically sufficient for this analysis). The sediment from the sample can extracted using a cut-off syringe or spatula. Do not include material more than 0.5 cm in diameter.

   d. Homogenize the mixture with a spatula.

   e. Insert the sulphide electrode into the solution and gently swirl it until meter reads **READY** (typically 2 – 5 min).

   f. Gently wipe the probe before the next sample. If an oily residue is observed on the probe, wash it with detergent before taking another sample.

3. Obtain an Eh measurement. Insert the probe into the homogenized sample described above, and wait until either the meter reads **READY** or the drift is 3 mV or less over a 2 sec period. Gently wipe excess sediment from the probe between sample measurements.

4. Record the temperature in the sediment sample.

5. Correct the Eh measurements by using sediment temperature and the correction factor for the filling solution in the probe supplied by the manufacturer.

6. Perform any additional measurement or analysis using the sediment sub-samples collected in Section C, step 1. Do not collect additional sub-samples for these analyses. Remove all unrepresentative material (e.g. shells, large worms, wood waste, rock) before filling the sampling receptacle See Appendix A for sample frequency and location.

7. Store all laboratory samples at 4º Celsius.

G. Biological Sampling

1. When collecting biological samples, scrape and rinse sediments from the grab into pre-cleaned containers. Save the rinse water* for infaunal sampling.

2. When sieving biological samples in the field:

   - Sieve each sediment sample, associated overlying water and rinse water through a 1.0 mm screen. Count, identify and record megafauna (e.g. large cnidarians, echinoderms and tube worms) then return them to the sea. Photograph specimens that need to be identified by taxonomists. Fix the remaining organisms in 10% buffered formalin. After 4 d, rinse over 0.5 mm screen and preserve in 70% isopropyl alcohol or ethyl alcohol.
   - Retain all coarse gravel and cobble less than 2.5 cm in diameter.
   - Remove epifauna adhering to rocks and other material that is greater than 2.5 cm diameter and include them in sieved sample.

   *Prior to use in sieving biological samples, rinse water must be filtered through a minimum 250 µm screen

3. For samples not sieved during the day they were obtained in the field use a 10% buffered formalin solution for preservation.

Reporting

Submit monitoring data by filling out templates supplied by WLAP.

4. Checking the Quality of Sediment Samples

Physical and Chemical QA/ QC

Free Sulfides

Take an additional sulphide measurement once every 20 samples, or once per batch if fewer than 20 samples are taken. See Section 7 for statistical approach to QA/QC.

Redox Potential

Take a triplicate measurement of E_h once every 20 samples, or once per batch if fewer than 20 samples are taken. See Section 7 for statistical approach to sulphide QA/QC.

TVS/ SGS/ Other Parameters

Obtain additional sediment from 1 of every 20 sub-samples, or once per batch if fewer than 20 samples are taken, and have duplicate analyses of required parameters completed.

Biological QA/ QC

There are 2 options for QA/ QC on biological samples:

1. Have certified facility staff submit QA samples to an expert contract taxonomist. The taxonomist's lab must have its own QA/ QC program.

2. Have certified facility staff complete the taxonomy on site. For every 10 grab samples, they must take an additional grab sample, screen it, and split it into 2 samples. They count and identify one of the samples themselves, while submitting the other one to a recognized lab for the same procedure.

   The results obtained by facility staff should match the lab results at a similarity level of at least 70%. Results from the contract lab must be reported to WLAP directly.

   The facility staff must be certified by an educational institute recognized for expertise in taxonomy. Staff must be certified in taxonomic identification of benthic organisms to the family level.

   Samples used by both the facility staff and the labs must be preserved and stored for a minimum of 5 years.

Reporting

Submit data by filling out templates supplied by WLAP.

5. Calibrating the Sulphide Electrode

A sulphide electrode should be calibrated before each sampling session, and recalibrated every 3 - 4 hr during the session.

Materials

- *Orion* model 290A meter and 9616BN silver/ sulphide electrode (*Accumet* and other brands are also acceptable.)
- Electrolyte Solution: Ag/ Cl reference electrode filling solution *Optimum Results A*
- Prepared solutions:

Solution	Preparation frequency
Sulphide Anti-Oxidant Buffer (SAOB)	every 3 to 4 hr
stock $S^{=}$ solution 10,000 µM (10^{-2} M Na_2S)	every 5 d
standard $S^{=}$ solution 1,000 µM (10^{-3} M Na_2S):	every 3 to 4 hr
standard $S^{=}$ solution 100 µM (10^{-4} M Na_2S)	every 3 to 4 hr
standard $S^{=}$ solution 10 µM (10^{-5} M Na_2S) use **only** for a 3- point calibration of sediment samples with low sulphide concentrations	every 3 to 4 hr
final calibration solutions	immediately before calibration

Preparing Solutions

A. Sulphide Anti-Oxidant Buffer (SAOB)

1. Materials

- 20.00 g NaOH (sodium hydroxide)
- 17.9g EDTA
- 8.75 g L-ascorbic acid
- de-aerated DI or distilled water.

2. Procedures

- In a 250 mL plastic screw top jar, mix the NaOH with the EDTA and dilute it to 250 mL with de-aerated DI or distilled water. This solution is stable for up to 7 d. (Larger or smaller volumes can be made up provided the ratios are maintained).
- Do not add the L-ascorbic acid to EDTA/ NaOH solution until just before sample analysis, since the solution is stable for only 4 hr after adding the L-ascorbic acid. Store SAOB buffer in the dark at 4°C.

- Once SAOB is added to a sediment sample or sulphide standard, take measurements within 30 min.

B. Stock $S^=$ solution 10,000 mM (10^{-2} M Na_2S)

1. Materials

- 0.2402 g Na_2S*9H_20 (pre-weighed and stored under nitrogen)
- de-aerated DI or distilled water

2. Procedures

- In a well-ventilated area, add 0.2402 g Na_2S*9H_20 to a volumetric flask and dilute to 100 mL using de-aerated DI or distilled water.
- Store this stock solution in an airtight dark glass bottle at 4°C. Provided the head space is minimized, this stock solution is stable for up to 5 d.

C. Standard $S^=$ solution 1,000 mM (10^{-3} M Na_2S)

1. Materials

- stock solution
- de-aerated DI or distilled water

2. Procedure

- In a 100 mL volumetric flask, pipette 10 mL of the stock solution and dilute to 100 mL using de-aerated DI or distilled water. Store in an airtight dark glass bottle at 4°C.

D. Standard $S^=$ solution 100 mM (10^{-4} M Na_2S)

1. Materials

- 1,000 µM solution
- de-aerated DI or distilled water

2. Procedure

- In a 100 mL volumetric flask, pipette 10 mL of the 1,000 µM solution and dilute to 100 mL using de-aerated DI or distilled water. Store in an airtight dark glass bottle at 4 °C.

E. Standard $S^=$ solution 10 mM (10^{-5} M Na_2S)

This solution is to be used only for a 3-point calibration for sediment samples with low sulphide concentrations.

1. Materials

- 100 µM solution
- de-aerated DI or distilled water

2. Procedure:

- In a 100 mL volumetric flask, pipette 10 mL of 100 µM solution and dilute to 100 mL using de-aerated DI or distilled water. Store in an airtight dark glass bottle at 4°C.

F. Final Calibration Solutions

1. Materials

- 25 mL SAOB buffer (containing L-ascorbic acid)
- 25 mL Stock $S^{=}$ solution 10,000 µM
- 25 mL Standard $S^{=}$ solution 1,000 µM
- 25 mL Standard $S^{=}$ 100 µM

2. Procedures

- Mix 25 mL of SAOB buffer and 25 mL Stock S= solution (10,000 µM) in a dark plastic 100 mL wide mouth bottle at 4°C.
- Repeat preceding step for 1,000 µM solution and 100 µM (or 10 µM concentration as necessary).
- Ensure that all calibration solutions are at the same temperature as the sediments being measured.

Procedures

The sulphide electrode should be recalibrated between each set of samples or once every 3-4 hr, whichever is less.

A. Prepare the probe for calibration

1. Remove the cover from the electrode and connect the electrode to the meter.

2. Check the level of the probe's filling solution, which should almost reach the filler hole. Add more solution if necessary. After filling a dry probe or topping up a low solution level, press down on the cap to wet the bottom O-ring and then tilt the container back to wet the top O-ring. Then add more solution to ensure the level almost reaches the filler hole.

B. Calibrate the probe for each standard solution

Calibrating the probe against 3 standard solutions (a 3-point calibration) is recommended. Start with the least concentrated standard (10 µM or 100 µM) and progress to the most concentrated standard (1,000 µM or 10,000 µM). Select standards with concentration ranges that bracket the expected sulphide concentration of the samples

1. Press the **mode** button until the display indicates concentration mode.

2. Place the electrode in the lowest standard and until the meter reading stabilizes before beginning calibration process.

3. Press the second function button and then the **calibrate** button. After a few seconds the lower field will read **P1**, indicating that the meter is ready for calibrating the first standard.

4. Press up arrow and **0.000** will appear.

5. Press up arrow again and decimal point will flash.

6. Press the up or down arrow keys to move the decimal place to the position you want (3 decimal places to the right for 100 µM and off the screen for 1,000 µM and 10,000 µM). Press **YES** when the decimal is in the correct position.

7. The first digit on the left will flash. Use the up or down arrow keys to change the digit, and press **YES** when the correct digit is displayed. To select 1,000 µM, press up or down arrow until first digit disappears, the press **YES**; for 10,000 µM press up or down arrow until **1** appears, then press **YES**. The next digit will then flash. Repeat the sequence for all digits until the readout displays the correct standard.

   Once you have entered the first standard, the lower field will indicate the next standard to calibrate (e.g. **P2** = second standard).

8. Rinse the electrode.

9. Repeat steps 4-7 until the 3 standards have been entered which completes the calibration.

C. Check the calibration

1. Press **MEASURE.** The meter will display the slope.

2. If the slope is between -27 and -33 record it on the data sheet. If the slope is outside this range, check the standards and calibrate the meter again.

3. Following calibration, rinse the electrode with DI or distilled water and blot it dry before measuring the first sample.

4. After taking the last sample in a series, rinse the electrode with distilled water and store in distilled water for short periods (up to one week). For longer periods, store it dry.

6. Standardizing the Redox Electrode

Materials

- *Orion* model 290A meter and 9678BN combination redox electrode (*Accumet* or other brands will work as well).
- electrolyte solution: Ag/ AgCl (silver chloride) reference electrode filling solution 900011 (correction factor at 20C is 204 mV).
- redox standard (an *Orion* off-the-shelf standard triiodide/ iodide redox couple or other standards recommended by the manufacturer)

Procedures

Standardization should occur every 4 hr or at the beginning and end of each transect:

1. Remove the cover from the electrode and connect the electrode to the meter.

2. Check the level of the filling solution. If necessary, add solution until it is at least one inch above the level of the solution being measured. After filling a previously dry probe or topping up the probe, push the cap and body together to leak some filling solution past the conical reference junction.

3. Place the electrode in the standard solution and wait until the reading stabilizes. Record the value of the standard and the meter reading at each standardization (for the triiodide/ iodide redox couple standard, the meter should read 220 mV – the potential of this standard solution).

4. The electrode is now ready for sampling. Insert it into the sample and record the mV reading after stabilization (when meter flashes **READY** or when drift is 3 mV or less over a 2 second period). Remove electrode and gently wipe off excess sediment prior to next measurement.

   Note: This raw data is uncorrected. To correct the data, follow the procedure in *Sediment Sampling: SECTION 3* F(5).

5. After use, rinse the electrode in DI or distilled water and store for short periods (a few weeks) in tap water. For longer periods, drain the electrode, rinse it in DI or distilled water, and store dry.

7. Performing Statistical Analyses

Preparations for Statistical Analyses

These procedures must be followed before the data are analyzed using inferential statistics.

A. Validate sampling stations

1. Validate reference stations

a. Confirm that there is more than one local reference station per facility.

b. Confirm that reference stations are 0.5-2.0 km from the facility.

c. Confirm that reference stations are at least 0.5 km apart.

d. Confirm that mean depth at local reference stations is within 25% of that at facility stations.

e. Confirm that mean % silt/ clay fraction at local reference stations is within 15% of that at facility stations.

2. Validate transects

a. Confirm that transects were laid along prevailing currents. Current meter directions should be within 20^{o} of transect directions.

B. Correct data entry errors

1. Identify dubious values (e.g. outliers)

a. Draw graphs (e.g. box plots, scatter plots)

b. Calculate summary statistics (e.g. $\bar{x}$, M, SD, max, min)

2. Make corrections

a. Contact data collectors for corrections

b. Make necessary changes

C. Perform QA/QC for physical, chemical, & biological data

1. Check SGS data

Confirm that 35% Relative Standard Difference has not been exceeded

2. Check $S^{=}$ and E_h data

Plot one variable against the other and look for outliers

3. Check TVS or TOC data

Confirm that 20% Relative Percent Difference has not been exceeded

4. Check Cu and Zn data

(to be added)

5. Check taxonomic data

Confirm Similarity is at least 70%. See Appendix C for summary of tests.

Statistical Methods to Determine If Requirements Have Been Met

These study designs and statistical tests are to be used to determine whether facilities are meeting chemical and biological requirements. See Appendix C for summary of tests.

A. Basic Study Designs

1. Existing facilities

2 basic designs are employed:

a. MCI design – data are collected at facility stations and compared against data collected at reference stations

b. Multiple Gradient design – data are collected at stations along multiple transects extending outward from the facility along prevailing currents, and related to distance from the facility

2. New facilities

Beyond BACI design – data are collected at facility stations and reference stations, in both baseline and operational periods, to see if effect of facility/reference depends on baseline/operational. Ideally there are multiple sampling times in both periods.

B. Meeting Chemical Requirements

1. Existing facilities

For stations located at or beyond the 30 m stations but within the tenure perimeter, first determine whether there has been a $S^{=}$ exceedance at any of these stations. Do this for each station by testing these hypotheses using a 1-sample t-test:

$H_O: \mu \leq 1300$ µM; $H_A: \mu > 1300$ µM (1-tailed)

$H_O: \mu \leq 6000$ µM; $H_A: \mu > 6000$ µM (1-tailed)

If there is evidence for an exceedance at a particular station, do analyses below to determine whether exceedance is due to fish farming or natural processes.

a. MCI design

For each station, perform Nested 1-way ANOVA to test this hypothesis:

$H_O: \mu_F \leq \mu_R$; $H_A: \mu_F > \mu_R$ (1-tailed) (F = facility, R = reference)

If the facility station mean is significantly greater than the reference station mean, there is evidence that the exceedance is due to fish farming, and the requirement has not been met.

Note that the above test is the same as a 2-sample t-test when design is balanced.

Note also that this analysis may be superfluous if facility values are far above reference values.

b. Multiple Gradient design

Perform NLR, SLR, or MLR, depending on relationships. For example, perform NLR to test this hypothesis:

H_O: $ß_1 \geq 0$; H_A: $ß_1 < 0$ (1-tailed)

If there is a significant non-linear decline outward from facility, then there is supporting evidence that the requirement has not been met.

Alternatively, use a *post hoc* test (e.g. Tukey's test), to make all possible pair-wise comparisons of distances, if more than one sample was taken at each distance. As above, a declining pattern provides supporting evidence that the requirement has not been met.

Again, note that these analyses may be superfluous if facility values are far above reference values.

For stations located at or beyond the perimeter of the tenure, do the above analyses without first testing for an exceedance.

2. New facilities

For stations located at or beyond the 30 m stations but within the tenure perimeter, first determine whether there has been a $S^=$ exceedance at any of these stations using a 1-sample t-test as described above.

If there is evidence for an exceedance at a particular station, do analysis below to determine whether exceedance is due to fish farming or natural processes.

a. Beyond BACI design

For each station, perform Asymmetric ANOVA to test the following hypotheses

H_O: There is no interaction between facility/reference and baseline/operational; H_A: there is an interaction (2-tailed).

If there is a significant interaction, there is evidence that the exceedance is due to fish farming, and the requirement has not been met.

Note that this analysis may be superfluous if facility values are far above reference or baseline values.

For stations located at or beyond the perimeter of the tenure, do the above analyses without first testing for an exceedance.

C. Meeting Biological Requirements

If a chemical requirement has not been met at a particular facility station, then biological analyses may be required for that station. Follow the methods below if biological analyses are required.

Use taxon richness (total number of taxa) as the measure of diversity to be analyzed, and total number of individuals or total percent cover, as measures of total abundance to be analyzed.

Data on biota from soft bottoms will be at the family, whereas those from hard substrata will be at class level.

1. Existing facilities

a. MCI design

For each station located at or beyond the 30 m stations but within the tenure perimeter, perform Nested 1-way ANOVA to test these hypotheses:

H_O: $\mu_F \geq \mu_R$; H_A: $\mu_F < \mu_R$ (1-tailed) (F = facility, R = reference)

Note that the above test is the same as a 2-sample t-test when design is balanced.

Report the results of these analyses in accordance with Section 6 (2)b of the Finfish Aquaculture Waste Control Regulation.

For stations located at or beyond the perimeter of the tenure, do a 1-way Nested ANOVA to test these hypotheses:

H_O: $\mu_F = \mu_R$; H_A: $\mu_F \neq \mu_R$ (2-tailed) (F = facility, R = reference)

If mean richness or mean total abundance at a particular station differs significantly from that at reference stations, then the requirement has not been met.

b. Multiple Gradient design

For each station located at or beyond the 30 m stations but within the tenure perimeter, perform NLR, SLR, or MLR, depending on relationships.

For example, perform NLR to test these hypotheses:

H_O: $ß_1 \leq 0$; H_A: $ß_1 > 0$ (1-tailed)

Report the results of these analyses in accordance with Section 6 (2)b of the Finfish Aquaculture Waste Control Regulation and indicate if there is a significant non-linear increase in richness or abundance outward from the facility.

Alternatively, use a *post hoc* test (e.g. Tukey's test), to make all possible pair-wise comparisons of distances, if more than one sample was taken at each distance. Again, report the results of these analyses in accordance with Section 6 (2)b of the Finfish Aquaculture Waste Control Regulation and indicate any increases.

For stations located at or beyond the perimeter of the tenure, do above analyses, except hypotheses should always be 2-tailed. If there are significant increases then the requirement has not been met.

2. New facilities

a. Beyond BACI design

For stations located at or beyond the 30 m stations but within the tenure perimeter, perform Asymmetric ANOVA to test these hypotheses for each station:

H_O: There is no interaction between facility/reference and baseline/operational; H_A: there is an interaction (2-tailed).

Report the results of the analysis for both richness and abundance in accordance with Section 6 (2)b of the Finfish Aquaculture Waste Control Regulation and indicate if there are significant interactions.

For stations located at or beyond the perimeter of the tenure, perform Asymmetric ANOVA to test the same hypotheses. If there is a significant interaction for either the richness analysis or the abundance analysis, then the requirement has not been met.

D. Analyses of Additional Variables

In addition to the analyses described above, analyses of other physical, chemical, and biological variables may also be done in a weight-of-evidence approach to determine whether requirements have been met.

Use contingency table analyses or ANOVA to determine whether gas bubbles, strong odours, black sediments, etc., are more common at facility stations than reference stations.

Use ANOVA and regression methods to analyze E_h, TVS, or TOC data, as was done above with $S^{=}$, to test for differences and trends.

Use contingency table analyses or ANOVA to determine if *Beggiatoa* or *Capitella* are more common at facility stations than reference stations.

In addition to the analyses of means described above, analyses of standard deviations may prove useful in further defining the nature of fish farm effects.

Use multivariate methods (e.g. MDS, ANOSIM) to determine whether community composition has been altered.

Statistical Power Analyses

A. Raising Statistical Power

Due to the variable nature of the data and small sample sizes, statistical power will often be low. Power can be increased by doing the following:

1. Increasing N

a. 1-sample t-tests

Increase number of grabs or quadrats per facility station.

b. MCI designs

For ANOVAs, increase number of reference stations by "borrowing" reference stations from facilities in the same geographic region, or from facilities coast-wide.

Alternatively, include nearby facilities belonging to the same company in same geographic region at similar stage of production cycle, along with their reference stations, in analyses.

Note that including additional reference stations and facilities in analyses will have a much greater effect on power than increasing number of grabs or quadrats at each station.

c. Multiple Gradient designs

For regressions, increase number of stations sampled per transect, or increase number of grabs or quadrats per station.

For *post hoc* tests, increase number of grabs or quadrats per station.

2. Increasing α

For all tests, consider using the more precautionary $\alpha = 0.10$ instead of the usual 0.05.

B. Estimating Desired Sample Sizes

To determine sample sizes (numbers of transects, stations, grabs, quadrats) needed to achieve desired statistical power for future monitoring, power calculations must be done.

$1 - \beta \geq 80\%$ is recommended.

For both Multiple Gradient designs and MCI designs, power calculations will be based on ES_{crit} values decided upon by the investigator.

When doing any power calculations, consider using the more precautionary $\alpha = 0.10$ instead of the usual 0.05, to raise power.

Appendix A: Design of Video Survey

Baseline Monitoring

Parameter	Sampling Units	Sampling Locations	Spatial Scale	Min # Replicates
Class richness and abundance of megafauna	Transects	Across entire tenure Reference stations	Length/width of tenure At least 100 m long	Enough to ID biophysical characteristics to 50 m resolution 2 at each station*
Class richness and abundance of macrofauna	Quadrats	Entire tenure reference station	1 x 1 m, with nine 33 x 33 cm sections As above	Enough to represent each substratum type 5 at each station

Notes

*1 transect runs perpendicular to shore.

Appendix B: Design of Sediment Sampling

Baseline Monitoring

Parameter	Sampling Units	Spatial Scale	Sampling Locations	Min # Grabs
$S^{=}$	Petite-Ponar, Ponar, Smith-MacIntyre, or van Veen grab, etc.	Any size	all stations	3 grabs per sediment type for each probable footprint. Minimum of 5 grabs if only 1 sediment type present.
E_h	Petite-Ponar, Ponar, Smith-MacIntyre, or van Veen grab, etc.	Any size	all stations	3 grabs per sediment type for each probable footprint. Minimum of 5 grabs if only 1 sediment type present.
TVS or TOC	Petite-Ponar, Ponar, Smith-MacIntyre, or van Veen grab, etc.	Any size	all stations	3 grabs per sediment type for each probable footprint. Minimum of 5 grabs if only 1 sediment type present.
SGS	Petite-Ponar, Ponar, Smith-MacIntyre, or van Veen grab, etc.	Any size	all stations	3 grabs per sediment type for each probable footprint. Minimum of 5 grabs if only 1 sediment type present.
Cu or Zn	Petite-Ponar, Ponar, Smith-MacIntyre, or van Veen grab, etc.	Any size	all stations	3 grabs per sediment type for each probable footprint. Minimum of 5 grabs if only 1 sediment type present.
Species richness and abundance of infauna and epifauna	Smith-MacIntyre, or van Veen grab, etc.	0.1 m^2	all stations	3 grabs per sediment type for each probable footprint. Minimum of 5 grabs if only 1 sediment type present.

Operational Monitoring

Parameter	Sampling Units	Spatial Scale	Sampling Locations	Min # Samples
$S^=$	Petite-Ponar, Ponar, Smith-MacIntyre, or van Veen grab, etc.	Any size	All stations (see Notes: below)	3 grabs at all stations**
E_h	Petite-Ponar, Ponar, Smith-MacIntyre, or van Veen grab, etc.	Any size	All stations (see Notes: below)	3 grabs at all stations**
TVS or TOC	Petite-Ponar, Ponar, Smith-MacIntyre, or van Veen grab, etc.	Any size	Only at stations at perimeter of c.s. and reference stations	3 grabs at each station located at perimeter of c.s., and 3 at each reference station
SGS	Petite-Ponar, Ponar, Smith-MacIntyre, or van Veen grab, etc.	Any size	Only at stations at perimeter of c.s. and reference stations	1 grab at each station located at perimeter of c.s., and 1 at each reference station
Cu or Zn	Petite-Ponar, Ponar, Smith-MacIntyre, or van Veen grab, etc.	Any size	Only at stations at perimeter of c.s. and reference stations	3 grabs at each station located at perimeter of c.s., and 3 at each reference station
Family richness and abundance of infauna and epifauna*	Smith-MacIntyre, or van Veen grab, etc.	0.1 m^2	All stations	5 grabs at each station, except 3 at each reference station

*Biological sampling will only occur if a $S^=$ requirement has not been met.

**If the mean of the $S^=$ measurements from the 3 grabs exceeds 1300 µM, an additional 2 grabs must be obtained from that station for $S^=$ and Eh.

Notes:

- Sampling station locations are: perimeter of containment structures; 30 m from zero metre station; perimeter of tenure; and reference stations.
- Within tenure, have at least one transect for each dominant current direction or an alternate study design, provided extent and magnitude of effects are represented.

Appendix C: Statistical Procedures

Main statistical methods for different facility station locations, and different variables. Information for richness and abundance applies to both soft and hard bottom sites. Note that a 1-way Nested ANOVA is equivalent to a 2-sample t-test when design is balanced.

A. Existing Facilities

	Free Sulphides	**Richness & Abundance**
Within Tenure	Do 1-sample t-test (1-tailed), then if necessary, do 1-way Nested ANOVA (1-tailed)	Do 1-way Nested ANOVA (1-tailed)
Tenure Perimeter	Do 1-way Nested ANOVA (1-tailed)	Do 1-way Nested ANOVA (2-tailed)

B. New Facilities

	Free Sulphides	**Richness & Abundance**
Within Tenure	Do 1-sample t-test (1-tailed), then if necessary, do Asymmetric ANOVA (2-tailed)	Do Asymmetric ANOVA (2-tailed)
Tenure Perimeter	Do Asymmetric ANOVA (2-tailed)	Do Asymmetric ANOVA (2-tailed)

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