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Monitoring methods to support area-based bivalve aquaculture management in the Pacific region

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Foreword

This series documents the scientific basis for the evaluation of aquatic resources and ecosystems in Canada. As such, it addresses the issues of the day in the time frames required and the documents it contains are not intended as definitive statements on the subjects addressed but rather as progress reports on ongoing investigations.

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ABSTRACT

The Pacific Shellfish Aquaculture Management Division (AMD) of Fisheries and Oceans Canada (DFO) requested recommendations regarding monitoring methodologies along with associated field and laboratory protocols that can be used by regulatory, industry and science personnel when carrying out environmental assessments. The sampling methods put forward in this report are intended to support a wide variety of approaches ranging from general area-based monitoring programs or local emerging issues associated with a significant knowledge gap. A suite of environmental variables that support bivalve aquaculture assessments was selected based on the following: 1) recommendations arising from government advisory processes and/or the scientific community; and 2) the ability of the indicator to detect potential shifts in ecosystem conditions and processes. The benthic variables selected include sediment texture, geochemical (e.g. organic, redox), macrofaunal, meiofaunal, and epifaunal attributes, while pelagic variables consist of both physical (temperature, salinity, dissolved oxygen, light) and biotic characteristics (phytoplankton, zooplankton). Relevant bivalve attributes include cultured and wild density, diversity, and condition indices. The pelagic and bivalve indicators represent a nutrient-seston-plankton-bivalve loop that can support a high-resolution, spatially-explicit, hydrodynamic-biogeochemical coupled model capable of evaluating ecological bivalve carrying capacity.

1. INTRODUCTION

In British Columbia (B.C.), bivalve aquaculture is located primarily on the west coast of Vancouver Island and in the Strait of Georgia, with the most productive sites associated with Baynes Sound, Cortez Island, and Okeover Inlet. Both carrying capacity assessments and potential management thresholds of indicators are site specific, reflecting the relevance of bay-scale hydrodynamics, sedimentary, and biological, characteristics on ecosystem function (Cranford et al. 2012; Filgueira et al. 2015b). Indices based on the comparison of key oceanographic and biological processes have been used as proxies for the carrying capacity of bivalve aquaculture sites (Filgueira et al. 2014). These indices (e.g. nutrients, plankton, and shellfish) compare the energy demand of bivalve populations (based on filtration rates) and the ecosystem's capacity to replenish these resources. Additionally, monitoring methodologies associated with potential carrying capacity indicators can provide a baseline for future ecosystem monitoring programs. Based on the information collected on long-term monitoring programs, regulatory management thresholds for ecological indicators can be established.

Monitoring methods and environmental sampling variables outlined in this technical report were adopted from: 1) regulatory monitoring programs associated with marine finfish aquaculture, Aquaculture Activity Regulation (AAR, 2019); 2) DFO Canadian Science Advice Secretariat (CSAS) processes (Wildish et al. 2005; Cranford et al. 2006); and 3) peer-reviewed research publications (e.g. Benthic methods: Sutherland et al. 2007a,c; 2016b; 2019a,b; Pelagic methods: Cranford et al. 2012; Filgueira et al. 2013, 2014). This technical report specifically addresses the needs of AMD as part of a CSAS request: "Recommend monitoring methodologies including field and laboratory protocols for use by regulatory, industry, and science personnel. Recommend indicators and identify/describe known associated changes to shellfish" (AMD, 2013). This handbook-style document will be divided into three sections pertaining to different habitat regimes: 1) Benthic soft-substrates: (sediment texture, organics, sulfide/redox, trace-elements, fauna, bivalve, and eelgrass); 2) Benthic hard-substrates (video/camera surveys); and 3) Pelagic setting (water properties, plankton, nutrients). Following a description of monitoring devices compatible with each habitat regime, each ecosystem variable will be characterized according to the following format: 1) literature review establishing monitoring relevance of each variable/indicator; 2) Management threshold where applicable; 3) field collection protocols; and 4) laboratory analysis.

A review of available bivalve carrying capacity models has been carried out as part of a DFO Gulf-Region CSAS process (Filgueira et al. 2015a), where a high-resolution, spatially-explicit model consisting of a coupled hydrodynamic-biochemical model was identified as the most efficient approach to assess ecological carrying capacity of bivalve aquaculture. Such an endeavour, is currently being undertaken in Baynes Sound (B.C.), which involves the coupling of hydrodynamic (Finite Volume Community Ocean Model (FVCOM)), biogeochemical (Bivalve Culture Ecosystem Model (BiCEM)), and bivalve (Dynamic Energy Model (DEB)) models.

2. BENTHIC SOFT-SUBSTRATE MONITORING METHODS

2.1. MONITORING DEVICES

2.1.1. GRAB DEPLOYMENT

Grab samplers including Van Veen, Ponar and Smith-McIntyre (Figure 1) can be deployed based on substrate suitability and the ability to penetrate the seabed (Lie and Pamatmat, 1965; Eleftheriou and Moore, 2005; Gage and Bett, 2007). In order to collect a suite of environmental monitoring variables from the same grab, the minimum grab surface area at the top end of the grab should be 0.1 m^2 (Sutherland et al. 2007a,c). Grabs are typically made up of 2 steel buckets or “jaws” hinged together and propped open in order to dig into and “bite” the seafloor upon impact. Subsequently, the grab automatically collects a sediment sample when the jaws close together upon withdrawal from the substrate. Undisturbed sediment samples can be collected from grabs that have a large surface area and a strong closing mechanism. The grab lid should be equipped with flap-doors that can be opened upon retrieval to access the sediment-water interface for sub-sample collection. During the descent to the seafloor, the winch wire angle should maintain a vertical position to permit vertical penetration and obtain an approximate GPS coordinate of the sediment sample at the seafloor. A grab table is used to secure the upright position of the grab on the boat deck to avoid tipping or shaking that may result in sample disturbance upon retrieval.

The integrity of the sediment sample should be ascertained prior to sampling. The grab should be rejected under the following conditions: 1) Cracks in the sediment column leading to loss of pore-water and fine grained material (e.g. organic matter, clays); 2) slumping of sediment; and 3) washout of sediment surface due to sloshing of overlying water during ascent. Sediment surface subsamples should be collected from the uppermost 2 cm of the sediment column immediately after the majority of overlying water is gently drained from the grab. The use of a pump and hose system with a high flow velocity may disturb the sediment surface and remove light organic material from the surface. Gravity-driven suction-hoses tend to have an intermediate flow that can avoid disturbance of the sediment surface. A few millimetres of water should be retained on the sediment surface to prevent desiccation of sediment and loss of porewater containing important environmental variables (e.g. organic content, pore-water sulfide concentrations). Surface sediments are emphasized since they 1) reflect recent changes in sedimentation patterns and relative inputs from riverine terrestrial and anthropogenic sources; 2) represent the active zone for metal accumulation via sedimentation and diagenetic processes; 3) host the bulk of micro-phytobenthos (microalgae) and meiofaunal communities within the sediment column (Dessai, 2008; Steyaert et al. 2007; Sutherland et al. 2007a,c; 2018b; Warwick 1981).

Figure 1: Smith-McIntyre grab resting on a table chute.

2.1.2. CORE DEPLOYMENT

While gravity cores can be deployed on well-sorted mud and some mixed sediments, sediment core collection on coarse sand texture classifications is not recommended (Figure 2). Insufficient clay content results in low cohesion and poor binding capability, often resulting in the loss of sediment during core ascent to the water surface. Further, coring systems equipped with a lander-style frames designed to stabilize a core in a vertical position (e.g. vibra-core, slow-core) are not compatible with steeply sloped substrates that are common to the B.C. coastline. Overall, grab deployments are favoured over coring methods due to: 1) a relatively larger surface area (grab: 0.1 m^2; core: 0.008 m^2) which would incorporate larger variability associated with patchiness; 2) ability to collect sediment samples for multiple environmental variables with 1 deployment based on larger surface area; 3) compatibility of grab methods across a larger range of sediment textures. In this regard, the collection of a suite of environmental variables across multiple core deployments may result in variability within the monitoring program.

Figure 2: Gravity core (left photo); Retrieval of sediment core from gravity core deployment (right photo).

2.2. SAMPLING VARIABLES

2.2.1. ECOSYSTEM VARIABLES

Benthic environmental variables ranging from sediment texture to faunal attributes that reflect organic enrichment and/or depositional events from anthropogenic inputs are listed below (Table 1).

Table 1: Benthic ecosystem variables in support of aquaculture monitoring.

Monitoring Variable	Field sample size	Lab sample size
Sediment grain size	500 mL	500 mL
Sediment porosity	100 ml	20 ml
Organic matter		
Organic carbon content		20 ml
Organic nitrogen		
Trace elements	100 ml	20 ml
Pore-water sulfide	60-cc syringe	15 ml
Dissolved oxygen	Buttons	Digital
Sulfide-oxidizing bacteria	Aerial coverage	0.1 m^2
Opportunistic Polychaete Complex	Aerial coverage	0.1 m^2
Macrofauna (> 1.0 mm)*	> 0.5 mm sieve	Entire grab volume
Meiofauna (0.063 – 0.5 mm)	60-cc syringe	Top 2 cm

* 0.5mm sieve can be used for special purposes (e.g. juvenile life stages)

2.2.2. SAMPLING ORDER FROM GRAB OR CORE DEVICE

In order to acquire representative measures of *in situ* conditions in a timely manner and avoid contamination and/or sampling artifacts during sediment subsampling from a single grab sample, the following order is recommended.

- Record GPS location during grab ascent and maintain vertical winch wire orientation to avoid: 1) displacement between grab and boat location; and 2) angled approach to seabed resulting in a tilted sediment sample.
- Drain overlying water to 1 cm of sediment surface via: 1) natural (gravity) drainage (preferred-less disturbance); 2) hose-siphon with intake placed away from sediment surface to avoid uptake and disturbance; 3) low-flow pump system with no sediment surface disruption.
- Record water temperature and take photo of grab surface with site label (station, date). Record description of sediment texture, colour, debris, algae and visible biota of grab sample.
- Remove large debris only if it is not embedded in sediment surface (e.g. wood bark) to avoid a disturbance of underlying pore-water spilling across sediment surface.
- Quantify aerial coverage of existing mat-forming indicator taxa (sulfide-oxidizing bacteria; opportunistic polychaetes).

- Insert redox probe directly into intact sediment to a depth of 1 cm, avoiding air pockets or sediment cracks to maintain constant contact between the surface area of the redox probe film and sediment pore-water. Be careful of creating sediment cracks during probe insertion that may facilitate pore-water drainage. Record redox values after 3 minutes to allow the readings to stabilize.
- Deploy 2 syringe-cores along the central axis of the grab (mid-axis) to obtain sediment core depths of 10 cm (Somerfield et al. 2005).
- One syringe-core sample will be used for sediment pore-water sulfide analysis, while the other syringe-core should be dedicated for meiofauna analysis. Deep syringe-cores will create a plug at the bottom end of the cores to avoid: 1) core slippage in the syringe barrel during retrieval; and 2) disturbance during the sectioning of the uppermost 2-cm layer. Do not retrieve the syringe-cores until the other surface sediment subsamples (top 2-cm) have been collected to avoid contaminating the surface sediments with potential fallout from deeper sediments during syringe-core retrieval (Figure 3).
- A 500 mL poly-propylene jar will be filled with sediment for grain size fractionation, while two 100 mL sediment samples will be collected and stored in two poly-propylene jars and analyzed for sediment porosity, total organic carbon, total organic nitrogen content, and trace-element concentrations. Putty knives (marked with a 2-cm line across the base) can be used in tandem to transfer surficial sediment from a grab mechanism to two clean 100-mL poly-propylene jars. The putty knives are used to capture an intact sediment subsample that retains the sediment sample and doesn't allow the pore-water to bleed out of the sample. All sediment samples will remain frozen during storage and transport back to the laboratory.
- Finally, gently retrieve the deployed syringe-cores containing a vertical sediment-column sample in order to extract the top 2-cm sediment layer (Sutherland et al. 2007c). Excavate the sediment from the entire side of the core-barrel at depth and seal the bottom of the syringe-core with a plug to retain pore-water while handling. Lift the syringe-core out of the grab sampler while maintaining a vertical orientation. Remove the plunger from the top-end of the core-barrel and insert it into the bottom end after removing the plug. Be careful to not let the sediment-core slip vertically within the core-barrel. Slowly push the plunger upwards to extrude the sediment sample 2-cm from the top-end. Quickly cut off the 2-cm sediment slice into an appropriate sampling jar and prepare each sample for sediment pore-water sulfide analysis or meiofauna preservation as outlined in the sections below (Sutherland et al. 2007c).

Figure 3: Syringe-cores deployed in a Ponar grab sample. A 2-cm deep surface scraping has been collected following syringe-core deployment. Note the black anoxic sediment underlying the oxic surface layer (~3 mm deep) (Left photo); Deployed syringe-cores and surface scraping within an intertidal sampling quadrat (0.25 m^2) (Right photo).

2.3. FIELD COLLECTION, MANAGEMENT REGULATORY THRESHOLDS AND LABORATORY ANALYSES

2.3.1. SEDIMENT GRAIN SIZE

Characterizing sediment texture is important when evaluating organic enrichment gradients, assessing benthic habitat function that supports benthic faunal communities, as well as delineating the controls governing trace metal abundance (Heip et al. 1985; Duplisea and Hargrave 1996; Sutherland et al. 2007c). Spatial patterns in sediment texture can identify hot spots associated with natural depositional and erosional settings (Pejrup 1988; Molinaroli et al. 2009a,b; Sutherland et al. 2018b). Sand-silt-clay and gravel-sand-silt proportions can be used to characterize depositional facies (Pejrup, 1988). Aquaculture activities may lead to changes in sediment texture due to alterations in: 1) ambient hydrodynamics depending on size and shape of physical structure of operation (e.g. redirection or baffling of currents); 2) local depositional or erosional processes; and 3) bio-deposition based on bivalve feeding preferences of available seston size and quality (Bernard, 1974, Sornin et al. 1988; Barille et al. 1993; Dupuy et al. 2000; Gangnery et al. 2001; Grant and Bacher, 2001; Cognie et al. 2003; Grant et al. 2005; Stevens et al. 2008; Forrest et al. 2009; Dowd 2003).

Depositional environments are characterized by fine sediments which are closely associated with higher organic content and trace-element concentrations (Volvoikar and Nayak, 2013; Fernandes et al. 2014; Noronha-D'Mello and Nayak 2015; Sutherland et al. 2007a,c; 2018b). This association is based on: 1) the preferential accumulation of fine-grained organic material in low-energy settings; 2) higher surface area to volume ratio of fine sediments and pore-water space; 3) the tendency of trace metals to preferentially bind to clay minerals and organic matter; 4) the links between organic matter accumulation, pore-water redox conditions, sulfate reduction and the accumulation of secondary metal sulfide (Luoma, 1990; Mayer and Rossi, 1982, Mayer 1994). Further, both natural and anthropogenic influences linked to physical disturbances, organic enrichment events, and/or anoxic events can lead to restructuring of meiofauna and macrofaunal communities (Warwick and Buchanan 1970; Coull and Chandler, 1992; Rosenburg, 2001; Widdlecombe and Austen, 2001; Demie et al. 2003; Sutherland et al. 2007c; Keeley et al. 2012; Rauhan Wan Hussin et al. 2012; Liu et al. 2015).

2.3.1.1. Field collection

Sediment can be collected from the grab using a clean trowel and a polyethylene jar (minimum volume of 500 mL). Depending on the sampling objective, sediment may be collected from: 1) the uppermost 2-cm of the sediment column in order to match the sampling depth interval associated with the collection of other sediment variables (e.g. sediment porosity, organic content, carbon and nitrogen concentration); or 2) within the entire grab sample if macrofauna is being considered which requires large-volume sediment samples. Sediment samples should be frozen during transport and storage prior to grain size analysis.

2.3.1.2. Laboratory analysis

For monitoring purposes, sediments are typically size-fractionated into 4 categories: gravel (> 2 mm); sand (2.0-0.063 mm); silt (0.063-0.002 mm), and clay (<0.002 mm) (AAR, 2019). Alternatively, additional grain size categories can be considered when project objectives require a higher sediment texture resolution: >2mm, <2mm, <1mm, <0.5 mm, <0.25 mm, <0.1 mm, <0.063 mm, <0.002 mm (Wentworth, 1929). In order to remove organic material prior to sediment grain size fractionation, a treatment of sodium hypochlorite (NaOCl) can be applied to each sample. Wet sieving is used to determine the sand fractions to: 1) reduce the risk of air-borne losses of fine sediments; and 2) break up sediment conglomerates that may be evident during dry-sieve methods (Murdoch et al. 1997). Pipette, hydrometer, or laser diffraction methods can be used to determine silt and clay contents (McKeague, 1978; Di Stefano et al. 2010; Fisher et al. 2017). Textural classes can be expressed as sand, silt, and clay proportions with a silt–clay boundary (0.002 mm) according to the Canadian System of Soil Classification (CSSC, 1998).

2.3.2. SEDIMENT POROSITY AND ORGANIC CONTENT

Porous, silty, organic-rich sediments are typical to depositional environments characteristic of aquaculture operations, where natural and waste particles settle within the dispersion umbra of a facility structure (Sutherland et al. 2007b,c). Sediment porosity (volumetric moisture content) represents the total interstitial volume or void space capacity between sediment grains within a bulk seabed sample (Munger 1963; Amos and Sutherland, 1994; Amos et al. 1996) where organic material accumulates or binds to sediment surfaces (Fernandes et al. 2014; Noronho-D'Mello and Navak 2015; Papageorgiou et al. 2010). Sediment porosity (e.g. moisture content) results from the combined or net influence of the following processes: 1) sedimentation (seabed accretion), 2) consolidation (compaction, dewatering), and 3) benthic transport (resuspension) (Lowe 1975; Grabowski et al. 2011; Shi et al. 2016). Increases in microbial respiration (oxygen consumption) and subsequent sediment pore-water sulfide generation (Holmer et al. 2005) linked to benthic organic enrichment, may impact faunal diversity and favour opportunistic taxa (Hargrave et al. 1993). In this manner, sediment porosity and organic content serve as quick and practical bulk measures to characterize depositional zones and organic accumulation on the seabed.

2.3.2.1. Field collection

After syringe-cores are inserted into the grab sediment sample, a 2-cm deep surface scraping is collected using a trowel and placed in a labelled 100-mL polyethylene acid-washed jar. These samples are kept frozen during storage and transportation back to the laboratory.

2.3.2.2. Laboratory preparation

Remove the sediment samples from the freezer and allow the sediment to thaw for 1-2 hours in the lab, or overnight in the fridge. The frozen sample jars are thawed with the lids secured tightly on the jar. Etch the top-side tab or underside of each aluminum dish (between 20 to 40 cm

diameter depending on sediment composition) with the appropriate sample identification information (ID). The etching/writing tool should leave a mark that will not wipe or burn off during both the drying and ash treatments. Etching the top-side tab is preferable over the aluminum pan underside as it reduces the potential for spillage while lifting and tilting the aluminum pan to confirm sample identification at each weighing step. Measure the mass (g) of each empty aluminum dish to 4-5 decimal places and record the sample ID and weight.

Due to differential settlement of sediment fractions and water that typically occur during storage and transportation from the field site, thoroughly mix a sample immediately prior to and during the transfer of a subsample into an aluminum dish. In the case of porous gel-mud samples, it is important to continuously stir the sample while pouring the gel-mud into the aluminum pan to obtain a representative subsample to avoid grain size fractionation. For well-sorted sediments, fill each aluminum pan so that it is two-thirds full. Repeat this procedure to provide duplicate samples. Add a triplicate sample to provide additional sediment for CHN analysis. For poorly-sorted rocky sediments, larger aluminum pans may be used, while standardizing volume or weight to accommodate larger sample sizes (25–45 g wet weight).

2.3.2.3. Laboratory analysis of sediment porosity (moisture content)

When interconnected void spaces are filled with marine pore-water, the fluid capacity or porosity can be determined through the loss of water from a sample based on differential wet and dry weight measurements. For example, total porosity (P) based on water loss can be determined from the following equation: P = 100 x (Vp/Vb) where pore volume (Vp) equals the difference between wet and dry sediment and bulk volume (Vb) represents the wet weight of each sediment sample. Weigh the combined wet sediment sample and pan immediately after sample transfer to avoid potential weight loss due to water evaporation (e.g. moisture content of bulk sediment). Place the sediment sample in a pre-heated drying oven at 55°C for 24h. After the 24-hour drying period, weigh a few individual samples over a period of time until a constant weight is achieved to ensure that all pore-water has evaporated. Each time a set of samples is removed from the oven it should be placed immediately in a sealed chamber containing desiccant for 2 hours. This step prevents the addition of added moisture weight due to condensation in the cooling process. Weigh the dry sediment and the aluminum pan. Determine both the wet and dry sediment weights after subtracting the aluminum-pan weight for each sample. Sediment porosity (water content) is calculated using the differential weight values between wet and dry measurements standardized by wet weight.

2.3.2.4. Laboratory analysis of organic content (loss on ignition)

Organic content represents the fraction of organic material in the pore-water volume within a bulk sediment sample and can be determined by ashing dried samples and calculating the differential ashed and dried weights. Place the dried sediment samples contained in the labelled aluminum pans identified in the above laboratory preparation section in a muffle furnace. Ash these sediment samples for 4 hours at 450°C or 2 hours at 550°C. Following the ashing step, partially open the muffle furnace door to partially cool the samples in the oven. Remove the sample pans from the muffle furnace and place in a desiccation chamber for 2 hours. Weigh the ashed sediment and aluminum pan. Determine the ashed sediment weight after subtracting the aluminum pan weight. Organic content is calculated using the differential weight values expressed as percent between dry and ashed weight measurements standardized by dry weight.

2.3.3. SEDIMENT TRACE-ELEMENTS

Chemical tracers of aquaculture activities are important when assessing potential environmental effects of aquaculture activities. Copper and zinc are examples of direct tracers of aquaculture waste material (feed and faecal pellets) as well as antifouling agents applied to netpen systems (Yeats et al. 2005; Sutherland et al. 2007a). Since certain trace-elements (e.g. cadmium, molybdenum, copper, zinc, and uranium) show preferential accumulation under reducing redox conditions, they can also serve as indirect tracers or sediment redox indicators of benthic organic loading events (Smith et al. 2005; Macdonald et al. 2008; Laing et al. 2009, Sutherland et al. 2011b). Sediment-redox trace-element indicators are suitable for environmental assessments of bivalve aquaculture relative to that of direct tracers (e.g., Zn, Cu) that have been used as feed/faecal tracers associated with finfish aquaculture assessments. In addition, sediment redox trace-element indicators can be used to complement existing sulfide/redox classification schemes designed to assess benthic impacts (Wildish et al. 2001; Hargrave et al. 2008). Yeats et al. (2005) and Sutherland et al. (2007a), for example, have shown that geo-normalization of trace-elements to Li content is an effective means to differentiate anthropogenic influences from the natural variability in the background (Loring 1990,1991; Aloupi and Angelidis 2001). Excess (i.e., above background) concentrations of trace-elements (TE) can be derived from this technique, defined as trace-element concentrations that fall above the upper confidence limit of a background TE-Li regression (Figure 4; Sutherland et al. 2007a). In addition, excess trace-elements can be categorized according to the Canadian Sediment Quality guidelines (CCME, 1995) and superimposed on a faunal-sulfide relationship to characterize the exposure of multiple stressors and cumulative effects on fauna (Figure 5; Sutherland et al. 2011b).

2.3.3.1. Management regulatory threshold

The Canadian Sediment Quality guidelines recommended by the Canadian Council of Ministers of the Environment (CCME) can be applied to sediment trace-element concentrations to assess if levels exceed the threshold effect level (TEL) and/or the probable effect level (PEL). Adverse biological effects would rarely occur below the TEL guideline and more likely to occur above the PEL upon exposure of biota to these trace-element concentrations (CCME, 1999).

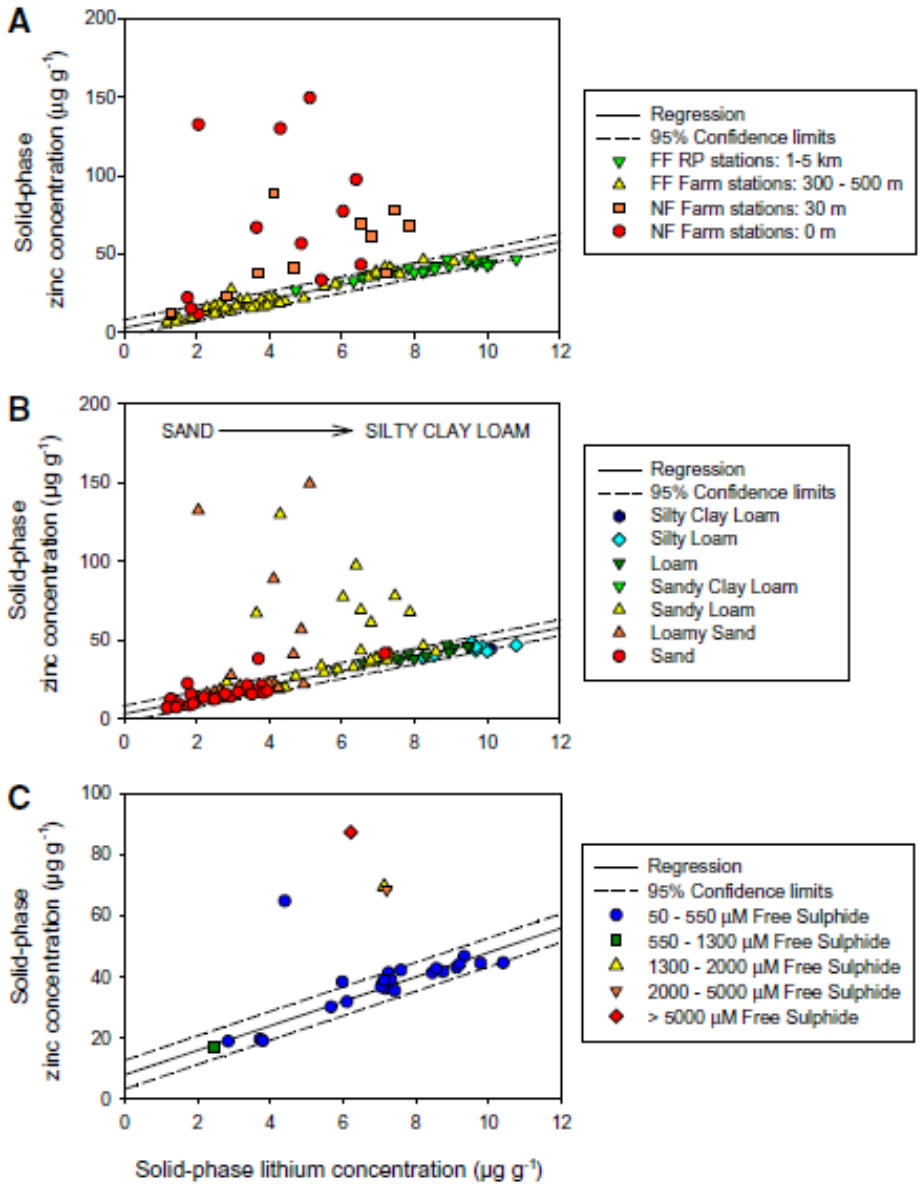

Figure 4: Background regression of solid-phase zinc and lithium concentrations with excess (above background) concentrations falling above the upper confidence limit (dashed line) of the regression (solid line). Data are colour-coded by farm distance (A) substrate type (B), and pore-water sulfide concentrations (C) (Sutherland et al. 2007a).

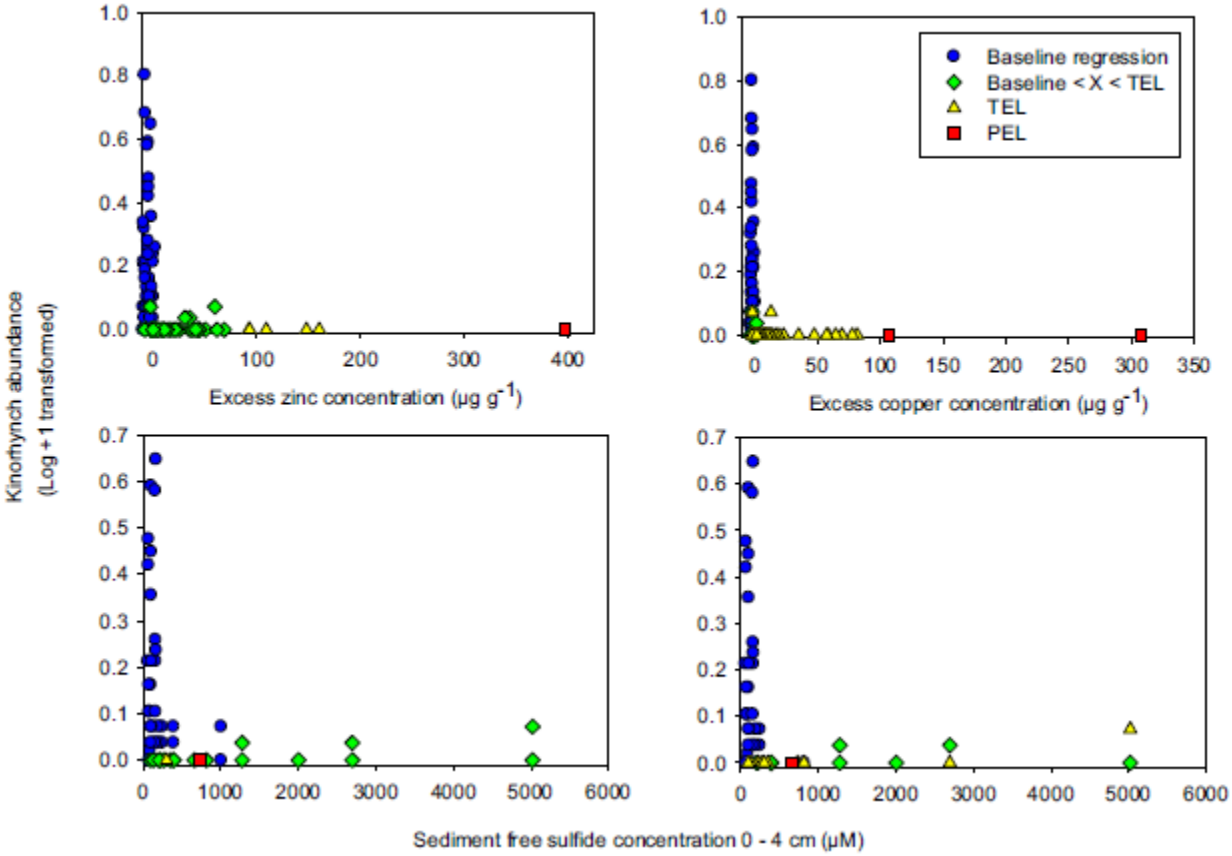

Figure 5: The relationship between meiofauna abundance (Kinorhynch) and excess (above background) trace-elements (Zn, Cu) after removing background concentrations (top graphs). The lower graphs show the interaction between Kinorhynchs and trace-elements according to sediment porewater sulfide concentrations (Sutherland et al. 2011b).

2.3.3.2. Field collection

A sediment sample designated for trace-element analysis will be collected using a acid-washed 100-mL polyethylene jar (Table 1). The protocols for acid-washing techniques should be chosen according to sample jar composition, target trace-element, and analytical method (Reimann et al. 1999). This sample will be collected in a similar manner to that sampled for organic variables stored in the first 100-mL jar. This sample should remain frozen during transportation to the laboratory and storage at the facility.

2.3.3.3. Laboratory analysis

Remove the sediment samples from the freezer and allow the sediment to thaw for 1-2 hours in the lab, or overnight in the fridge. The frozen sample jars are thawed with the lids secured tightly on the jars. Due to settling of the sample during transportation and storage, the sample will need to be mixed thoroughly before and during transfer of samples. Label small acid-washed glass beakers (15 mL) in preparation for the drying process. In order to avoid metal contamination, metal-laden pans (aluminum) should not be used. Samples should be sent to an accredited laboratory capable of analyzing trace-elements according to the US EPA method ICP-AES 200.15 using ultrasonic nebulization (US-EPA, 1994). The digestion process follows that of partial digestion or SALM method (Strong Acid Leachable Methods) where a sample is passed through a 1-mm sieve, dried at < 60°C, and then digested in a mixture of concentrated nitric

(HNO_3) and hydrochloric (HCl) acids at 90°C for 2 hours. The extracts are then analyzed for trace-element content via inductively coupled plasma atomic emission spectroscopy (ICP-AES) for the majority of elements and inductively coupled plasma mass spectrometry (ICP-MS) for trace elements (e.g. Sb, La, Tl, Sn and U). Analytical service packages may consist of a suite of trace-elements consisting but not limited to the following: aluminum (Al), antimony (Sb), arsenic (As), barium (Ba), cadmium (Cd), calcium (Ca), chromium (Cr), cobalt (Co), copper (Cu), iron (Fe), lead (Pb), lithium (Li), magnesium (Mg), manganese (Mn), molybdenum (Mo), nickel (Ni), phosphorus (P), potassium (K), silicon (Si), sodium (Na), strontium (Sr), sulphur (S), thallium (Tl), tin (Sn), titanium (Ti), uranium (U), vanadium (V) and zinc (Zn). Mercury typically requires a separate analytical method that consists of digestion with HCl/HNO_3 and analysed by cold vapour atomic absorption spectrophotometry.

2.3.4. SEDIMENT PORE-WATER SULFIDE

Benthic organic enrichment events have been associated with both marine finfish and bivalve aquaculture activities (Dahlback and Gunnarsson, 1981; Tenore et al 1982; Carter 2004; Nizzoli et al. 2006; Cranford et al. 2006, 2009; Carlsson et al 2009; Hargrave, 2010; McKindsey et al. 2011) likely due to the deposition of organic waste material in the form of feed pellets, faeces, dead fish, and/or gear-fouling material. The accumulation of organic matter served to enhance benthic oxygen demand, and under these conditions, elevated concentrations of sediment pore-water sulfide can develop as a result of a shift from microbial aerobic respiration to anaerobic respiration involving sulfate reduction (Holmer et al. 2005). This in turn can promote shifts in the diversity of macrofauna (Stenton-Dozey *et al.* 2001; Brooks and Mahnken, 2003) and meiofauna (Sutherland et al. 2007c; Bouchet et al. 2007) along aquaculture-derived organic enrichment gradients.

Management regulatory threshold

Sediment sulfide impact classification systems (e.g. threshold intervals) have been developed in Pacific and Maritimes settings associated with benthic finfish (Sutherland et al. 2007c; Hargrave et al. 2008; AAR, 2019) and bivalve studies (Cranford *et al.* 2006). The B.C. finfish environmental monitoring protocols include the following sulfide impact categories: <700 uM (OxicA); 700 – 1300 uM (OxicB); 1300 uM (Hypoxic); 4500 uM (Anoxic). Although a variety of methods are being developed as potential candidates for sediment sulfide assessments (e.g. UV-spectrophotometry; Cranford et al. 2017), this document outlines the ISE method employed to date in Canada based on recommendations from peer-reviewed publications and CSAS processes (DFO, 2005; Wildish et al. 2005; Cranford et al. 2006, 2012; Sutherland et al. 2007c).

Laboratory preparation and *in situ* analysis of sediment pore-water sulfide concentration

It is important to note that dissolved sulfide measurements should only be used in soft sediments with relatively higher proportions of silt-clay and water content, similar to those in which the method and thresholds were established. Mixed, coarse, and/or loose sediments that do not maintain a sediment fabric and retain pore-water sulfide during the collection and transfer of a sediment sample will result in non-representative dissolved sulfide estimates (MER Assessment corporation, 2008).

Sulfide calibration standards (sodium sulfide non-ahydrate) should be prepared in the laboratory prior to field work. A Thermo Scientific ORION™ (6916BNWP) Silver/Sulfide Ion Selective Electrode (ISE) should be filled with Optimum results “A” filling solution (Orion 900061) 24 hours prior to use to allow the electrode to equilibrate. The calibration of the Thermo Scientific ORION Silver/Sulfide electrode (6916BNWP) should take place immediately prior to sediment sample collection based on the limited life-span associated with the calibration solutions (3 hours; Wildish et al. 1999; AAR, 2019). It is important that sediment pore-water sulfide concentration

be analyzed at the time of sample collection in the field due to the volatile nature of sulfides and the potential for oxidation artifacts during sample storage and transportation back to the laboratory (Wildish et al. 1999). The method for extracting a top 2-cm sediment slice from a deployed syringe-core in the deep, centre portion of the grab sample is described in Section 2.2.2 “Order of priority for subsample collection from grab mechanisms” or within Sutherland et al. (2007c). The 2-cm slice of the sediment surface will be placed directly into an equal volume of sulfide anti-oxidant buffer (SAOB) in a 100-mL polyethylene acid-washed jar. The sediment-SAOB sample should be mixed thoroughly prior to the collection of sulfide measurements and gently swirled until and after the probe stabilizes. It is important to avoid scratching the probe plate at the probe tip by avoiding contact between the probe, jar, and sediment during the swirling process. Sediment pore-water sulfide concentration ($\Sigma H_2S = H_2S + HS^- + S^=$) is recorded using the silver/sulfide electrode equipped with a Thermo Scientific Orion ISE compatible and portable meter.

2.3.5. SEDIMENT REDOX POTENTIAL

Sediment reduction-oxidation potential (redox) can be measured to determine the redox potential discontinuity depth (RPD) which reflects a marked decrease in sediment oxygen availability at a certain layer within the sediment column (Nilsson and Rosenburg 1994). Cost-effective sediment pore-water sulfide and redox methods were developed for monitoring soft sediments surrounding finfish farms in Canada (Wildish et al. 1999, 2001). Redox potential (E_{NHE}) is measured using a platinum electrode, where negative values are associated with hypoxic/anoxic conditions, while positive values are associated with oxygenated sediments. Although redox potential is considered a monitoring variable in the AAR (2019), it is only used in tandem with sediment pore-water sulfide concentrations to validate oxic-anoxic classifications through a $\log_{10}$(S)-linear(Eh) inverse relationship (Figure 6) (Sutherland et al. 2007c; Wildish et al 2005). Redox potential is not used on its own to assess sediment quality based on the following factors: 1) high variability in results between probes (Wildish et al. 2004); 2) poor precision in oxic sediments (Wildish et al. 1999; Brooks and Mahnken, 2003; Giles, 2008); and 3) platinum film scoring or poisoning (precipitate buildup) with increased use.

2.3.5.1. *In situ* field measurements

Redox potential (E_{NHE}): measurements are collected using a Thermo Scientific ORION combination platinum-redox Standard Hydrogen Electrode (SHE; Orion 9678BNWP) in series with a Thermo Scientific Orion ISE meter (similar to that used for sulfide measurements). The redox electrode is filled with filling solution (4 M KCl with Ag/AgCl; Orion 900011) 24 hours prior to use. The redox potential electrode is calibrated with the Thermo Scientific Orion™ ORP standard (967901) and can be used to periodically check proper functioning of the electrode. The redox electrode can be inserted directly into the sediment without breaking the sediment fabric that might result in cracking of sediment, drainage of pore-water and exposure to air (oxygen). The redox value is recorded after an electrode equilibrium period of 3 minutes. Sediment temperature is recorded at the time of measurement and used to correct redox potential values. If precipitates build up on the electrode film-plate (Wildish et al. 2004), clean the platinum plate with finishing paper supplied by the manufacturer.

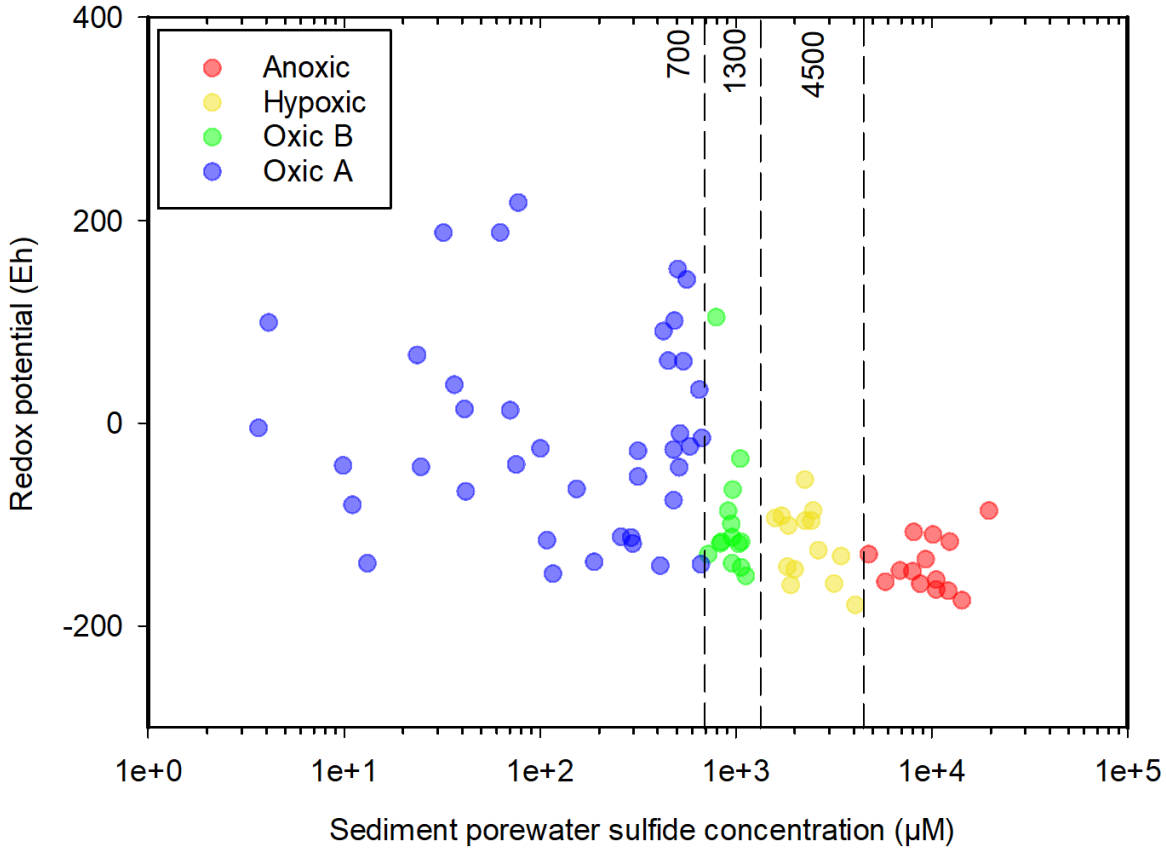

Figure 6: Relationship between sediment porewater sulfide concentration and redox potential (E_{NHE}) according to a sulfide impact classification system (AAR, 2019).

2.3.6. SULFIDE-OXIDIZING BACTERIA

Sulfide-oxidizing bacteria are considered to be a primary indicator of organic-rich, hyper-sulfidic substrates associated with aquaculture activities within deep fjords (Krost et al. 1994; Sutherland et al. 2018a), gulfs and bays (Weston, 1990; Macleod et al. 2004), as well as sublittoral maerl-beds (Hall-Spencer et al. 2006). Mat-forming bacteria require a steep oxic-anoxic gradient on a vertical scale of mm in proximity to the sediment-water interface (Jorgensen and Revsbech 1983; Teske and Nelsen 2006; Preisler et al. 2007). Although the establishment of a sulfide chemocline at the surface interface of soft-sediments is common, sulfide-oxidizing bacteria are opportunistic in that they will also inhabit both mixed- and hard-substrates (e.g. surrounding the base of boulders, crevices; Sutherland et al. 2018a). It is important to note that sulfide-oxidizing bacteria can also occur in natural settings characterized by oxygen-minimum sediments (Jorgensen et al. 2010; MacGregor et al. 2013) as well as areas affected by non-aquaculture inputs made up of wood waste deposits (Elliott et al. 2006) and sewage outflows (Kim et al. 2007). Sewage outflows as well as log transport and storage (booming) occur in the Pacific region. Cumulative impacts of multiple anthropogenic influences and the appropriate selection of reference sites should be considered when assessing sulfide-oxidizing bacteria and Opportunistic Polychaete Complexes (OPC) as indicators of aquaculture activities.

2.3.6.1. Management regulatory threshold:

For soft sediment sampling, the presence or absence of sulfide-oxidizing bacteria in a grab sample is a reporting requirement according to Annex 8 of the Aquaculture Activities Regulation

(AAR, 2019). Reporting requirements and a regulatory threshold are applied to sulfide-oxidizing bacteria spatial coverage as part of video transect surveys associated with hard substrates. For further information, see Section 3.0 of this report, “Benthic hard-substrate monitoring methods”.

2.3.6.2. Field collection

Since sulfide-oxidizing bacteria are restricted to the sediment–water interface of soft substrates due to their reliance on mm-deep, steep oxic-anoxic gradients (Jorgensen and Revsbech 1983), percent coverage estimates of bacterial mats can be quantified both visually and analytically from: 1) subtidal sediment using a grab mechanism (minimum surface area: 0.1 m^2; depth: ~16 cm); or 2) intertidal sediment using a quadrat frame (minimum surface area: 0.25 m^2). In terms of visual estimates, the percent coverage of bacterial mats can be recorded *in situ* according to the following spatial increments: sparse, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100%. The mat texture can be described by a combination of the following descriptors: colour, thickness (thick, thin), and porosity (solid, lattice, veiled). Photos or videos of the survey area can also be collected and analyzed (Sutherland et al. 2018a; 2019a).

2.3.6.3. Laboratory analysis

Photo-media software (e.g. Photoshop) can be used to filter bacterial mats by colour, thickness, and/or texture to distinguish the mats from background substrates and digitize their spatial coverage. The spatial estimates derived from photos should be standardized to *in situ* visual estimates to groundtruth information. Alternatively, since sulfide-oxidizing bacteria (e.g. *Beggiatoa*) have large cells and filaments, sediment surface scrapings can be collected and examined microscopically and phylogenically (PCR amplification; 165 rRNA gene sequencing) for distribution/abundance and taxonomic community assessments, respectively (Preisler et al. 2007; Jorgensen et al. 2010). Microscope examination can be carried out by suspending a known weight of wet sediment and determining the counts (abundance) and dimensions (biomass) of the cells from a known volume on a microscope slide.

In terms of DNA methods, *Beggiatoa*-like cells that are occupied by sulphur granules, a central vacuole, and low cytoplasmic volume may be responsible for low amounts of chromosomal DNA available for DNA extraction. This scenario may explain a lack of detection of DNA-derived, *Beggiatoa*-like taxa that was detected visually through camera surveys of the seabed (Bissett, Bowman, and Burke 2006; Schulz and Jorgensen, 2001). However, Dowle et al. (2015) suggested that 1) DNA-derived detection may have been possible through a higher frequency of sample collection; or 2) although the mat-forming bacteria were detected through DNA analysis, it was present in the form of a close-relative of *Beggiatoa* spp. Further research is required to elucidate the methods (e.g., collection, storage, preservation, isolation, and sequencing) that will provide an appropriate detection resolution of sulfide-oxidizing bacteria using these meta-barcoding techniques. In addition, these non-visual, DNA-derived approaches may be costly or not practical for a regulatory monitoring or management program, depending on objective and resources.

2.3.7. OPPORTUNISTIC POLYCHAETE COMPLEX (OPC)

Similar to sulfide-oxidizing bacteria, OPCs can occur in: 1) naturally-organic-rich settings (Pearson and Rosenberg 1978; Pearson et al. 1983; Ramskov and Forbes 2008; Rabalais et al. 2013); 2) sewage outfalls based on non-aquaculture inputs (Blackstock et al. 1986); and 3) organic-rich gradients associated with finfish aquaculture activities (Macleod et al. 2004; Tomassetti and Porrello 2005; Paxton and Davey 2010; Martinez-Garcia et al. 2013). OPCs are found on sediment surfaces (epifauna), within the sediment column (infauna), and veneers overlying rocky ledges (Emmett et al. 2007; Sutherland et al. 2018a, 2019a). Unlike sulfide-oxidizing bacteria, OPCs are not restricted to zones with steep redox gradients at the water-

sediment interface. The population reproductive cycle and response to sharp changes in organic inputs can influence their presence or absence and should be considered when monitoring OPCs (Pearson et al. 1983; Ramskov and Forbes 2008; Soto et al. 2009). The "boom and bust" presence of the population can pose challenges with their use as an indicator and should be monitored over time.

2.3.7.1. Management regulatory threshold

For soft sediment sampling, the presence or absence of OPCs in a grab sample is a reporting requirement according to Annex 8 of the Aquaculture Activities Regulation (AAR, 2019). Reporting requirements and a regulatory threshold are applied to OPCs as part of video transect surveys associated with hard substrates. For further information see Section 3.0 of this report, "Benthic hard-substrate monitoring methods".

2.3.7.2. Field collection

Percent coverage estimates of OPC mats can be quantified per surface unit area from subtidal sediment collected from a grab mechanism (minimum area of 0.1 m^2) or intertidal sediment using a quadrat frame placed on the substrate (minimum area: 0.5 m^2). Similar to sulfide-oxidizing bacteria, photos or videos of the survey area can be collected and analyzed at a later time (Sutherland et al. 2019a). In terms of subsurface estimates of infaunal OPCs in soft-sediments, refer to the macrofauna methods outlined in the section below. When determining OPC estimates associated with hard-bottom substrates, refer to Section 3.0 of this report, "Benthic hard-substrate monitoring methods".

2.3.7.3. Laboratory analysis

Photo-media software (e.g. Photoshop) can be used to filter individual annelids of the OPCs by colour, shape, and/or texture to: 1) distinguish them from background substrate; and 2) digitize their spatial coverage per survey area (%). The spatial estimates derived from photos should be standardized with *in situ* visual estimates. Regarding infauna OPCs, individuals can be identified morphometrically using microscopy or barcoding through DNA sequencing. Both methods require a high level of taxonomic sorting of taxa to reduce analytical costs for both the morphometric and barcoding identification methods.

2.3.8. MACROFAUNA (>0.5 MM)

Macrofaunal communities have been shown to shift in diversity, abundance, and/or biomass when exposed to benthic organic enrichment (Pearson and Rosenburg, 1978; Diaz and Rosenburg, 1995). In addition, changes in macrofaunal metrics have been detected due to the influence of organic enrichment gradients associated with bivalve aquaculture (Cranford et al. 2006). OPC taxa are common macrofaunal indicators associated with hyper-sulfidic and anoxic conditions associated with organic deposition and accumulation (Pearson and Black, 2001; Harstein et al. 2004; Dumbauld et al. 2009; Forrest et al. 2009; Frechette, 2012). Further, a polychaete to amphipod ratio can be used as an indicator of organic enrichment as it represents a tolerant (polychaete) vs. sensitive (amphipod) characterization of a changing habitat (Mangion et al. 2017). This ratio is considered a benthic biotic index (benthic opportunistic polychaetes and amphipods; BOPA) as part of an Ecological Quality Status (EQS) developed for the European Water Framework Directive (WFD, 2000/60/EC). It has recommended that this index should be restricted to the inclusion of locally-known polychaete indicators of organic enrichment (e.g. *Capitella spp*.) in order to increase the sensitivity of this index to habitat changes (BOPA-Fish Farming; Aquado-Gimenez et al. 2015). Use of the BOPA index is less time-consuming and requires less expertise to sort samples, identify taxa, and analyze data,

especially when considering other biotic indexes rely on a full macrofaunal taxonomic assessment (e.g. Shannon, Simpson, AMBI indices).

In order to quantify macrofauna, organisms need to be separated from the sediment grains using a sieve that does not exceed a 1-mm pore size (Rumohr, 2009). The 1-mm sieve is typically used to assess macrofauna communities for environmental monitoring programs to avoid logistical and time issues associated with clogging and subsequent overflowing of sieves. A 0.5 mm sieve can be used for specific objectives targeting: 1) smaller-sized adult organisms; 2) juvenile life stages of target taxa with short life cycles; and 3) flexible and/or vermiform-shaped taxa (> 1mm) that may pass through the 1.0 mm sieve. Figure 7 shows a sieve table where a stacked sieves consisting of a 1.0 cm and 0.5 mm sieves are used to collect macrofauna. Grab samplers may also be used to assess the burying habitat of Pacific Sand Lance that take advantage of benthic habitats as part of their life style (Robinson et al. 2013).

2.3.8.1. Field collection (subtidal)

Sediment that is collected using a grab mechanism can be emptied into a labelled bucket and transferred to the sieve table immediately for processing. A series of sieve screens stacked on top of a catchment tray supported by a rack stand are used to sieve various size fractions simultaneously. This system reduces the risk of spilling and sample loss when transferring samples between sieves and into sample buckets (ICES, 1994). A two-sieve stack overlying a catchment tray (1 m x 1 m) is recommended to handle large sample volumes (grab surface area: 0.1 m^2; Pohle and Thomas, 1997) and silt-laden sediment found near aquaculture operations and Pacific region deep-water seabeds.

The top sieve, made up of a 1.0-cm mesh size, will catch gravel, wood, shell fragments, algae, and megafauna, that may harm smaller organisms caught by the 1.0 mm sieve. The material found on the top sieve may harbour smaller organisms and should be washed gently to allow these organisms to pass through the 1.0-cm sieve. The second sieve will consist of a 1.0-mm mesh size, which is used to define the macrofauna size category in monitoring programs (Rumohr, 2009). Filtered sea-water (100 µm filter pore-size) is used to gently wash sediment and macrofauna through the stacked sieve system simultaneously without introducing new organisms (e.g. copepods, larvae) to the benthic sample. The water pressure should be gentle to avoid damaging fragile organisms (e.g. polychaetes) and pushing soft-bodied organisms (e.g. vermiform) through the sieve mesh, thereby creating artefacts in the size fractionation process. To reduce the clogging of a sieve once the sediment settles on the surface, water can be introduced to fluidize the sample and agitate the sieve to suspend sediment and allow water and biota to pass through the mesh pore spaces. Polychaetes and other fragile organisms should be picked up from the sieve by hand using tweezers to avoid damaging organisms with the use of scraping tools on the sieve surface. Macrofauna are typically preserved in 4% formaldehyde (10% formalin) and buffered with Borax (sodium tetraborate) to avoid dissolution of shell material (Rumohr, 2009). Replicate samples can be collected as per the study design and resources.

Figure 7: Sieving of a macrofauna grab sample using stacked sieves (1.0 cm, 0.5 mm) placed on a sieve table. In the right photo, the 1.0 cm sieve is in the background, while the macrofauna sample is being collected from the 0.5 mm sieve.

2.3.8.2. Field collection (intertidal)

A push core (Inner diameter: 10 cm; Height: 25 cm) can be used to collect infaunal macrofauna in the intertidal zone (Sutherland et al. 2013). The core barrel is pushed into the sediment until the lower edge reaches 20 cm (8 inches) into the substrate. The 20-cm sampling depth-interval can be marked on the outside of the core barrel to facilitate accurate core-depth penetration. The sediment outside of the core-barrel is excavated to the base, where it is capped at the bottom before retrieving the sediment sample and barrel from the beach.

2.3.8.3. Laboratory analysis

Macrofauna can be identified morphometrically using microscopy or barcoding through DNA sequencing. Both methods require a high level of taxonomic sorting to reduce analytical costs for both identification processes. In terms of the enumeration of macrofauna, the organisms ae scanned under 10x and 40x magnification using a stereomicroscope (e.g. Leica Wild M3Z microscope). Identification can take place using published dichotomous keys (e.g. Kozloff, 1996) and handbooks (e.g. Harbo, 1999). Macrofauna abundance is standardized according to the area and depth of sediment collected from the grab or core barrel specifications.

2.3.9. MEIOFAUNA (0.063 – 0.5 MM)

The influence of aquaculture operations on meiofauna can be expressed as a decrease in abundance and shift in taxa richness (Duplisea and Hargrave, 1996; La Rosa et al. 2001; Mirto et al. 2000). In a B.C. setting, several taxa groups (Kinorhyncha, Crustacea, and Polycheata) have shown responses to sulfide gradients and have been recommended as indicators of aquaculture activities (Sutherland et al. 2007c). Relative to macrofauna, meiofaunal analysis is cost effective, practical (small volume; 10.6 mL^{-3}), characterized by fewer morphological taxonomic groupings, low taxonomic resolution, and requires less enumeration time per sample. The nematode-to-copepod ratio, which represents a ratio of tolerant taxon (nematodes) to a more sensitive taxon (harpacticoid copepods), has shown potential in characterizing aquaculture-derived organic enrichment gradients (Sutherland et al. 2007c). The application of environmental DNA (eDNA) meta-barcoding methods has shown that meiofauna-sized taxa (e.g. Foraminifera) may represent a useful bio-indicator of aquaculture-environmental

interactions (Pawlowski et al. 2016). He et al. (2019) used eDNA metabarcoding to characterize the relationship between foraminifera along organic-enrichment gradients in association with aquaculture activities in B.C. However, eDNA may detect “ghost” taxa represented by preserved extracellular or test material (e.g. foram calcareous skeleton) in water or sediment environments (Pawlowski et al. 2018), thereby, reducing indicator sensitivity along a benthic enrichment gradient. Recent research has shown that eRNA may serve as a more sensitive indicator regarding correlations: 1) with morphological indices associated with ecological change (Laroche et al. 2016; 2017; Pawlowski et al. 2014, 2018); and 2) between eRNA-derived relative abundance and picoeukaryote cell abundance relative to that of eDNA (Giner et al. 2016). Since this method is an emerging research field with fast-evolving methods, it is advised to seek expertise prior to implementing an up-to-date technique that provides a sensitive measure for a certain monitoring objective and environmental setting.

2.3.9.1. Field collection

Meiofauna samples are extracted from the top 2-cm of a modified syringe-core deployed in a grab sample or in an intertidal sampling quadrat (Somerfield et al. 2005; Sutherland et al. 2007c). The method for extracting the top 2-cm sediment slice from a deployed syringe-core in the deep, centre portion of the grab sample is described in the Section 2.2.2 “Order of priority for subsample collection from grab mechanisms”. Each 2-cm sediment subsample is placed in a labelled 50-mL jar and either preserved in 4% formaldehyde or placed immediately in a freezer. In terms of eDNA analysis, a sample extracted from a syringe-core should be preserved in ethanol.

2.3.9.2. Laboratory methods

Morphometric analysis: Meiofauna can be extracted from sediments using a suspension and decantation process following size fractionation (0.5 and 0.063 mm) and staining with Rose Bengal (Warwick and Buchanan, 1970). The sample retained on the 0.063 mm sieve is transferred to a 250 mL graduated cylinder (height: 33-cm) and filled to a volume of 240 mL (32 cm height) using filtered seawater (0.45 µm filter membrane). The sample is suspended in the cylinder and allowed to stand for 60 seconds for the settlement of large particles. The organisms in the supernatant are retained when they are passed through a 0.063 µm sieve. This sample resuspension and decantation procedure is repeated three times. The decanted sample is then scanned under 10x and 40x magnification using a stereomicroscope and meiofauna are enumerated. Meiofauna abundance is standardized according to the volume of sediment collected within the syringe-core barrel (Depth: 2.0 cm; Inner Diameter: 2.6 cm).

eDNA analysis: All sampling gear needs to be cleaned of DNA material and stored in containers to avoid contamination of sampling. The syringe-core samples can be preserved in 50 mL jars containing 95% ethanol and stored at -20°C once transferred to the laboratory. Samples can be prepared for Foraminifera and benthic metazoan analysis according to He et al. (2019, 2021a,b).

2.3.10. BIVALVE ABUNDANCE, RECRUITMENT, AND CONDITION INDEX

B.C. contributes significantly to Canada’s total bivalve production in terms of harvest landings (clams: 67%; oysters: 60%) and dollar value (clams: 85%; oysters: 39%DFO, 2019). High production areas could result in a potential competition for food (phytoplankton) and space between cultured and natural bivalve populations. Localized phytoplankton depletion has been documented in high bivalve production zones across Canada associated with mussels (Cranford et al. 2014; Grant et al. 2008) and oysters (Guyondet et al. 2013; Powell et al. 1995). Bivalves are a key component of an ecological carrying capacity assessment. Since the cultured biomass plays the most significant role in ecosystem dynamics, precise information on standing

stock biomass over time is required. The underlying rational is that the performance of the cultured standing stock signals food availability, namely phytoplankton, a key component of carrying capacity assessments (see above). Therefore, a poor performance of the cultured population signals that the available food is not enough to maintain maximum shellfish growth, which could be an indicator of seston depletion. While Filgueira et al. (2015) recommends that condition index (relationship between shell length and tissue weight) is a simple and reliable indicator for ecological shellfish carrying capacity assessment, they also note that recent modelling efforts show that shell length and tissue weight can be used as independent indicators for these assessments. In addition, these metrics are simple, practical, and cost-effective monitoring variables, which support shellfish Dynamic Energetic Budget models.

Certain high-production bays (e.g. Baynes Sound) harbour vast intertidal zones that support large cultured and natural bivalve populations (clams, oysters) along with suspension cultures. The intertidal population may also contribute to localized phytoplankton depletion, a reduction in bivalve health status (condition index), and subsequent self-thinning of the population. If self-thinning takes place within the smaller clams, a population recruitment failure may take place. It is therefore important to assess the abundance, recruitment potential, and condition index of bivalve populations. Condition index, for example, is based on the relationship between bivalve tissue weight (ash-free dry weight) and shell length and has been deemed a reliable ecological indicator for bivalve aquaculture density and carrying capacity (Filgueira et al. 2013). Further modelling exercises working in this direction have suggested that tracking shell growth rate and meat growth rate could also be used as indicators to assess carrying capacity. Shell growth rate has the advantages of simplicity and being an indicator that does not require sacrificing the shellfish, facilitating the logistics in the monitoring process.

2.3.10.1. Field collection and lab analyses

Bivalve abundance

The number of oysters within a wooden quadrat can be recorded in a field notebook, placed in a labelled Ziploc bag and stored in a cooler for transport back to the laboratory. Clams can be collected from a 0.25 m^2 quadrat by digging out the sediment to a depth of 20 cm and placing it on a 1 m x 1 m sieve (1-cm mesh size) placed beside the quadrat (Figure 8). A tarp is placed under the sieve tray to capture sediment that falls through mesh screen, which will be used to refill the quadrat dig-hole. A bucket of water can be used to separate clams from sediment by washing sediment through the sieve. The clams are placed in labeled Ziploc bags before being stored in a cooler for transportation to a freezer facility. A stereomicroscope with a magnification of 10x and 40x can be used to sort and enumerate bivalve taxa groups in each sample. Bivalve abundance is standardized according to the quadrat surface area (0.25 m^2) and recorded sediment depth. Bivalve taxa identification can be carried out according to Harbo (1999).

Figure 8: Sieving bivalve from sediment collected from a 0.25 m^2 sampling quadrat.

Clam recruitment

Sediment surface scrapings (< 4 cm) are collected from a smaller quadrat (0.05 – 0.1 m^2). These samples are sieved through 0.25 and 1.0 mm mesh sizes (VWR® U.S.A. Standard Testing Sieves). These tiny clams are collected off the sieve surfaces, placed in labelled 50 mL jars, and stored in a cooler for transportation to the laboratory freezer. The clams are examined under 10x and 40x magnification using a stereomicroscope and plotted on a size-frequency and age-frequency distribution to determine recruitment patterns (Sutherland et al. 2019b).

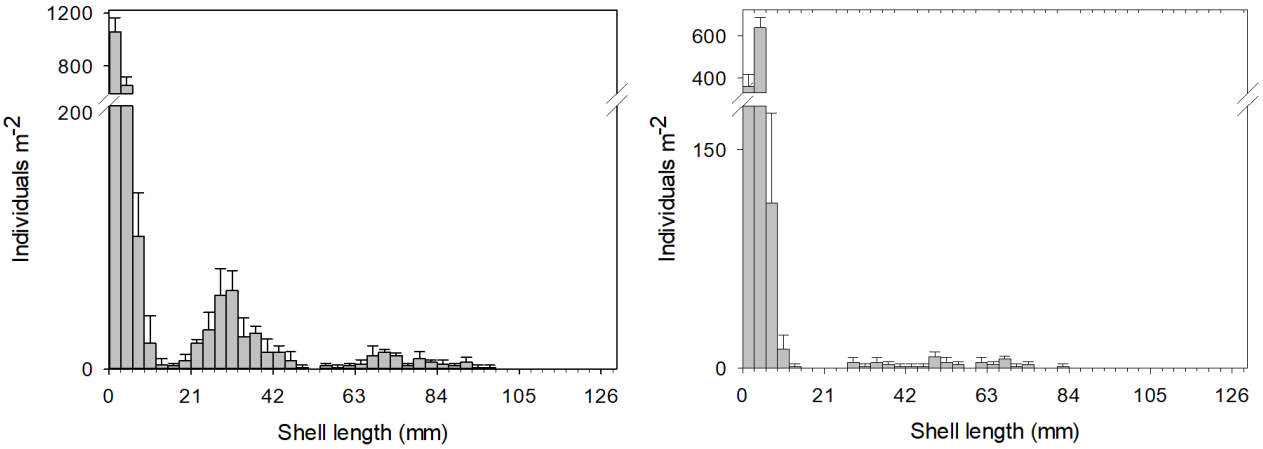

Figure 9: Frequency distribution graph showing the relationship between total clam abundance and shell length. Both graphs show strong recruitment with the left graph showing several juvenile and adult cohorts, while the right graph reveals a collapsed adult population (Sutherland et al. 2019b).

Bivalve condition index

Bivalve tissue can be dissected from its shell and placed into a labelled and pre-weighed aluminum pan. The tissue is then dried using a VWR® 1370 GM Gravity Oven at 55°C for 48 hours or until a constant sample weight is achieved. Samples are desiccated for 2 hours prior to dry weight determinations to avoid potential condensation during the cooling process. Inorganic content is calculated using the differential weight values between dry and ashed measurements

standardized by dry weight. Dried samples are ashed at 550°C for 2 hours in a Thermolyne 1400 furnace for this calculation.

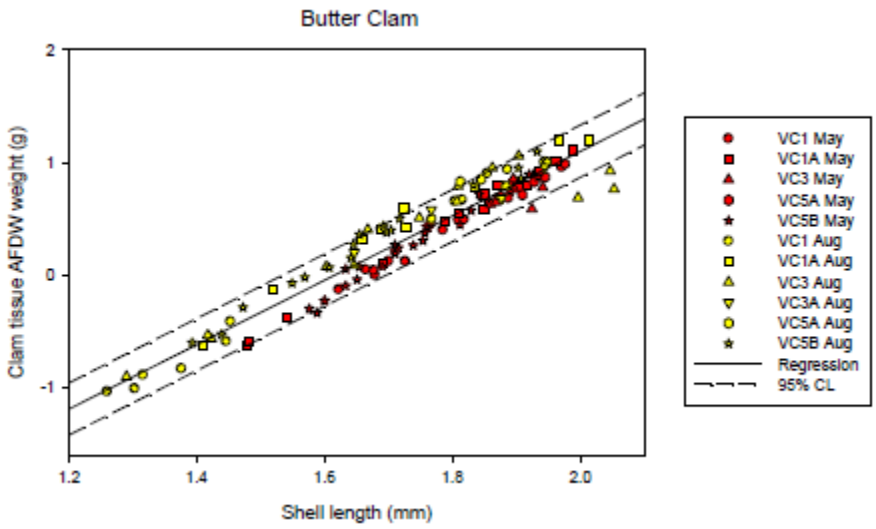

Figure 10: Clam condition index: Relationship between the inorganic portion of clam tissue biomass and shell length of Butter clams (Saxidomas gigantea). Note that: 1) majority of data fall within the confidence limits of the regression identifying a healthy population; 2) the tissue weight increased between May and August relative to shell length; and 3) new recruits appeared in August. Axes are log transformed. AFDW = Ash-free dry weight (Sutherland et al. 2019b).

2.3.11. MACROALGAE

While macrolgae have been identified as effective indicators of nutrient enrichment in coastal estuaries (Ahn et al. 1998; Robinson et al. 2005). More specifically, certain algal taxa, (e.g. *Ulva linza*, *Ulva intestinalis*) that experience increased nutrient uptake, growth rates, and capacity to store nutrients are considered to be effective bio-indicators of eutrophication (Fong et al. 1998, Fong and Zeldar, 2000; Bat et al. 2001; Cohen and Fong 2006). The production of many intertidal algal species may change rapidly in both space and time and often bloom following pulsed influxes of nutrients (Fong and Zeldar, 2000). For example, *Ulva* species have been shown to display a spatial pattern in relation to trends in stable nitrogen isotope when it blooms in areas of high nitrate and ammonium (Gartner et al. 2002, Teichberg et al. 2008). In addition, seasonal increases in the uptake of dissolved nutrients by algae (ammonia, nitrate/nitrite) can be influenced by increasing temperatures as well as changing nutrient availability which may be experienced in the summer season (Fong and Zeldar 1993; Fong et al 1993,1994). Furthermore, these indicator algae also sequester trace-metals, which in turn may be used to identify spatial patterns in anthropogenic inputs (Villares et al. 2001; Wildish et al. 2005).

2.3.11.1. Field collection

The surface coverage of algae (%) is recorded for each sampling quadrat. Algae should be carefully removed from the sediment surface of a 0.25 m^2 quadrat (Figure 11). Algal samples should be stored in a cooler for transportation by boat to a freezer back at the laboratory.

2.3.11.2. Laboratory analysis

Algae samples should be rinsed with filtered seawater, separated into taxa categories, and prepared for a variety of analyses (biomass (dry weight) and trace-element content). Algae can be dried in a conventional oven at 55° C for 24 hours or until a constant weight has been

achieved. Dry weights can be determined using a sensitive balance with a 5-decimal range (e.g. Sartorius). Biomass can be determined for the following categories depending on the monitoring objectives: total algae; algal taxa, or individual algae.

Figure 11: Before (left) and after (right) removal of macroalgae within a sampling quadrat.

2.3.12. EELGRASS

Eelgrass beds provide important nursery habitats for juvenile salmon and crabs, refuge from predators, and a substrate for invertebrates that serve as prey for higher trophic levels (Kitting, 1984; Short and Wyllie-Echeverria, 1996; Chambers et al., 1999). In addition, ducks and geese rely on eelgrass as a staple food source (Baldwin and Lovvorn, 1994; Vermeer et al., 1994). Eelgrass habitat may influence its environment by reducing current and wave action, as well as increasing sedimentation of particles, organic deposition and sediment stability. Further, eelgrass beds may promote a shift in redox potential as well as biofilm and invertebrate communities (Orth et al., 1984; Heck et al., 1995; Heiss et al., 2000; Gacia et al., 2003; Carr et al., 2010). Eelgrass beds are considered to be sensitive habitats by Fisheries and Oceans Canada (Vandermeulen, 2005) following the definition of habitat sensitivity outline by ICES (2002). Coastal developments, including port developments, have influenced eelgrass beds (Sutherland et al. 2013; Murphy et al. 2021). In B.C., the native (*Zostera marina*) and invasive (*Zostera japonica*) eelgrass taxa are typically segregated spatially on shorelines due to differential preferences for low- and high-tide conditions, respectively (Nomme and Harrison, 1991). Precision Identification (2002, 2004) and Neckles et al. (2012) provide reviews of eelgrass ecology and outline standardized methods to classify, map, and monitor eelgrass habitat at different spatial scales, while Murphy et al. (2021) and Shafer et al. (2014) review management and conservation approaches based on science assessments of eelgrass ecology.

Emerging research regarding remote-sensing surveys designed to estimate coastal topography (tidal flats), seabed bathymetry, and vegetation cover (eelgrass) can provide high-resolution eelgrass estimates distributed across a varying landscape at large spatial scales (Sutherland et al. 2007; O'Neill and Costa. 2013; Barrel et al. 2015; Collins et al. 2016; Webster et al. 2014; Forsey et al. 2020. In order to capture both landscape and vegetative coverage, a suite of survey methods can be selected in various combinations from the following techniques according to project objective: 1) multi-spectral satellite imagery (vegetation); 2) aerial-born LIDAR (Light Detection and Ranging: topography, bathymetry, vegetation), Red-blue-green photographic images; and 3) subsea sonar (acoustic Multibeam and Biosonics® (single-beam)). While remote sensing surveys rely on manual benthic and water-column surveys to ground-truth

techniques, they provide a means of capturing large and inaccessible areas at a high frequency (O'Neill and Costa, 2013). Further, satellite imagery may allow for hindcasting to provide a history of eelgrass coverage as a baseline for current and future studies. While the field and laboratory work consist of manual survey techniques, these emerging remote techniques, that are rapidly evolving, can be considered in combination of future monitoring efforts involving manual surveys.

2.3.12.1. Field collection

Table 3 provides a guide to combine various eelgrass metrics according to a monitoring objective, where certain metrics can be 1) measured *in situ*; 2) collected for laboratory analysis; or a 3) combination of both types of data collection. In addition, Figure 12 provides a schematic diagram of eelgrass (*Zostera marina*) structures including leaf (blade), sheath, and root-rhizome components. Eelgrass can be reported as density (Leaf Area Index) or biomass (dry weight) according to sampling area. Given the patchy nature of eelgrass, it is important to collect replicate estimates of these measures at each sampling station. In terms of Leaf Area Index, the following variables should be recorded: shoot density (number of shoots cm^{-2}), number of blades (leaves) per shoot, blade density (number of blades cm^2), and blade length Leaf Area Index (LAI; Precision Identification, 2002; 2004) can be calculated based on the cumulative area of eelgrass blades as follows:

LAI_{blades} = [Avg shoot length (cm)] x [Avg blade width (cm)] x [blade density (No. area)]

The advantage of measuring LAI is that it is not an intrusive method to the habitat and does not rely on laboratory analysis. However, eelgrass LAI and biomass estimates can be challenging when macroalgae are present and entangled with eelgrass (Figure 13).

Table 2: Eelgrass monitoring metrics for assessing health status.

Monitoring Metric	In situ data collection	In field sample collection	Level of resolution required
Shoot density	Shoot number by quadrat	Collect all shoots in core sample	Basic level: Distinguish shoot by the leaf sheath enclosing the base
Flowering shoot frequency	Flowering shoot number by quadrat	Collect all shoots by core sample	Proportion of flowering shoots to total shoots
Shoot size	Measure length and width of each leaf	Measure length and width of each leaf	Measured to sheath height or maximum length at shoot base in sediment
Above-ground shoot biomass	N/A	All shoots in core sample	Separate and dry individual shoots
Below-ground rhizome biomass	N/A	Entire rhizome-root in core sample	Separate and dry individual rhizome-root associated with individual shoots
Leaf Area Index: amount of leaf area per ground area	Count and measure shoot leafs per quadrat	Count and measure shoot leafs per quadrat	Requires length and width of all leaves per shoot. May require subsampling in dense areas or low tide restrictions

In terms of biomass, both the root-rhizome mat and the shoot-blade-canopy of eelgrass can be included in estimates, since many invertebrate species show close associations with these two distinct portions of seagrasses (Orth et al., 1984). If so, each shoot/blade and connected root system should be extracted carefully, placed in a labelled Ziploc bag, and stored in a cooler for transportation to the laboratory (Xu et al. 2018, 2020; Figure 12).

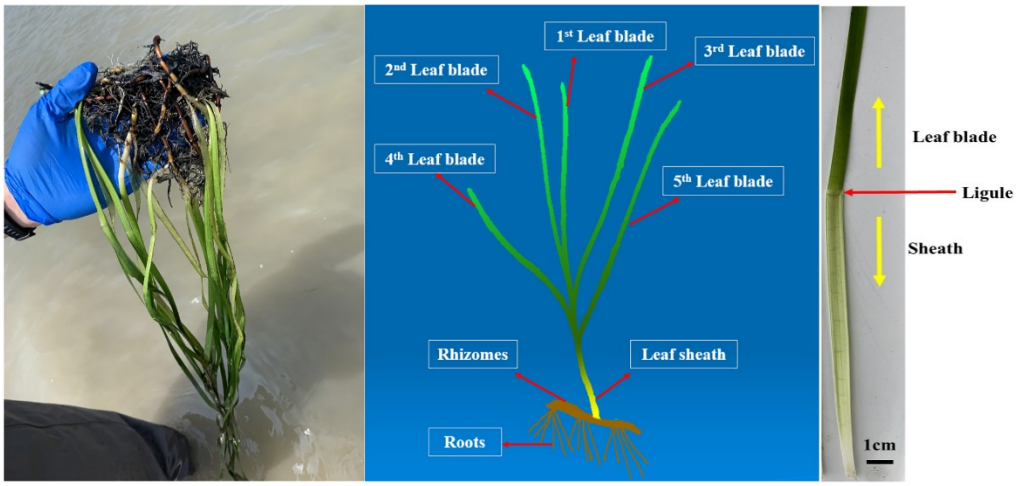

Figure 12: Zostera marina L. (left) and eelgrass structure (centre), showing above-ground (leaf blades and sheath) and below-ground (roots and rhizomes) tissues. The image on the right shows the division point between sheath and leaf blade (shoot) (Xu et al. 2020).

Figure 13: A 0.25 m^2 sampling quadrats at an intertidal area inhabited by eelgrass (left) and a mixture of eelgrass and macroalgae (right).

2.3.12.2. Laboratory analysis

Regarding eelgrass biomass, each eelgrass shoot and root system needs to be separated from entwined macroalgae and rinsed carefully with water to remove debris and tiny epifauna. The washed shoot and root-rhizome system can be separated and placed into a drying oven at 55°C for 24 hours or until a constant weight is achieved.

3. BENTHIC HARD-SUBSTRATE MONITORING METHODS

Hard-bottom substrates that are deemed “non-grabbable” cannot be sampled using conventional coring and grab methods. Non-grabbable seabeds are dominated by bedrock (true hard-substrates) and/or compact (mixed-bottom substrates) devoid of a sediment fabric that would retain fine-sediments and pore-water sulfide upon retrieval of a grab/core mechanism. In this regard, video methods have become part of regulatory monitoring programs on solid bedrock and mixed-bottom substrate environments where traditional chemical indicators cannot be collected (AAR, 2019). Video surveys can be carried out using a remotely-operated vehicle

(ROV) in deep-water settings or through SCUBA-diver assistance in shallow waters (Crawford et al. 2001; Sutherland et al. 2016a; 2018a; 2019a). Continuous survey transects (ROV) are recommended over discrete sampling (drop camera) that may not address heterogeneity between stations (patchy nature of substrate texture as well as motile or mega-benthos) that is typical of the benthic conditions in B.C. Drop-drift camera surveys may serve as a compromise between continuous and discrete (point sampling) video surveys by increasing areal coverage to increase robustness of abundance estimates of large or rare taxa as well as the level of patchiness within the area of interest.

Primary indicators of organic enrichment events on hard-bottom substrates associated with aquaculture consist of mat-forming taxa (e.g. sulfide-oxidizing bacteria, OPC) that can be seen on the seabed surface (Macleod et al. 2004; Emmett et al. 2008; Ross et al. 2016; Sutherland et al. 2018a). The two biotic indices identified in the AAR (2019), that represent benthic organic enrichment, can also be applied to benthic monitoring for bivalve aquaculture-environmental interactions. Biotic indices should include percent areal coverage of: 1) indicator species (sulfide-oxidizing bacteria, OPC); and 2) abundance of common and transitional epifauna within a video image frame (Emmett et al. 2007; Sutherland et al. 2018a). Abiotic indices may include the percent coverage of substrate type (e.g. compact sand, pebble, cobble, boulder) according to the Canadian Wentworth Scale (Wentworth, 1929) as well as other natural (e.g. shell-hash) and/or anthropogenic material (e.g. farm debris: deposited shells).

3.1. FIELD COLLECTION, MANAGEMENT REGULATORY THRESHOLDS, AND LABORATORY ANALYSES.

3.1.1. Field methods

3.1.1.1. Continuous video surveys along transects

Benthic video collection should follow Section 3 of the AAR (2019) that outlines minimum technical requirements for ROV equipment (e.g. lights, lasers) as well as survey protocols for continuous video assessments associated with finfish aquaculture. In general, a video survey starting point and trajectory is typically initiated from the edge of an aquaculture structure and aligned with a dominant and/or sub-dominant current direction as part of compliance monitoring. However, in order to avoid depth-confounding effects on biotic diversity estimates, which typically exhibit a strong vertical zonation distribution within the steeply-sloped inlets of B.C. (Levings et al. 1983, Leys et al. 2004), it is recommended that video surveys follow a constant bathymetric contour within a dominant current direction (Sutherland et al. 2016b). In order to delineate between water-depth and aquaculture influences on taxa distribution, it is important to stay within a 10m depth-range across both continuous and discrete video surveys. In terms of long-distance surveys, ROV transects should be divided into individual 80-m transects (maximum distance) to avoid the potential for veering off a desired bearing that can take place over longer survey lengths (Emmett et al. 2007).

Weighted-buoys are deployed at geo-referenced locations at transect distances of 0 and 80 m from the netpen system. Transect end-points are established using the GPS start point to produce a “dead-reckoning” end-point location based on a designated bearing. At the 0 m location, the ROV-camera should descend on a vertical line until it has reached the seafloor. After orienting its position on a desired bearing, the camera is remotely driven at a speed of 0.2 m s^{-1} and a height of 0.5 metre above the seafloor. Once the ROV reaches the buoy anchor deployed at the end of the transect, it ascends to the surface of the water. A second video transect can be repeated in a similar manner to the first transect. The video image should be displayed in real time on the boat deck to assist the ROV operator and should be equipped with an overlay of time and location of camera (dGPS latitude, longitude, and depth above seafloor).

The use of tandem forward facing (FF) and downward facing (DF) cameras for surveying transects along with tandem viewing screens for combined viewing of FF and DF videos will reduce the potential loss of information due to: (1) alternating blackout periods of each camera type passing over wall vs. ledge dominated substrates; and (2) preference for detecting taxa based on size, structure, shape, and/or contrast with background substrate (Figure 14) (Sutherland et al. 2019a).

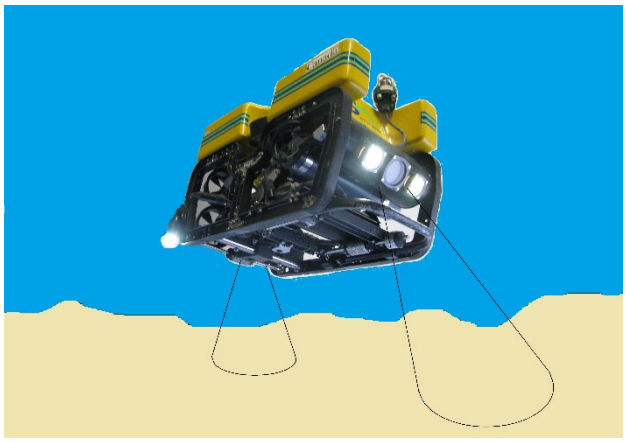

Figure 14: Diagram of a remotely operated vehicle (ROV) equipped with tandem forward-facing and downward-facing camera orientations for surveying the seabed (Sutherland et al. 2019a).

Drop-drift video surveys

Video surveys can be carried out at discrete stations along a linear transect or grid design that will help characterize an organic enrichment gradient, a depositional field or a bay-wide assessment. A tow- or drop- camera can be used to either collect 1) 5 replicate still photos (point-sampling), or 2) 3-minute drift videos at designated stations based on the local conditions and program objective (Sutherland et al. 2011a). The 3-minute drift video provides a larger areal coverage of a seafloor to accurately estimate large, rare, or patchy taxa abundance that may not be represented with smaller photo images. Although the camera height above the seafloor should be prescribed and standardized across a monitoring program, it can be modified for programs according to the detectability required for targeted indicator epifauna that range in size or contrast from the background (eg. mat-forming, small, or camouflaged taxa). One disadvantage of this system is that wavy conditions and steep slopes create challenges during tow/drop camera deployments in terms of maintaining a focal length relative to the seafloor.

3.1.2. Management regulatory threshold

In terms of benthic video assessments associated with finfish aquaculture, a regulatory performance threshold exists for surface mat-forming indicators (*Ssulfide-oxidising bacteria, OPC)* of benthic organic enrichment on mixed- or hard-bottom sustrates. This threshold consists of a minimum 10% spatial coverage of 1) any four video-segments (2 m length) within 100 m to 124 m from the finfish cage edge; 2) two contiguous video-segments are within 116 m to 124 m from the finfish cage; and 3) a minimum of two or more contiguous video-segments are within 124 m to 140 m from the finfish cage edge (AAR, 2019; Guidance Document: Section 11). Additional research is required to develop a regulatory threshold for bivalve aquaculture associated with different waste outputs and an area-based monitoring approach. In addition, researchers have suggested other epifaunal taxa that may serve as indicators or organic enrichment (e.g. plumose anemones; Emmett et al. 2007; Sutherland et al. 2018a).

3.1.3. Laboratory analysis

In terms of continuous ROV surveys, each video transect is divided into 2-m long segments to provide a high-resolution spatial analysis of sediment texture and epifaunal biodiversity. In terms of quantifying individual taxa, individuals can be counted as they crossed a line placed at a mid-horizon of the video image over a 2-m video distance. The video segment length is determined using the time stamp and the fly speed (0.2 ms^{-1}). When dealing with the entire epifaunal community, the data can be converted and standardized to 4-m segments to provide greater areal coverage for larger organisms (e.g., seastars, anemones, etc.). Literature sources recommended for BC taxa identification include Harbo (1999); Lamb and Edgell (2010), and Lamb and Hanby (2005).

In terms of quantifying mat-forming taxa (sulfide-oxidising bacteria, OPC) or substrate coverage, each 2-m segment can be divided into manageable time (distance) components where the percent coverage estimates are recorded and averaged across the entire 2-m segment. Substrate coverage is reported according to the Wentworth scale (Wentworth, 1929) based on the following categories: sand-mud (< 4 mm); pebble (4 - 64 mm); cobble; (64-256 mm); boulder (> 256 mm). Additional substrate categories may include bedrock (> image area); rock wall (vertical slope); and shell-hash (broken shell-sediment mixture). Table 2 provides descriptions of substrate categories and identifies compatible sampling devices.

Table 3: Description of substrate categories for benthic video surveys (Sutherland et al. 2019a).

Parameter	Fine sediment (FS)	Mixed substrate (MS)	Rock wall substrate (RWS)
Sediment composition	> 85% mud, sand, veneer < 15% MS and/or RWS	Various combinations of mud, sand, veneer, pebbles, cobbles, boulders, bedrock, rock wall, shell-hash, and/or skeletal sponge matrix	> 85% rock wall substrate < 15% FS and/or MS
Seabed slope	Low grade	Low to high grade	Vertical rock wall
Sampling type	Grab / core / video	Grab / video	Video

4. PELAGIC MONITORING METHODS

This section will review pelagic monitoring variables that provide ecosystem-level interactions with bivalve aquaculture populations. The interactions of pelagic variables are complex within the dynamic ecosystem shown in Figure 15 (Cranford et al. 2006), where some relevant indicators may not have quantitatively defined regulatory thresholds. Nonetheless, it is important to include these pelagic variables in some combination to uphold the ecosystem structure that provide control points of bay-wide carrying capacity assessments.

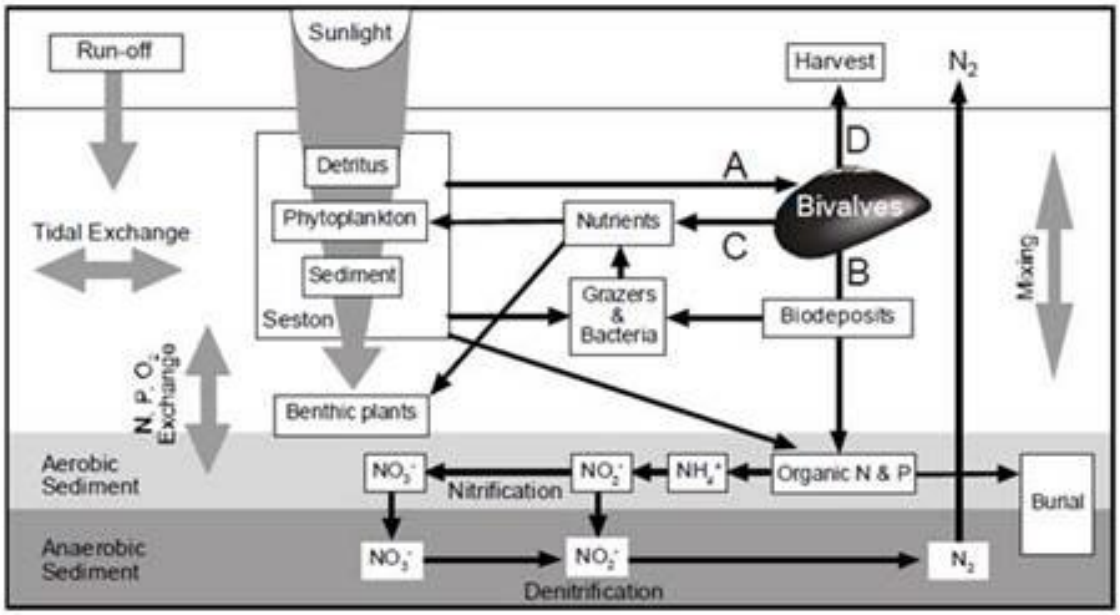

Figure 15: A conceptual diagram of the interaction between bivalve (shellfish) aquaculture and pelagic variables within a coastal ecosystem setting (Cranford et al. 2006).

4.1. FIELD COLLECTION, MANAGEMENT REGULATORY THRESHOLDS, AND LABORATORY ANALYSES

4.1.1. TEMPERATURE, SALINITY, DISSOLVED OXYGEN, AND WATER CURRENTS

Temperature, salinity, dissolved oxygen (DO), and water currents provide information regarding water-column stability (stratification) and water renewal events, the latter which results in oxygen replenishment (Lazier 1963; Strickland and Parsons 1972). DO is considered an important water quality metric and provides information regarding the health status of benthic and pelagic environments (e.g. hypoxia, anoxia) (Levin et al. 2009; Devlin et al. 2007; Pavlidou et al. 2015). Although mobile or transitory fish and bivalves can avoid hypoxic settings, non-motile or resident benthic taxa and cultured bivalves cannot avoid oxygen depletion events. Organisms may exhibit stress when exposed to hypoxic conditions associated with DO levels ranging between 2 and 5 mg l^{-1} (Diaz and Rosenberg, 1995; U.S. EPA, 2000, 2003; Vaquer-Sunyer and Duarte, 2008). This dissolved oxygen threshold range varies depending on taxa and duration of exposure.

A biophysical suitability assessment for aquaculture sustainability was carried out along the B.C. coastline according to minimum DO concentration (100% saturation) to ascertain the biophysical suitability of certain inlets for aquaculture production (Caine et al. 1987; Ricker et al. 1989). These ratings were based on existing DO levels and documented sublethal hypoxic effects associated with Canadian taxa as reported by Davis (1975). These DO classifications consisted of good (8.5 $mg \cdot L^{-1}$), medium (6.4 $mg \cdot L^{-1}$), or poor (4.6 $mg \cdot L^{-1}$). A DO depletion index was developed to serve as a ratio between DO depletion induced by: 1) fish respiration or microbial respiration associated with phytoplankton bloom degradation; and 2) a chosen farm-wide ventilation threshold level (DO replenishment) (Page, 2005; Page et al. 2005). Research is required to test this concept at a bay-wide approach in regards to bivalve cultured by both suspended raft and intertidal rack systems.

4.1.1.1. Field deployment

1) Time-series at a single station: a fixed multi-probe sonde deployment at a specified depth (e.g. chlorophyll maximum). Sonde includes temperature, salinity, depth, oxygen, turbidity (seston), and chlorophyll. Logging frequency depends on deployment time (e.g. 10 min interval); 2) Vertical profiles across a spatial grid: Conductivity-Temperature-Depth (CTD)-rosette deployments. Collection of water from rosette Niskin bottles and subsequent filtration methods for seston, chlorophyll, and nutrients are listed below; and 3) Water currents are commonly measured by acoustic Doppler current profilers (ACDP) or acoustic Doppler velocimeters (ADV), where the former meter can provide a water-column profile sectioned into data bins, while the latter meter provides high-resolution, point-source current velocities at a desired depth. Current meter data-logging specifications and mooring design both require input from an experienced oceanographer to 1) secure data collection compatible with monitoring/modelling objective; 2) consider both temporal coverage regarding both neap/spring periods over a 1-year period and spatial coverage with side-mounted boat profilers; and 3) develop and deploy current meter mooring according to site-specific conditions.

4.1.2. SUSPENDED PARTICULATE MATTER

Seston represents living and non-living suspended particulate matter (SPM) in the water column that is made up of inorganic material (e.g. silt, clay) and biological matter (e.g. plankton, nekton, flocculated organic material). Bivalves use the biological matter in the seston as food source (Suspended particulate organic matter (SPOM)). The specific characteristics of the seston determine the feeding behaviour of bivalve and, consequently, a good understanding of seston composition is key for individual bivalve bioenergetics. In addition, the spatial and temporal variability of seston is cornerstone to scale the individual processes up to the bay-scale level and understand the potential for production capacity of a bivalve aquaculture site. Bivalves can significantly alter seston concentration, which in turn may have negative consequences on both wild and cultured bivalves as well as other secondary producers (Cranford et al. 2006; Filgueira et al. 2015a). Understanding available sources of seston is key to determine if the cultured biomass relies on external sources of seston or on local production within the cultivation area (e.g. bay). Given the relevance of seston for other species within the food web, the utilization of seston has been commonly used as an indicator of the potential negative effects of bivalve aquaculture sites on the ecosystem, and consequently as a benchmark for ecological bivalve carrying capacity. In terms of interaction of bivalve aquaculture in the environment, DEPOMOD has been used to predict the dispersal of particulate aquaculture wastes around cultured bivalve sites (Weise et al. 2009), while carrying capacity studies have detected bay-wide or localized changes in seston concentrations (Grant et al. 2008; Guyondet et al. 2013; Cranford et al. 2014; Filgueira et al. 2015a). Seston, coupled with phytoplankton, can serve as a key variable in an area-based aquaculture management approach when assessing the carrying capacity of a bay.

4.1.2.1. Field collection

Pre-weigh Advantec filters GF75 (25 mm diameter, 0.3 µm pore size) and place in labelled Petri dishes. Deploy and retrieve Niskin bottle deployed to a designated depth and transfer a 1-L water sample to a clean labelled Nalgene Bottle. Filter 800 – 1000 mL of the water sample on to pre-weighed Advantec filter GF75 with a 25-mm diameter and a 0.3 µm pore size. Once filtration is complete, carefully return the filter to a labelled Petri dish and place in a freezer. Record the volume filtered and filter number on water sampling curation data sheet.

4.1.2.2. Laboratory analysis

Unfreeze the filters and place each filter and Petri dish in an oven set to 55°C. Allow the filters to dry for at least 24 hours or until a constant weight is achieved. The seston concentration is

determined by the weight of dry material on the filter standardized by the water volume filtered. Seston concentrations can be used to calibrate turbidity sensors that provide a higher temporal resolution. Suspended particulate organic matter (SPOM), an indicator of total food available for bivalves, can be determined by combusting dry SPM samples at 500°C for 5 hours. SPOM is calculated as the difference between dried and ashed weights, standardized by the dried weight for each sample (Guyondet et al. 2015).

4.1.3. PHYTOPLANKTON PRODUCTION AND COMMUNITY ANALYSIS

Phytoplankton is considered to be the main food source for bivalves, and consequently has been used as a potential indicator of depletion by bivalve populations (Filgueira and Grant, 2009; Guyondet et al. 2013). Furthermore, size fractionation of the phytoplankton population into nanoplankton (> 0.3µm) and picoplankton (0.3 – 2.0 µm) may be required given that bivalves can select food particles based on size (Cranford et al. 2006). Bivalves take food particles from the water at the level of the gill, where specialized cirri and cilia from the filaments generate water currents and capture particles. In general, retention efficiency is low for small particles and rapidly increases up to 100% efficiency with increasing particle size. The differential retention efficiency could shift the size spectrum of phytoplankton (e.g. picoplankton vs. nanoplankton) as a consequence of the grazing pressure from both bivalve and/or other secondary consumers (e.g. zooplankton, ciliates). In turn, there are confounding variables that can affect the ratio of picoplankton:nanoplankton such as seasonality of nutrient availability.

It has been suggested that the removal of micro-zooplankton by bivalve filtration, results in an increase of picoplankton, which serves as micro-zooplankton prey. This shift in phytoplankton structure suggests that both picoplankton and micro-zooplankton can serve as indicators of an ecosystem shift in addition to phytoplankton depletion (Figueira et al. 2015b). Thus, diel migration of phytoplankton within the upper water column may pose challenges in detecting phytoplankton depletion within the bivalve production layer. In this regard, monitoring programs need to account for vertical movement of phytoplankton above the pycnocline in deep bays in the Pacific region where 3D movement occurs. Examining broad phytoplankton taxa groups (diatoms, flagellates, and other taxa) will allow one to account for the 1) seasonal succession of spring, summer, and fall blooms and 2) vertical-migration of the flagellate community (Haigh and Taylor, 1990, 1991; Haigh et al. 1992). Despite these confounding variables, the ratio of picoplankton:nanoplankton matches at the regional scale with other indicators of aquaculture pressure (e.g. shellfish condition index) (Cranford et al. 2006). Primary production is key to determine if the utilization of phytoplankton by the cultured bivalve biomass exceeds the ecological threshold that can cause an impact on the ecosystem, a concept commonly coined as ecological carrying capacity. The Bivalve Standard of the Aquaculture Stewardship Council (ASC, 2019 proposed a criterion that no more than one third of phytoplankton primary production should be used by cultured shellfish would lead to the same conclusion. Accordingly primary production, dissolved nutrients and zooplankton are collectively considered key indicators supporting carrying capacity assessments (Mackas and Harrison, 1997).

4.1.3.1. Field collection

Seawater samples compatible with phytoplankton collection can be collected using Niskin bottles, pipe samplers, or pumps (Sutherland et al. 1992). In order to preserve fragile phytoplankton, it is important to avoid the use of rotary pumps and the shaking of samples vigorously prior to preservation or filtration. Once the phytoplankton sample is obtained, processing should commence immediately in semi-dark conditions to reduce artefacts associated with light shock and predation in the sample bottle. In terms of phytoplankton size fractionation estimated as chlorophyll concentration, seawater (150 mL) should be filtered through a GF75 filter (Diameter: 25 mm; pore size: 0.3 µm) for a total phytoplankton

(nanoplankton + picoplankton) estimate and another 150 mL of water filtered through a PCTE polycarbonate filter (Diameter: 25 mm; pore size 2 µm) to determine the picoplankton size fraction (0.3 – 2 µm). Each filter is folded and placed in an individual labelled scintillation vial and placed in a freezer. Record date, sampling station/depth, and volume filtered for each sample. In terms of phytoplankton taxa groups, seawater (100ml) is transferred into an amber 125 ml jar, where 5 drops of Lugol's Solution are added to achieve a tea-coloured sample. After the lid is securely placed on the jar, the sample is gently tipped back and forth to allow for gentle mixing of phytoplankton within the jar.

4.1.3.2. Laboratory analysis

Phytoplankton size-fractionation

A fluorometer (Turner^{10AU}) is used to carry out chlorophyll measurements. The filters are removed from the freezer and placed in the dark. Ten mL of acetone (90% acetone:10% distilled water) is placed in each scintillation vial and stored in a dark, cool fridge for 24 hours to allow for the acetone solution to extract the chlorophyll. After the extraction period, the acetone in each vial is transferred to a cuvette with the glass-fibre filter left behind in the scintillation vial. The cuvette is wiped free of moisture and inserted into the fluorometer. After the chlorophyll reading on the display is recorded, 3 drops of 10% HCl is added to the acetone in the cuvette. A second reading is recorded for a phaeopigment estimate. The fluorometric readings are converted to chlorophyll and phaeopigment according to Parsons et al. (1972). *Phytoplankton taxa groups:* Phytoplankton taxa are analysed using the Utermohl technique inverted microscope (Hasle, 1978), where the counts are converted into cells L^{-l}.

4.1.4. PHYTOPLANKTON PRIMARY PRODUCTIVITY

Although bivalves can use organic detrital matter as a food source, phytoplankton has been identified as their main food source. Therefore, bivalve aquaculture tends to occur in sheltered bays associated with high levels of primary productivity to provide a sufficient food source (Dame and Prins, 1998). In the context herein, "primary production" is defined as the measure of the standing stock (e.g. chlorophyll concentration/biomass), while "primary productivity" is defined as the rate at which phytoplankton is produced, in terms of carbon mass uptake per unit time per unit volume (Harrison et al. 1991). These definitions have been adopted to be consistent with phytoplankton literature of coastal B.C. (St. John et al. 1992; Harrison et al. 1999; Grundle and Varela, 2009).

In order to assess the ecological bivalve carrying capacity of a bay, it is important to know how phytoplankton growth is affected by seasonal and anthropogenic influences (Guyondet et al. 2015). For example, phytoplankton diversity shifts seasonally between diatom- and flagellate-dominated communities which are characterized by different productivity rates (Furnas, 1990). In order to avoid phytoplankton depletion in a bay, one needs to examine productivity rates in terms of water column growth factors (e.g. temperature, dissolved nutrients) and predation by zooplankton and bivalves. Thus, primary production, primary productivity, dissolved nutrients, and zooplankton are considered potential indicators supporting bivalve carrying capacity assessments in protected bays. The following sections outline methods for the quantification of dissolved nutrients and zooplankton.

4.1.4.1. Field collection

Collect a water sample at a designated depth within the water-column using a dark Niskin-bottle. The sampled water should remain in the Niskin bottles (fresh, in the dark) for a minimum of 20 minutes (dark adaptation) before collecting a water subsample. At each sampling station, collect water in 2 clear and 2 darkened 500 mL polycarbonate bottles (labelled by station).

Transfer 650 mL directly from the Niskin bottle into each of the 4 prepared polycarbonate bottles until the bottles are filled to the top (650 mL volume in total). Place bottles in a cooler on the boat deck. Repeat the transfer of water from Niskin to polycarbonate bottles for the water samples collected at other depths.

4.1.4.2. ^{13}C incubation

Add 600 µL of the ^{13}C solution into each bottle [6g of $NaH_{13}CO_3$ (99% 13°C) in 250 mL of de-ionized water]. Close the labelled bottle and secure the screw cap. Record the approximate time that the ^{13}C was injected into the bottles as well as the time of the deployment and retrieval of the bottle mooring following the 24 hour incubation period. Secure all bottles on the deployment line by feeding the hose clamp through the rope-twine and securing the hose clamp around the neck of the bottle (Figure 16). The bottles should be at a similar depth in which the water was collected. Label the floatation buoy with contact information. Install HOBO light and temperature loggers beside the sample bottles attached to the line. Transport deployment equipment to the location of water collection and carefully lower mooring block over the side keeping the line tight to ensure bottle attachment. Deploy mooring for 24h. Keep bottles in a dark cooler while transporting them back to a filtration station.

Figure 16: Primary productivity mooring showing 3 sets of duplicate light/dark incubation bottles at various water depths located above within, and below the chlorophyll-max layer (Left photo). A close up view of incubation bottles attached to mooring rope (Right photo).

4.1.4.3. Filtration

Only GFF (Glass fiber filters) should be used for primary productivity (^{13}C analysis). Polycarbonate filters (2.0 µm; PCTE) are used only to remove a phytoplankton fraction of the sample that will be filtered by the GFF. Carry out the following for each bottle (dark and light): 1) ***Particles > 0.3 µm (Total phytoplankton):*** Filter 300 mL of sample directly on a 25-mm

Advantec GFF filter. Throw away the filtrate which consists of water that passes through filter. Place filter in a labelled individual scintillation vial and place in the freezer; 2) ***Particles 0.3 > 2.0 µm (Picoplankton)*****:** Filter an additional 300 mL on a 2-5mm polycarbonate filter (2.0 um; PCTE) and transfer the filtrate to a clean Erlenmeyer flask. Throw away the polycarbonate filter. Take the filtrate and re-filter it, this time through an Advantec GFF filter (0.3< 2.0µm portion of the phytoplankton population). Throw away filtrate and rinse Erlenmeyer flask with distilled water. Put filter in a labelled individual scintillation vial and place in the freezer for storage and transportation. Repeat this procedure for the next sample bottle.

4.1.4.4. Laboratory analysis

In the laboratory, add 100 µL of 0.5 N HCl to each sample vial to remove any residue of carbonates. Let acid evaporate overnight in fume hood. Dry filters at 55°C for 24 hours or when a constant weight is achieved and send samples to a laboratory for ^{13}C analysis.

4.1.5. DISSOLVED NUTRIENTS

Nutrients play a large role in phytoplankton dynamics as they can either limit or promote primary productivity based on seasonal and anthropogenic inputs in the Strait of Georgia (Harrison et al. 1983; St. John et al. 1992; Sutton et al. 2013). Dissolved nutrients are a key variable in bivalve carrying capacity assessments that focus on a nutrient-phytoplankton-seston-bivalve loop within a high-resolution, spatially-explicit, hydrodynamic-biogeochemical model (Filguiera et al. 2015a). Understanding the source of nutrients available for the autochthonous phytoplankton population is relevant to understand if phytoplankton productivity relies on new inputs or nutrient recycling. The cultured population itself can act as a source of nutrients via excretion or remineralization of faeces, which can act as a reservoir of nutrients in periods in which natural abundance of nutrients is limiting, concomitantly altering the natural phytoplankton dynamics (Ibarra et al. 2014). The type of available nutrients can also influence the phytoplanktonic community (see above). Therefore, nutrient dynamics is relevant not only for primary productivity (see above) but it could be also useful to understand potential effects of the cultured population on the ecosystem. The common dissolved nutrients traditionally measured in environmental monitoring programs associated with phytoplankton population studies consist of nitrate (NO^{-3}), nitrite (NO^{-2}), ammonia (NH_4), phosphate (PO_4), and silicate (SiO_3). Dissolved nutrients are differentiated from particulate nutrients by filtration through a 0.45 µm filter.

4.1.5.1. Field collection

Dissolved nutrients are obtained by collecting the filtrate of seawater that has passed through a 0.45 µm filter. The filtrate can be stored in acid-washed 30-mL Nalgene bottles or falcon tubes and stored in a freezer prior to laboratory analyses.

4.1.5.2. Lab analyses

Frozen samples can be submitted to the Institute of Ocean Sciences for the analysis of dissolved nutrients (Barwell-Clarke and Whitney, 1996). Nitrate +Nitrite: Nitrate is reduced to nitrite by a copper-cadmium reductor column. The nitrite ion reacts with sulfanilamide under acidic conditions to form a diazo compound. This compound then couples with N-1-napthylethylenediamine dihydrochloride to form a reddish-purple azo dye which can be measured on a colourimeter. Silicate: The procedure is based on the reduction of silicomolybdate in acidic solution to molybdenum blue by ascorbic acid. Oxalic acid is introduced to the sample stream before the addition of ascorbic acid to eliminate the interference from phosphate. Orthophosphate: A reagent stream combining an acidified solution of ammonium molybdate, antimony potassium tartrate and ascorbic acid forms a phosphomolybdenum blue complex, which can be measured on a spectrophotometer.

4.1.6. ZOOPLANKTON

The predation of phytoplankton by both bivalve and zooplankton can potentially reduce phytoplankton populations below natural levels and result in “phytoplankton depletion” (Grant et al. 2008; Guyondet et al. 2013; Cranford et al. 2014; Filgueira et al. 2015a). The competition between zooplankton and bivalves would favour bivalves due to the unbalanced biomass favouring bivalves, rendering potential negative effects that may be more relevant for zooplankton than for bivalves as a whole. This unbalanced biomass has been used to remove zooplankton from ecosystem models with focus on the shellfish-phytoplankton interaction. The rationale is that the effect of zooplankton in phytoplankton populations is negligible compared to the effect caused by the cultured population. Contrarily, removing zooplankton is not feasible in food-web models given that zooplankton constitutes a key link for energy transfer between phytoplankton and higher trophic levels. In addition, shellfish could potentially predate on zooplankton, although the motility of zooplankton can minimize this potential trophic interaction as it was demonstrated for the case of lobster larva and mussels (Sonier et al. 2018). Direct feeding on zooplankton, or indirect changes via shifts in the food web could affect zooplankton populations (e.g. Nielsen and Maar 2007; Maar et al. 2008). Although the effects on zooplankton populations caused by bivalve aquaculture have not been the main focus of monitoring and carrying capacity studies, potential effects on the larvae of valuable species has recently increased the interest for bivalve-zooplankton interactions.

4.1.6.1. Field collection

Water-bottle, pumping systems, and nets can be used to collect zooplankton depending on their size, life stage, and swimming speed (Sameoto et al. 2000).In terms of zooplankton nets, length, mouth area, mesh size and tow speed need to be considered in a sampling design to prevent: 1) net avoidance behaviour from fast-swimming copepods; 2) net clogging, flushing, and bow wake during ascent; and 3) damaging more fragile taxa. Vertical net tows are commonly used in the deeper basins of the Pacific region, while horizontal or oblique tows are suited for shallow systems where one can target a chlorophyll-max layer. The ICES Zooplankton Manual (2000) can also be referred to for zooplankton size classifications, doubling times, nomenclature, morphology, fragility, and preservative (ICES, 2000; Figure 1.1, 1.2, 1.3, 1.4-1.12; Table 1.1) as a guide for choosing the appropriate collection method. The Institute of Oceans Science (Fisheries and Oceans Canada) have supported a large, archived zooplankton database that can be referred to when determining the local taxa and seasonal life stages of interest for a bay-specific monitoring program. In terms of estimating zooplankton abundance using eDNA analytical techniques, it is important to seek expertise prior to implementing monitoring methods due to fast-evolving development of these techniques associated with this emerging field of research (Djurhuua et al. 2018).

4.1.6.2. Laboratory analysis

Zooplankton samples can be analyzed by microscopy, coulter counter, Flowcam, and/or eDNA analytical techniques. Refer to the ICES Zooplankton Manual (2000) for the appropriate technique according to zooplankton size and objective of the monitoring program.

5. SUMMARY

The above recommended variables can be used in any combination depending on the monitoring objectives, nature of the estuary, and localized settings within in an estuary. Table 3 outlines environmental variables that would support different environmental monitoring theme assessments. End-users may choose to mix and match variables depending on their objective and site of concern. When developing a future monitoring program, study design may include

the following aspects: 1) temporal and spatial frequency; 2) reconnaissance surveys to identify reference sites using select indicators/variables; 3) reconnaissance surveys to determine replication requirements for each monitoring variable (power analysis); and 4) cost-effective and practical methods (Margalef, 1958; Raffaeilli et al. 2003; Solan et al. 2003; Wildish et al. 2005; Tweddle et al. 2018; Kuhn et al. 2019). Based on existing knowledge gaps, future research can highlight1) further validation of management regulatory thresholds surrounding mat-forming indicators, such as, sulfide-oxidizing bacteria and OPC in a variety of substrates and settings; 2) establishing thresholds for key indicator variables; 3) micro- and macro-plastic assessments establishing site-specific baseline reference areas, and 4) examining multiple stressors in a cumulative effects environmental setting. Finally, ecological bivalve carrying capacity assessments of aquaculture-laden bays would incorporate a suite of pelagic variables that make up the nutrient-phytoplankton-seston-bivalve loop required for the application of a hydrodynamic-biogeochemical model (Filgueira et al. 2015a). It is important to note that these monitoring variables may not have significance regarding a general ecosystem objective if measured in isolation of the other pelagic variables.

Table 4: Benthic and pelagic sampling variables classified according to ecosystem monitoring themes.

VARIABLES/INDICATORS	BIVALVE MONITORING THEMES/OBJECTIVES				
	Ecological carrying capacity	Benthic organic enrichment	Pelagic eutrophication	Sensitive habitat (eelgrass)	Physical installations (net, raft)
Soft-substrate variables					
Sediment grain size	-	BOE	-	SH	PI
Sediment porosity/organics	-	BOE	-	SH	PI
Sediment trace-elements	-	BOE	-	SH	PI
Sediment porewater sulfide	-	BOE	-	SH	PI
Sediment redox	-	BOE	-	SH	PI
Sediment nutrient influx/eflux	ECC	BOE	-	-	-
Sulfide-oxidizing bacteria	-	BOE	-	SH	PI
Opportunistic polychaete complex	-	BOE	-	SH	PI
Macrofauna (>0.5 mm)	-	BOE	-	-	PI
Meiofauna (0.063 - 0.5 mm)	-	BOE	-	SH	PI
Bivalve abundance/diversity	ECC	BOE	-	-	PI
Bivalve recruitment (Intertidal)	ECC	BOE	-	-	PI
Bivalve condition index	ECC	BOE	-	-	PI
Macroalgae	-	BOE	PE	-	PI
Eelgrass	-	BOE	PE	SH	PI
Hard-substrate variables					
Substrate composition	-	BOE	-	-	PI
Epifaunal abundance	-	BOE	-	-	PI
Sulfide-oxidizing bacteria	-	BOE	-	-	PI
Opportunistic polychaete complex	-	BOE	-	-	PI
Pelagic methods variables					
Temperature, salinity, oxygen	ECC	-	PE	SH	PI
Suspended particulate matter	ECC	-	PE	SH	PI
Phytoplankton production	ECC	-	PE	-	-
Phytoplankton primary productivity	ECC	-	PE	-	-
Dissolved nutrients	ECC	-	PE	SH	-
Zooplankton	ECC	-	PE	-	-
Water currents	ECC	-	PE	SH	PI

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