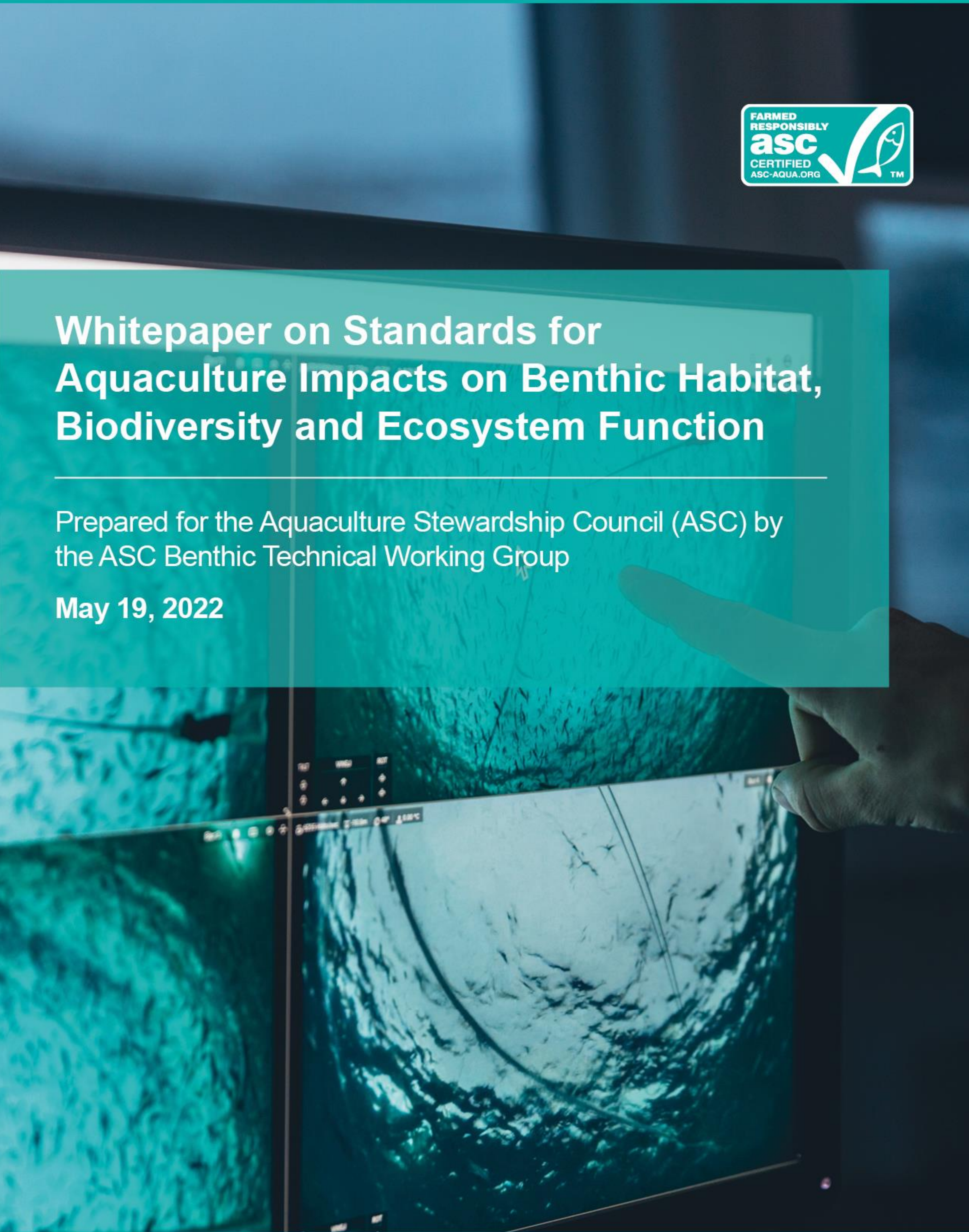

Whitepaper on Standards for Aquaculture Impacts on Benthic Habitat, Biodiversity and Ecosystem Function

Prepared for the Aquaculture Stewardship Council (ASC) by the ASC Benthic Technical Working Group

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1. About the Aquaculture Stewardship Council (ASC) Standards

The Aquaculture Stewardship Council is an independent, not-for-profit organization that operates a voluntary, independent third-party certification and labelling program based on a scientifically robust set of standards.

ASC Vision:

A world where aquaculture plays a major role in supplying food and social benefits for humanity whilst minimizing negative impacts on the environment.

ASC Mission:

To transform aquaculture towards environmental sustainability and social responsibility using efficient market mechanisms that create value across the chain.

The ASC Standards set strict requirements for responsible farming, which encourage seafood. The standards define criteria designed to validate the environmental sustainability and social responsibility of the aquaculture sector. These standards have been developed based on the following seven basic Principles:

1. Comply with all applicable national laws and local regulations.
2. Conserve natural habitat, local biodiversity and ecosystem function.
3. Protect the health and genetic integrity of wild populations.
4. Use resources in an environmentally efficient and responsible manner.
5. Manage disease and parasites in an environmentally responsible manner.
6. Develop and operate farms in a socially responsible manner.
7. Be a good neighbor and conscientious citizen.

A new ASC Farm Standard is being developed to better align all the current species-specific standards and as part of the commitment to continuous improvement based on the evolution of both science knowledge and best aquaculture practices.

A revision of the current ASC benthic organic enrichment monitoring requirements is under development to address the potential impacts of solid organic waste products from aquaculture, including waste feed and/or faeces, on benthic habitat, biodiversity and ecosystem function. Organic enrichment of the seabed is a key environmental concern across the open water marine and freshwater aquaculture industry.

Purpose: *The purpose of the revised requirements is to provide a credible, efficient, and measurable performance-based environmental management system designed to minimize, mitigate, or eliminate negative impacts from seabed organic enrichment.*

ACS certification is meant for farms that adhere to this principle.

This whitepaper provides the rationale for a revised ASC benthic organic enrichment monitoring requirements and ecological quality decision-support system. These revised requirements place limits on both the magnitude and spatial scale of alterations and disruptions to benthic habitat, biodiversity and ecosystem function. A range of abiotic and biotic indicators are described herein that can serve as proxies for numerically classifying the Ecological Quality Status (EQS) of open-water farms.

The following sections include a summary of the peer-reviewed research relevant to this ASC revision. It is recognized that a large amount of international work has already gone into the management and regulation of benthic impacts associated with open-water aquaculture. Established regulatory environmental management frameworks and associated monitoring programs were considered during the development of these revisions. Although the revised requirements for benthic organic enrichment monitoring may be more stringent than some current regulatory requirements (see Section 9), this higher level is needed to achieve both global best practices and to achieve the overall mission of the ASC.

The focus of the ASC is on minimising the impact of aquaculture rather than setting prescriptive methods for measuring this impact. Some international regulatory monitoring standards for benthic organic enrichment may already meet or even exceed the goals of the revised ASC requirements. Flexibility is therefore provided to allow operators to submit a farm-specific benthic monitoring program if they can show that it goes beyond the revised requirements of the ASC (see Section 8). The ASC will determine, through an internal and external expert review process, if the proposed farm-specific monitoring programs meet stringent ASC requirements. Approval of farm-specific monitoring programs will be limited to exceptional and well documented cases. Operators are encouraged to adopt the multi-tiered monitoring systems presented in Section 5.3 (fish cage systems), Section 6.3 (freshwater fish cage systems) and Section 7.3 (mollusc systems) that were designed by the ASC to address all mandatory requirements for benthic organic enrichment monitoring. The multi-tiered systems was designed as a practical monitoring approach that increases in complexity and cost only if a preliminary rapid screening shows that the magnitude and spatial extent of impacts exceeds predetermined habitat and biodiversity status limits.

2. Benthic Organic Enrichment: The Case for Aquaculture Monitoring

The response of benthic organisms and sediments to the deposition of solid organic aquaculture wastes is well known. Although these effluents have little direct environmental impact beyond some localized smothering of sessile organisms, the subsequent chemical transformation of organic matter through natural biogeochemical processes can seriously affect benthic habitats and communities beneath and adjacent to farms. If these impacts are of sufficient magnitude and spatial scale, they risk altering ecosystem function. For these reasons, solid organic waste introduced into aquatic and marine systems is considered a deleterious substance requiring management intervention.

A broad range of basic and applied research projects conducted over several decades has led to the development and broad acceptance of the following science conclusions:

1. The deposition and enrichment of sediments of all types with solid organic matter wastes stimulates aerobic decomposition processes, resulting in an increase in the biological oxygen demand (BOD) of sediments. Organic matter inputs can initially increase macrofaunal biodiversity around farms through the provision of an additional food resource. However, if sediment BOD increases beyond the capacity of local physical processes to resupply oxygen into the sediment from the water column, hypoxic to anoxic conditions will develop in the sediments.

2. In the absence of oxygen, microbes continue to decompose the excess organic matter through several anaerobic respiration processes that occur in a characteristic sequence. Quantitatively the most important of these in marine systems is sulfate reduction in which sulphate is reduced to sulfide gases (H_2S, SH^- and S^{2-}; referred to as total free sulfide or S^{-2}) that dissolve in sediment porewaters. When the available sulfate is depleted, methanogenesis begins. In freshwater systems, methane production plays a more quantitatively important role in organic matter decomposition due to the low sulfate content in lakes. These reduced end-products of decomposition create a chemical oxygen demand in sediments that further exacerbates negative benthic effects from the elevated BOD.

3. The production of S^{-2} during organic matter decomposition can impact benthic macrofauna communities and cause associated changes in ecosystem function. S^{-2} is highly toxic to most invertebrate species and the toxicity effect is compounded by the presence of sediment hypoxia/anoxia.

4. Structural changes in benthic macrofaunal communities resulting from a progressive increase in organic enrichment are well known. Macrofauna species exhibit different sensitivities to hypoxic and sulfidic conditions. Moderate organic enrichment can stimulate the colonization of tolerant taxa, but additional oxygen depletion and S^{-2} accumulation cause a decrease in abundance, biodiversity and biomass. Even highly tolerant opportunistic species eventually decline with increasing S^{-2} concentrations.

5. Ecosystem function is defined by the multitude of processes that control the flow and cycling of materials to system components. Functionality will differ over space and time. The introduction and decomposition of excess organic matter affect energy/carbon supply to consumers and thereby affects biotic communities and trophic networks. Depending on the magnitude and spatial extent of effects on ecosystem components, organic wastes may significantly disrupt natural ecosystem function.

6. Numerical modelling has a proven capacity to accurately simulate the major physical processes that control the deposition rate and spatial distribution of deposited organic matter across a wide range of farm environmental settings.

7. The prediction of biological impacts related to any given waste deposition rate is complex owing to the wide range of site-specific physical, geochemical and biological processes that collectively control the capacity of the environment to assimilate organic waste inputs. Recent studies have shown that carbon deposition rates causing significant benthic community impacts can vary by several orders of magnitude depending on the farm location. This environmental variability causes uncertainty in model predictions of benthic impacts from aquaculture.

Environmental management frameworks consist of a linked series of activities that identify, critically evaluate, and address predictions of potential environmental threats. Given that environmental impact predictions are subject to some level of uncertainty, owing to unforeseen factors and gaps in knowledge, monitoring programs are essential to ensuring that actual effects do not exceed predictions. The revised ASC benthic organic enrichment monitoring requirements link monitoring data to a common ecological quality rating and a predetermined course of action designed to minimize, mitigate or eliminate negative impacts from seabed organic enrichment.

3. Indicators of Benthic Organic Enrichment Impacts

The literature on benthic community responses to organic enrichment is extensive and the general conclusion is that once the natural capacity of the environment to mitigate waste inputs through physical waste dispersal and aerobic degradation processes is exceeded, further deposition of organic matter triggers a succession of habitat and community alterations that are remarkably consistent regardless of biogeographic location. Figure 1 illustrates the classic benthic community response to increasing organic enrichment. The organic enrichment gradient, initially proposed by Pearson and Rosenberg (1978), has been validated through extensive research in all benthic habitats (e.g. Nilsson et al., 1991; Diaz and Rosenberg, 1995; Nilsson and Rosenberg, 2000; Gray et al., 2002; Diaz et al., 2004), including those supporting finfish and bivalve aquaculture farms (e.g. Macleod et al., 2008; Hargrave et al., 2008; Hargrave et al., 2008b; Keeley et.al., 2012; Cranford et al. 2020). A multitude of metrics are available to quantify the Ecological Quality Status of the seabed and Appendix 1 includes descriptions of some of the more common metrics used to numerically classify benthic effects. The following summary briefly outlines the types of metrics that are available as well as some of the known advantages and limitations. Considerable information on this topic is available in the scientific literature (e.g. Salas et al., 2006, Pinto et al., 2009, Keeley et.al., 2012).

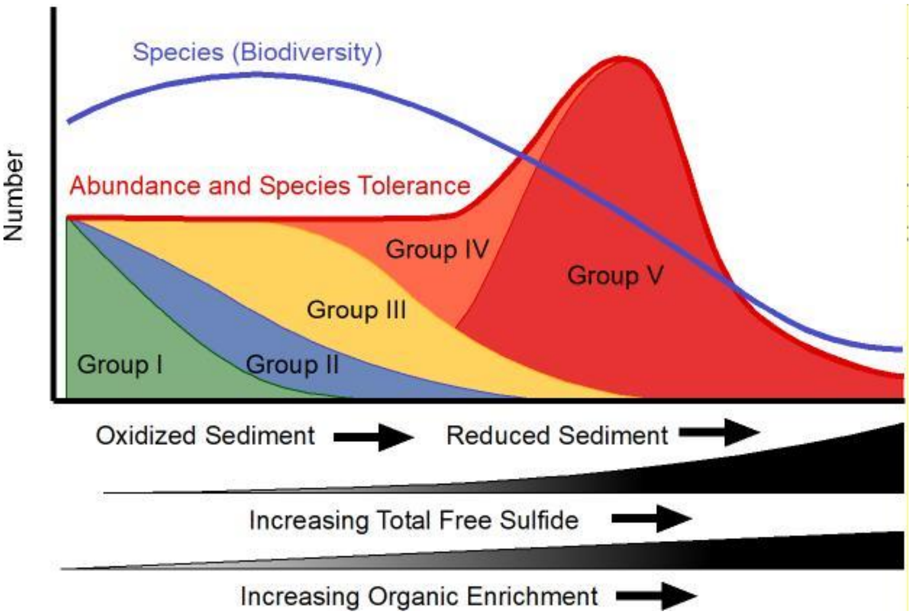


Figure 1. Generalized patterns of benthic habitat and invertebrate community alterations as they are related to organic enrichment (after Nilsson and Rosenberg (2000) and Borja et al. (2000)).

3.1 Biotic Indices

The simplest measure of community composition at a sampling location is species richness (*S*; total number of species per area). High species richness has been shown to positively enhance community productivity and to aid in resisting disturbances (contributes to resilience in ecosystem functionality). However, this index does not consider species abundance, which is an important component of diversity. The total abundance of infauna present in a sampled area (*N*) also provides valuable information on the community response to increasing organic enrichment, but while the abundance of some species declines as oxygen becomes depleted and free sulfide concentrations increase, others initially increase in abundance before eventually declining in numbers. Despite the differential responses of individual species groups, *S* and *N* eventually decline to zero at high free sulfide concentrations. The non-linear relationship between *N* and organic enrichment complicates the use of this metric as an impact classification tool.

The Shannon (*H'*) and Simpson (*D and 1/D*) metrics are abstract mathematical approaches that attempt to combine both species abundance and richness into single indices. These compound indices are often employed for monitoring aquaculture impacts but it has been argued they should not be used owing to inherent biases related to their sensitivity to rare and abundant species. A change in species rarity/abundance can represent either a positive or negative effect depending on the species' ecological role. These mathematical biases make it difficult to interpret results obtained using these indices without a detailed analysis of more specific community data.

The succession of benthic macrofauna community alterations in relation to the degree of organic enrichment is attributed to the wide range of tolerances of different species to increasing hypoxic and sulfidic sediment conditions. The classic response illustrated in Figure 1 has led to the identification and use of 'indicator' species groups as metrics of general community impacts. Specific polychaete taxa, such as *Capitella capitata*, are highly tolerant to S^{2-} and can rapidly colonize sulfidic sediments. The abundance of *C. capitata,* as a proportion of total species, provides useful information on the degree of impact to organic enrichment. Similar metrics are available that focus on the presence of sensitive groups, such as amphipods and molluscs, while others serve as compound indices that compare the relative proportions of sensitive and tolerant species and groups. An elaborate form of this multispecies tolerance approach for classifying benthic community health is AZTI's Marine Biotic Index (*AMBI*) in which over 10,000 benthic species have been ranked into five groups (Fig. 1) based on tolerance to disturbance. Multiple studies across a wide range of benthic habitats have identified *AMBI* to be effective for establishing the ecological quality status of sediments.

The Infaunal Trophic Index (*ITI*) approaches the problem of identifying ecological status by examining the basic feeding strategies of community members. Changes in the dominance of suspension- and deposit-feeders provide an indication of the amount of particulate organic matter present in the benthic environment. *ITI* responds to the presence/absence of four trophic groups known to exhibit different sensitivities to the supply of organic matter and sediment BOD, producing a single number describing the overall trophic condition of the benthic community. Although currently recommended for use in some of the ASC standards, science-based support for *ITI* has moderated owing to reports of difficulties in establishing the real diet of species and the lack of a clear separation between different feeding strategies. *ITI* results are also believed to be dependent on water depth and sediment grain size. A general requirement for any metric is that it be independent of site-specific characteristics.

Macroinvertebrate community assessments could be significantly enhanced by recent advances in DNA-based tools for benthic species identification. Molecular approaches, including DNA-barcoding, DNA-metabarcoding and eDNA metabarcoding, are very promising approaches for benthic biomonitoring and are currently in use in Norway and are ready for implementation in New Zealand. The ASC strongly supports incorporating new and innovative technologies in the ongoing development of monitoring standards once practical protocols for sampling, analysis and interpretation become standardized and broadly available.

Numerically characterizing benthic communities with a single number is a complex multidimensional concept and numerous studies designed to identify a single 'ideal' index have been unsuccessful. The above discussion highlights how the use of a single index may result in misleading or incorrect conclusions. Environmental management decisions need to be linked to the results of more than one environmental performance index. Several biotic indices have been developed that mathematically combine the results of several indices (e.g. *M-AMBI* and the Infaunal Quality Index; Appendix 1). Defining ecological status based on the results of several univariate and multivariate indices is not believed to be overly cumbersome for monitoring programs given that most biotic indices rely on a common taxonomic data set.

The total number of species in a sample generally increases with the area sampled such that comparing index values based on different sized samples will lead to bias. The proper use of most biotic indices therefore requires that the area of all benthic samples be standardized. Species richness and abundance are also dependent on the mesh size used to separate macrofauna from sediment. A standard grab or core sampler with a sampling area of 100 cm^2 and a mesh size of 1.0 mm for is required under the revised requirements to minimize both sediment processing time and taxonomic analysis cost. Analysing biotic samples to higher taxonomic levels such as families, rather than species, is also an acceptable cost-reduction measure, but only in cases where sediment quality assessment criteria are shown to be equivalent to the Ecological Quality Status (EQS) system (see Section 4). For example, the family-level Benthic Quality Index (*BQI-family*) has been shown to be highly correlated with several species-level indices and applicable EQS thresholds have been developed (Dimitriou et al. 2012; Appendix 1).

Note: see Appendix 1 for references on biotic indices.

3.2 Abiotic Indices

Several geochemical variables are closely associated with, and even responsible for the biological effects of solid organic enrichment on the benthic macrofauna community. Given that adverse effects of organic matter degradation on benthic macroinfauna directly result from increasing hypoxic and sulfidic conditions in surface sediments, dissolved oxygen (DO) and total free sulfide (S^{2-}) concentrations have been recommended as practical tools for aquaculture monitoring (Hargrave et al., 2008; Cranford et al., 2017). DO concentrations in surface sediments are informative of the initial community effects caused by aerobic waste degradation processes. Once DO is depleted, sediment S^{2-} levels begin to increase, directly impacting resident communities. Relationships between S^{2-} concentrations and multiple biotic indices described in Section 3.1 have been shown to apply across a broad biogeographical range and at both finfish and bivalve culture facilities (Cranford et al., 2020; Appendix 1). S^{2-} can be rapidly measured in the field and is considered a reliable and practical proxy of biological effects from organic enrichment.

The use of S^{2-} as an aquaculture monitoring tool has been subject to some discussion in the scientific literature owing to concern regarding methodological issues (e.g., Brown et al., 2011; Cranford et al., 2017) that have since been resolved (Cranford et al., 2020). The ion-selective electrode protocol for S^{2-} analysis, which has been the standard analytical approach for aquaculture monitoring, was shown to be unreliable owing to contamination issues, lack of analytical robustness, low precision, calibration instability and multiple other procedural artifacts (Appendix 2). The development of a rapid analysis method (direct UV spectrophotometry) that can be performed on the sampling vessel has resolved these analytical issues. New empirical relationships between S^{2-} concentrations, *Eh* data and multiple biotic indicators of macroinfauna community structure have been developed that have been shown to apply across a wide range of bottom types (silt, sand, gravel and mixed grain sizes) and farmed species (salmonids and bivalves; Cranford et al., 2020). Consequently, the UV method has been adopted as the ASC standard protocol for total free sulfide analysis of surficial sediments (See Appendix 2).

A geochemical variable that is closely related to *DO* and S^{2-} concentrations in surficial sediments is the redox potential (*Eh*; also known simply as redox). *Eh* measurements provide information on the dominant microbial processes in sediment responsible for mineralizing waste organic matter, including sulfate reduction. *Eh* measurements serve to support and confirm S^{2-} data by indicating that sulfate reduction is the dominant waste degradation process in the sediment sample (See Appendix 3.2). An ORP electrode is used to measure the oxidation/reduction potential of the sediment. The *Eh* (also called Eh_{NHE}) is then calculated in relation to a reference hydrogen electrode by adding a constant that is specific to the electrode filling solution and temperature (US EPA, 2013; Appendix 3). This correction is necessary for ORP measurements to be comparable between farms and with other indices.

pH measurements are used in combination with *Eh* data in Norway and Chile aquaculture monitoring programs where the relationship between these two variables is used to help classify the magnitude of fish farm impacts on benthic habitat and communities (Hansen et al. 2001, Schaaning and Hansen, 2005). Data presented in Schaaning and Hansen (2005; shown in Appendix A1.3) indicate that *pH* measurements above 7.2 are classified as having an acceptable benthic organic enrichment impact. However, pH values as high as 7.8 may be classed as “transitory”. While *pH* is valuable for identifying highly degraded sediment quality conditions, its capacity to categorize intermediate organic enrichment stages appears limited.

The above abiotic indices of benthic community alterations from excess organic enrichment have two major advantages over biotic indices. First, they can be measured rapidly in the field using diver, grab or core samples; thus, facilitating timely decisions on farm impacts (Hargrave et al. 2008; Cranford et al., 2020). Second, they can be measured at significantly lower costs than biotic metrics of macrofauna community structure (Wildish et al. 2001).

4. The Ecological Quality Status (EQS) System

The capacity to make consistent decisions related to the impact of organic wastes from aquaculture on benthic macroinfauna communities requires a decision-support system that classifies the degree of farm impacts revealed by benthic monitoring. Classifications are pre-defined based on specific abiotic and biological quality elements that collectively describe the health/ecological status of the benthic macroinfauna community. Several classification systems have been described for grouping community characteristics according to the degree of disturbance (minor to severe; normal to extremely polluted; normal to azoic; oxic to anoxic, etc.). The Ecological Quality Status (EQS) classification system is widely reported in the scientific literature; is currently in use for conducting regulatory sediment quality assessments in multiple countries; and underpins some of the current ASC standards (e.g., the Salmon Standard). EQS groups are defined using normative descriptions of the associated macrofaunal community (Table 1).

Table 1. Standard descriptions of benthic macrofauna assemblages for each of the five Ecological Quality Status (EQS) classes.

EQS Group	Definition
High Status	No or very minor disturbance. Species abundance, richness and diversity is high and sensitive taxa dominate. Opportunistic taxa are absent or of negligible abundance. Geochemical quality elements indicate aerobic conditions with low free sulfide toxicity.
Good Status	Slight disturbance: The level of diversity and abundance of invertebrate taxa is slightly reduced. Most of the sensitive taxa are present but slightly reduced. Opportunistic taxa are present but negligible in abundance. Geochemical quality elements indicate aerobic sediment conditions with a slight increase in free sulfide levels.
Moderate Status	Moderate disturbance: The level of diversity and abundance of invertebrate taxa is moderately reduced. Sensitive taxa have negligible abundance or are absent. Tolerant and first-order opportunistic taxa co-dominate in abundance. Geochemical quality elements indicate a moderate increase in anaerobic conditions with free sulfide levels known to be lethal to sensitive and indifferent taxa.

Poor Status	Major disturbance: Evidence of major alterations to the values of the biological quality elements. Diversity is greatly reduced with sensitive and indifferent taxa showing negligible abundance or are absent. Tolerant taxa are sub-dominant to first-order opportunistic taxa. Geochemical quality elements indicate a major increase in anaerobic conditions and sulfide concentrations lethal to most taxa.
Bad Status	Severe disturbance: Evidence of severe alterations to the values of the biological quality elements and in which large portions of the relevant biological communities normally associated with undisturbed conditions are absent. First-order opportunistic taxa dominate but are greatly reduced in abundance. Geochemical quality elements indicate a severe increase in sulfide concentrations that are lethal to all taxa.

The revised ASC requirements are designed to be applied globally and it is therefore important that the EQS classifications defined in Table 1 apply to all benthic habitats suitable for aquaculture. Similarly, any of the indices employed to monitor changes in the status of benthic communities must respond consistently regardless of the habitat receiving aquaculture organic waste. The monitoring requirements reported herein are based on the widely accepted Pearson and Rosenberg (1978) qualitative model of benthic community responses to increasing organic enrichment and the multiple methodologies proven capable of quantifying the magnitude of these responses.

The operational use and interpretation of abiotic or biotic indicator data obtained during a farm monitoring program requires that numerical boundaries be predefined for the five EQS groups (High, Good, Moderate, Poor, and Bad) described in Table 1. Table 2 reports operational EQS thresholds for many of the commonly employed indicators of organic enrichment. Appendix 1 provides details supporting the setting of these group boundaries, including links to the published (peer-reviewed) literature and relationships between biotic and abiotic indicators.

The use of normalized index values (index value at farm site divided by reference value) as a basis for determining the effect of the farm on benthic ecological status was considered in the development of the revised recommendations. This approach would place less constraint on farms operating in regions not defined as either High or Good status. However, linking farm compliance decisions to relative changes in impact metrics, instead of the actual impact magnitude, is equivalent to permitting the EQS group definitions described in Table 1 to be altered for each farm. This approach lacks consistency in environmental protection and is contrary to the purpose of the ASC to minimize negative impacts. Adopting a relative impact scheme would allow some farms to push benthic conditions beyond pre-defined EQS conditions judged to be acceptable.

Table 2. Abiotic and biotic indicator limits for each of the five Ecological Quality Status (EQS) groups describing the impacts of marine organic enrichment on benthic macroinfauna communities (Table 1). See Appendix 1 for index details and information sources.

Indicator	EQS Classification				
	High Status	Good Status	Moderate Status	Poor Status	Bad Status
Total Free Sulfide (S^{2-}; µM)*	0 to 75	75 to 250	250 to 500	500 to 1100	> 1100
Redox potential (Eh_{NHE})	>0		0 to -100	-100 to -150	<-150
pH***	>7.5		7.1 to 7.5	6.8 to 7.1	<6.8
Richness (*S*%; % of max *S*)	>80	50 to 80	35 to 50	15 to 35	<15
Shannon's diversity (*H'*)**	>4	3 to 4	2.5 to 3	1 to 2.5	<1
Opportunistic Taxa (*GrV; %*)	<20	20 to 40	40 to 60	60 to 80	>80
Polychaete/Amphipod Ratio (*BPOFA*)	<0.031	0.031 to 0.126	0.126 to 0.187	0.187 to 0.237	>0.237
AZTI's Marine Biotic Index (*AMBI*)	<1.2	1.2 to 3.0	3.0 to 3.9	3.9 to 4.8	>4.8
Multivariate *AMBI* (*M-AMBI*)	>0.83	0.83 to 0.59	0.59 to 0.47	0.47 to 0.35	<0.35
Benthic Habitat Quality (*BHQ*)	8 to 15	6 to 8	4 to 6	2 to 4	<2
Simplified Richness (S_{50})	>16	11.7 to 16	7.5 to 11.7	5.4 to 7.5	<5.4
Benthic Quality Index (*BQI*)	>16.0	12.0 to 16.0	8.0 to 12.0	4.0 to 8.0	<4.0
Benthic Quality Index (*BQI-family*)	>20.8	9.2 to 20.8	5.7 to 9.2	1.9 to 5.7	<1.9
Infaunal Trophic Index (*ITI*)***	>97	51 to 97	29 to 51	1 to 29	<1
BENTIX	>0.67	0.5 to 0.67	0.42 to 0.49	0.33 to 0.41	<0.33
Norwegian Quality Index (*NQI1*)	>0.86	0.68 to 0.86	0.43 to 0.68	0.20 to 0.43	<0.20

Norwegian Sensitivity Index (*NSI*)	> 27.4	23.1 to 27.4	18.8 to 23.1	10.4 to 18.8	< 10.4
Indicator Species Index (*ISI_{2012}*)	>9.6	7.5 to 9.6	6.2 to 7.5	4.5 to 6.2	<4.5
Alternative Classification Schemes: **Enrichment Stage (*ES*)** **Oxic Status**	1 Oxic A	2 Oxic B	3 to 4 Hypoxic A	4 to 5 Hypoxic B	6 to 7 Anoxic

* Measured by UV spectrophotometry. See Appendix 2 and 3.

** Not recommended for the ASC Standard. See Appendix 1.

*** EQS definitions have not been validated. See Appendix 1.

5. Marine Fish Cage Systems

5.1 Revised Benthic Organic Enrichment Monitoring Requirements

The revised requirements are intended to encourage producers to minimize the impacts of aquaculture over a broad spatial scale. The requirements described below are directed primarily at marine and brackish water farms overlying sediments that can be sampled using grab or core sampling methods suitable for the type of seabed present. This includes clay, silt, sand, and gravel bottom types. Hard bottoms, which are rarely regulated under international legislations, require a different approach for assessing organic enrichment impacts and are currently exempted from the revised requirements. If the seabed cannot be effectively sampled owing to the presence of hard substrates, then the farm is classed as having “hard bottom”. Bottom video or other evidence is required to support this seabed classification. Maerl beds represent a unique type of biogenic benthic habitat that consists of soft sediment sediments covered by coralline alga. Maerl beds are highly productive, support diverse benthic communities and provide critical ecosystem goods and services. Consequently, the revised requirements do not support the presence of farming activities in and around maerl beds, or other sensitive habitat containing threatened or regulated species.

It is generally accepted that the main factors controlling the spatial scale of benthic organic enrichment impacts around fish farms are farmed biomass, sediment type, water depth and hydrographic conditions. In a review of spatial trends in benthic impact parameters around fish farms, Giles (2008) showed that near-azoic conditions beneath fish cages progressively decrease in impact with distance from the cages and suggested that impacts are largely confined to a radius of approximately 40 to 70 m. Mayer and Solan (2011) examined fish farm impacts on sediment chemistry in Scotland and observed that the ‘immediate footprint’ of the farms extended 25 to 50 m from the cage edge. Spatial analysis of benthic impact indices for samples collected at Maine fish farms indicated that impacts to the benthos rarely extend more than 60 meters from the cages. Some other studies indicated that benthic effects may occur at a greater distance in some regions. For example, Keeley et al. (2013) reported enrichment and ‘moderate’ impacts between 80 and 150 m distance from some fish farms in New Zealand.

The wide range in farm-specific benthic responses to aquaculture enrichment shows that it is not trivial to anticipate when and where the benthic community will be affected (Kalantzi and Karakassis, 2006, Borja et al., 2009). A problem with comparing spatial impact trends between studies is that no common benthic quality classification system has been employed internationally to permit direct comparison of results obtained with a multitude of indicators. In addition, the scale of the impact zone may vary over the course of the farmed species grow-out and not all studies were conducted during a similar phase of the husbandry cycle. Organic enrichment monitoring should occur during the period when the benthic impact is expected to be highest (i.e. worst-case scenario). This period can occur around the time of peak feeding, at peak biomass, at the time of harvest, or during the period of maximum water temperature when waste degradation processes are most rapid. International regulatory agencies consistently require monitoring when organic loading is anticipated to be highest (Table 3).

Table 3. Examples of international regulatory requirements related to the timing of benthic surveys.

Region	Survey Timing
Australia	Annual sampling of sediments in Autumn (~March)
Canada	At least once during the production cycle or every 24 months for farms with finfish continuously on site. Within 30 days of peak feeding or peak biomass in British Columbia. Between July 1 and Oct. 31 in other provinces (close to peak feeding).
Chile	Variable rules apply but generally conducted in the last year of the production cycle and up to two months before starting the harvest.
Ireland	Annual survey during peak biomass or at least within 30 days after the end of harvesting a year class.
New Zealand	Annual survey to coincide with the period of maximum biological impact. This period tends to coincide with highest water temperature.
Norway	B-Investigation: At half of maximum load or at maximum load depending on previous site conditions. C-Investigation: During the first two months with maximum load and until two months after harvest.
USA (Maine)	Once per growing cycle during year of maximum biomass for the facility

The revised requirement for the timing of organic enrichment monitoring surveys at marine fish cages is as follows:

- Companies applying for ASC certification must provide information on when the maximum impact on the benthos is predicted to occur for their farm(s). Based on this preliminary information, one of the following monitoring requirements will apply:
    - Surveys are to be conducted during the final year of each production cycle at the facility and within 30 days after peak feeding or peak biomass.
    - In the case of multiple peaks in feeding/biomass occurring in any year, sampling will take place within two weeks of the maximum annual water temperature.
    - In the case of sustained biomass in the months before harvest, surveys should take place within two weeks prior to the final harvest date.

The revised monitoring requirements focus solely on the region outside the boundaries of the farm, as defined by the edges of the animal holding structures, with the objective of limiting the area of seabed impacted by organic enrichment. This is consistent with monitoring in several countries where sites at increasing distances from the farm edge are allowed different degrees of benthic impact (Holmer et al., 2008; also see Section 8).

The revised sampling program requires monitoring stations to be established within each of three EQS compliance zones and at “reference” sites around the farm. A minimum of triplicate sediment sampling is to be conducted at four permanent stations located along transects extending outward from all four sides of the farm (16 sampling locations). The four transects are to be located orthogonal to the predominant direction of the current flow. Sampling stations along each transect are to be located within the following sampling zones:

Zone 1: 10 to 30 m from edge of holding structures

Zone 2: 31 to 100 m

Zone 3: 101 to 150 m

Zone 4: 250 to 500 m (Reference Sites

It is advised that the sampling sites be located at the outer-most distance within each zone. However, the revised monitoring requirements allow each company some flexibility to align the sampling program with local regulatory monitoring requirements. The above monitoring zones were established based on (1) knowledge of the spatial distribution and magnitude of organic enrichment effects observed around marine farms, and (2) compatibility with regulatory requirements in major farming countries. A minimum of two impact indicators from Table 2 (or approved alternate) is required to quantitatively determine the EQS condition at each sampling station based on the applicable indicator thresholds provided in Table 2.

The EQS determined for Zone 4 represents the “reference” condition for the farm. The reference data are used for comparison with data from the three monitoring zones and are not defined herein as representing baseline/natural conditions. The identification of true reference sites that represent unimpacted baseline conditions at a farm is often complicated by spatial variations in sediment type, water depth, and interactions with other anthropogenic and natural variables. Natural interannual temporal variability can also affect benthic systems. The sampling gradient approach described is meant to provide sufficient spatial data to permit an assessment of how the farm interacts with conditions in the surrounding environment, while also providing information on temporal variability within the “reference zone”. In cases where the potential impact zone of a farm may overlap with another farm (e.g., the reference site falls within 200 m of the adjacent farm) the overlapping transect location or direction may be adjusted to help avoid potential farm interactions. The same applies for any transect/station that would intersect with dry land. Transect directions may also be altered to avoid sampling in areas where water depth changes rapidly along the transect. In all cases, four sampling transects are required, with each being as close to 90 degrees from each other as possible.

The current monitoring requirements within the ASC species-specific standards require farm sites to utilize an Allowable Zone of Effect (AZE) to define sampling locations and allow farms to either use an AZE predefined by ASC (i.e., a set distance from the cages) or an AZE defined using a robust and credible modelling system (the latter is mandatory in the Salmon and Seriola/Cobia Standards). A shortcoming inherent with the use of AZE modelling as a tool for environmental management is that it only predicts how far away from the farm a predefined impact may occur, without considering if the spatial scale of the impact is acceptable. This is contrary to the purpose of the ASC revised requirements to consistently minimize negative effects from aquaculture. The sampling zone, or impact gradient, approach does not preclude the continued use of deposition models to define sampling locations, as long as the predicted site-specific AZE does not fall outside the above sampling zone boundaries. Permitting the extension of these boundaries would conflict with ASC definitions of the acceptable spatial scale of impacts defined in Section 5.2.

A preliminary rapid assessment of farm impacts in each zone may be permitted, using practical and low-cost abiotic indicators, to determine if the farm requires the full monitoring program described above (see Section 5.3).

5.2. Benthic EQS Objectives and Farm Compliance Framework

Decisions on farm compliance are to be based on EQS classifications measured using (1) the revised zonal monitoring requirements, (2) the average EQS for all samples collected around the farm within each sampling zone, and (3) the average EQS for all samples collected in the Reference Zone (Zone 4). Zonal EQS averages are to be determined across all replicate samples, transects and impact indicators. This multi-direction sampling and data analysis approach was adopted to treat farms equitably regardless of whether the area of seabed impacted was spread uniformally around the farm, or if it occurs primarily in one or two directions. A long, narrow zone of effect in a single direction can occupy a smaller area of seabed than a farm that has a more localized zone of effect around the whole farm.

Table 4 provides the revised EQS objectives (i.e., farm compliance thresehoIds) applicable to the three Monitoring Zones. Similar to the current ASC requirements, the maximum benthic impact permitted around a marine fish farm is characterized by a Moderate EQS classification (defined in Tables 1 and 2). The revised requirements include increasingly strict EQS objectives with distance from the farm edge. These are detailed in Table 4.

Table 4: Revised Ecological Quality Status (EQS) objectives for benthic sampling zones around marine and brackish water fish farms.

Reference Zone EQS Classification	Farm Zone	EQS Objective
High EQS	Zone 1	Moderate EQS must be achieved by 30 m
	Zone 2	Good EQS must be achieved by 100 m
	Zone 3	High EQS must be achieved by 150 m
Good EQS	Zone 1	Moderate EQS must be achieved by 30 m
	Zones 2 and 3	Good EQS must be achieved at 100 and 150 m

Moderate EQS	Zone 1, 2 and 3	Moderate EQS must be achieved in all zones
Poor or Bad EQS		Not an acceptable farm site

Each farm needs to demonstrate that it is meeting the EQS requirements described in Table 4. These requirements are not subject to any exemption from a potential cumulative effect from neighbouring farms. The ASC does not expect the average EQS in any Monitoring Zone to be of higher status than the average condition measured in the Reference area (Zone 4). Consequently, the farm EQS objectives shown in Table 4 can vary depending on the average EQS of the Reference. However, a mean EQS classification within Zones 1, 2 or 3 that exceeds the Moderate EQS threshold will be considered non-compliant with the revised benthic organic enrichment requirements.

The monitoring data required to inform farm compliance decisions against the revised requirements may be obtained using the ASC Benthic Monitoring Program (described below in Section 5.3) or through a user-defined program approved by the ASC (described in Section 8). In both cases, the monitoring is to be conducted annually by personnel that are either independent of the company owning the farm or approved by regional/ national regulators. Personnel performing this work are required to undergo training and demonstrate competence and proficiency in the use of all required methodologies and technologies employed under the revised requirements.

5.3. ASC Benthic Monitoring Program

A standardized monitoring program is presented here that would simplify the process by which companies can apply for ASC certification and ensure continued compliance within the revised requirements for organic enrichment monitoring. This program is recommended and is expected to be the typical approach. However, companies may apply to use a different monitoring methodology as described in Section 8. The program described in this section is particularly relevant to small companies in countries where there is limited regulation in place. This preferred ASC monitoring program was developed based on three guiding principles:

1. **Comprehensive.** The program is capable of documenting both the magnitude and scale of benthic impacts against acceptable EQS limits using proven methodologies.
2. **Practical.** Not all farms exhibit an environmental risk that warrants a costly and complex monitoring program.
3. **Responsive.** Rapid analysis of monitoring data allows timely decisions to be made regarding farm certification or the need for increased monitoring complexity.

The ASC benthic monitoring program employs a tiered approach in which the number of monitoring stations and the complexity/cost of sample analysis increases in relation to a risk assessment or preliminary monitoring data. The operator may decide to begin monitoring at any of the following monitoring tiers based on the past performance of the farm. Each monitoring tier is summarized as follows:

Table 5: Benthic Monitoring Program - Tiered Assessment Approach

Program	Description	Indicators	Locations
Tier 1	**Rapid screening:** Low-cost farm impact screening using practical, near-real-time abiotic measurements to determine the risk for organic enrichment impacts.	S^{2-} and *Eh*	30, 100, 150 and 500 m distances in predominant current direction.
Tier 2	**Impact delineation:** Enhanced spatial analysis of abiotic impacts around the farm using practical monitoring tools.	S^{2-} and *Eh*	Same as Tier 1 but including sampling in three additional directions.
Tier 3	**Biotic impact:** Comprehensive characterization of biotic impacts around the farm.	3 biotic metrics from Table 2	Same locations as Tier 1 and Tier 2.

Tier 1 offers a simplified starting point to help operators keep assessment time and costs to a minimum, and rewards good performance. Triplicate sediment samples are to be collected within each specified distance from the farm in the predominant current direction (Fig. 2). Each sediment sample will be analysed immediately onboard the survey vessel for total free sulfide (S^{2-}; in triplicate) and redox potential (*Eh*: single measure) in surface sediments (0 to 2 cm depth) using the rapid field analysis methods given in Appendix 3. The results of Tier 1 sampling are to be interpreted immediately onboard the sampling vessel. If the results (mean values for each monitoring zone) are within acceptable EQS thresholds for each monitoring zone (Tables 2 and 4), no further monitoring is required for that period. If an unacceptable EQS classification is determined for any of the three sampling zones, monitoring will immediately proceed to Tier 2.

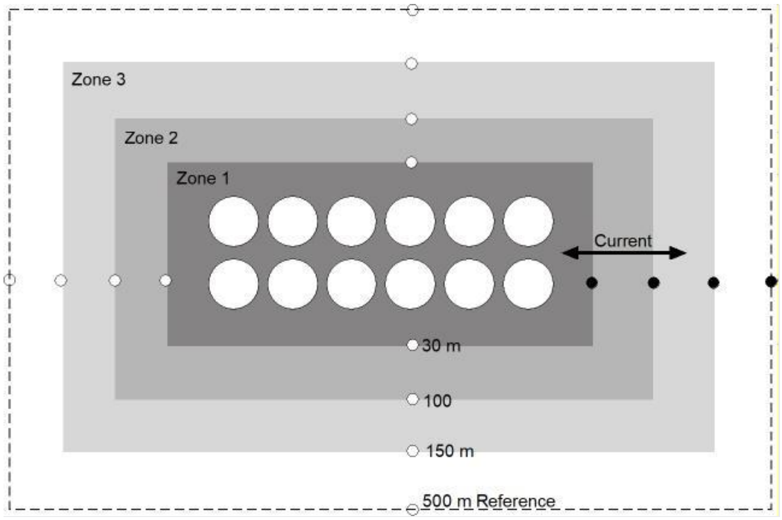


Figure 2. Schematic of sampling locations and acceptable EQS Zones under Tier 1 (●), 2 (● and ○) and 3 (● and ○) monitoring programs for caged fish aquaculture. The Ecological Quality Status monitoring zones are shown with sampling sites located at the outer zonal boundary.

Tier 2 monitoring provides more detailed information on spatial impacts around the farm while still providing the operator with a practical farm assessment approach. Like Tier 1, it can be conducted rapidly using the same abiotic indicators. If the average indicator value for each sampling zone (calculated using all three replicates and all four sampling directions) falls outside the acceptable limit, the risk for benthic community impacts is assessed to be high. The operator then has two options:

a) accept that the farm is non-compliant with the revised requirement and take remedial action, or

b) immediately proceed to Tier 3 sampling to further characterize spatial impacts by employing biotic indicator monitoring.

Tier 3 sampling is meant for assessing sites shown to be of high risk to the structure and function of benthic communities. It provides companies with an option to appeal the results of Tier 2 sampling by conducting a direct assessment of farm organic enrichment impacts on benthic communities across the three sampling zones. Triplicate grab samples collected at the same locations as described for Tier 1 and 2 are to be screened through a 1.0 mm mesh and all organisms preserved for taxonomic analysis. The results of a minimum of three of the biotic metrics listed in Table 2 will be used to determine the EQS of the community in each sampling zone. Decisions on farm compliance will be based on the dominant EQS classification identified within each sampling zone (Fig. 2). Of the 12 classifications provided (3 biotic metrics times 4 transects), six or more unacceptable EQS classifications (see Table 4) will results in a decision of non-compliance.

6. Freshwater Fish Cage Systems

6.1 Freshwater Benthic Impacts and Indicators

While considerable progress has been made over the years to develop and revise robust ASC benthic standards for marine environments, the same cannot be said for freshwater fish cage aquaculture in lakes; including trout, pangasius, tilapia and the freshwater stage of salmon. None of the existing ASC freshwater standards include significant levels of benthic monitoring, particularly when compared to the rigorous monitoring protocols required for marine aquaculture. One explanation for this disparity has been that the diversity of freshwater aquaculture systems (water depth, eutrophication status, turbidity, thermoclines) makes it difficult to develop a standard approach. However, the development of freshwater standards has largely been hampered by a relative lack of scientific literature as most freshwater aquaculture environmental impact studies have focused on water quality rather than benthic impacts.

The revised requirements for monitoring marine benthic impacts at freshwater farms in lakes follows the approach originally set out by Pearson and Rosenberg (1978) in their model of benthic invertebrate responses to organic enrichment gradients (see Section 3 and Fig. 1). This qualitative model describes changes in invertebrate abundance (numerical density), diversity and biomass along an organic enrichment gradient. The quantitative model described in Tables 1 and 2 serve as a means to numerically classify marine aquaculture benthic impacts based on abiotic and biotic indicator variables and provide decision support for managing benthic impacts at marine farms (see Section 4). The organic enrichment gradient and related abiotic and biotic thresholds of effect have been validated through extensive research in all marine benthic habitats.

Several studies have evaluated the impacts of freshwater fish farms in riverine environments. Hettige et al, (2020) investigated benthic macroinvertebrate assemblages in the Selangor River, Malaysia and found that they were affected by effluents from aquaculture farms, as did Soofiani et al (2012) in Iranian rivers. Loch et al (1996) compared taxa richness of stream insects sensitive to water quality above and below three commercial trout farms in North Carolina. Taxa richness was higher 1.5 km downstream from the farm outfalls but was still not as high as above the outfalls while pollution-tolerant species were noted just below the outfalls.

The benthic impacts of tilapia cages in northern Lake Victoria have also been examined. Nabirye et al. (2016) concluded that benthic macroinvertebrate species diversity differed significantly between the reference site and one of the tilapia cage sites (an array of 50, high density, low-volume cages) where the pollution tolerant Chironomus sp. (lake fly larvae) and Melanoides tuberculata (a freshwater snail) dominated. At another nearby tilapia cage site macro-benthic abundance was consistently higher at a sampling site between the cages compared to downstream or upstream of the cages (Mbweza-Ndawula et al., 2013).

In a study on a tilapia cage farm in Lake Kariba, Zimbabwe, there was accumulation of nutrients under the cages (Troell and Berg, 1997). Significantly higher porewater concentrations of nutrients were found under the cages compared to controls. However, these were not of the same magnitude as studies in temperate waters. Higher temperatures resulted in greater turnover of nutrients and the presence of wild fish resulted in lower levels of carbon and nutrients in sediments than would be seen in temperate waters. This was supported by Gondwe et al (2012) who traced organic wastes from a tilapia cage farm in Malawi. They found that there was minimal accumulation of wastes under the cages but high uptake of carbon and nitrogen stable isotopes that were included in experimental feeds by wild fish aggregating round the cages.

Rooney and Podemski (2009) studied freshwater invertebrate benthic effects at an experimental rainbow trout farm in northwestern Ontario, Canada. The farm was established to examine effects of cage-based trout aquaculture in a small lake. The scale of operation was small compared to commercial farms but the stocking density relative to the size of the water body and its flushing rate was higher than what would normally be permitted by regulatory authorities. After 2 months, invertebrate abundance was reduced under the fish cage (2,542 individuals/m2) compared to 45 m away (16,137 individuals/m2). Taxa richness (number of species) was also depressed but changes in biomass were variable, as they could be skewed by the individual size of the invertebrates. After two production cycles, abundance was correlated with distance from the cage. The effects of farming were localized and dissipated within 15 m of the cage edge. They concluded that invertebrate abundance demonstrated the most potential for incorporation into monitoring schemes at new farms but species richness could be a valuable monitoring metric at new farms. This suggests that the biotic indicators used for marine aquaculture (Table 2) should also be relevant in freshwater environments.

Wetton (2012) studied the benthic waste exposure gradient around commercial freshwater fish farms in Ontario and further confirmed that the Pearson and Rosenberg (1978) organic enrichment gradient model also applies to the freshwater environment. Invertebrate density, richness and biomass patterns along the sedimentation gradient from the commercial farms were similar to the model described by Pearson and Rosenberg (1978). The reported threshold of significant benthic community effects based on carbon sedimentation (2.0 - 3.0 g C m-2 day-1) was also similar to that at marine caged fish farms. Above this deposition rate, adverse effects on freshwater benthic invertebrate abundance and richness rapidly increase. In general, the rates of carbon, nitrogen and phosphorus sedimentation were highest beneath the cages and declined exponentially with increasing distance. Waste sedimentation rates were significantly elevated within 5 to 15 m from the cages and significantly elevated sediment waste concentrations were observed as far as 30 m.

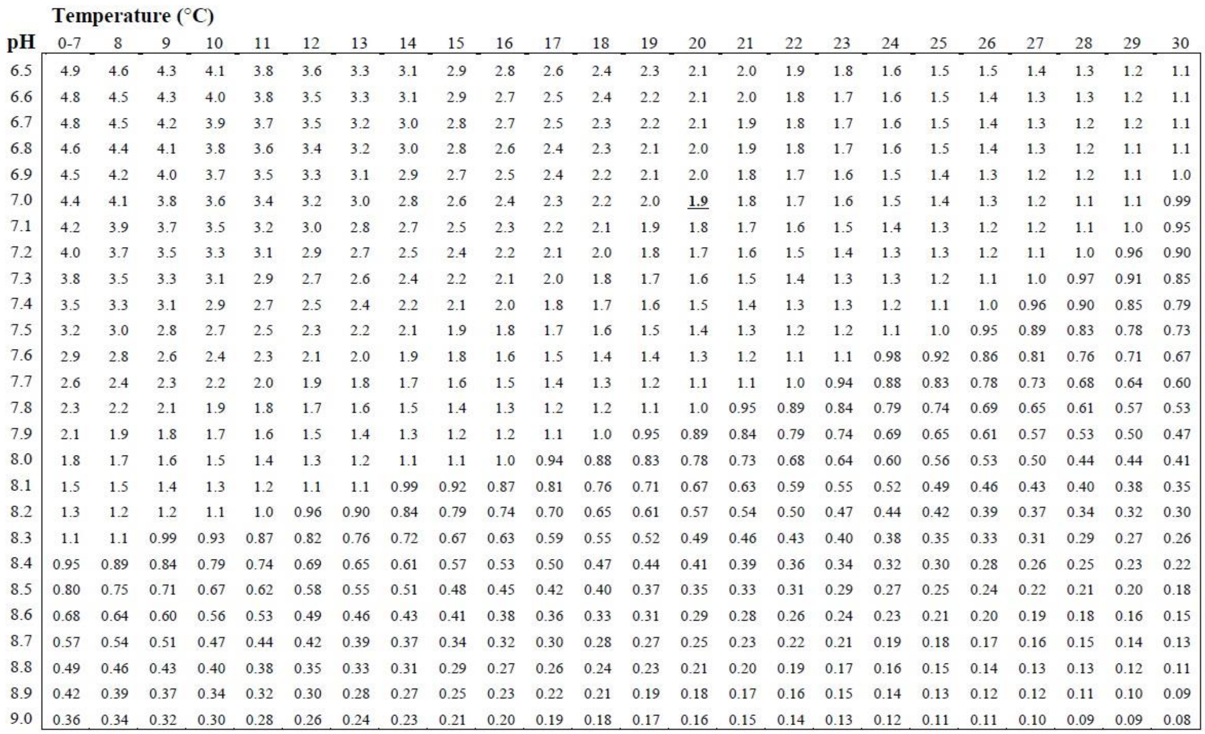
The recommended revision to the current ASC standards for freshwater fish culture, including pangasius, trout, tilapia and salmon smolt, is based on a similar benthic monitoring and EQS classification approach as described above for marine farms (Section 5) with one major difference. It is not appropriate to utilize total free sulfide (S2-) concentrations as an indicator for monitoring and classifying benthic impacts at freshwater farms because sulfide reduction is a relatively minor process in the mineralization of organic matter in freshwater systems. Rooney and Podemski (2010) showed that sulfide levels remained below detection limits in pore-water samples around freshwater fish cages. An alternative practical geochemical indicator is required to conduct rapid site assessments such as described in Tier 1 monitoring at marine fish farms in Section 5.3. Dissolved oxygen levels are naturally low in diffusion-dominated muddy surficial sediments and suboxic levels in coarse sediments do not significantly impact benthic communities because they can obtain oxygen at the sediment-water interface. Total phosphorous accumulates in sediments around fish farms, but is more of a concern for pelagic impacts than for the benthic community. Copper and zinc concentrations under fish cages can reach levels that could cause adverse biological impacts. Copper originates in antifouling coatings applied to the cage while zinc exists in the fish feeds.

Rooney and Podemski (2010) demonstrated that pore-water total ammonia was elevated (60+ mg/l) under the cage after one month, reaching a plateau of 77 mg/l by month 16. pH readings displayed a gradient rising from ~pH 5 to pH 8 with increasing distance from the cage. The toxicity of total ammonia is influenced by pH as the proportion of the toxic un-ionised fraction increases with pH. This means that although total ammonia concentrations are generally higher at the fish cages, the levels of un-ionised ammonia can be similar at the cages and at sampling points away from the cages. Rooney and Podemski (2010) recommended that ammonia and pH be used for monitoring because they are responsive, sensitive and biologically relevant. The pH level in sediment porewater has also been used in some marine aquaculture monitoring programs in conjunction with redox potential (Eh) measurements (see Appendex 1.3) and threshold EQS values for both variables are included in Table 2 for marine aquaculture monitoring from Schanning and Hanson (2005). Both pH and Eh tend to be highly variable in oxygenated sediments characterized by High and Good EQS status, but more stable readings are obtained as organic enrichment increases (see Appendix 1.2 and 1.3). All geochemical metrics are known to vary substantially with sediment depth. The transition between oxic and anoxic states tends to be close to the sediment surface in muddy marine and freshwater sediments, even in undisturbed environments, as a result of the limited diffusion-dominated exchange of oxygen across the sediment-water interface. The customary approach for sediment chemical monitoring has been to determine values representative of the upper 0 to 2 cm of sediment and measurements by gently mixing this region in grab samples prior to taking readings. pH, Eh and total ammonia are readily measured in real-time in the field and all three metrics are recommended for Tier 1 (rapid assessment) and Tier 2 (impact spatial delineation) monitoring programs at freshwater farms. Instructions for the measurement of total ammonia nitrogen in extracted porewater can be found in a standard water-analysis manual such as *Standard Methods for the Examination of Water and Wastewater* and are reported by regulatory agencies such as the US EPA. A recommended practical methodology is presented in Appendix 3.3.

Rooney and Podemski (2009) showed that TAN levels greater than approximately 10 mg/L corresponded with severe reductions in species richness, abundance and biomass under freshwater fish cages. This roughly corresponds with the US EPA criterion maximum concentration acute threshold of 17 mg N/L at pH 7 and 20°C in porewater. This threshold concentration in sediment porewater appears to correspond with a Bad EQS benthic classification. The upper boundary to a Moderate EQS classification is assumed to coincide with the US EPA chronic criterion for total ammonia nitrogen of 1.9 mg/L at pH 7 and 20^{o}C (US EPA, 2013). This US EPA criterion for total ammonia nitrogen are well supported for both fish and benthic invertebrates (Kinsman-Costello et al., 2015).

In water, ammonia occurs as NH4+ and NH3, which together are called total ammonia nitrogen (TAN). NH3, called un-ionized ammonia, is the form more toxic to aquatic species. Both water temperature and pH affect which form of ammonia is predominant at any given time in an aquatic system. The impact of pH and temperature on the speciation of ammonia is an important consideration when determining if the **Moderate EQS threshold (1.9 mg/L TAN at pH 7 and 20oC)** for lake sediments has been exceeded. As a result, the recommended threshold is not a specific value, but rather a range of values that apply to the ambient pH and temperature of the sediment at the sampling site (Table 6).

Table 6. Temperature and pH dependent concentration values for total ammonia nitrogen (mg/L) describing the threshold between Moderate and Poor Ecological Quality Status. The highlighted value is the threshold that applies to sediments with 7.0 pH and 20^{o}C. The applicable threshold for measurements taken at other ambient sediment conditions are shown.



	Temperature (°C)																							
pH	0-7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
6.5	4.9	4.6	4.3	4.1	3.8	3.6	3.3	3.1	2.9	2.8	2.6	2.4	2.3	2.1	2.0	1.9	1.8	1.6	1.5	1.5	1.4	1.3	1.2	1.1
6.6	4.8	4.5	4.3	4.0	3.8	3.5	3.3	3.1	2.9	2.7	2.5	2.4	2.2	2.1	2.0	1.8	1.7	1.6	1.5	1.4	1.3	1.3	1.2	1.1
6.7	4.8	4.5	4.2	3.9	3.7	3.5	3.2	3.0	2.8	2.7	2.5	2.3	2.2	2.1	1.9	1.8	1.7	1.6	1.5	1.4	1.3	1.2	1.2	1.1
6.8	4.6	4.4	4.1	3.8	3.6	3.4	3.2	3.0	2.8	2.6	2.4	2.3	2.1	2.0	1.9	1.8	1.7	1.6	1.5	1.4	1.3	1.2	1.1	1.1
6.9	4.5	4.2	4.0	3.7	3.5	3.3	3.1	2.9	2.7	2.5	2.4	2.2	2.1	2.0	1.8	1.7	1.6	1.5	1.4	1.3	1.2	1.2	1.1	1.0
7.0	4.4	4.1	3.8	3.6	3.4	3.2	3.0	2.8	2.6	2.4	2.3	2.2	2.0	1.9	1.8	1.7	1.6	1.5	1.4	1.3	1.2	1.1	1.1	0.99
7.1	4.2	3.9	3.7	3.5	3.2	3.0	2.8	2.7	2.5	2.3	2.2	2.1	1.9	1.8	1.7	1.6	1.5	1.4	1.3	1.2	1.2	1.1	1.0	0.95
7.2	4.0	3.7	3.5	3.3	3.1	2.9	2.7	2.5	2.4	2.2	2.1	2.0	1.8	1.7	1.6	1.5	1.4	1.3	1.3	1.2	1.1	1.0	0.96	0.90
7.3	3.8	3.5	3.3	3.1	2.9	2.7	2.6	2.4	2.2	2.1	2.0	1.8	1.7	1.6	1.5	1.4	1.3	1.3	1.2	1.1	1.0	0.97	0.91	0.85
7.4	3.5	3.3	3.1	2.9	2.7	2.5	2.4	2.2	2.1	2.0	1.8	1.7	1.6	1.5	1.4	1.3	1.3	1.2	1.1	1.0	0.96	0.90	0.85	0.79
7.5	3.2	3.0	2.8	2.7	2.5	2.3	2.2	2.1	1.9	1.8	1.7	1.6	1.5	1.4	1.3	1.2	1.2	1.1	1.0	0.95	0.89	0.83	0.78	0.73
7.6	2.9	2.8	2.6	2.4	2.3	2.1	2.0	1.9	1.8	1.6	1.5	1.4	1.4	1.3	1.2	1.1	1.1	0.98	0.92	0.86	0.81	0.76	0.71	0.67
7.7	2.6	2.4	2.3	2.2	2.0	1.9	1.8	1.7	1.6	1.5	1.4	1.3	1.2	1.1	1.1	1.0	0.94	0.88	0.83	0.78	0.73	0.68	0.64	0.60
7.8	2.3	2.2	2.1	1.9	1.8	1.7	1.6	1.5	1.4	1.3	1.2	1.2	1.1	1.0	0.95	0.89	0.84	0.79	0.74	0.69	0.65	0.61	0.57	0.53
7.9	2.1	1.9	1.8	1.7	1.6	1.5	1.4	1.3	1.2	1.2	1.1	1.0	0.95	0.89	0.84	0.79	0.74	0.69	0.65	0.61	0.57	0.53	0.50	0.47
8.0	1.8	1.7	1.6	1.5	1.4	1.3	1.2	1.1	1.1	1.0	0.94	0.88	0.83	0.78	0.73	0.68	0.64	0.60	0.56	0.53	0.50	0.44	0.44	0.41
8.1	1.5	1.5	1.4	1.3	1.2	1.1	1.1	0.99	0.92	0.87	0.81	0.76	0.71	0.67	0.63	0.59	0.55	0.52	0.49	0.46	0.43	0.40	0.38	0.35
8.2	1.3	1.2	1.2	1.1	1.0	0.96	0.90	0.84	0.79	0.74	0.70	0.65	0.61	0.57	0.54	0.50	0.47	0.44	0.42	0.39	0.37	0.34	0.32	0.30
8.3	1.1	1.1	0.99	0.93	0.87	0.82	0.76	0.72	0.67	0.63	0.59	0.55	0.52	0.49	0.46	0.43	0.40	0.38	0.35	0.33	0.31	0.29	0.27	0.26
8.4	0.95	0.89	0.84	0.79	0.74	0.69	0.65	0.61	0.57	0.53	0.50	0.47	0.44	0.41	0.39	0.36	0.34	0.32	0.30	0.28	0.26	0.25	0.23	0.22
8.5	0.80	0.75	0.71	0.67	0.62	0.58	0.55	0.51	0.48	0.45	0.42	0.40	0.37	0.35	0.33	0.31	0.29	0.27	0.25	0.24	0.22	0.21	0.20	0.18
8.6	0.68	0.64	0.60	0.56	0.53	0.49	0.46	0.43	0.41	0.38	0.36	0.33	0.31	0.29	0.28	0.26	0.24	0.23	0.21	0.20	0.19	0.18	0.16	0.15
8.7	0.57	0.54	0.51	0.47	0.44	0.42	0.39	0.37	0.34	0.32	0.30	0.28	0.27	0.25	0.23	0.22	0.21	0.19	0.18	0.17	0.16	0.15	0.14	0.13
8.8	0.49	0.46	0.43	0.40	0.38	0.35	0.33	0.31	0.29	0.27	0.26	0.24	0.23	0.21	0.20	0.19	0.17	0.16	0.15	0.14	0.13	0.13	0.12	0.11
8.9	0.42	0.39	0.37	0.34	0.32	0.30	0.28	0.27	0.25	0.23	0.22	0.21	0.19	0.18	0.17	0.16	0.15	0.14	0.13	0.12	0.12	0.11	0.10	0.09
9.0	0.36	0.34	0.32	0.30	0.28	0.26	0.24	0.23	0.21	0.20	0.19	0.18	0.17	0.16	0.15	0.14	0.13	0.12	0.11	0.11	0.10	0.09	0.09	0.08

6.2 Farm Compliance Decision Framework

Decisions on freshwater farm compliance are based on the same procedures as described in Section 5.2 for marine farms. However, the EQS objectives are simplified owing to lack of available information on total ammonia nitrogen thresholds separating all of the five EQS classifications. The number of monitoring zones is reduced to two (Zone 1 = 0 to 30 m, and Zone 2 = 31 to 100 m) and the reference sites are closer to the farm, compared to marine farms, at 150 m. The best available knowledge indicates that the upper boundary to a Moderate EQS classification coincides with TAN concentrations shown in Table 6 and at Eh and pH thresholds of -100 mV and 7.1, respectively. The recommended EQS objectives for freshwater farms are detailed in Table 7.

Table 7: Revised Ecological Quality Status (EQS) objectives for benthic sampling zones around freshwater fish farms relative to the EQS classification of the reference site.

Reference Zone EQS Classification	Farm Zone	EQS Objective
Good to High EQS	Zone 1	Moderate EQS must be achieved by 30 m
Moderate EQS	Zone 1 and 2	Moderate EQS must be achieved in both zones
Poor or Bad EQS		Not an acceptable farm site

The EQS objectives identified in Table 7 will not be implemented until after two monitoring cycles to allow for an internal assessment of the recommended thresholds and methodologies. This assessment will consider feedback from aquaculture companies on the revised benthic monitoring/management approach for caged fish farms in lakes.

6.3 ASC Benthic Monitoring Program

The three-tiered monitoring program described in Section 5.3 for marine farms is also recommended for freshwater fish farms in lakes, with the exception of the types of geochemical indicators to be applied. The Tier 1 and 2 standardized monitoring programs consist of Eh, pH and total ammonia nitrogen (TAN) measurements in surficial sediments (0 to 2 cm depth) and the optional Tier 3 program requires additional benthic commumity analysis based on the use of three of the biotic indicators from Table 2. The sampling zones and distances recommended for monitoring in freshwater systems also remain the same as described in Section 5.1 and 5.3 for marine systems. The sampling stations shown in Figure 2 will apply with the following exceptions:

1. The 150 m stations will be used as the reference stations and no sampling is required at the 500 m distances, which applies only to marine farms.

2. Any sampling station that intersects with land will be excluded.

Companies applying for ASC certification must provide information on when the maximum impact on the benthos is predicted to occur for their farm(s). Based on this preliminary information, one of the following monitoring requirements will apply:

- Surveys are to be conducted during the final year of each production cycle at the facility and within 30 days after peak feeding or peak biomass.

- In the case of multiple peaks in feeding/biomass occurring in any year, sampling will take place within two weeks of the maximum annual water temperature.

- In the case of sustained biomass in the months before harvest, surveys should take place within two weeks of the final harvest date.

Monitoring is to be conducted by highly trained personnel that are independent of the company owning the farm.

7. Marine Mollusc Systems

7.1. Revised Benthic Organic Enrichment Monitoring Requirements

The effects of bivalve mollusc farms on marine benthic environments are relatively well known (see reviews by Dame 1996, Cranford et al. 2006, 2009 and 2012, Ortero et al. 2006, Hargrave et al. 2008, Norkko and Shumway 2011, McKindsey et al. 2011, Shumway 2011). Benthic organic loading from bivalve aquaculture stems from the consumption and re-packaging of natural suspended particulate material into larger faeces and pseudofaeces that settle to the seabed. This particle re-packaging diverts primary production and energy flow from planktonic to benthic food webs. The mortality and fall-off of cultured bivalves and fouling organisms from suspended aquaculture structures cause additional benthic organic loading. The magnitude of benthic effects from these organic deposits is highly site-specific and can range from positive effects, associated with an increase in food availability to benthic species or an increase in habitat structure, to significant negative benthic effects characterized by diminished sediment geochemical conditions and impoverished benthic communities (Figure 1).

Compared with bivalve aquaculture, the farming of other molluscs is relatively small. This includes the farming of abalone. Abalone are herbivores and most sea-based culture systems use algae as the main food. The literature on the environmental performance of the abalone culture industry is sparse. Open systems used for abalone farming allow for the deposition of organic matter beneath culture arrays, but the impacts are localized and unlikely to significantly alter the structure and functionality of the ecosystem (e.g. Oh et al. 2015). However, a large-scale farm may result in excess impacts from biodeposits, particularly if the farm is located in shallow, poorly flushed areas, sensitive habitats or marine protected areas.

The magnitude and spatial scale of benthic impacts from bivalve aquaculture depend on factors controlling the rates of consumption of suspended food particles and waste production by the stocked animals (e.g., the density of culture, individual feeding rates and concentration of food particles), local waste dispersion processes (hydrographic regime), and the capacity of the benthic environment to assimilate deposited wastes through natural geochemical and microbial processes. Physical waste dispersion processes, in combination with water depth, influence the distribution of organic matter accumulating on the seabed and are critical in determining the scale of benthic effects. The highest risk for bivalve aquaculture to impact benthic habitat, biodiversity and ecosystem function through organic enrichment is associated with farms containing high stocking densities located in shallow, poorly flushed areas.

Bivalve culture activities are highly diverse and include suspended and bottom culture husbandry methods. The density and biomass of bivalves held in bottom culture (in- and on-bottom) are limited by available space, thus limiting organic waste deposition. Waste production by culture activities located near the water surface has a similar space limitation. Suspended culture activities that take place over a significant fraction of the water column greatly increase both the biomass of animals that can be grown in a certain area and the vertical flux of organic wastes to the seabed. This type of intensive and extensive aquaculture primarily includes raft and long-line methods. Bivalve culture areas where the organic waste assimilative capacity has been shown to be surpassed, resulting in significantly disturbed benthic conditions, are primarily limited to these types of suspended culture. However, bivalve aquaculture activities in shallow, low current areas, or in ecologically sensitive and protected marine habitats also require efforts to document their environmental performance and minimize or eliminate negative impacts from seabed organic enrichment. Coastal seagrass meadows are recognized as sensitive structured habitat that provides important nursey areas for fish and shellfish. Excess seabed organic enrichment from aquaculture biodeposition may lead to an increase in toxic free sulfide levels that impair the ecophysiological functioning and growth of seagrass (e.g., Lamers et al. 2013).

Under the revised ASC requirements, monitoring is required for all suspended bivalve aquaculture farms (bivalves held at multiple depths in the water column). All other bivalve and abalone culture activities are exempt from the revised monitoring requirements, except if they are conducted under the following conditions:

1. The average water depth in a subtidal farmed area is less than 3 m and the average current speed is less than 10 cm per second. These conditions indicate a limited capacity to physically disperse biodeposits.

2. The farmed area is within the natural distribution of a seagrass meadow or within the boundaries of an area protected by regional legislation.

Unlike finfish cage culture, the negative effects of benthic organic enrichment from mollusc culture are primarily contained within the area defined by the presence of animal holding structures. In some regions, bivalve farm operations can comprise a significant fraction of the coastal zone. The revised monitoring requirement for bivalve culture therefore focuses on determining the magnitude of effects within the boundaries of the aquaculture facility, with reference stations positioned in the surrounding region. A minimum of triplicate sediment sampling is to be conducted at permanent stations located inside the perimeter of the farm and at reference stations located in areas of similar bottom type outside the farm. A minimum of two impact indicators from Table 2 (or approved alternate) is required to quantitatively describe the conditions at each sampling station. The EQS classification of each sampling location is to be determined from the average classification across all metrics and sample replicates. A preliminary rapid assessment of farm impacts may be permitted, using practical and low-cost abiotic indicators, to determine if the farm requires a more comprehensive monitoring program.

For mollusc farms containing a single cohort, monitoring is to be conducted in the final year of production within 30 days after peak biomass. Farms containing more than one production cycle (several cohorts present with the potential for multiple peaks in biomass) are to be surveyed annually within 30 days from the time of maximum water temperature. Mollusc farms exhibiting three consecutive years of acceptable benthic performance will be permitted to switch to a five-year sampling frequency as long as there have been no significant changes to farming practices.

7.2. Farm Compliance Decision Framework

The revised organic enrichment requirements for mollusc aquaculture place a limit on negative impacts on benthic habitat, biodiversity and ecosystem function by setting the acceptable Ecological Quality Status classification of surficial sediment (0-2 cm from the surface) within the farm boundaries to “Moderate” (Table 2).

The current ASC standards for bivalve and abalone farms assess certification compliance based solely on total free sulfide (S2-) monitoring. The revised requirements utilize this same indicator, although the sediment samples are to be analysed using the revised protocol (Appendix 3) and new EQS classification thresholds (Table 2). Eh measurements are also now included in the revised requirements to support the S2- data in the determination of the farm organic enrichment classification (see relationship between these two indicators in Appendix 1, Section A1.2).

Table 8: Revised abiotic requirement for marine mollusc systems.

Indicator	Revised Requirement
The acceptable concentration of total free sulfide in surficial sediment (0 to 2 cm depth)	≤ 500 µM
The acceptable redox potential in surficial sediment (0 to 2 cm depth)	≥ -100 mV

Indicator threshold requirements shown in Table 8 represent the boundary between Moderate and Poor EQS classifications. Bivalve farms within areas shown to exhibit a “Poor” or “Bad” EQS classification at the reference sites are not considered acceptable in the revised requirements.

The monitoring data required to inform farm compliance decisions against the revised EQS requirement may be obtained using the ASC benthic monitoring program for mollusc aquaculture systems (Section 7.3) or through a user-defined program approved by the ASC (Section 8). In both cases, the monitoring is to be conducted by highly trained personnel that are independent of the company owning the farm.

7.3 ASC Benthic Monitoring Program

The revised requirements for monitoring mollusc farms (when applicable) display many similarities to the caged fish program, with the exception of focusing the sampling effort on detecting organic enrichment impacts inside the farm boundaries as opposed to sampling adjacent to the fish cages. This is necessary because of the need to conserve natural habitat, local biodiversity and ecosystem function across the relatively broad spatial scale of some bivalve culture systems. The revised monitoring requirements were developed based on the three guiding principles (comprehensive, practical, and responsive) described in

Section 5.3 and utilize the same tiered sampling and analysis approach. Tier 1 consists of a rapid screening of benthic EQS using practical, near-real-time geochemical indicators (S^{2-} and *Eh)* across a limited spatial scale. Broader-scale Tier 2 sampling for analysis of the same indicators is only conducted if the Tier 1 results indicate that the farm does not comply with the revised ASC requirements described in Section 7.2. The Tier 3 survey uses the same sampling design as Tier 2, but employs a minimum of 3 biotic indicators. This Tier 3 program is only employed if Tier 2 sampling indicates that the farm is not in compliance and if the company wishes to appeal this decision.

The sampling design under the revised requirements utilizes a “gradient” sampling approach in which seabed samples are collected at seven stations situated 10 m apart from each other along transects that extend across the farm boundary. Tier 1 employs a single transect that runs in the direction of the predominant current direction. Tier 2 and 3 sampling is conducted at three additional orthogonal transects (Fig. 3). If a farm boundary is contiguous with another farm, the additional transects can be relocated to a location that crosses both farm and reference conditions.

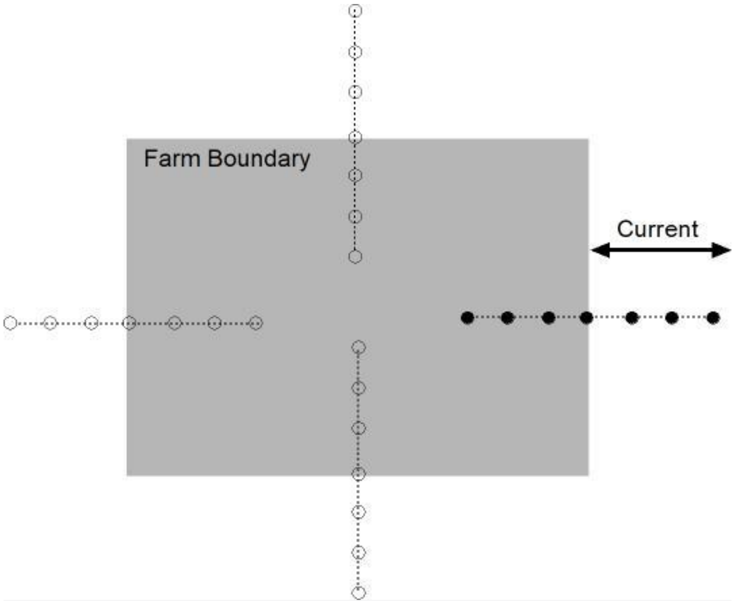


Figure 3. Schematic of sampling locations at bivalve farms under Tier 1 (●), 2 (● and ○) and 3 (● and ○) monitoring programs. Sampling locations on each transect are 10 m apart with the middle station located on the farm boundary.

Triplicate seabed samples are to be collected at each station and the surficial sediment (0-2 cm depth) is analysed for S2- and Eh following the protocols described in Appendix 3. Under the Tier 3 program, triplicate seabed samples collected at each station are screened through a 1.0 mm mesh and all organisms preserved for taxonomic analysis. The EQS for each sampling station is calculated as the average classification across all indicators and replicates. Compliance will be determined based on the average EQS classification for all stations inside the farm, including the farm boundary stations (Table 8). The average EQS calculated for each of the three distances outside the farm boundary will be used to assess how the farm is interacting with external benthic conditions.

8. User-Defined Benthic Monitoring Program Requirements

The revised benthic organic enrichment monitoring requirements include some flexibility for operators to use an approach that aligns with regional regulatory requirements while demonstrating the capacity to detect the same EQS thresholds described in the farm compliance framework (Sections 5.2, 6.2 and 7.2) across all spatial monitoring zones. This non-prescriptive approach to monitoring is meant to recognize the in-depth monitoring and regulation of aquaculture in some jurisdictions/countries and to foster innovation. Although the ASC does not mandate the use of the benthic monitoring programs described in Sections 5.3, 6.3 and 7.3, the onus is on the operator to make a highly detailed and convincing case to the ASC that their proposed farm monitoring program meets the following requirements.

The user-defined monitoring approach must be aligned with the overall purpose of the revised benthic organic enrichment monitoring requirements (see page 3).

a) This requires statements from the operator clearly outlining their environmental policy and how their monitoring approach is capable of minimizing, mitigating or eliminating negative benthic habitat, biodiversity and ecosystem effects from seabed organic enrichment.

    - The program needs to quantify both the magnitude and spatial scale of benthic impacts from organic enrichment adjacent to the farm using proven methodologies.

b) Provide information on the sampling design including all sampling locations and distances to farm edge, benthic sampling methodologies employed, and the number of replicates.

    - Provide a rationale for reference station selection that aligns with ASC intent of quantifying spatial and annual temporal interactions between the farm and the surrounding natural benthic environment.

    - Provide a rationale for the timing of monitoring that is in line with the maximum potential for benthic impacts. Although annual sampling is expected, any proposal to reduce the frequency of sampling would require a strong justification.

    - Describe all impact indicators to be employed and the sample preparation and analysis procedures.

c) The user-defined monitoring program needs to address benthic ecological quality objectives that are at least as stringent as those described in the revised ASC requirements outlined in Sections 5 (marine fish cages), 6 (lake fish cages) and 7 (marine molluscs).

- Describe the farm-management decision framework to be employed, including quantitative benthic indicator thresholds that drive these decisions and the rationale for selecting these thresholds.

- Compare and demonstrate compatibility between the user-defined site impact classifications and the EQS classification system as defined in Tables 1 and 2.

The user-defined monitoring program submitted by operators will be pre-screened within the ASC for compatibility with the purpose, rationale, intent and general requirements of the revised requirements. Those programs that appear to meet general criteria will be reviewed externally by a panel consisting of international science experts in aquaculture-environment interactions to ensure that they fulfill the overall purpose and specific requirements. Given the comprehensive and stringent amendments to the monitoring requirements detailed in this whitepaper, approval of user-defined programs is anticipated only in rare cases. The ASC encourages all companies to implement the ASC Benthic Monitoring Programs detailed in Sections 5, 6 and 7.

9. Comparison with International Standards

9.1. Current and Recommended Requirements

The current ASC Salmon Standard (Ver. 1.3) addresses organic enrichment effects on benthic habitat biodiversity and ecosystem function based on a prescriptive approach that relies on measurements of *Eh* or S^{2-}, *S*, *AMBI*, *BQI* and *ITI*. In previous versions of this standard, the allowable zone of effect (AZE) was defined as 30 meters from the cages, however, the current ver.1.3 requires a site-specific AZE determined by modelling. An Ecological Quality Classification of “Good” was required outside the AZE. This classification was defined as “the level of diversity and abundance of invertebrate taxa is slightly outside the range associated with the type-specific conditions. Most of the sensitive taxa of the type-specific communities are present.”

The revised benthic organic enrichment monitoring requirements for marine fish cage systems differ from the current requirements in that they are designed to better address the spatial scale of the impact. The expected Ecological Quality Status in Zone 2 remains equivalent to the current requirements (Good status at ≥ 30 m). However, some of the indicator EQS boundaries shown in Table 2 have been altered based on the results of recent peer-reviewed research. The addition of monitoring in Zones 2 and 3 addresses the need to prevent disturbances on sensitive and ecologically important taxa. The revised requirements also provide for greater flexibility in the selection of monitoring stations and impact indicators to better mesh with regional regulatory monitoring protocols.

The revised requirements remove the requirement for deposition models to determine monitoring locations based on the prediction of the Allowable Zone of Effect (AZE) for the following two reasons. First, monitoring is meant to address uncertainty in impact predictions so linking monitoring to modelling contradicts the intent. Second, the organic waste deposition rate threshold believed to define a significant adverse effect is now known to be highly variable and site-specific (Giles, 2008, Keeley et al., 2013, Bravo and Grant, 2018) owing to variations in the capacity of local physical, chemical and biological processes to assimilate these wastes. Any predicted AZE based solely on physical particle deposition modelling can be expected to entail a high degree of uncertainty.

9.2. Canada

Under the *Fisheries Act* and Aquaculture Activities Regulations (AAR), impacts on benthic habitat adjacent to fish farms are regulated based solely on total free sulfide (S^{2-}) monitoring data. *Eh* data are also obtained but are not employed in the decision framework. The revised requirements encourage the use of these geochemical indicators, however, prescribing certification or regulatory decisions to just one indicator is contrary to widespread science recommendations.

Methodologies outlined in the Canadian AAR Monitoring Standard strictly require the use of the Ion Selective Electrode (ISE) method for measuring S^{2-}. For reasons given in Appendix 2, the ISE protocol is not judged suitable for use under the revised requirements owing to multiple known analytical and user biases shown to be inherent with the ISE method, and the site-specific nature of these biases (see Appendix 2). These biases were not well known when the AARs were implemented.

The prescribed location of monitoring sites in the AAR Monitoring Standard varies for different regions in Canada. Regulatory decisions for fish farms in eastern Canada are based primarily on data collected at the farm edge (0 m distance) where the impact is specified to not exceed the Hypoxic A threshold. This is equivalent to the revised requirements of not exceeding the Moderate EQS threshold defined for Zone 1 (up to a distance of 30 m). Monitoring in British Columbia is conducted at 30 and 125 m distances where the regulatory thresholds are defined by Oxic B and Oxic A conditions, respectively. This is also equivalent to the acceptable EQS conditions defined in Section 6 for Zones 2 and 3. With the exception of the ISE-based S^{2-} concentration thresholds, the Canadian organic enrichment classification boundaries shown in Hargrave (2008) are equivalent to the EQS boundaries shown in Table 2 (note the same EQS and Oxic classification limits for *Eh* and biodiversity indicators).

Unlike the revised ASC requirements, all bivalve aquaculture farms are exempted from any benthic monitoring under the Canadian Aquaculture Activities Regulations.

9.3. USA

The *Clean Water Act* regulates discharge of pollutants, including salmon faeces and uneaten feed, into US waters with regulatory delegation to State authorities. The Finfish Aquaculture Monitoring Program (FAMP) collects information on benthic habitat characteristics and effects, including changes in community structure and function. Benthic monitoring sampling is carried out immediately adjacent to and at various distances from selected cage systems on a schedule such that each cage system is monitored in alternating years. Beginning in 1998, benthic sampling was focused on sites having potentially greater impacts on the benthos and greater emphasis was placed on near-cage sampling.

The State of Maine Department of Environmental Protection (DEP) General Permit for Net Pen Aquaculture (2014; https://www.maine.gov/dep/water/wd/net-pen-aquaculture/MEG130000-2014permit.pdf) includes sediment and benthic monitoring requirements and limitations within and outside the sediment mixing zone (defined as 100 ft (~30 m)). The permit relies on monitoring of the Shannon-Wiener Diversity Index (*H'*), total abundance composed of *Capitella capitata* and sulfide concentrations at 35 m distance from the pens. There is also a separate requirement to demonstrate compliance with sulfide regulations at 5 m distances prior to restocking a facility. These sampling site locations are compatible with the revised requirements for Zone 2 monitoring.

The acceptable DEP permit thresholds (≤4,000 µM sulfide for farm restocking and ≤3,000 µM for general monitoring) are equivalent to 'Bad' EQS conditions and are therefore not comparable to the revised requirements. Although a USEPA approved method is required for sulfide measurements under the DEP permit, there is no stipulation to remove the non-toxic solid component as is required in the revised requirements. Consequently, total sulfides appear to be monitored as opposed to the total dissolved (free) sulfides responsible for benthic community impacts. The *C. capitata* (*GrV*) metric allowable limit of ≤50% at 35 m distance in Class SC waters is equal to permitting a Moderate EQS, which is less stringent than the revised requirements (Good EQS in Zone 2).

Net pen operations in Washington State require a National Pollutant Discharge Elimination System (NPDES) permit. NPDES permitting requires project proponents to conduct baseline studies, implement best management practices, monitor for benthic impact, and limit thresholds further reducing the risk. State rules WAC 173-204-412 apply to marine finfish rearing facilities and sediment quality compliance and monitoring requirements. Regulatory limits for marine sediment organic enrichment are based on TOC concentrations and benthic infaunal effects within and including the distance of 100 feet (~30 m) from the outer edge of the net pens. Marine facilities that exceed the sediment quality conditions beyond 30 m must begin an enhanced sediment quality monitoring program to include benthic infaunal abundance. This enhanced spatial monitoring is consistent with the revised requirements.

The maximum biological effects level allowable at 30 m distance stipulated in state rule WAC 173-204-420 is:

- <50% of the reference taxa consists of Class Crustacea, Phylum Mollusca or Class Polychaeta and the test sediment abundances are statistically different (t-test, $p \leq 0.05$) from the reference sediment abundances.

This limit is somewhat equivalent to the revised requirements that strives to confine a Moderate EQS benthic condition inside Zone 1. However, given that first-order opportunistic polychaetes co-dominate or dominate in Poor to Bad EQS conditions, comparison of the ASC and State quality thresholds is somewhat confounded.

9.4 Norway

The Norwegian Activities Regulations employs three types of aquaculture monitoring programs that each include several indicator variables (Hansen et al. 2001; Norwegian Standard NS 9410:2016). Each investigation differs in complexity, accuracy for detecting benthic effects from organic enrichment, and frequency of sampling. The B-investigation focuses only on the actual footprint of the farm (beneath and between cages). The C-investigation is the most complex and focuses on the regional scale. Unlike the ASC spatial sampling requirements (three sampling zones inside 150 m from cage edge), the Norwegian C-investigation can extend outward to approximately 500 m with three sampling sites selected within this range based on a pre-investigation. Whereas the ASC monitoring approach aims to ensure that organic enrichment impacts at all farms are within threshold values at three set distances, the Norwegian program is designed to ensure that threshold values are not exceeded at variable sampling distances for each farm. Although the sampling effort within both the ASC and Norwegian monitoring programs appears similar, the sampling designs can differ markedly.

Monitoring parameters described in the Norwegian Standard (NS 9410) include abiotic (*pH*, *Eh*, *TOC, TN*), biotic (macrofauna to taxonomic level), and sensory (gas, colour, odor, sludge thickness and consistency) parameters. The environmental condition (quality classification) of the sediment is determined based on a scoring system. According to this standard, Level 1 classification indicates no effects of the aquaculture on benthic conditions, while Level 4 indicates that aquaculture has a serious negative effect on the environment requiring additional monitoring and regulatory intervention.

Although type-C monitoring under the Norway Standard utilizes similar methods and variables as the revised requirements, the approach to classifying benthic quality based on monitoring data differs markedly. An inter-calibration is needed to determine the equivalency of site classifications and related acceptable impact thresholds. Unlike the revised requirements, which requires annual monitoring, the frequency of employing a type-C investigation in Norway is at the discretion of the local authority.

Given the extensive nature of aquaculture/environment regulation and monitoring in Norway (including spatial sampling of multiple abiotic and biotic indicators), it is anticipated that the Norwegian program can be enhanced to comply with the revised requirements.

The Faroe Islands generally follow the Norwegian standard.

9.5 Scotland

The Water Environment (Controlled Activities) (Scotland) Regulations 2011 identifies substances having an unfavorable influence on the oxygen balance (i.e. organic enrichment) among the main pollutants from finfish aquaculture. The Scottish Environment Protection Agency (SEPA) launched a new regulatory framework in 2019 that is still evolving but reflects the changing nature of the industry away from very sheltered, non-dispersive locations towards larger farms in more exposed locations[1]. The new regulatory framework is based on allowing a mixing zone in which wastes from a floating cage fish farm are not fully mixed and dispersed. The regulatory framework limits the maximum area of the mixing zone to the area lying within 100 metres of the pens in all directions. By the edge of this mixing zone, deposition of organic waste discharges must be sufficiently low for the status of sea life on the sea bed to be assessed as good. The 100 m appears to have close compliance with the revised ASC operational objective for good EQS to be achieved by 100 m. The new framework also mentions improved auditing, including a quality assurance scheme, to increase public confidence in monitoring by operators.

The status of the seabed is assessed where possible by collecting Infaunal Quality Index (IQI) data along a minimum of four transects, each comprised of a minimum of seven stations. The monitoring stations are oriented to determine the distance from the farm representing good quality status along each transect. IQI is a multivariate biotic metric based on taxa number, AMBI and Simpson’s evenness and good status is defined by an IQI threshold of ≥ 0.64. Although it includes biotic metrics employed in the ASC revised requirements (Table 2), this index represents an Ecological Quality Ratio (EQR) in which benthic community health at each sampling station is measured relative to the health of the community at the reference stations. This differs substantially both conceptually and quantitatively from the EQS classification system employed by the ASC (see Section 4), resulting a the potential for different farm compliance decisions under both systems. For example, it is possible for farms with reference sites classified as poor under the EQS system (an unacceptable classification in Table 4) to have a good status at 100 m under the Scottish EQR classification approach.

Where environmental standards have not yet been developed for all seabed habitats, such as rocky seabeds, visual imagery will be used to assess their condition within the mixing zone while appropriate biological standards will be progressively developed.. If the impact area is greater than that permitted, enforcement action could be taken.

9.6 Republic of Ireland

The Department of Agriculture, Fisheries and Foods Monitoring Protocol No. 1 for offshore finfish farms describes benthic monitoring requirements. Two levels of monitoring during peak biomass are described in relation to production tonnage and mean current speed. Level I consist of visual observations, *Eh* and organic carbon measurements beneath and at multiple distances out to 100 m from the cages. Control stations are required at least 500 m away from the cages. Level II monitoring requires additional measurements of macrofauna abundance at the same locations. Monitoring Protocol No. 1 is highly consistent with the revised requirements

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[1] https://www.sepa.org.uk/media/433439/finfish-aquaculture-annex-2019_31052019.pdf

and the tiered sampling approach has much in common with the ASC benthic monitoring program described in Section 6.3.

The Marine Environment and Food Safety Services (MEFSS) Benthos Ecology Group of the Marine Institute (Ireland) has been responsible for the review and assessment of existing and proposed activities that may have an influence on the marine environment and benthos monitoring. Their recommendations have been used in reporting organic enrichment impacts under the Water Framework Directive and Marine Strategy Framework Directive. These directives utilize the Ecological Quality Status system employed in the revised requirements.

9.7 New Zealand

Fish farms in the Marlborough Sounds are monitored pursuant to a marine monitoring adaptive management plan and the results are measured against defined environmental quality standards. Analytes measured in grab samples include grain size, infauna and epifauna, organic matter, *Eh* and sulfides (ISE method). An ‘impact zones’ approach is utilized that provides an upper limit to the spatial extent and magnitude of seabed impacts. Type 2 monitoring (default level) is conducted annually in the predicted zone of maximal effect beside the pens, at the predicted outer limit of effects, and at reference stations. The sampling distances vary between farms in accordance with the physical properties of the site. Additional sampling (Type 3) is carried out after five years of operation to map the distribution and extent of organic enrichment and compare the predicted and actual footprint.

The legislated environmental quality standards vary to some extent between farms in accordance with the consent conditions that were imposed when the farm was permitted. However, most farms are managed in accordance with the Marlborough Best Management Practice Guidelines (MPI, 2018). Under this framework, benthic effects are determined using overall Enrichment Stage (ES) which is derived from multiple geochemical and biological parameters (See Appendix 1.18). The level of acceptable impact reduces with distance from the net pens. The ES and EQS group classifications are conceptually similar and corresponding groups are shown in Table 2.

Overall, the Marlborough Sounds tiered sampling design, impact zone approach, and site classification system is consistent with the revised requirements although the location of sampling sites is defined by model predictions. However, the New Zealand Tier 3 sampling design permits detection of the actual farm footprint.

9.8 Australia

There is no national aquaculture monitoring standard, and there are significant differences in the way that aquaculture is regulated and administered across States. These differences are primarily associated with differences in culture species, farming technologies and location, making it difficult to generalize on compatibility with the revised requirements. Tasmania has the most detailed environmental monitoring requirements. The Tasmanian environmental monitoring program is currently under review, but the current provisions are outlined in the Finfish Farming Environmental Regulation Act 2017 (Finfish Act), with finfish farms operating under Environmental Licences issued by the Environmental Protection Authority (EPA). The Environmental Licence consolidate all environmental conditions into a single instrument. There is requirement for not just local but also broadscale assessments, with comprehensive baselines required to establish the prevailing conditions for each farm/ lease/ region. Modelling is used in advance to determine allowable zones of effect (AZEs) based on anticipated production loads and seasonal hydrodynamics for each site, with monitoring sites/times determined on that basis. Farm based monitoring is undertaken annually and timed to align with peak production levels. It is designed to support management and detect potential impacts at both the near field (close to cages) and far field (broadscale) levels.

Monitoring consists of a combination of on-farm monitoring, broad scale monitoring and assessment of sediment and water quality. Tasmania's regulations are heavily focussed on visual benthic criteria both within the lease area, at a 35 m compliance limit from the lease boundary and at control site(s) in accordance with the requirements specified in the relevant marine farming licence. There is a tiered response to monitoring with initial adverse results triggering a more detailed survey. Similar to the revised requirements, the tiered response provides for more detailed sediment chemistry and biological monitoring to be undertaken should unacceptable impacts be observed. Significant research has recently been undertaken to ground-truth visual assessments with biotic/ abiotic sampling. The Broadscale Environmental Monitoring Program (BEMP) is a regionally based monitoring program, undertaken in most Marine Farm Development areas, that aims to document broadscale spatial and temporal trends for key environmental parameters, and thereby contextualise the assessment of the environmental effects of finfish aquaculture in the region. The BEMP generally includes monthly water quality sampling; annual/bi-annual surveys of seafloor fauna and chemistry, and in relevant areas seagrass and deep and inshore reef communities. Annual BEMP reports are prepared each year and detail all environmental monitoring undertaken and the results of that monitoring. The BEMP sediment component monitors sediment chemistry and invertebrate communities in areas well beyond the farms with a view to ensuring system wide sustainability and acknowledging the broad suite of influences in the farming systems that can/ will influence the carrying capacity.

9.9 Chile

The Undersecretariat of Fisheries has identified Aquaculture Appropriate Areas (AAAs) within which aquaculture development licences can be issued to industry after approval by the Undersecretariat of the Armed Forces. The Environmental Assessment Service undertakes baseline environmental assessments for all new licenses and the National Fisheries and Aquaculture Service controls the monitoring of ongoing farm operations. Environmental standards have been set to help ensure the sustainability of farms and it is the responsibility of the farmers to undertake environmental monitoring through an approved monitoring company. Monitoring is predominately focused on controlling anoxic conditions in sediments. Audits and compliance are monitored by the National Fisheries and Aquaculture Service and the Directorate General of Maritime Territory and Marine Merchant. The government can retract a farm licence if environmental laws have been breached (Alvial et al., 2012).

Benthic sampling is conducted at eight sites uniformly located around the perimeter of farms and at two reference stations during the time of maximum biomass accumulated during the calendar year or production cycle. Benthic organic enrichment indicators include organic matter, Eh, pH, and diversity (Shannon-Wiener (H'), dominance (Simpson, D) and uniformity (Pielou, J'). Acceptability thresholds are defined as ≤ 9% for organic matter and ≥ 7.1 and 50 mV for pH and Eh, respectively. These limits equate to requiring a minimum of Good (based on Eh) to Moderate (based on pH) EQS status at sampling stations (see Table 2). The Eh threshold is more stringent than stated in the revised ASC benthic organic enrichment monitoring requirements. Acceptability thresholds for biodiversity are not listed in the Environmental Regulations for Aquaculture from the Ministry of Economy Development and Reconstruction.

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Appendix 1

Information Supporting EQS Classification Boundaries for Indicators of Benthic Macrofauna Community Health

Note: The multi-indicator results from Cranford et al. (2020) were employed as a test data set to compare EQS classifications indicated by abiotic and biotic indicators across a wide range of habitats and aquaculture practices.

A1.1. Total Free Sulfide (S^{2-})

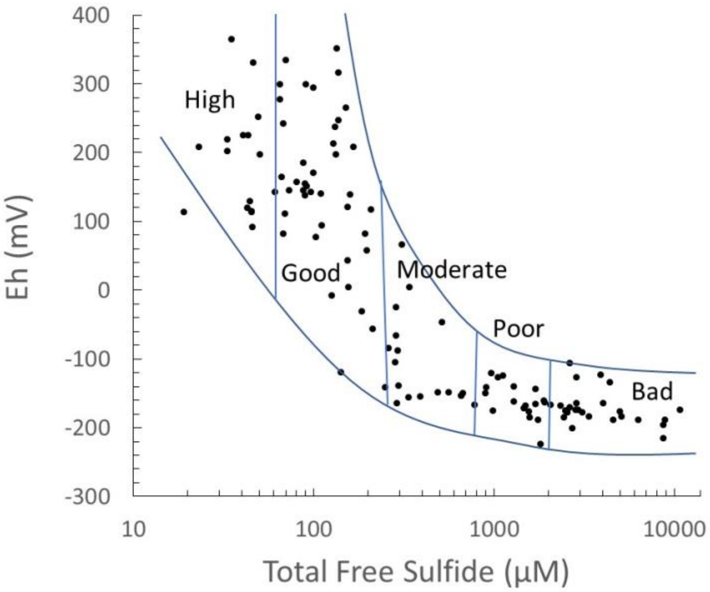
S^{2-}: The total concentration (µM) of hydrogen sulfide, bisulfide and sulfide dissolved in sediment porewater.

S^{2-} concentration in surficial sediments (0 and 2 cm depth) serve as a practical proxy for benthic community impacts owing to the high toxicity of hydrogen sulfide, bisulfide and sulfide in porewater (see Appendix 2 for recommended methodology). S^{2-} levels in the test data set were measured in grab and core samples using the direct UV spectrophotometry method and confirmed using the methylene blue method. EQS group boundaries for S^{2-} concentrations (µM) were derived based on an intercalibration with five biotic indices (Cranford et al. 2020).

A1.2. Redox Potential (Eh)

Eh: Redox potential (also referred to as Eh_{NHE}), in the present context, is a measure of the tendency of organic matter deposited on the seabed to be oxidized or reduced by different microbial processes.

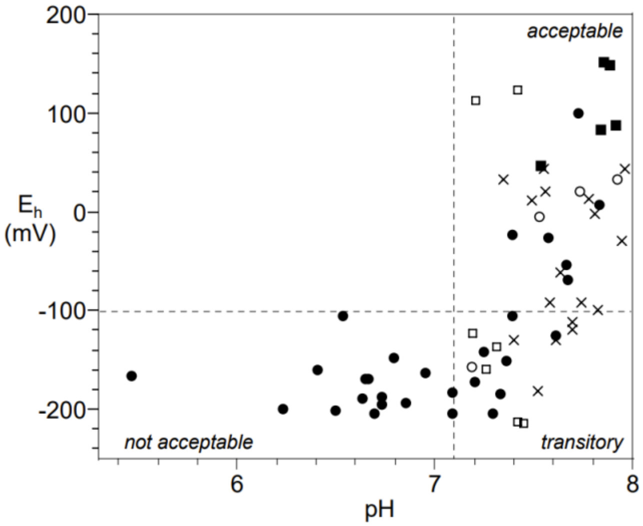
This geochemical indicator of sediment toxicity is used as a tool to support S2- measurements by confirming that surficial sediments are anaerobic and that sulfate reduction is the dominant microbial process mineralizing organic matter (Eh < 0 mV). Eh is easily measured using a oxidation-reduction probe (ORP) electrode and the results are expressed normal to a hydrogen electrode using a correction that depends on temperature and the electrode filling solution (Wildish et al. 1999; see Appendix 2). Eh values above 0 mV tend to be highly variable and of limited use for discriminating between Good and High EQS. Similarly, Poor and Bad status are not well discriminated owing to the relatively narrow Eh range that defines these conditions. The following graphs shows the correspondence between Eh and S2- EQS class limits for the test data (adapted from Cranford et al. (2020) and including additional unpublished data (Cranford, personal communication). The EQS classes shown in the following graph are based on S2- thresholds. The Eh data primarily serve as a verification of S2- results.



A1.3. pH

pH: A scale used to specify the acidity or basicity of an aqueous solution.

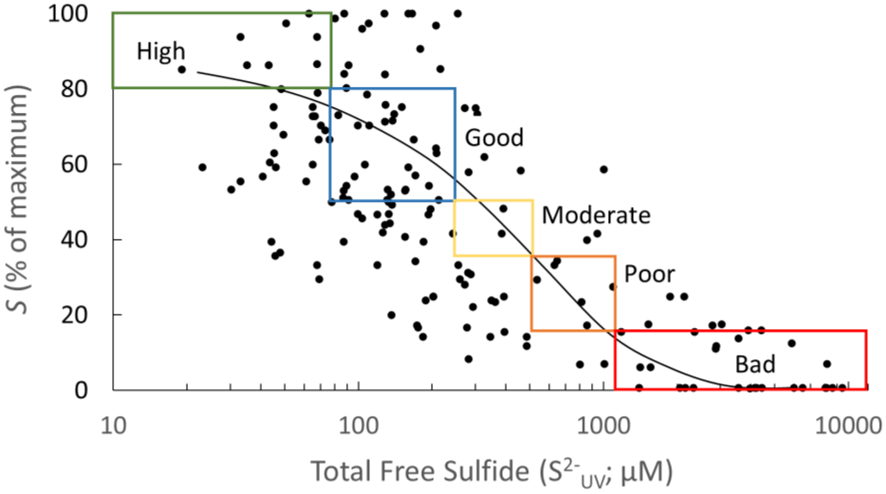
Schanning and Hansen (2005) reported the relationship shown below between *pH* and *Eh* in sediments adjacent to salmon aquaculture sites in Norway. This relationship is used in a component of the aquaculture management system in Norway as a practical tool for monitoring environmental effects at net cage locations. The graph below from Schanning and Hansen (2005) shows that *pH* values between 7.2 and 8.0 indicate "acceptable" organic enrichment status. However, the *Eh* threshold values indicate that sites within this *pH* range can be classed as having Good, Moderate, Poor and Bad EQS classifications. The threshold EQS values for *pH* shown in Table 2 are based on central tendencies in this relationship with *Eh*, but require additional validation.



A1.4. Species Richness (*S* and *S*%)

S: Number of species in a sampled area. ***S%:*** Number of species as a percentage of the observed maximum number for a sampled area.

Establishing EQS class limits for *S* is relatively challenging given that this variable ranges between 0 and ∞ and depends on the area of seabed sampled. The situation is improved when richness is expressed as a percentage of maximum *S*. This maximum was set at 80 species for a sampled area of 100 cm^2. The *S%* class limits shown in Table 2 are based on species reduction data (*1/S%)* reported by Keeley et al. (2012) after adjusting these limits to correspond with the EQS system. The following graph shows the correspondence between these *S%* thresholds and total free sulfide thresholds reported by Cranford et al. (2020).

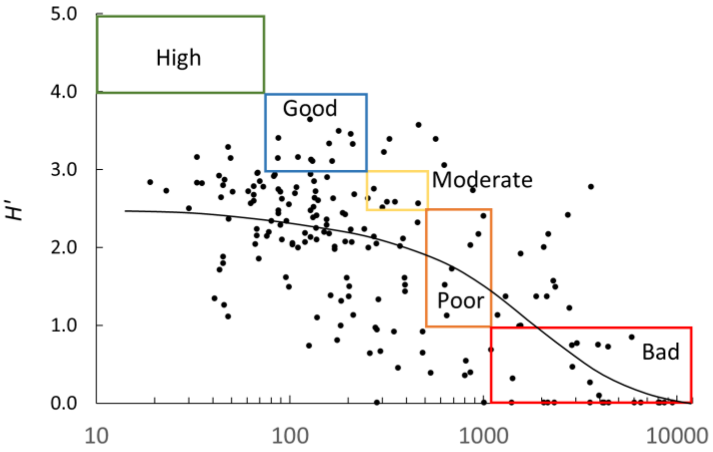


A1.5. Classical diversity indices: Shannon's diversity (H') and Simpson's dominance (1/D)

H': Diversity index representing the uncertainty about the identity of an unknown individual.

1/D: Diversity index representing the probability that two randomly chosen individuals belong to different species.

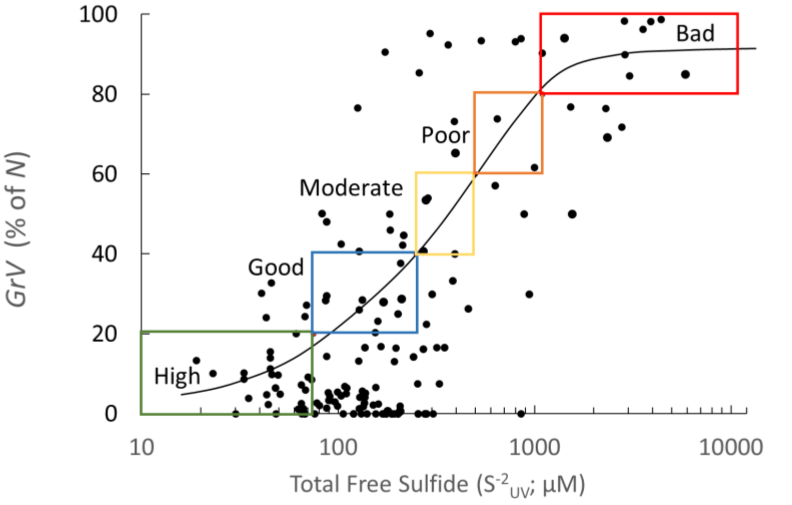
Class limits for *H'* have not been defined for the EQS system but were estimated in Table 2 based on information in Hargrave et al. (2008) and Keeley et al. (2012). The dependence of *H'* on sample area is likely responsible for the poor relationship between the *H'* and S^{2-} class limits and the test data shown below (different size grab samples). No thresholds have been set for *1/D* and a similarly high dependence on sample area is expected. Consequently, both *H'* and *1/D* are not recommended as part of the revised requirements.



A1.6. Group V Opportunistic Species Dominance (GrV)

GrV: Proportion of first-order opportunistic species (Group V) relative to total macrofauna abundance.

GrV requires limited taxonomic experience and analysis effort. This index is based on a theoretical model describing the pollution tolerance responses of five species groups (e.g. Borja et al. 2000). Thresholds in Table 2 are from Cranford et al. (2020).

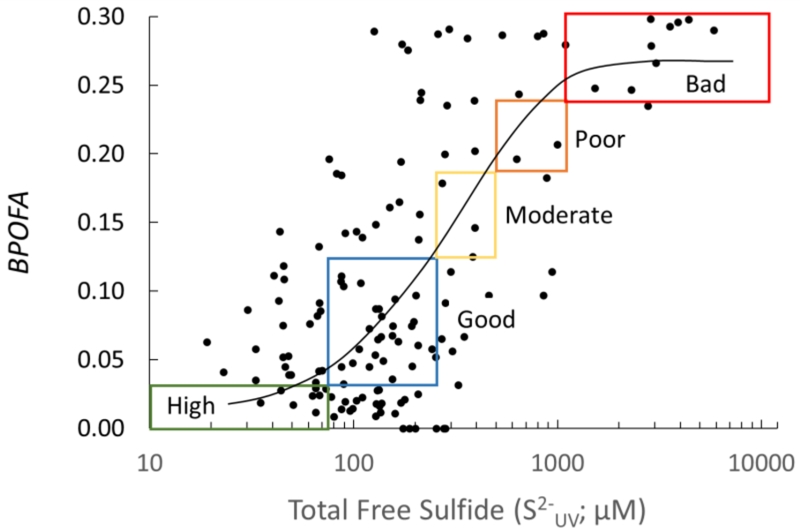


A1.7. Benthic Polychaete Opportunistic Families Amphipods (BPOFA)

BPOFA: Abundance ratio of benthic opportunistic polychaete and amphipod families.

BPOFA is a relatively simple taxonomic indicator based on the ratio of the number of pollution tolerant and pollution sensitive families in the community. The EQS thresholds reported for

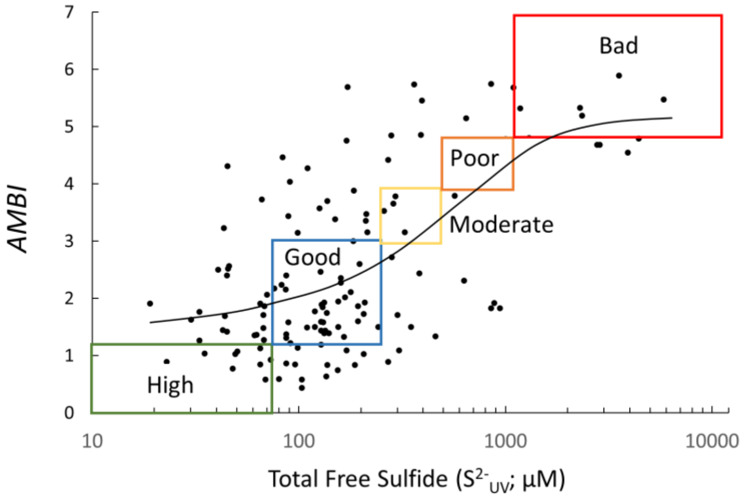
BPOFA by Dauvin et al. (2016) were employed in the calibration of the free sulfide thresholds (Cranford et al. 2020).

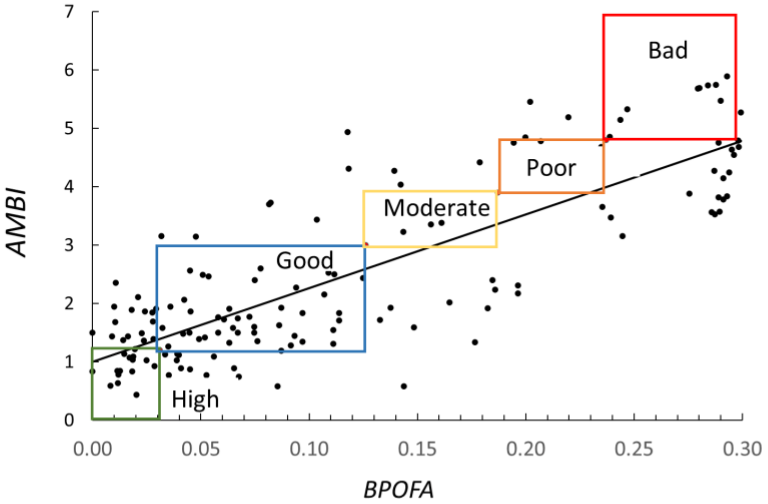


A1.8. AZTI's Marine Biotic Index (AMBI)

AMBI: The proportional abundance of five ecological groups of benthic taxa that are predefined by their degree of tolerance to ecological stress.

AMBI was developed as a general indicator of the response of benthic communities to natural and anthropogenic disturbances in coastal and estuarine environments (Borja et al., 2000) and the free software, species tolerance list and instructions are readily available at www.ambi.azti.es. It has frequently been used within the context of the EU Water Framework Directive for determination of EQS. Following *AMBI* guidelines, the original EQS classifications were augmented with empirical data on closely related taxa groups to better describe the impact of aquaculture organic enrichment on benthic communities (Cranford et al. 2020). These thresholds are presented in Table 2 and are shown below in relation to EQS thresholds for S^{-2} and *BPOFA*.

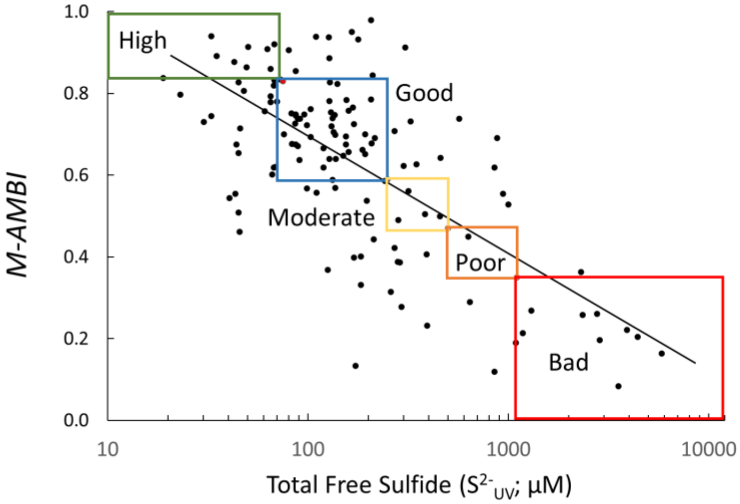




A1.9. AZTI's Multivariate Marine Biotic Index (M-AMBI)

M-AMBI**:** A multi-variate index that incorporates *AMBI*, *S* and *H'* data through factor analysis together with discriminant analysis.

M-AMBI is a multivariate index that integrates three of the above indices (*AMBI*, *H'* and *S*) through multivariate factor analysis (Muxika et al., 2017). The *M-AMBI* algorithm is included in the user-friendly and freely-available *AMBI* software. As with *AMBI*, the EQS thresholds in Table 2 were adjusted according to empirical data on major tolerance group responses to sulfide toxicity. This index performed well in classifying the test data set.



A1.10. Infaunal Quality Index (*IQI$_{v.IV}$*)

IQI$_{v.IV}$: A multi-metric index that combines weighted data on the proportions of sensitive and opportunistic taxa (*AMBI*), Species evenness (Simpson's *1-D*) and taxonomic diversity (*S*).

IQI$_{v.IV}$ was developed by the Water Framework Directive UK Technical Advisory Group (Philips et al. 2012). This metric classifies EQS relative to natural reference conditions. The use of a sliding scale of EQS definitions for each farm site is not consistent with the ASC approach of using standard EQS definitions (Table 1) that apply everywhere. Consequently, this index is not included in Table 2.

A1.11. Benthic Habitat Quality (*BHQ*)

BHQ: An observation index based on scoring physical structures in surficial sediments and the depth of the redox potential discontinuity.

BHQ is derived from sediment profile imaging (SPI). The EQS classes in Table 2 are from Rosenberg et al. (2004). See Hargrave (2010) for BHQ relationships to other indices of benthic organic enrichment.

A1.12. Relative Richness (*S50*)

S50**:** The number of species identified among a random sample of 50 individuals.

Although referred to by Hargrave (2010) as the *BQI* index, the name has been changed here to prevent confusion with the *BQI* index developed by Rosenberg et al. (2004). The EQS class limits for *S50* in Table 2 are derived here from the empirical relationship with *Eh* (Table 4 in Hargrave, 2010). Hargrave (2010) provides relationships between *S50* and several other indices of benthic organic enrichment.

A1.13. ES50 and the Benthic Quality Index (BQI)

***ES_{50}*:** The expected number of species (*ES*) among 50 individuals.

***BQI*:** A multi-metric index that combines information on observed and expected species richness (*S* and ES_{50}) and abundance (*N*).

BQI-family: Same as *BQI* but based on taxonomic identification to the family level.

This ES_{50} richness and *BQI* indices of benthic quality are described in Rosenberg et al. (2004). The ES_{50} is calculated according to Hurlbert's (1971) formula. The EQS thresholds for *BQI* and *BQI-family* in Table 2 are from Rosenberg et al. (2004) and Dimitriou et al. (2012), respectively.

A1.14. Infaunal Trophic Index (*ITI*)

***ITI*:** an index of the overall trophic condition of the benthic community based on the presence/absence of four trophic groups that exhibit different sensitivities to organic enrichment.

ITI class distinctions are described as "Normal" (*ITI* values 100-60), "Modified" (60-30) or "Degraded" (30-0). Ruellet and Dauvin (2007) estimated the EQS class boundaries shown in Table 2, however, these do not appear to have been validated and do not agree well with empirical data (e.g. Hargrave, 2010). Until this classification issue is resolved, *ITI* is not recommended for ASC monitoring.

A1.15. Bentix Index

Bentix: The proportional abundance of three ecological groups of benthic taxa that are classified by their degree of tolerance to ecological stress.

Bentix is conceptually similar to AMBI in that it is based on a library of species sensitivities. Instead of defining five sensitivity groups, Bentix is based on three (Simboura and Zenetoc, 2002). Bentix is reported to be simple to use while being independent of habitat type, sample size and taxonomic effort. The species list is available at www.hcmr.gr/english_site/services/env_ aspects/bentix.html. EQS threshold values shown in Table 2 for Bentix (modified scale based on a 0 to 1 range) are from Simonini et al. (2009).

A1.16. Norwegian Quality Index (NQI1)

NQI1: A multi-metric index that combines weighted values for AMBI, total infauna abundance (N), and species richness (S).

NQI1 is similar in concept to IQI v.IV except that it is calculated using absolute values for the component indices rather than values relative to a reference site. EQS limits in Table 2 are from Husa et al. (2014).

A1.17. Norwegian Sensitivity Index (*NSI*)

NSI: The average species sensitivity value of all individuals in a sample.

NSI is similar to the BQI index (Rosenberg et al. 2004) but also assigns sensitivity values to each species using a continuous scale instead of using a constant value for species groups as in AMBI (Rygg and Norling, 2013). Another difference from BQI is that NSI is based on the ES100 species diversity indicator instead of ES50. NSI results along a pressure gradient are well correlated with AMBI values (Rygg and Norling, 2013). EQS values in Table 2 are from Rygg and Norling (2013).

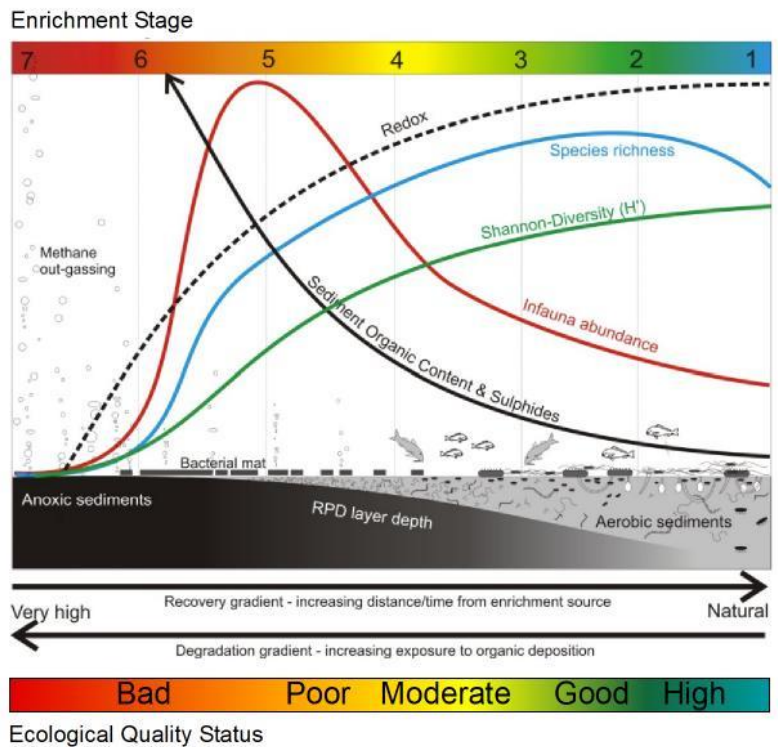
A1.18. Indicator Species Index (*ISI*)

ISI: The average of the sensitivity values of species occurring in a sample.

The methodology is described in Rygg (2002) and is based on the stress level endured by species defined by that species ES_{100} diversity value. This index is incorporated in calculation of the *NSI* index above. This index tends to follow a similar pattern with ecological stress as the *H'* diversity index (Rygg 2002) and EQS values in Table 2 are from Rygg and Norling (2013).

A1.19. Enrichment Stage (ES)

ES: A benthic organic enrichment impact classification system that reflects a progression from unimpacted (ES = 1) to highly impacted (*E*S = 7) conditions.



The ES groups largely correspond with the Eco-Groups defined in Borja et al. (2000) and therefore each group largely corresponds with EQS class thresholds. *ES* values are assigned based on best professional judgement after review of site physical, geochemical and taxonomic data. Qualitative descriptors of ES classes described by Keeley et al. (2012b) and illustrated above (based on Keeley, 2013) were compared with descriptions in Table 1 and the corresponding class boundaries are provided in Table 2.

Appendix 2

Position on Total Free Sulfide Analysis

Current ASC Standards have included total free sulfide (S2-) measurements for monitoring the effects of organic enrichment on benthic habitat, biodiversity and ecosystem function. S2- is highly toxic to benthic macroinfauna and directly interconnected with ‘Moderate”, “Poor” and “Bad” benthic organic enrichment EQS classifications. Geochemical measures, including S2-, provide a practical means for monitoring biotic impacts compared with taxonomic analysis, which requires greater technical expertise, much higher analysis and interpretation costs, and long delays to receive monitoring results. The ASC therefore considers S2- to be a key indicator for monitoring the effects of aquaculture organic enrichment on benthic habitat, community structure and ecosystem function.

The tiered monitoring programs described in Sections 5.3 and 7.3 require the ability to rapidly conduct S2- measurements onboard the sampling vessel. Immediate information on the potential magnitude and spatial scale of benthic impacts from the farm facilitates rapid decisions to be made on the requirement for additional spatial sampling. Given the importance of S2- data in the revised monitoring requirements, the protocol for S2- analysis must meet all of the following strict requirements:

1. Capacity for rapid sample processing and analysis of surficial sediments (0 to 2 cm depth) onboard the sampling vessel.
2. Exhibit high accuracy and precision in detecting total free sulfides ($H_2S + HS^- + S^{2-}$) in surficial sediments.
    - The methodology must not be prone to contamination by other non-target chemicals in marine sediments such as iron sulfide and pyrite.
    - S^{2-} samples cannot be exposed to the atmosphere prior to analysis to prevent loss through oxidization and volatilization.
3. High analytical robustness is required (the results must not be prone to slight variations introduced by different users and measurement conditions).
4. The methodology requires a low limit of detection (LOD) and quantification (LOQ) and must be capable of measuring a wide range of free sulfide concentrations. This is necessary to permit quantification of all Ecological Quality Status classifications.
5. Minimize the use of toxic chemicals to prevent user exposure.
6. The instrument calibration must exhibit high stability (not prone to variation over the time-scale of farm sampling and analysis).
7. Instrument calibration standards should be based on an ISO certified reference material to ensure consistency in results between users/farms.

8. The methodology needs to be applicable to a wide range of sediment collection methods, but primarily to grab samplers.
9. The methodology should be relatively practical compared with alternative approaches:
    - rapid and user friendly under field conditions,
    - does not require a high degree of technical expertise, and
    - can be performed at a relatively low ongoing cost.

The standard approach for measuring S2- in surficial sediments has been the Ion Selective Electrode (ISE) method (see current ASC Standards requirements) owing to the relative simplicity compared with other analytical options available. However, numerous users have stated that the ISE method exhibits low analytical robustness. For example, early studies at aquaculture farms led Brooks and Mahnken (2003) to state that "…experience in British Columbia clearly points out that subtle differences in protocols and/or techniques can result in significant differences in results…". The low analytical robustness of the standard ISE method is also demonstrated by the need for frequent calibration of the probes, difficulties to achieve accurate standardizations at low concentrations, probe sensitivity to temperature (Hargrave et al. 2008), and instability of the calibration stock standard (sodium sulfide nonahydrate crystals readily oxidize and therefore require purity verification prior to each use).

The ISE analysis protocol is conducted on sediment/porewater slurries with the expectation that the presence of the particulate fraction would not interfere with the analysis of S2-, which is dissolved in the porewater fraction. However, as stated by Hargrave (2010), "…exposure of sediment with high concentrations of pyrite to alkaline conditions will increase apparent S concentrations if particulate sulfides are solubilized." The ISE protocol requires the use of highly alkaline conditions (pH = 13). ISE measurement errors related to the mobility of mineral sulfides were documented by Brown et al. (2011), who stated that "…the accepted [ISE] protocol can lead to significant bias of free sulfide measurements, with orders of magnitude higher concentration detected in the buffered sediment-porewater slurry than in porewater samples isolated and analysed separately." These authors concluded that the poor accuracy of the ISE method was likely caused by dissolution of particulate sulfides and/or sulfur present in the sediments under the required intense alkaline conditions. Cranford et al. (2017) verified this conclusion and showed that the standard ISE protocol is also prone to errors from S2- volatilization and oxidation. Brodecka-Goluch et al. (2018) reported that volatilization and oxidation resulted in the loss of one third of S2- in porewater samples. S2- is highly reactive, and is rapidly oxidized by oxygen, especially when exposed to light or in the presence of heavy metals. These studies have shown that the wide range of potential biases inherent with the standard ISE protocol can render the data uninterpretable.

A critical property of any indicator of organic enrichment that is to be applied globally, or even regionally, is that the EQS thresholds must apply regardless of the farm location. The relationship observed by Brooks and Mahnken (2003) between S2- (ISE method) and total number of macrofauna taxa contributed to the development of the "oxic" organic enrichment classification scheme reported in Hargrave et al. (2008) that was employed in current ASC Standards. These data were collected largely at four salmon farms in British Columbia characterized by sandy sediments (muddy sand to sandy gravel; Table 9 in Brooks, 2001). However, sampling across a much broader range of geographic locations and sediment types (silt to gravel) did not reveal any consistent relationship between the ISE S2- data and any macrofauna impact indicator (Cranford et al. 2020). Geographic variability in particulate sulfur contamination of the ISE data is suspected to have confounded the detection of consistent relationships with biotic indicators.

Alternative S2- methods include methylthymol blue colourimetry, iodometric titration, ion chromatography, capillary ion electrophoresis, and ultraviolet spectrophotometry. The latter method measures bisulfide (HS-) directly without the need to convert it to some other detectable form (Guenther et al. 2001). Prior to analysis, any H2S and S-2 in the porewater sample is converted to HS- by adjusting the sample pH to between 8 and 10. Direct methods are inherently analytically robust owing to the limited number of user steps and reagents employed for the analysis. The UV method exhibits very high sensitivity to S2- with a reported detection limit of <1 µM (Guenther et al. 2001). Cranford et al. (2017) adapted this method as a practical approach for rapidly measuring S2- in porewater samples from surficial sediments at aquaculture sites. Further adaptations and validation of the UV spectrophotometry method against the methylene blue method are reported in Cranford et al. (2020). That study also provided a revised EQS system for classifying organic enrichment impacts on marine benthic macrofauna based on S-2 monitoring. Sampling was conducted at 12 aquaculture farms in eastern and western Canada and in New Zealand with sediment types ranging from silt to gravel. Unlike the ISE results, the UV spectrophotometric data revealed consistent relationships between S-2 and multiple biotic indicators (shown in Appendix 1).

A key element in the evolution of the revised requirements is the incorporation of the best available science knowledge and methodology. The UV spectrophotometric approach for total free sulfide analysis at aquaculture sites (see Appendix 3) is relatively new but has been extensively validated both analytically and as a proxy for biotic impacts. It is also the only methodology available that meets all nine of the essential criteria listed above.

1. Experience with the UV method under routine farm monitoring conditions has shown that grab or core samples can be analysed onboard the sampling vessel within 5 min. For example, 65 grab samples were collected, processed and analysed in triplicate in one day.
2. Contamination is avoided through exclusion of the particulate fraction and by preventing porewater contact with the atmosphere.
3. High analytical robustness is a general property of direct optical methods.
4. The protocol has the capacity to measure 0 to >20,000 µM concentrations with a LOD and LOQ that permits quantification of all EQS classifications.
5. The protocol does not require use of toxic reagents. The only ‘reagent’ employed is buffered distilled water.
6. Instrument calibration is highly stable and only requires monthly confirmation of instrument performance.
7. Calibration is conducted with an ISO Certified Reference Material.

8. The method was developed to be compatible with all types of core and grab samples.
9. The method is rapid and relatively simple compared with all known alternatives including the ISE method. Cost is primarily associated with the initial acquisition of a UV spectrophotometer suitable for field use ($5,000 to $15,000 US). Ongoing annual costs for the calibration standard and expendables is estimated at less than $200 US per farm survey.

ASC strives to continuously improve monitoring standards by incorporating new and innovative technologies, but only after they are proven to be reliable and practical alternatives to current practices. S-2 data analysed using the standard ISE protocol have proven to be unreliable and are no longer considered acceptable for addressing the ASC benthic organic enrichment monitoring requirements. The EQS thresholds for S2- shown in Table 2 are based on data analysed using the UV protocol provided in Appendix 3. Although the decision to change the S2- standard operating procedure will mean that environmental monitoring consultants will require retooling and training, and that the current ISE S2- data will be considered obsolete. This change in methodology is decisively founded in science.

Appendix 3

Standard Operating Procedures for the Field Analysis of Abiotic Indicators Employed in Tier 1 and 2 Monitoring Programs

A3.1. Total Free Sulfide (S-2) Analysis in the Field by Direct UV Spectrometry

The methodology includes both the field extraction and analysis of porewater in surficial sediments (grabs or cores) as described in Cranford et al. (2017) and as modified in Cranford et al. (2020).

Materials List

- UV Spectrophotometer suitable for field use (e.g. IMPLEN C40 mobile nanophotometer).
- Quartz cuvette: 200-2500 nm spectral range, pathlength 10 mm, 1.4 ml capacity (e.g. Helma Analytics No 104-B-10-40). Note that quartz is required.
- 5 cm RizoCera porewater extractors (https://www.rhizosphere.com/rhizocera).
- 10 cc syringe.
- Stainless steel compression springs that fit inside the 10 cc syringe.
- 100 µL gas-tight syringe (e.g. https://www.hamiltoncompany.com/laboratory-products/syringes/80630).
- 1 mL pipettor or bottle dispenser for rinsing cuvette and for sample dilutions.
- Ammonia hydroxide, 0.44M or similar concentration.
- pH strips for adjusting the dilution water (potable water will suffice) to between 8 and 10.
- Sulfide WP - Certified Reference Material (available from Sigma: QC1034-20 mL) for instrument calibration at one-month intervals.
- 1 and 5 L pipettors and 10 to 20 mL vials for preparing standards.
- Lint-free optical wipes (e.g. Kimwipes) for cuvette cleaning surfaces.

Porewater Extraction

1. Drain water in sediment sampler to sediment surface.
2. Using syringe containing a stainless spring, depress plunger, attach RhizoCera, and insert into sediment surface at a 45° angle. Release plunger to start automatic porewater extraction from 0 to 2 cm depth.
3. After approximately 2 min, the syringe should contain sufficient porewater (0.5 to 1 mL).
4. Remove the syringe from the sediment and remove the RhizoCera. Discard the water in the syringe as this is only used to flush out the RhizoCera.

5. Insert the 100 µL syringe needle directly into the interior of the RhizoCera and withdraw the 100 µL sample.
6. Rinse any sediment from the exterior of the RhizoCera before reusing.

Note: The interior of the RhizoCera is flushed automatically between samples during the extraction procedure.

UV Spectrophotometric Analysis

1. Turn on the spectrophotometer and, if available, select data output for the 230, 240 and 250 nm wavelengths. Otherwise save the full sample scan.
2. Add small amounts of ammonium hydroxide to 1 L of dilution water until the pH is between 8 to 10. This volume of buffered dilution water is sufficient for daily use.
3. Rinse the quartz cuvette and add 1 mL of the buffered water.
4. Clean the outside of the cuvette with a lint-free wipe and place in instrument. Zero the instrument using this blank solution. Instrument blanking should be performed regularly.
5. Add the 100 µL porewater sample to the cuvette containing 1 mL of buffered water, invert to mix, and record the absorbances at the three wavelengths. Most instruments have the capacity to save the full scan.
6. Remove the cuvette, rinse with buffered water and prepare for next sample.
7. Calculate the total free sulfide concentration using the absorbance values and the regression equations determined by the calibration procedure below. Although absorption data are provided for three wavelengths, S^{2-} is only calculated using the lowest wavelength that provides absorbances below 2. If the absorbance at 230 nm is >2, then use the 240 nm absorbance, etc.

Instrument Calibration

The calibration is highly stable and only needs to be conducted once a month to ensure the instrument has not been damaged. An ISO Certified Reference Material (CRM; Sulfide WP) of known concentration is used as the stock solution for preparing five working standards by serial dilution (1:2, 1:5, 1:10, 1:50 and 1:100).

1. Dilute the stock CRM solution to prepare the five known concentrations using pipettors and the buffered water.
2. Blank (zero) the instrument and then analyze the standards using the same procedure as the samples, including dilution with 1 mL of buffered water. Record the results for the three selected wavelengths (230, 240 and 250 nm), omitting any absorbances greater than 2.0.

3. Calculate the three calibration equations (one for each wavelength) using regression analysis (x = absorbance at selected wavelength and y = standard concentration in µM units) while excluding any absorbance values above 2.0.

Note: The following S^{2-} concentration ranges typically apply for the three wavelengths:

230 nm: 0 to 2,000 µM (suitable for quantifying all EQS conditions from High to Bad)

240 nm: 2,000 to 4,000 µM

250 nm: 4,000 to 10,000 µM

Note: 260 nm can be used for higher concentrations

A3.2. Redox Potential (*Eh*) measurement

Eh can be measured directly in the grab/core using an Oxidation Reduction Potential (ORP) probe that uses a silver/silver chloride or platinum reference electrode. The ORP probe must be calibrated, operated and maintained according to strict manufacturer specifications. ORP measurements (referred to as ORP, $E_{Ag/AgCl}$ or E_{Pt}), are by themselves ambiguous and it is only through specifying the reference scale can the data be interpreted by the user. ORP measurements converted to a hydrogen scale are reported as "*Eh*" and some publications designate the same measurements as Eh_{NHE}. ORP data (mV) obtained in the field with Ag/AgCl or Pt electrodes are converted to the hydrogen scale as follows:

$$Eh = \text{ORP (mV)} + \text{half-cell potential of reference electrode},$$

where the half-cell potential of the Ag/AgCl or Pt reference electrode is related to the molarity of the filling solution and measurement temperature.

Half-cell potential of Ag/AgCl reference electrode

T (°C)	Molarity of KCl filling solution				
	1.5M	3M	3.3M	3.5M	4M
5	254	224	220	219	219
10	251	220	217	215	214
15	249	216	214	212	209
20	244	213	210	208	204
25	241	209	207	205	199
30	238	205	203	201	194

1. The ORP probe can be inserted directly into the sediment surface inside the core/grab to ~1 cm depth after mixing the sediment around the probe location to 2 cm depth. Ensure full contact between the ORP electrode tip and wet sediment.

2. Record the sample temperature.

3. The ORP mV reading should stabilize within 1-2 min. If redox conditions are not controlled by single oxidation-reduction reactions, as in oxic sediments, there is often a slow, continuous drift of electrode potentials. An arbitrary time (3-4 min) can be chosen to record mV readings if they do not stabilize in less than this time. Potentials in reduced sediments usually stabilize more rapidly.

4. Correct the ORP potential (mV) relative to the normal hydrogen electrode as described above using manufacturer information on the electrode filling solution and data on sediment temperature.

A3.3. Total ammonia nitrogen measurement

Total ammonium nitrogen (TAN) consists of the ammonium ion (NH_4^+) and un-ionized ammonia (NH_3). NH_3 makes up a higher proportion of TAN at higher pH and is typically associated with most of the toxic effects of TAN. As with total free sulfide analysis, TAN is measured using porewater samples extracted from surficial sediments (0 to 2 cm depth). The extraction procedure is described in Appendix 3.1 and utilizes RhizoCera samplers inserted to a depth of 2 cm in grab samples. Subsamples should be collected without unnecessary exposure to air. Avoid trapping bubbles of air when filling and capping plastic sample vials.

The Eh, pH and temperature of the sediment sample is measured directly in the grab sample (stirred upper 2 cm of sediment) using Oxidation Reduction Potential (ORP), pH and temperature probes while the porewater is being extracted in another section of the grab.

Acceptable methods for TAN analysis include spectrophotometry, fluorometry, and electrochemical detection. The gas sensing ISE method (Standard Method 4500-NH_3 Nitrogen D and E) is an approved approach for TAN analysis, but it should be recognized that it can also be challenging to perform correctly. The major drawbacks with this method is that it requires at least 50 ml of sample and collection of that quantity of porewater for routine monitoring is not practical under field conditions. The ISE technology has additional disadvantages including high maintenance, frequent calibration, poor performance at low TAN concentrations, and frequent replacement of the sensor system.

Low sample volumes can be accurately analyzed using a variety of manual and automated colorometric methods. The phenate method (Standard Method 4500-NH_3 F and G) reacts alkaline phenol and hypochlorite with ammonia to form indophenol blue. The color intensity is measured photometrically to determine the final concentration. The salicylate method (EPA 350.1) reacts at pH 12.6 with hypochlorite ions and salicylate ions in the presence of sodium nitroprusside as a catalyst to form indophenol. The amount of color formed is directly proportional to the ammonia in the sample. Results are read at 690 nm. It is preferred that porewater samples should be analysed as soon as possible after sampling (i.e., within an hour). However, samples can be stored in plastic bottles up to one month in a freezer at below -18°C. Before determination of ammonia, samples should be allowed to defrost slowly, preferably overnight, in darkness.

Hach® Company gained US EPA Equivalence on a simple salicylate method for use in wastewater based on the TNTplus™ Ammonia platform. This is a simple, cost-effective, 15-min test, requiring no calibration and just 0.5 mL of porewater. Independent analysis (Guadalupe-Blanco River Authority, Seguin, Tx) reported the limit of quantification of this Test-In-Tube 831 kit was 1 mg/L, which is sufficient for detecting TAN concentrations exceeding the EQS threshold (Table 6). During analysis, the pH of the water sample must be between pH 4–8 and the temperature of the water sample and reagents must be between 20–23 °C. The equipment required consists of a Hach DR3900 spectrophotometer (approximately $5,500 US) and Hach TNTplus 831 Low Range (1-12 mg/L NH_3-N) reagent kits, which each contain 25 test vials (approximately $70 US per kit).

The TAN concentration, pH, Eh and temperature reported for sediment collected at each sampling site will used to assess caged fish farm compliance for lake systems (see Tables 6 and 7).