

# **Sediment grain size sampling and analysis within the Canadian Aquaculture Monitoring Program**

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AQUACULTURE MONITORING PROGRAM

by

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## EXECUTIVE SUMMARY

The Department of Fisheries and Oceans (DFO) national Aquaculture Monitoring Program (AMP) was initiated in 2017. Its mandate is to assess interactions between aquaculture and the environment using consistent protocols across the country. The purpose of this technical report is to provide advice on Sediment Grain Size (SGS) sampling and analysis methods to develop and implement an appropriate and optimal monitoring program. In the context of aquaculture monitoring, SGS in this report is defined as the size distribution of the inorganic particles after removal of organics and disaggregation. SGS plays a major role in sediment biogeochemistry and ecosystem function.

Deleterious substances tend to attach to the smaller particles. Flocs are aggregations of smaller particles which form under certain conditions. Flocs sink much faster than the unflocculated smaller particles which sink so slowly that they are unlikely to reach the bottom. The presence of aquaculture increases the likelihood of flocculation and thus may lead to the accumulation of deleterious substances and enhance the deposition of smaller particles leading to habitat alteration. For these reasons flocs are important and must be sampled and measured correctly. Appropriate sampling equipment must be used and for some environments, new sampling methods and equipment might be required.

Flocs are collections of particles of size range less than 16  $\mu\text{m}$ , resulting in a grain size distribution in this size range which is very smooth and without modal peaks. The Coulter Counter (CC) which has historically been used by DFO for grain size analysis correctly displays this distribution. Models to determine the fraction of bottom sediment deposited in the form of flocs can be applied to these size distributions to determine changes in the amount of material being deposited in flocs. The LS 13 320 laser diffraction instrument (LS) can analyse 3-4 times as many samples in a day as the CC. The LS uses a light scattering model which assumes modes exist and incorrectly resolves the part of the size distribution where the impacts are likely to be found. The assumption of modal trends, the need to input the refractive indices for the particles being measured and the suspending liquid, and application of diffraction models that assume spherical particles leads to poor correlation between the CC and LS data. In addition, samples from different depositional environments have different correlations. Size distributions from the LS can not be used to determine changes in the amount of material being deposited in flocs. As a result, it is not recommended that the LS replace the CC in the AMP.

In the effort to move towards an operational long-term consistent phase, this report complements Milligan et al. (2024) to provide direct science advice regarding the use of SGS for the AMP and its limitations. More specifically it addresses procedures and standards for sample collection and SGS analysis to reach three ultimate AMP goals: (A) characterizing bottom type and benthic habitat, (B) monitoring accumulation of fine-grained sediment and (C) providing the SGS data required to model the transport and deposition of aquaculture inputs. This document provides advice for internal and external AMP data users to ensure an accurate utilization and interpretation of SGS in the AMP database.

## ABSTRACT

Milligan, T., Law, B., Zions, V., O’Laughlin, C., and Lacoursière-Roussel, A. 2025. Sediment grain size sampling and analysis within the Canadian Aquaculture Monitoring Program. Can. Tech. Rep. Fish. Aquat. Sci. 3654: ix + 41 p.

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The grain size distribution of the inorganic fraction of bottom sediment can be used to characterize aquaculture sites, assess changes in sedimentation resulting from aquaculture, and to study the transport and deposition of deleterious substances. This report provides science-based advice to the Aquaculture Monitoring Program (AMP) of Fisheries and Oceans Canada on best practices related to sampling methods, laboratory analysis, and data interpretation for sediment grain size (SGS). This report complements Milligan et al. (2024) that concluded that the Beckman Coulter LS 13 320 laser diffraction instrument (LS) should not replace the Coulter Counter (CC) within the AMP to determine if changes to SGS are occurring. The LS is unable to determine the amount of material deposited in flocs limiting it to site assessment and monitoring the accumulation of fine-grained inorganic sediment in individual areas. It does not have the ability to compare different regions. The LS would not be effective for developing a nationally consistent database nor to study and model the transport of deleterious substances. Due to the natural variation of SGS, annual sampling of surficial sediments is not recommended. Instead, cores with undisturbed sediment water interfaces should be collected to determine changes in sedimentation over time.

## RÉSUMÉ

Milligan, T., Law, B., Zions, V., O’Laughlin, C., and Lacoursière-Roussel, A. 2025. Sediment grain size sampling and analysis within the Canadian Aquaculture Monitoring Program. Can. Tech. Rep. Fish. Aquat. Sci. 3654: ix + 41 p.

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La distribution granulométrique de la fraction inorganique des sédiments benthiques peut être utilisée pour caractériser les sites d’aquaculture, évaluer les changements dans la sédimentation résultant de l’aquaculture et étudier le transport et le dépôt de substances nocives. Ce rapport fournit des conseils scientifiques au Programme de surveillance de l’aquaculture (PSA) de Pêches et Océans Canada sur les meilleures pratiques relatives aux méthodes d’échantillonnage, aux analyses de laboratoire et à l’interprétation des données pour la granulométrie des sédiments (SGS). Ce rapport complète le rapport de Milligan et al. (2024), qui a conclu que l’instrument de diffraction laser LS 13 320 de Beckman Coulter (LS) ne devrait pas remplacer le compteur Coulter (CC) dans le cadre du PSA pour déterminer si des changements dans la SGS se produisent. Le LS est incapable de déterminer la quantité de matière déposée sous forme de floccs, limitant ainsi son utilisation à l’évaluation des sites et aux suivis de l’accumulation de sédiments inorganiques dans des zones spécifiques. Le LS n’a pas la capacité de comparer différentes régions. Il ne serait pas efficace pour développer une base de données nationale cohérente, ni pour étudier et modéliser le transport de substances nocives. En raison de la variation naturelle de la SGS, un échantillonnage annuel des sédiments de surface n’est pas recommandé. Plutôt, il est préférable de prélever des carottes de sédiments avec des interfaces sédiment-eau intactes pour déterminer les changements de sédimentation au fil du temps.

# 1 INTRODUCTION

Aquaculture alters sedimentation through the introduction of feed and faecal material, or, in the case of shellfish, the production of feces and pseudo feces. Sedimentation is defined as the overall process of particle transport to, emplacement on, removal from and preservation in the seabed (McKee et al., 1983). Two stages are encompassed in this description. The first is the initial deposition and temporary emplacement on the seabed and represents a relatively short period of sediment formation. The second is sediment accumulation which represents a much longer time and includes particle removal by physical erosion, biological resuspension, and chemical dissolution (McKee et al. 1983). The sedimentation of aquaculture wastes has been the focus of environmental monitoring as it often leads to anoxia (Chang et al., 2011) and loss of biodiversity (Wildish and Pohle 2005). Wildish and Pohle (2005) found in Letang Inlet NB *“that the macrofaunal changes are far field effects resulting from the increased sedimentation (quality and amount) associated with intensive mariculture.”*

Most monitoring methods have concentrated on the immediate area of disruption, normally within tens of meters of an aquaculture site. Measurements of redox potential (eH) and sulphide, organic content, and biodiversity are used to monitor the accumulation of wastes and identify exceedance of regulatory limits (Chang et al., 2011; Cranford et al., 2017, 2022; DFO-MPO, 2018). Sediment grain size (SGS) has frequently been identified as a key parameter for monitoring changes in aquatic environments because it can provide a record of the processes governing sedimentation in both the near and far field. In aquaculture, grain size is used to describe initial environmental conditions and, potentially, changes over time. SGS co-varies with sedimentary organic matter content, pore water composition, and bacterial community composition (Hargrave et al. 1997) and can be used to characterize local hydrodynamics. Characterizing benthic substrates is fundamental to the site selection process but also provides the baseline for monitoring the dispersal of wastes. With more accurate analysis of the size distribution of the inorganic particles in a sediment, depositional conditions can be described in detail and can be used to monitor changes in sedimentation over time (Kranck and Milligan, 1985; Milligan and Law, 2005; Law et al., 2014).

For both finfish and shellfish aquaculture monitoring, SGS has direct implications for organic enrichment, sulfide build-up, benthic fluxes, and the overall assimilative capacity of the benthos (Martinez-Garcia et al. 2015). At its simplest, fine-grained muddy sediment is found in low energy environments and coarse sands and gravels are found in high-energy environments. Analysis of the size distribution of the inorganic particles in sediment can be used to describe a site’s geomorphic setting and local fluid dynamics. It’s a controlling factor in sediment geochemistry and the transport and deposition of contaminants. Ecosystems with different bottom SGS react differently to organic enrichment, contaminant retention, bioremediation, bioturbation, and bio-irrigation processes (Martinez-Garcia et al. 2015). Cations derived from mineral and

pollution sources are preferentially adsorbed onto clay, which has the highest surface area to volume ratio of any particle size class. Contaminants also adhere to organic coatings on particles. Thus, most trace metals and many other contaminants associate closely with the fine particle fraction of sediments (Tye et al., 1996; Zwolsman et al., 1996; Milligan and Loring, 1997; Milligan and Law, 2013;). In aquatic environments, this material is transported in the form of flocs, agglomerations of both organic and inorganic particles (Kranck, 1973; Eisma, 1986; Milligan and Loring 1997; Milligan and Law, 2013; Seelen et al., 2018).

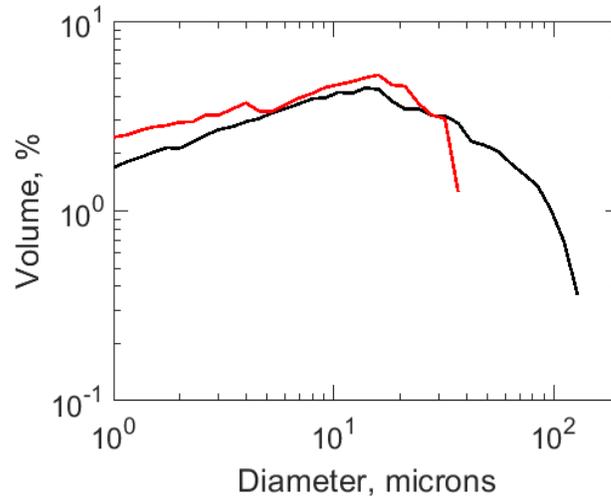


Figure 1: Coulter Counter (CC) disaggregated inorganic grain size distributions of the material deposited beneath a salmon cage (black line) and in suspension near bottom at the same location (red line). 71% of the inorganic particles in the top 1 cm are < 16  $\mu\text{m}$  and 84% in the suspended sediment are < 16  $\mu\text{m}$ .

Inorganic particles deposited with excess feed and feces under an aquaculture cage have the same size distribution as those in the overlying water because aquaculture does not introduce new inorganic particles into the environment (Fig 1). Instead, the addition of waste from finfish aquaculture increases sedimentation of inorganic sediment from suspension through the enhancement of flocculation. Flocculation is the term used for the process in aquatic environments that brings together colloids, organic material, and small inorganic particles to form loosely packed agglomerations of particulate material called flocs (Kranck, 1973, McCave, 1984, Eisma, 1986, Milligan and Kranck 1992; Milligan et al., 2007). Through flocculation, the settling velocity of fine-grained inorganic sediment and associated organic material is increased by several orders of magnitude (Sternberg et al., 1999; Fugate and Friedrichs, 2003; Fox et al., 2004). Flocculation rate is nonlinearly dependent on particle concentration and the contact efficiency or stickiness of the particles (Winterwerp, 1998, 2002; Milligan and Law, 2005; Milligan et al., 2007). Increasing concentration or stickiness accelerates the rate of floc formation. Turbulence enhances flocculation by increasing encounter rates, but beyond a certain level turbulence disrupts flocs (Milligan and Hill, 1998; Winterwerp, 1998, 2002). Increased particle concentration and stickiness caused by aquaculture can lead to rapid floc formation, increasing the number, size, and settling velocity of the flocs

(Milligan and Hill, 1995). Law et al. (2008) identified 16  $\mu\text{m}$  as the upper size limit for inorganic particles incorporated into flocs. With shellfish, high concentrations of organisms produce large numbers of feces and pseudo feces, the natural by-product of feeding (McKindsey et al., 2011). As shellfish are suspension feeders, deposited material also consists primarily of inorganic particles  $< 16 \mu\text{m}$ . With the addition of more cohesive material, the natural equilibrium between settling and erosion can be tipped in favour of the accumulation of this  $< 16 \mu\text{m}$  fraction leading to a fining of the inorganic grain size (Law et al. 2008).

Flocs are easily suspended and susceptible to breakup, making their dispersion dependent on local circulation patterns and intensities (Figure 2). Because they are easily suspended and break up under stress, flocs composed of fine-grained sediment and associated organic material will continue to be transported until they reach a location where bottom stress is low, leading to increased flux of both organic and inorganic material into the far field (Milligan and Law, 2005; Woodruff et al., 2013; Lavallee et al., 2020). This flux of sediment to the bottom can impact habitat quality (Wolanski and Spagnol, 2000; Wildish and Pohle, 2005; Bilotta and Brazier, 2008) and the presence of flocs in suspension can impact water quality and light availability (Boss et al., 2009).

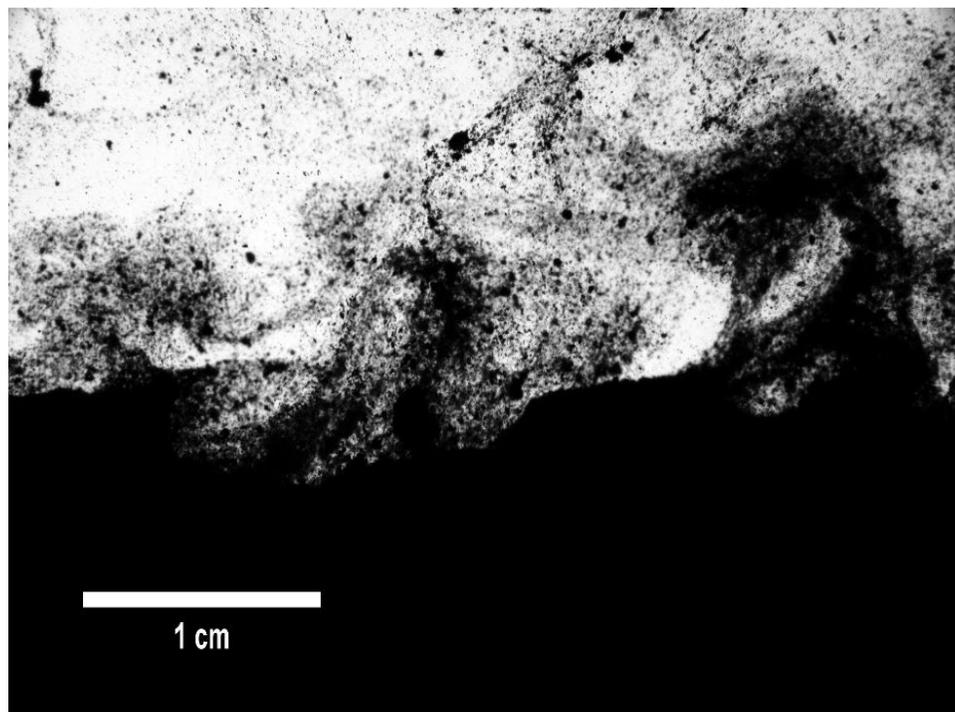


Figure 2: Silhouette image of flocculated sediment being suspended by a silhouette camera landing on the bottom.

Flocs incorporate surface-active contaminants through adherence to particle surfaces and bacterial exudates (Milligan and Loring, 1997; Milligan and Law, 2013; Reich et al, 2019; Hamoutene and Salvo 2020). Their settling velocity, on the order of  $1 \text{ mm s}^{-1}$ , is

orders of magnitude greater than that of the component particles. This allows contaminants to accumulate at the sediment-water interface. Because flocs are composed largely of organic material, they are a preferred food source for filter feeding organisms (Cranford et al., 2005; Lander et al., 2013) which can enhance contaminant uptake (Brown et al., 1986; Lyons et al., 2005; Kach and Ward, 2008). Physical alteration by structures associated with aquaculture can disrupt natural flow patterns which can lead to either deposition of fine-grained material when flow decreases (McKindsey et al., 2011) or scour in high current areas when flow is accelerated (Wu et al 2014). Suspension and transport of deposited material is dependent on the frequency and duration of events where the critical erosion stress of the deposit is exceeded. This can vary from virtually no resuspension in low-energy muddy environments to semidiurnal in tidal current regimes to seasonally in locations exposed to storm-driven wave events. Accumulations of cohesive material on the bottom can increase erosion shear stress and inhibit resuspension (Law et al., 2008, 2016, 2019). Dispersion of suspended material is governed by local current patterns and intensities and re-suspended waste particles will deposit where bottom stress falls below the erosion shear stress for the material. This can occur in the interstitial spaces of coarse sand, gravel, and cobble substrates (Law et al. 2016; Hamoutene et al., 2016, 2018; Marcos 2020).

## **2 SCIENCE BASED ADVICE**

### **2.1 Goal Description**

The purpose of this document is to provide advice and recommendations to ensure SGS sampling and analysis methods meet the requirements for a national AMP. Recommendations provided are specific to using SGS for three potential long-term goals of AMP:

- (A)** Characterizing benthic substrates to better understand variation observed in other parameters of interest for habitat classification and siting criteria.
- (B)** Monitoring the accumulation of fine-grained inorganic sediment resulting from aquaculture through flocculation or deposition of feces and pseudo feces.
- (C)** Used as an indirect parameter to model probability of post-deposition particle resuspension and transport of aquaculture inputs including organic material (OM), trace metals, pesticides, and therapeutants. Through the process of flocculation, surface-active contaminants are transported and deposited with fine sediment particles making SGS and its link to the size and settling velocity of flocs a key parameter for modelling.

Monitoring for changes in sedimentation due to aquaculture requires sediment sampling and grain size analysis methods to accurately determine changes in the transport and deposition of flocculated sediment. To be consistent, the program must include standardized methods for sampling bottom sediment that are appropriate for SGS and must achieve a level of accuracy that allow comparisons of impacts from a wide range

of depositional conditions. Assessing the accuracy of the current SGS technologies is essential to provide technical advice on the method and limits of SGS data to provide nationally consistent information around aquaculture sites.

## 2.2 Sediment Sampling Methods

Sediment sampling methods must be appropriate for the time scale of the disturbance being monitored (Table 1). For bottom sediments, the time scale of the disturbance determines the type of sample required. To characterize the sedimentary environment at a site, a sample that integrates an extended period is appropriate for capturing the net depositional conditions. In many coastal environments, sedimentation rates are on the order of 0.1 - 1 cm y<sup>-1</sup> so sampling the top 2 cm could integrate from 2 - 20 years of sedimentation. However, to monitor a site, it is critical that the sample includes recently deposited material from the sediment water interface and that the depth to which sediment is sampled represents a time scale corresponding to the expected impact of the disturbance (Milligan and Law, 2013). Ideally, the time scale of the deposit would be determined for bottom sediment samples to ensure that the sample is representative of the disturbance being monitored. This can be done with Pb<sup>210</sup> and Cs<sup>137</sup> dating of cores (Smith et al., 2005). Sediment initially deposited on the seabed can be mobile therefore methods that include material from the sediment water interface are required (Figure 2) (Kalnejais et al., 2007; Milligan and Law, 2013). In hard bottom areas, the substrate is unlikely to change, but aquaculture wastes may be deposited within the interstitial spaces in cobble bottom and specialized samplers will be required to sample them (Hamoutene et al., 2018).

The type of sampler used and the depth within the sample that is collected for analysis has a direct impact on the representativeness of the collected samples (Kalnejais et al., 2007; Milligan and Law 2013). For bottom sediment, sampling methods may be broken down into three basic categories: imaging, grabs, and cores. Within these three categories there are instruments with varying levels of effectiveness to address AMP goals.

Table 1: Specifications and availability of bottom sediment samplers in Fisheries and Oceans Canada, 2019.

	<b>Mini Slo-Corer</b>	<b>Haps Corer</b>	<b>Gomex Box Corer</b>	<b>Wildco Box Corer</b>	<b>Large Bore Gravity Corer</b>	<b>Eckman Grab (small)</b>	<b>Eckman Grab (large)</b>	<b>Van veen Grab</b>	<b>Ponar Grab</b>
<b>Site characterization</b>	Y	Y	Y	Y	Y	Y	Y	N	N
<b>Retain sample on recovery</b>	Usually	If no shell/rocks	If no shell/rocks	If no shell/rocks	Usually	If no shell/rocks	If no shell/rocks	If no shell/rocks	If no shell/rocks
<b>Slow entry</b>	Y	Y	Y	N	N	N	N	N	N
<b>Sharp leading edge</b>	Y	Y	Y	Y	Y	Y	Y	Y	Y
<b>Maximum diameter (cm)</b>	10	12.7	25 X 25	15 X 15	14.5	15 X 15	23 X 23	36 X 28	23 X 23
<b>Surface area of sample (cm<sup>2</sup>)</b>	79	127	625	225	165	225	529	1,008	529
<b>Volume of top 0.5 cm<sup>3</sup></b>	39	63	313	113	83	113	265	504	265
<b>Accurate partitioning of depth for subsampling</b>	Y	Y	N	N	Y	N	N	N	N
<b>Vessel capacity - Weight with sample (kg)</b>	70	100-160	81 max	49 max	200+	10	20	68-90	42

### **2.2.1 Imaging techniques**

Techniques include photographic and video surveys and side scan or multibeam imaging of the bottom. Several methods have been developed for photographic and video surveys of aquaculture sites that are best suited to qualitative assessment of the bottom (Crawford et al., 2001; Wildish et al, 2005; Sutherland et al., 2007, 2019). Recent advances in image processing have made a more quantitative assessment possible using structure from motion and other techniques (Figueira et al., 2015; Mohammed et al., 2020). This technique could be adapted to aquaculture monitoring (DFO, 2014). Sidescan sonar and multibeam are also capable of assessing bottom habitat and substrate type although in many cases these techniques require samples to be taken to validate post processing (Tlusty et al., 2000; Wildish et al., 2005; Brown et al., 2011). However, none of these methods can provide a sample for SGS analysis essential to reach goals B and C.

### **2.2.2 Grabs (Eckman, Ponar, Shipek, Peterson, van Veen, Smith-McIntyre)**

Grab samplers all rely on the same basic principle of landing on the bottom open and then closing over the sediment sample, although they vary greatly in size and ease of use. Some, like the lightweight Eckman grab, are best suited to sampling soft mud at shallow depths whereas others are better for consolidated sediment and sand. This largely is a factor of the weight of the grab although spring mechanisms are used to assist in sampling (e.g. Eckman, Shipek). Sample size and depth of penetration depend on sediment texture, area of the grab, and its weight. The size of the vessel required for deploying different grabs is weight dependent with the Ponar and small van Veen being deployable from small vessels, and in some cases, like a small Eckman in shallow water, by hand. In sands and gravels much larger grabs are required to penetrate to sufficient depth to recover a representative sample.

When using grabs, it must be assumed that flocculent material at the sediment-water interface will be lost on contact (Figures 2,3). Grabs also contain varying amounts of overlying water which drains during recovery and opening carrying with it the mobile surface layer. In muddy substrates, grabs like the Eckman can maintain a relatively undisturbed surface. The depth of the disturbance caused by sampling will vary with the type of substrate, size of the grab, and the force with which it impacts the bottom (Blomqvist, 1991).

Distortion of the sediment profile from closing the jaws frequently occurs during sampling thereby compromising the effectiveness of grabs for vertical subsampling. Except for the Eckman grab in soft sediment locations, a vertical profile of grain size with depth in the sediment will be difficult to determine.

The disturbance of the sediment-water interface and distortion of the vertical structure by grabs means they are best used for site description (Goal A).



Figure 3: Image of the surface sediment being suspended by the impact of a van Veen grab.

### 2.2.3 Corers (Gravity corer, HAPS, Multicorer, Box Corer, Slo-corer)



Figure 4: Gravity corer. Image courtesy of KC Denmark A/S.

Cores provide a historical record of sediment deposition that can be used to observe changes in SGS and associated contaminants over time. Each is based on the same basic principle: push a core barrel into the sediment. With the recognition of the importance of the sediment-water interface and preservation of the vertical structure within the core, new designs such as the Slo-corer and the HAPS corer have been developed that control the penetration and removal of the core barrel to prevent disturbance of the surface layer and contraction of the sediment. Cores have several advantages over grabs: there is less distortion of the vertical structure of the sediment, they can be vertically sectioned with precision, they can be dated using radioisotopes, and, more recently, their surfaces can be eroded to provide samples of the mobile layer. Gravity corers are some of the simplest (Figure 4). They are weighted and lowered to the bottom at a speed governed by the nature of the bottom sediment, with less weight and less speed being required for soft sediments. Most will have a core catcher at the end of the barrel to retain the sediment and a poppet valve at the top that closes on

retrieval to provide suction for withdrawing the core barrel without loss of the core. Gravity corers with this configuration will disturb both the surface of the sediment and along the length of the barrel. They can also cause shortening of the core due to compaction.



Figure 5: Box corer. Image courtesy of KC Denmark A/S.

Box corers are intermediate between grabs and corers (Figure 5). They deploy a large open box structure instead of a core barrel. They are open at the top and bottom which reduces the bow wave and are closed by a spade mechanism as they are lifted from the bottom. They are very large, heavy pieces of equipment and limited to vessels with several tons lifting capacity. The advantage of the box corer is that it can sample the surface with minimal disturbance and smaller core samples can be taken from the box and sectioned. It is often able to retrieve samples in coarse sediment.

Cores taken by divers by pushing a tube into the bottom and capping it before withdrawal can avoid disturbance of the surface layer and have the added advantage of being used where structures interfere with the deployment of other equipment. They are restricted in their use due to depth limitations and associated risk.

New corer designs focus on preserving the sediment-water interface and lessening compaction of the sediment. Most use open core barrels without catchers to reduce the bow wave and they delay the deployment of the core barrels until the unit has landed on the bottom. The barrel descends after landing with the depth of penetration being controlled by the amount of weight pushing on the core barrel. Mechanisms close the top of the core after penetration and seal below the core as the barrel clears the bottom on retrieval. The HAPS corer and the Multicorer are examples of this type of corer and are commercially available (Figure 6).

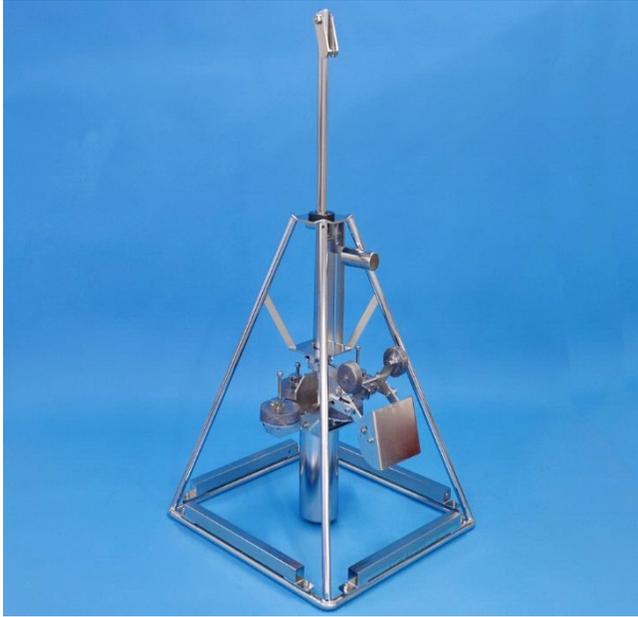


Figure 6: HAPS corer (left) and Multicorer (right). Images courtesy of KC Denmark A/S.

The Bothner style Slo-corer has the additional feature of slowing the rate at which the barrel contacts the surface of the sediment (Figure 7). This ensures that the sediment-water interface remains undisturbed during sampling. The barrels for the Slo-corer have been designed to interface with the Gust erosion chamber to determine erosion shear stress and provide samples of the flocculent material. The large Slo-corer uses a 300 kg weight to drive the core barrel into the bottom and can retrieve cores in sand. The mini Slo-corer uses 70 kg and is deployable from smaller vessels.



Figure 7: Bothner style large Slo-corer (left) and mini Slo-corer (right).

The Slo-corer and mini Slo-corer are not commercially available, but construction plans are available within DFO. Others like the box corer require large vessels which are

difficult to schedule or not available. Table 2 lists the types of corers available within DFO, their locations, and their operating limitations.

#### **2.2.4 Summary**

If the purpose of analysing SGS within the AMP is to use consistent methods to observe or predict changes to the environment related to aquaculture, the appropriate sampling equipment is required in all regions. All corers can be used to address Goal 2 with the understanding that gravity cores will lose the most recently deposited material. Goal 3, the transport and deposition of contaminants, requires corers that do not disturb the sediment water interface. None of the above sediment samplers can sample gravel or cobble bottom without loss of the flocculant material deposited in the interstitial spaces. New samplers will need to be designed if these environments are to be monitored. Aquaculture wastes deposited on sand can be susceptible to rapid removal and transport during increased bottom stress events (Law and Hill, 2019). Sampling sandy environments will also require new sampler designs and an appropriate sampling strategy that can capture the erosion of these wastes, which can be cohesive, and to observe where they are deposited (Law et al., 2016).

Table 2 provides a compilation of sampling challenges encountered during some recent sampling activities undertaken by the AMP.

Table 2: A compilation of sampling challenges encountered during some recent sampling activities undertaken by the Aquaculture Monitoring Program (AMP) that is being developed by Fisheries and Oceans. \* indicates this volume is for the top 1cm of the sediment collected by the grab or core.

Province	Region	Gear used by AMP	Substrate type	Depth limitation	Amounts sampled*	Challenges and solution
<b>Newfoundland and Labrador</b>	South Coast of NL	Tall Eckman grab (6"x6"x9")	Hard substrate with patches of soft sediment	Depths are > 30m with important slopes and in some situations depths greater than 100 m	125 – 1475 mL	<p><i>Challenges:</i></p> <ul style="list-style-type: none"> <li>the absence of a sediment layer due to hard substrates in some areas prohibits grabs.</li> <li>hard substrates can cause damage to the sampling gear and depth and slope can affect its closure.</li> <li>The small size of the grab and its low weight combined with drifting due to weather and depth introduces an angle delaying closing and issues in the precision of the sampling point</li> </ul> <p><i>Solutions:</i></p> <ul style="list-style-type: none"> <li>add weights to the jaws</li> <li>using video cameras to assess the substrate type at every station before deploying gear helps in limiting issues.</li> </ul>
<b>Nova Scotia</b>	SW Nova Scotian shore (Jordan Bay, Liverpool, etc.)  -Bras d'Or Lakes	Slo-Corer	-Very fine, compacted sands or mixed bottom (i.e., mud, sand, gravel, cobble, shell debris) in SWNS  Generally mud or mixed bottom in Bras d'Or Lakes	-Relatively shallow: depths < 20m with flat or gentle slopes.  -Some sites in the Bras d'Or Lakes have depths ~50-90 m.	< 100 ml	<p><i>Challenge:</i> Mixed bottom in some areas can make the use of a corer challenging.</p> <p><i>Solution:</i> The use of a large Ekman grab (9"x9") can be considered as an alternative.</p>
<b>New Brunswick</b>	Bay of Fundy	HAPS corer	Generally mud or mixed bottom (i.e., mud, gravel, cobble, shell debris)	Relatively shallow: depths < 20m with flat or gentle slopes.	126ml	<p><i>Challenge:</i> Mixed bottom in some areas can make the use of a corer challenging.</p> <p><i>Solution:</i> The use of a large Ekman grab (9"x9") can be considered as an alternative.</p>
<b>British Columbia</b>	Clayoquot Sound	VanVeen Grab	Generally mud	Depends on site (10 – 100m)	1008ml	<p><i>Challenge:</i> losing the top layer at grab closure (issues with national consistency of sampling).</p> <p><i>Solution:</i> The use of a corer should be considered as an alternative.</p>
<b>British Columbia</b>	Discovery Islands	VanVeen Grab	Mixed Bottom	Depends on site (30 – 200m)	1008ml	<p><i>Challenge:</i> the absence of a sediment layer due to hard substrates in some areas prohibits grabbing. Important depths and high currents make grabs challenging.</p> <p><i>Solutions:</i> Solutions proposed with the NL context could be applicable.</p>

## 2.3 Grain size analysis methods

### 2.3.1 Sieve and hydrometer (S&H)

The sieve and hydrometer method for grain size analysis has replaced sieve and pipet as the ASTM standard for SGS based on particle settling (Krumbein and Pettijohn, 1938). It is a long-established and commercially available method using sieves to determine the > 63  $\mu\text{m}$  fraction and particle settling in still water using a hydrometer for the < 63  $\mu\text{m}$  fraction. Hydrometer analysis calculates the size of particles from the speed at which they settle out of suspension from a dispersant suspension using Stokes Law, with the largest particles settling fastest. For hydrometer analysis, readings of the specific gravity of the suspension are recorded at set times (ASTM D7928-21e1). In pipette analysis, withdrawals are made from the suspension at times set by the class size settling velocity. Subsamples are dried and weighed. In both methods, the percent concentration in each size class is calculated from the loss of mass between time steps. Results are normally presented in phi units ( $\phi$ ) from the Udden-Wentworth scale:

$$\phi = -\log_2 d$$

where  $d$  is the diameter in millimeters.

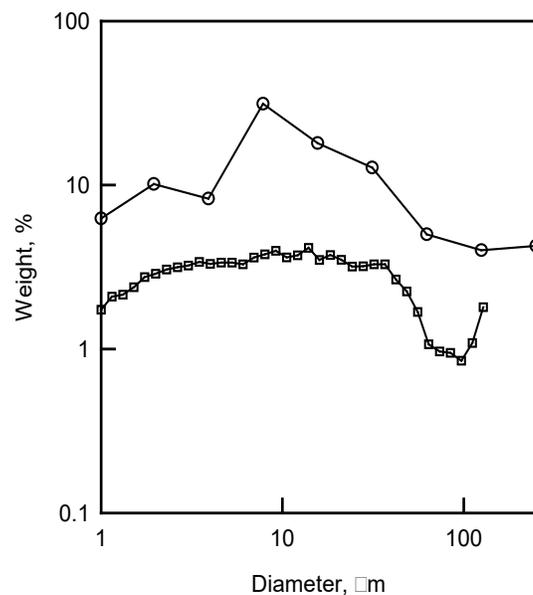


Figure 8: Comparison of sieve and hydrometer and CC SGS analysis for a sample from the Gulf Region. Resolution for the CC is  $1/5 \phi$  (diameter doubles every 5 points) and for S&P it is  $1 \phi$  (diameter doubles at every point).

#### Advantages:

- The method is commercially available and provides historical continuity.

#### Disadvantages:

- Because it relies on settling of particles in still water, samples can take 24 hours to resolve smaller sizes limiting the number of samples that can be run in a day. It is low resolution, 1  $\phi$  in most cases (Figure 8). The lack of resolution means it is unsuitable for looking at changes in the < 16  $\mu\text{m}$  size class of particles identified by Law et al., (2008) as the size range of inorganic particles incorporated into flocs. Because of its low resolution of grain size, it is only suitable for Goal A, habitat classification and site surveys.

### 2.3.2 Beckman Coulter LS 13 320 (LS)

The LS interprets particle size distributions by measuring the angle and intensity of light scattered by particles in suspension as a laser beam passes through the suspension. Small particles scatter light at large angles relative to the laser beam and large particles scatter light at small angles. The LS does not directly measure the size of particles, rather it uses algorithms based on the theories of light scattering to interpret the angle and intensity of light scattering from the particles to yield information about the particle size distribution. Both Fraunhofer and Mie theory are used (Beckman Coulter, 2011). Fraunhofer diffraction theory describes the maximal and minimum light scattering angles as a function of an object's size at any given wavelength independent of the optical properties of the particle or suspension. Fraunhofer tends to be the standard method as it a simpler form of scattering theory. However, particles must be much larger than the wavelength of the light (typically > 30  $\mu\text{m}$ ) and non-transparent (Beckman Coulter, 2011). Mie is a much higher precision derivation of light scattering theory which can resolve the < 50  $\mu\text{m}$  fraction with greater accuracy. However, it requires input of the refractive indices, both real and imaginary, for the particles being measured and the suspending liquid (Ozner et al., 2010). Mie theory accounts for all possible interactions between particles and light but is applicable only to spheres (Beckman Coulter LS 13 320 manual. The LS can only achieve 25%-50% peak-to-peak resolution of mono sized particles (Beckman Coulter, 2016).

#### Advantages:

- Laser diffraction instruments have a wide particle size range, nominally from ~0.4  $\mu\text{m}$  to > 2000  $\mu\text{m}$ , which varies with the model and manufacturer. The Beckman Coulter LS 13 320 in use in DFO has 92 logarithmic channels over a size range from 0.375 – 2000  $\mu\text{m}$ . A specialized PIDS (Polarization Intensity Differential Scattering) module can extend this range to 116 channels starting at 0.017  $\mu\text{m}$ . PIDS uses the polarization effects of light scattering with wavelength dependence at high angles to interpret the smallest particle sizes (Beckman-Coulter, 2011).
- Only small amounts of sediment are required for analysis, between 0.01 and 10 g for the LS 13 320, depending on the sample module being used and the size of the particles being analyzed. When the volume of material in log size classes is constant, the number of particles in each class increases logarithmically as diameter decreases so much less material is required for fine-grained mud than sand. The LS 13 230 sets minimum and maximum limits for obscuration of the laser by particles in suspension to ensure the proper concentration of particles is in the sensing zone.

- LD instruments have a large capacity for grain size analysis with ~30 samples per day being a reasonable output.

Disadvantages:

- Typical muddy bottom sediment creates unfavorable conditions for particle size analysis in the < 16  $\mu\text{m}$  fraction (see section 3.4) (Milligan et al., 2024).
- Platy and rod like particles can be under or overestimated.
- Samples with high refractive indices (e.g.  $\text{CaCO}_3$ ) must be accounted for.
- Results cannot be directly compared to earlier work with a CC.
- Results cannot be used to determine changes in floc deposition (Milligan et al., 2024).
- Technical expertise is required to maintain quality. The instrument requires continuous operator oversight to prevent erroneous data from incorrect obscuration levels, laser alignment, and dirty surfaces.

### 2.3.3 Beckman Coulter Multisizer 3 and 4 (CC)

Coulter Counters determine the number and volume of individual particles suspended in an electrolyte using the Coulter Principle (Coulter, 1953). Particles in dilute suspension are drawn through a small aperture of known diameter by vacuum. A constant current is maintained between two electrodes located on either side of the aperture. As a particle transits the aperture, it displaces an equivalent volume of electrolyte causing fluctuations in impedance which are detected as voltage pulses proportional to the size of the particle. The concentration of particles in the suspension is adjusted to avoid coincidence, where more than one particle occupies the sensing zone at the same time. Within the CC, a pulse height analyzer counts and sizes the pulses and assigns them to a pre-set number of logarithmic equivalent spherical diameter size classes, or channels, that are calibrated using beads of known diameter (Milligan and Kranck, 1991).

Each CC aperture has a size range of approximately 2–50% of its diameter. The latest models of the Coulter Counter, MS3 and MS4, have 256 and 300 logarithmic equivalent spherical diameter channels respectively over this size range. The channels are binned to produce a  $1/5 \phi$  class size where diameter doubles every 5 channels. This reduces the noise caused by low numbers of larger particles when spread over the total number of channels. Binned channels with < 10 counts are rejected. Multiple aperture tubes are required to analyze sediment, typically 30, 200 and 400  $\mu\text{m}$ . The SGS from the three tubes overlap and are blended to produce a nominal size range of 1–194  $\mu\text{m}$ . Larger and smaller apertures are available to extend this size range, but they require specialized techniques.

Advantages:

- The CC needs a very small amount of sediment for analysis as very low concentrations of particles are required to avoid coincidence. This allows for very precise vertical sampling of cores, ~ 1 mm (Wheatcroft et al., 2006).

- Water samples can be filtered for grain size analysis of suspended sediment (Hill et al., 2000; Lavallee et al., 2020).
- Its requirement for very small samples makes it effective for analysis of material from the sediment-water interface either from direct sampling or from erosion of the surface (Milligan and Law, 2013).
- The CC accurately measures the volume of individual particles.

Disadvantages:

- Grain size analysis with a CC is time consuming, reducing productivity to around 10 samples per day.
- Errors are introduced when the concentration of particles exceeds a coincidence limit.
- Most sediment samples will require significant reduction in volume before analysis which, like all subsampling protocols, must be carried out accurately.
- Platy and rod-like particles may be under or overestimated depending on their orientation and the assumption that they are spherical.
- Technical expertise is required to maintain quality as spurious counts from electrical noise can interfere with counting. The instrument requires continuous operator oversight to prevent erroneous data from things like blockages.

## 2.4 Results from CC and LS comparison

Within DFO, the CC has been used in the study of particle dynamics in aquatic environments since the 1960s (Sheldon and Parsons, 1967; Kranck, 1973). The hypothesis that the size distribution of the inorganic fraction of bottom sediments is composed of grains settled either in flocs or as single grains was developed using CC data (Figure 9) (Kranck, 1980; Kranck and Milligan 1985; Curran et al., 2004). The inorganic grain size distribution of bottom sediment determined by CC has been used to determine the amount of flocculation in aquatic environments (Milligan et al., 2024). Based on this model, it was shown that the amount of material deposited in flocs increased with the introduction of open cage salmon aquaculture in Letang Inlet, NB (Milligan and Law, 1995).

Weathering of rocks produces Rosin-Rammler size distributions which appear as straight lines when plotted as log-log (Krumbein and Tisdell, 1940; Kranck, 1980; Kranck and Milligan, 1985). During transport and deposition, these straight-line distributions are modified and when deposited the resulting sediment will consist of material settled as flocs, as single grains, and, in some cases, sediment that has been mixed into the sediment during higher energy events that can transport coarse grains in suspension (figure 9) (Kranck and Milligan, 1985). The slope  $m$  of the straight-line Rosin Rammler

distribution is preserved within flocs (figure 9). Fundamental to the floc model is the need to resolve this unsorted, straight-line portion of the particle size distribution.

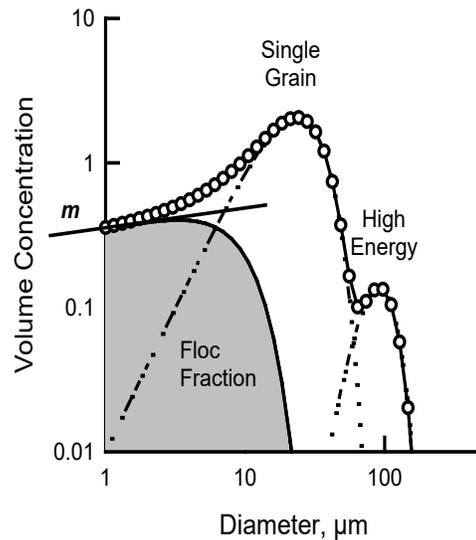


Figure 9: Idealized grain size distribution of the volume concentration of inorganic particles in a bottom sediment sample (circles) analyzed with a Coulter Counter, showing floc fraction, the contribution from floc deposition (grey), and single grain settling (dashed line) determined with the model of Kranck and Milligan (1985) as modified by Curran et al (2004). The high energy peak represents particles that might be introduced during high bottom stress events, possibly as bed load.  $m$  (solid line) is the slope of the unsorted parent suspension described by Krumbein and Tisdell (1940) and Kranck and Milligan (1985). Floc fraction varies with the amount of floc deposition.

The need for lower costs and an increased number of analyses made the LS an attractive alternative to the CC for SGS within the AMP. Laser diffraction is becoming a standard method for SGS (Norton, 2019; ISO 13320-2020) but it was unclear if the LS was an appropriate replacement for the CC in the AMP. A comparison of 581 samples from 5 different areas in Atlantic Canada analyzed with both the CC and LS found that the LS should not replace the CC within the AMP (Milligan et al., 2024). As shown and discussed in Milligan et al. (2024), the fundamental differences for determining size distributions result in very different looking SGS distributions for Sillikers clay, used as a lab standard for particle size analysis and in experiments (Kranck, 1980; White et al., 2022). Sillikers is a fine-grained glacial marine deposit of variably shaped clays and fine silt composed mainly of kaolinite and illite with 0.13% carbon.

Coulter counters can achieve size resolution of a mixed solution of mono-sized particles to better than 5% but the LS can only achieve peak-to-peak resolution of 25%-50% (Beckman Coulter, 2016). As a result, peaks appear much smoother with LS, and they tend to overlap. In Sillikers clay, volume concentration from the CC increases smoothly to a modal peak at  $\sim 20 \mu\text{m}$  and then decreases regularly (Figure 10). This size distribution consists of a floc settled portion,  $< 10 \mu\text{m}$ , and a single grain portion defined by the mode and the negative slope  $> \sim 30 \mu\text{m}$ . In contrast, the LS has positive and negative slopes that define two modal peaks at  $\sim 8 \mu\text{m}$  and  $\sim 25 \mu\text{m}$ . Where the slope of the CC distribution between 1 and  $10 \mu\text{m}$  defines a straight line, the LS changes slope

in a regular manner, defining a valley, suggesting that the straight line portion of the distribution is  $< 2 \mu\text{m}$ .

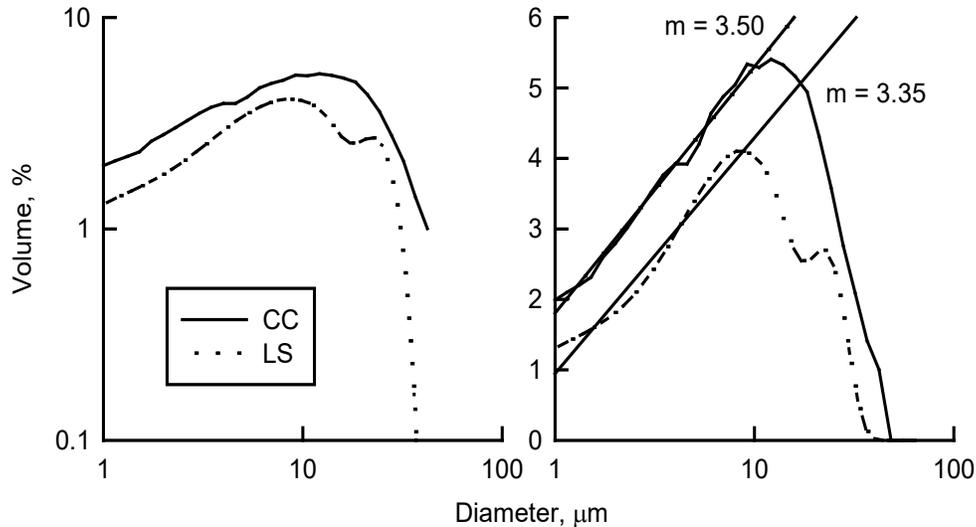


Figure 10: SGS of Sillikers Clay analyzed with the CC and the LS. Both log-log and semi-log plots of percent volume are presented to emphasize the difference between the size distributions. The LS has two smooth modal peaks where the CC consists of single mode. The slope of the  $< 10 \mu\text{m}$  fraction in the LS is inconsistent suggesting that the straight-line portion of the curve is  $< 2 \mu\text{m}$ .

Similar smooth size distributions consisting of multiple modal peaks with volume regularly distributed about them are found in all sediment samples analyzed with the LS. The presence of a well-defined modal hump in the  $< 16 \mu\text{m}$  fraction in the LS data prevents use of the model developed by Kranck et al. (1992a, b) and Curran et al. (2004) used in Milligan and Law (2005) to determine the fraction of material deposited as flocs, floc fraction (Milligan et al., 2024). Milligan et al. (2024) applied the Curran et al. (2004) model to 120 samples from 5 different bays in the Gulf region with varying depositional conditions. The LS failed to show the expected variations in floc deposition that were seen in the CC data (Milligan et al., 2004).

As an alternative to floc fraction, % sortable silt (%SS) and the % volume of particles  $< 16 \mu\text{m}$  ( $\%<16$ ) were used to characterise the size distribution of the samples analyzed with the CC and LS. % SS was proposed by McCave et al. (1995) and McCave and Hall (2006) to determine paleo ocean current speed based on the assumption that particles  $> 10 \mu\text{m}$  can be sorted but particles  $< 10 \mu\text{m}$  are winnowed away in proportions equal to their presence in the sediment. The  $< 10 \mu\text{m}$  fraction moves as flocs so all size classes below  $\sim 10 \mu\text{m}$  are mobilized at the same time (McCave et al., 1995; Law et al., 2008). % SS has been used to study flocculation in near shore sediments, where biological effects on floc formation can dominate and bottom stress is high (Chang et al. 2005, 2007; Molinaroli et al. 2009; Flemming et al. 2024). In a similar manner, but focusing on the erodibility of mud rather than the sorting of silt, Law et al. (2008) showed that in cohesive sediment, grains up to  $16 \mu\text{m}$  erode at approximately the same rate and in the

same proportion in which they occur in the sediment. Increased floc deposition would result in an increase in this fraction. Figure 11 shows the values for floc fraction, % SS and % < 16 $\mu$ m calculated using SGS analyzed with a CC for the core described in Milligan and Law (2005). Milligan et al. (2024) found that values for %SS and % <16 from the CC and the LS are correlated but not interchangeable and the relationship between the instruments changes with the area being sampled. Reduced Major Axis regression values were different for each area sampled and all areas were different from the overall relationship between the CC and the LS (Milligan et al., 2024).

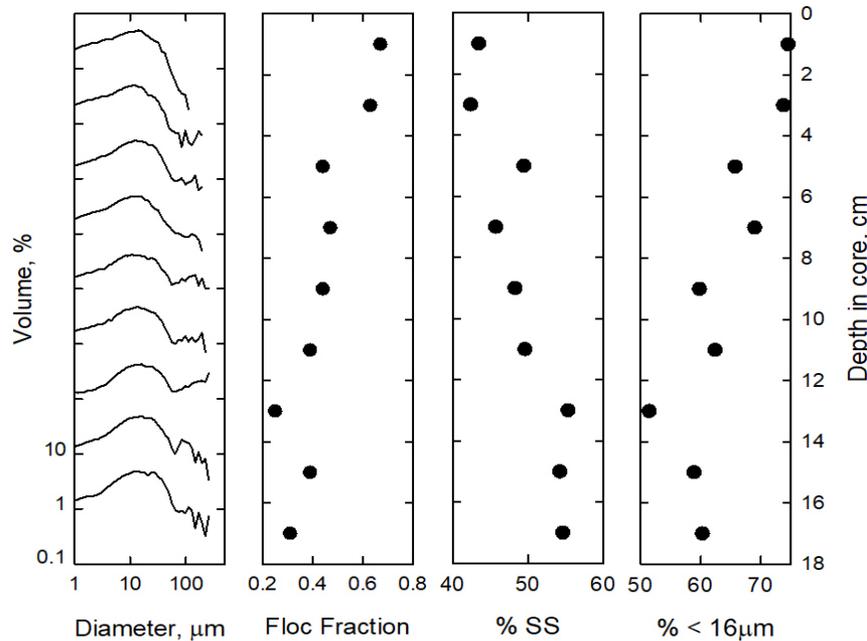


Figure 11: Plots of SGS analysed with a CC with values for floc fraction, %SS and %<16 for the core collected in Back Bay, NB. Log-log plots of SGS are offset vertically to represent depth in the core (Milligan et al., 2024).

The reason for the area dependence for the relationship between CC and LS can be clearly seen in samples from Letang Inlet and Placentia Bay NL (Figure 12). Samples run on the CC have two distinct size distributions where the LS distributions are very similar. In accordance with Krumbein and Tisdell (1942) and Kranck and Milligan (1985) the Placentia Bay and Letang samples run on the CC exhibit a region of unsorted particles with a straight-line distribution from 1- ~10  $\mu$ m, but with very different slopes (Figure 12). In the LS analysis, the two areas have very similar distributions in both log-log and cumulative plots. The CC has no mode in the NFLD sample where the LS has two at ~8  $\mu$ m and ~30  $\mu$ m. This is due to how the LS interpolates the diffraction pattern (Milligan et al., 2024). The CC measures individual particles whereas the LS interprets the pattern of light scattered by particles using a proprietary manufacturer's algorithm which assumes that particle size distributions have a mode and that particles are distributed about that mode in a regular manner. The inorganic fraction of bottom sediment in low energy environments consists primarily of broad, unsorted size distributions of small diameter particles with equal volume in equal logarithmic size classes. Larger grains are sometimes present. In unsorted sediment the number of particles increases exponentially with decreasing particle size (Milligan et al., 2024).

Low resolution of mixed particle sizes, exponentially increasing numbers in the smallest size classes, and varying indices of refraction in the particles leads to the creation of smooth modal peaks in the LS analysis of inorganic bottom sediment (Kowalenko and Burbain, 2014; Milligan et al., 2024).

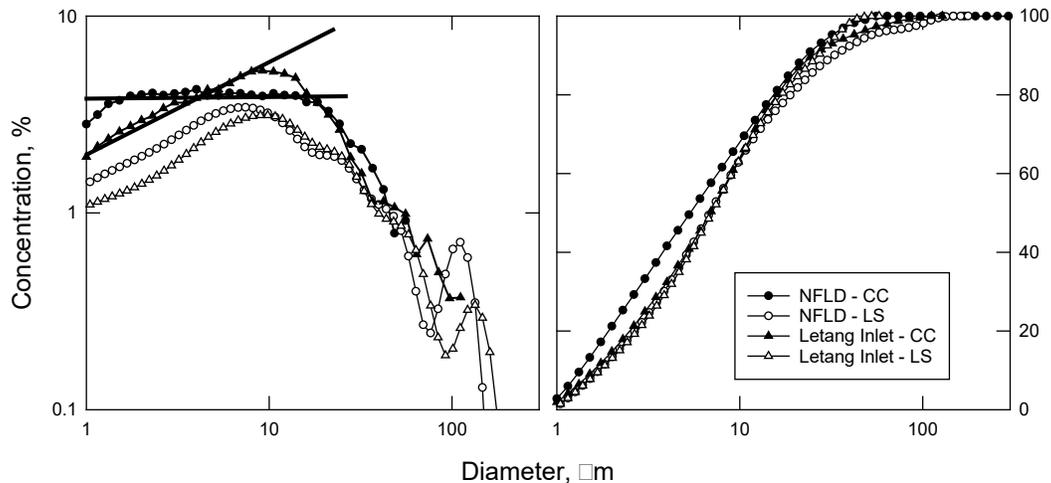


Figure 12: Log log and cumulative size distributions for a sample from Letang Inlet NB and Placentia Bay NFLD analyzed with a CC and a LS. Heavy line in the log-log plot is linear regression fit for diameters between 1 and 10  $\mu\text{m}$  (Milligan et al., 2024).

Further evidence of how interpolation of the scattering pattern in the LS can modify size distributions and produce very different particle size distributions was shown during still water settling of Sillikers clay and after sieving samples from Letang Inlet using different screen sizes (Milligan et al., 2024). In a duplication of the Kranck (1980) experiment on the still water settling of Sillikers clay, the CC data showed the expected different evolutions in the size distribution over time depending on if the suspension was flocculated or unflocculated (Milligan et al., 2024). In the unflocculated suspension only the largest particles settled over time and the distribution and concentration of the smaller particles remained constant. In the flocculated suspension the CC showed that all sizes were removed in equal proportions, maintaining the same size distribution as concentration decreased. In the LS data from both the unflocculated and flocculated suspensions, the size distribution in the smaller size classes changed over time and new modal peaks appeared (Milligan et al., 2024). When samples from Letang Inlet were analysed and then screened at 63  $\mu\text{m}$  and 16  $\mu\text{m}$ , the size distribution from the CC did not change other than to remove particles greater than the nominal screen size. With the LS, the size distributions in the smallest sizes changed and modes greater than the screen size appeared (Milligan et al., 2024). The interpolation of the scattering pattern of particles in the LS does not accurately represent the actual particle size distribution of disaggregated inorganic particles but does capture the variation over a broad region of the distribution. As % SS and % <16 are both integrated over many channels, the smoothing of the size distribution and creation of a modal peak does not have a large impact on these values. However, the inability of the LS to differentiate between different slopes in the fine-grained portion of the size distribution introduces the apparent area dependence making it unsuitable for comparison between areas. The LS

can be used to observe changes in the fine-grained fraction of inorganic bottom sediment with the understanding that it does not have the same accuracy for the < 16  $\mu\text{m}$  fraction as the CC and it is unable to determine the fraction of material deposited in flocs. As surface area is greatest in the smallest size classes, underestimation of this fraction would impact any effort to predict the fate of surface-active contaminants using LS data. For these reasons it is recommended that the LS does not replace the CC in the AMP.

## **2.5 Results from repeat sampling**

The effectiveness of SGS multi-year and seasonal sampling and analysis was evaluated using AMP data from Letang Inlet and independent survey data from a tidal flat at Kingsport NS (Milligan et al., 2024). Samples were collected at the same locations, three times over ~1.5 y and analysed with both the CC and LS (Figures 13, 14). Samples from Kingsport were collected at low tide with greater than 0.5m precision. Samples from Letang were collected with an Eckman grab from a vessel using GPS positioning with precision, at best, on the order of 3 m.

Kingsport and Letang showed variations in both % SS and % < 16 with no seasonal or annual pattern with either instrument. As the fine fraction is mobile, seasonal changes in bottom stress may result in removal or deposition of this fraction, as will the duration of time after an increase in bottom stress. On tidal flats, the variability is due to wave action and sediment supply (Law et al., 2013; 2019). While not as extreme as a tidal flat, a similar response would occur in most locations with seasonal variations in bottom stress (Law and Hill, 2019). Variations in sediment texture can also result from lack of precision in station location when sampling from a vessel, especially where bottom slope is large as sedimentation will vary with depth. Natural variation in SGS in surficial sediment samples will exceed the variations that might be attributed to aquaculture. Using cores to look at temporal variations in deposition eliminates error due to sample position as it integrates depositional conditions at a single point over time. In place of temporal sampling of surficial sediment, cores should be used to identify changes in sedimentation.

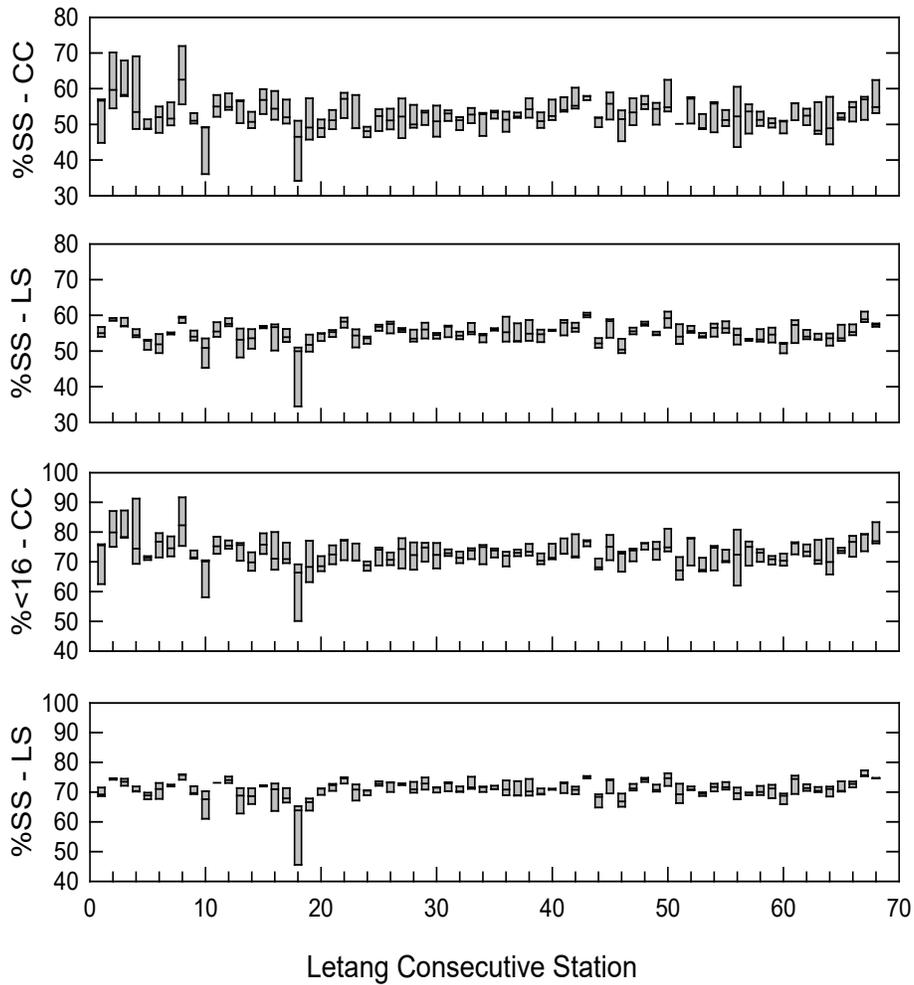


Figure 13: Box plot of % SS and % <16 data from the CC and LS for samples collected at Letang Inlet NB 2019/03/12, 2019/10/02 and 2020/09/29. For illustration, consecutive station numbers represent individual sample locations.

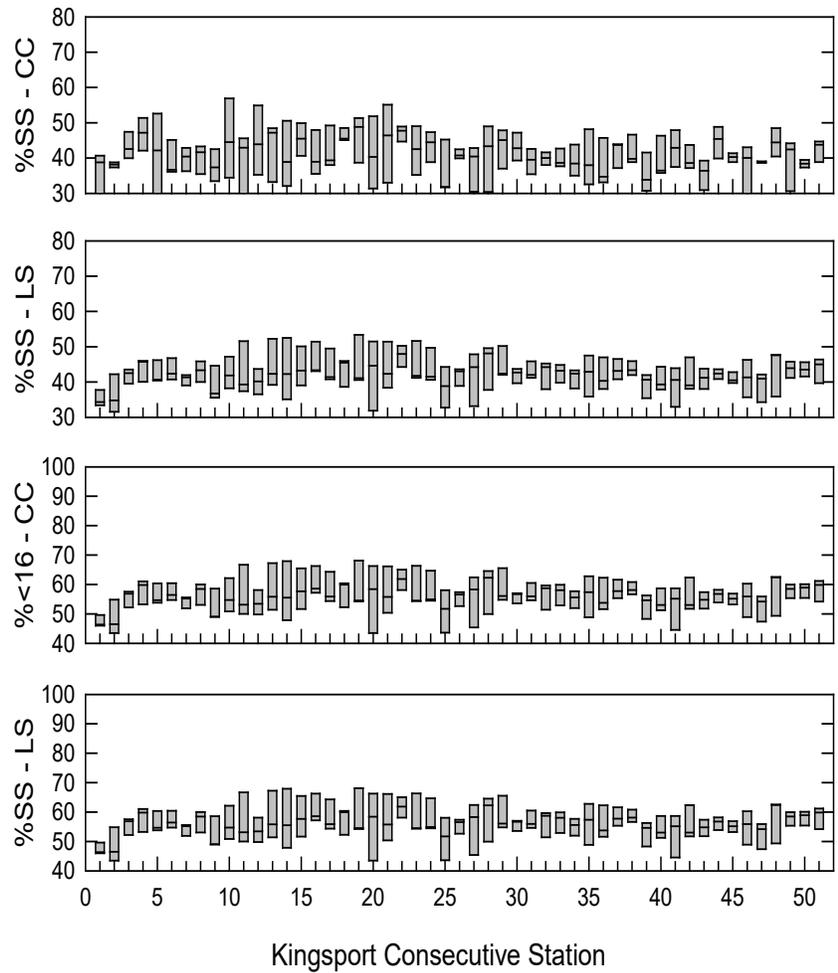


Figure 14: Box plot of % SS and % <16 data from the CC and LS for samples collected at Kingsport NS tidal flat 2019/03/10, 2019/10/19 and 2020/09/10. For illustration, consecutive station numbers represent individual sample locations.

### 3 Conclusion

This report provides advice on the application of appropriate methods for Canadian aquaculture site assessment and habitat description, monitoring sediment accumulation, and measurement and prediction of dispersal of aquaculture inputs. This document informs on the best practices and limits for the use of SGS in the DFO AMP to reach the three potential program goals.

Aquaculture operations do not introduce inorganic particles but can enhance the deposition of existing particles in suspension through flocculation. This report focuses on the size analysis of inorganic particles < 16 µm identified by Law et al. (2008) as the upper size range of inorganic particles incorporated into flocs.

- SGS should be used to evaluate long term changes in sediment accumulation in low energy environments where fine sediment accumulates.
- Dated core samples should be used to determine changes to sediment deposition.
- To sample particles < 16 µm incorporated into flocs, sampling methods that do not disturb the mobile layer at the sediment water interface such as the Slo-corer are required. If other samplers are used it must be understood that the samples will not include recent sedimentation.
- Natural variation in SGS from surficial sediment samples can exceed variations due to aquaculture. Annual monitoring of SGS is not recommended.
- A Slo-corer and Gust erosion chamber should be used to parameterize the processes controlling the transport of aquaculture inputs for modelling purposes (Milligan and Law, 2013). It should also be used if the recently deposited material must be sampled. These samplers are available in only some regions.
- Currently samplers capable of collecting the <16µm fraction from sand, gravel, and cobble bottoms do not exist within DFO. This gap should be addressed if the AMP is to be a National program.

To monitor SGS, more specifically the disaggregated inorganic grain size, laser diffraction, Beckman-Coulter LS 13 320, was proposed in 2018 by the AMP-WG as an alternative to the Coulter Counter because of its greater efficiency, approximately 30 samples per day vs 10 samples with a Coulter Counter, and larger size range, 0.4-2000 µm. An evaluation of the CC vs the LS was carried out to assess if the LS could replace the CC in the generation of an accurate National SGS database.

- The LS is unable to determine floc fraction; but comparison of % sortable silt and % <16 µm values derived from samples analyzed with a CC and a LS shows that both instruments can be used to observe changes in the amount of fine sediment deposited.
- Data cannot be used interchangeably between CC and LS.
- Sites cannot be discriminated with the LS. For aquaculture monitoring, this limits the LS to monitoring individual areas without the ability to compare different regions.

- For predictive modelling and observation of the transport and deposition of aquaculture inputs understanding of the sediment dynamics in terms of the amount of flocculation is required. This information can only be obtained with a CC.
- Analysis with the LS can underestimate the surface area in the smallest size classes. This could impact prediction of the fate of surface-active contaminants.
- We therefore do not recommend the LS 13 320 to be used to develop a national consistent database to monitor aquaculture impacts.

Annual sampling will fail to resolve changes in accumulation of the < 16µm fraction due to high seasonal variation. Surficial sediment samples should be collected every 3-5 years. Cores should be collected and sectioned to determine long term changes to the depositional environment.

Regardless of which instrument is used to determine SGS, technical expertise is required to ensure quality control and assurance. Both instruments require an in-depth knowledge of operation to ensure that they are working correctly. This will require consistency for technical support.

The frequency and method of sampling for SGS should be governed by the science question being asked, not by the need for regular reporting of SGS.

## 4 Recommendations

Optimal SGS sampling and sample processing for the AMP are summarized in Table 3.

### 4.1 Goal A. Characterizing bottom type and benthic habitat

A general survey of bottom sediment grain size in and around an area of interest can be used to characterize benthic ecosystems and habitats. In this context, samples are collected and processed to describe the benthic environment, not to evaluate changes over time. A low-resolution of SGS is sufficient and sampling is limited to an initial assessment without the requirement to be repeated over time. Interpretation of the data is limited to baseline conditions, site/station selection and classification, differentiating between erosional and depositional sites and preference habitats (i.e. bioindicators). Imaging, grabs, and corers are all appropriate tools to assess bottom types. When using grabs to collect a sample, the assumption must be that disturbance of the sediment-water interface does alter the overall assessment of depositional conditions. If the sediment-water interface is lost, samples cannot be used for a baseline to monitor changes to SGS and surface-active contaminant transport and deposition. Visual imaging is an appropriate primary tool for assessing benthic habitat and can be used to observe accumulations of material on hard bottom substrates (Muschenheim and Milligan, 1996; Hamoutene et al., 2016) and evolution of benthic biota ((e.g. Sanz-Lázaro and Marín 2011; Valdemarsen et al., 2015). Sieve/hydrometer/pipette provides sufficient resolution of SGS for site assessment and habitat classification but not for use as a baseline to observe changes in the <16 µm fraction. Laser diffraction provides a

rapid, high-resolution analysis of SGS, but uncertainties exist related to the accuracy for the  $< 16 \mu\text{m}$  fraction.

## **4.2 Goal B. Monitoring accumulation of fine-grain sediment**

Annual sampling for SGS as part of a monitoring program is not recommended. CC and LS analysis of samples collected on 3 occasions over  $\sim 1.5\text{y}$  in Letang Inlet, Kingsport, and Rant Point show very large variations in % SS and %  $< 16\mu\text{m}$ . Natural seasonal variation and inaccuracy in precisely occupying the same location can lead to variations in SGS that exceed predicted changes in SGS from aquaculture. Cores should be used to monitor accumulations of fine-grained sediment and should be the preferred method for sampling in the AMP. Milligan and Law (2005) showed that the amount of floc deposited material ( $< 16 \mu\text{m}$ ) increased synchronously with the development of open cage salmon aquaculture in Letang Inlet at the same time as trace metal concentrations increased (Yeats et al., 2005). It is essential that sampling methods retain an undisturbed surface layer which limits samplers to diver-collected cores, HAPS or Multicorers, and Slo-corers (Milligan and Law, 2013). In unconsolidated, cohesive, muddy sediments, Eckman grabs have been effective but any grab with a disturbed surface must be rejected. Cores should be extruded and sectioned to provide temporal resolution of the SGS and other sediment characteristics of interest (eg. porosity, %organic content, persistent organic pollutants). Methods that provide temporal resolution of changes to sedimentation (Milligan and Law, 2005) are presently outside of the scope of monitoring. They can be used, however, to resolve observed changes in SGS if required. Radioisotope dating of cores can provide a timeline for the evolution of the SGS (Smith et al., 2005; Kirchner, 2010; Little et al., 2015) and x-raying and splitting cores can provide extremely accurate sampling of layers within the sediment (Wheatcroft et al., 2006).

SGS analysis requires high resolution of the  $< 16 \mu\text{m}$  fraction. While the LS can observe variations in the  $<16 \mu\text{m}$  fraction, it does not have the precision required to differentiate between how sediment accumulates in different areas. Its inability to resolve different source slopes in different geographical regions limits it to overall changes in sediment accumulation. If the goal is to understand how sediment is moving within an environment, then analysis requires the CC. This is especially critical for studying the transport and deposition of deleterious substances.

In areas with hard substrate, new methods need to be developed to sample flocculant material (Hamoutene et al., 2018).

## **4.3 Goal C. Monitoring and modelling the transport and deposition of aquaculture inputs**

Aquaculture inputs in this context include organic material, trace metals, pesticides, and therapeutants. Direct deposition of feed and fecal material is limited to the near field but is susceptible to resuspension and dispersal in the form of flocs (Law et al., 2016,

2019). Ideally, to observe the transport and deposition of aquaculture derived inputs and to collect data to support modelling, the focus of the study should be the flocculent material at the sediment-water interface and the surface sediment to a depth appropriate to the timescale of the disturbance. In most soft bottom areas this would be limited to the top 1-2 mm. On hard bottom, flocculent material resting on the bottom or in the interstitial spaces should be sampled. New devices to sample flocculent material on hard bottom need to be developed (Keeley et al. 2021). At this time, sampling for aquaculture inputs is limited to cores with undisturbed sediment-water interfaces that can be eroded (Tolhurst et al., 2000; Dickhudt et al., 2009; Elliot et al., 2012; Milligan and Law, 2013; Reid et al, 2016; Seelan et al., 2018; Reich et al., 2019).

The far-field transport and deposition of aquaculture inputs is not presently part of the AMP. Sampling is limited to stations at varying distances from sites. Given that the standard method is a grab and a subsample from the top 1-2 cm, only historical accumulation at a site can be measured. Given the importance of understanding the dispersal of aquaculture inputs and their potential interaction with benthic organisms, methods have been developed to ensure that sampling reliably captures the mobile layer. The Gust microcosm erosion chamber has been successfully used in several environments to measure the mobility and concentration of contaminants in surface sediment (Figure 19) (Milligan and Law, 2013; Droppo et al. 2007, Seelan et al., 2018). The Gust chamber is an erosion simulator that comprises a polycarbonate housing with a rotating stirring disk, a removable lid, and water input and output connections (Dickhudt et al., 2009). It fits directly on top of a core tube (Figure 15). By controlling both the rotation rate of the stirring disk and the rate at which water is pumped through the device, a uniform shear stress can be applied across the sediment surface. Shear stress at each level is maintained until an attached turbidity meter records values that correspond to the concentration of the background water which is pumped into the chamber. The Gust chamber provides both erosion shear stress, estimates of sediment mobility, and samples for later analysis of grain size and contaminant load.



Figure 15: Left: Sediment core with undisturbed sediment-water interface taken with the mini Slo-corer. Note the very clean overlying water with no indication of suspension. Right: Gust erosion chamber mounted on the top of an undisturbed core in preparation for erosion.

Other methods for suspending material from the sediment-water interface are available. For example, Walker and Grant (2009) used a simple paddle system based on the design of Tsai and Lick (1986) to look at the erodibility of sediment in association with mussel aquaculture. Samples from this type of system could conceivably be used for contaminant transport. Sutherland and Amos (2020) used Sea Carousel to examine differences in erosion shear stress for different substrates in British Columbia. At this point in time, sea carousel is unable to sample the suspension at different stresses, but modifications could be possible.

Table 3: Technical recommendations for each goal separately. Please refer to the specific sections to have details and data supporting the advantage/limits of each sampling/collection (Section 2.2) and sample processing/analysis (Section 2.3) methods

Suitable bottom types		Bottom type characteristics		Monitoring accumulation of fine-grained sediment		Provide data to study contaminant transport	
<b>Regulation goal:</b>		Baseline data Site selection		Harmful alteration of habitat		Introduction of deleterious substances Harmful alteration of habitat	
<b>Purpose:</b>		Site classification - erosional or depositional Establish baseline conditions Understand bioindicators (i.e. preference habitats)		Observation of changes to depositional environment Link to changes in flora and fauna		Observation of transport of contaminants from aquaculture Quantifying concentration of contaminant Link to changes in flora and fauna	
2.2. Sampling methods		Benefits	Disadvantage	Benefits	Disadvantage	Benefits	Disadvantage
<b>Photography (video and still)</b>	Rocky to coarse/cobble	Rapid assessment of depositional conditions and organism assemblages Image analysis of coarse bottom	Qualitative only, except where image analysis possible  No physical sample	Not applicable		Not applicable	
<b>Grab (van Veen, Eckman, Ponar)</b>	Most environments	Standard technique, readily available Deployable from small vessel	Loss of material from the sediment water interface Loss of sample in gravels or where shells present No sediment profile	Not recommended (Loss of material from the sediment water interface)		Not recommended (Loss of material from the sediment water interface)	
<b>Diver core</b>	Most environments	Precise location  Intact sediment water interface.  Can be sectioned to provide sediment profile Can be used for observation of short timescale disturbance	Depth limited  Cost  Occupational health and safety risk	Precise location  Intact sediment water interface.  Can be sectioned to provide sediment profile Can be used for observation of short timescale disturbance Can be used with Gust erosion chamber to erode surface layer	Depth limited  Cost  Occupational health and safety risk At present, no method to erode surface of core	Precise location  Intact sediment water interface.  Can be sectioned to provide sediment profile Can be used for observation of short timescale disturbance	Depth limited  Cost  Occupational health and safety risk At present, no method to erode surface of core
<b>Haps Corer</b>	Soft sediment	Intact sediment water interface.  Can be sectioned to provide sediment profile Can be used for observation of short timescale disturbance	Weight and size require vessel with appropriate equipment for deployment Ineffective in coarse sediment <i>Not readily available</i>	Intact sediment water interface.  Can be sectioned to provide sediment profile Can be used for observation of short timescale disturbance	Weight and size require vessel with appropriate equipment for deployment <i>Not readily available</i> At present no method to erode surface of core	Intact sediment water interface.  Can be sectioned to provide sediment profile Can be used for observation of short timescale disturbance	Weight and size require vessel with appropriate equipment for deployment Ineffective in coarse sediment <i>Not readily available</i>  At present no method to erode surface of core

<b>Hydraulically damped corer - Slo-corer, multi-corer</b>	Soft sediment	Intact sediment water interface.	Weight and size require vessel with appropriate equipment for deployment	Intact sediment water interface.	Weight and size require vessel with appropriate equipment for deployment	Intact sediment water interface.	Weight and size require vessel with appropriate equipment for deployment
		Can be sectioned to provide sediment profile	Ineffective in coarse sediment	Can be sectioned to provide sediment profile	Ineffective in coarse sediment	Can be sectioned to provide sediment profile	Ineffective in coarse sediment
		Can be used for observation of short timescale disturbance	<i>Not readily available</i>	Can be used for observation of short timescale disturbance	<i>Not readily available</i>	Can be used for observation of short timescale disturbance	<i>Not readily available</i>
		Can be used with Gust erosion chamber to erode surface layer (Slo-corer)	Weight and size require vessel with appropriate equipment for deployment	Can be used with Gust erosion chamber to erode surface layer (Slo-corer)	Weight and size require vessel with appropriate equipment for deployment	Can be used with Gust erosion chamber to erode surface layer (Slo-corer)	Weight and size require vessel with appropriate equipment for deployment
<b>Box corer</b>	Soft sediment	Relatively undisturbed sediment water interface. Drainage of box can result in loss of sediment water interface	Not readily available	Relatively undisturbed sediment water interface. Drainage of box can result in loss of sediment water interface	Not readily available	Relatively undisturbed sediment water interface. Drainage of box can result in loss of sediment water interface	Not readily available
		Core subsamples from box can be sectioned to provide sediment profile		Core subsamples from box can be sectioned to provide sediment profile		Core subsamples from box can be sectioned to provide sediment profile	
		Can be used for observation of short timescale disturbance		Can be used for observation of short timescale disturbance		Can be used for observation of short timescale disturbance	
		Core subsamples from box can be interfaced with erosion chamber		Core subsamples from box can be interfaced with erosion chamber		Core subsamples from box can be interfaced with erosion chamber	
		Can sample coarse bed (with loss of fines)					

**2.3 Sample processing:**

<b>Image analysis</b>	Gravel/cobble environments	Rapid assessment of depositional conditions and organism assemblages	Presently qualitative	Not applicable	Not applicable
<b>Sieve and hydrometer (pipette)</b>	1 – 2000µm	Readily available ISO/ASTM standard technique	Time consuming for small particle size	Not recommended due to low resolution of < 16 µm fraction	Not recommended due to low resolution of < 16 µm fraction
		Compatible with historical data	interlab/interanalyst variability due to environmental conditions and measurement error		
			Low resolution (1 phi). Large sample size		
<b>Laser diffraction</b>	0.4 - 2000 µm fraction	Rapid	Questionable accuracy for material < 16 µm	Use with caution. Can not determine fraction of sediment deposited in flocs. %<16µm and sortable silt % possible parameters. Different regions can not be compared.	Not recommended at this time
		High precision	Different makes/models can give different results		
		High resolution	Technical expertise required		
		Small sample size			

<b>Coulter Counter, i.e. Multisizer</b>	1 - 400 microns	High precision	Slow	Accurate measurement <16 µm fraction	Slow	Accurate measurement <16 µm fraction	Slow
		High resolution	Technical expertise required	Determines fraction of sediment deposited in flocs	Technical expertise required	Determines fraction of sediment deposited in flocs	Technical expertise required
		High accuracy		Small sample size allows fine vertical resolution of cores		Small sample size allows fine vertical resolution of cores	
		Small sample size		Integration with erosion of sediment water interface		Integration with erosion of sediment water interface	

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## ACRONYMS AND GLOSSARY

**AMP:** DFO Aquaculture Monitoring Program

**CC:** Coulter Counter

**DFO:** Department of Fisheries and Oceans Canada

**SGS:** Sediment grain size: Physical properties of inorganic particle size. Analytic approach to classify sediment types. Other commonly used properties of sediment are sorting and shape (roundness and sphericity) including:

Textural class	$\mu\text{m}$	mm	$\phi$
Gravel	> 2000	> 2	> -1
Very coarse sand	2000 - 1000	2 - 1	-1
Coarse sand	1000 - 500	1 - 0.5	0 - 1
Medium sand	500 - 250	0.5 - 0.25	1 - 2
Fine sand	250 - 125	0.25 - 0.125	2 - 3
Very fine sand	125 - 62.5	0.125 - 0.0625	3 - 4
Silt	62.5 - 4	0.0625 - 0.004	4 - 8
Clay	< 4	< 0.004	> 8

**ASTM:** American Society for Testing and Materials, is an international standard organization that develops and publishes voluntary consensus technical standards for a wide range of materials, products, systems, and services.

**ISO:** International Organization for Standardization

**LD:** Laser diffraction

**%SS:** sortable silt %

**Gust Chamber:** erosion device developed and validated by G. Gust capable of measuring erosion at different shear stress and collecting samples of material eroded. (Tollhurst et al. 1999; Law et al., 2008)

**Electro-resistance particle counting:** The principle on which Coulter Counters were designed

**Sillikers clay:** A fine-grained glacial marine deposit composed mainly of kaolinite and illite from Sillikers NB.

## **AUTHOR CONTRIBUTIONS**

ALR, BL, TM, and FP conceived the framework of this science-based advice. ALR and BL contributed resources. BL, VZ and SN developed and led the laboratory analyses. CO'L and SN worked on data management. TM analyzed the data. TM and ALR wrote the manuscript, while BL, FP, CO'L and DH helped to draft and improve the manuscript. All authors edited the manuscript and approved the final version.

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