

Best Management Practice guidelines for salmon farms in the Marlborough Sounds:

Part 1: Benthic environmental quality standards and monitoring protocol (Version 1.1 January 2018)

New Zealand Aquatic Environment and Biodiversity Report No 219

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Glossary

Term / Definition acronym

%OM Percent organic matter of sediments, also sometimes referred to as 'ash free dry weight' or 'loss on

ignition'.

AMBI AZTI's Marine Biotic Index (Borja et al. 2000)

AMP Annual monitoring plan

AMR Annual monitoring report

Anaerobic Processes in the absence of oxygen.

ANZECC Australia and New Zealand Environment and Conservation Council (ANZECC 2000)

Azoic Without life; but in this context, refers to the absence of macrofauna.

BMP Best Management Practice(s)

Bol Board of Inquiry appointed by the New Zealand Environmental Protection Authority

BPJ Best Professional Judgement

BQI Benthic Quality Index (Rosenberg et al. 2004)

CE-Ref Potential cumulative effects station - An optional additional monitoring station situated in an area that is

potentially predisposed to organic accumulation or is otherwise of concern

d Margalef's diversity index.

DEPOMOD Depositional model – originally proposed in Cromey et al. (2002)

EPA New Zealand Environmental Protection Authority

EQS Environmental Quality Standards

ES Enrichment Stage as measured by a combination of environmental indicators. See details within,

Appendix B and Keeley et al. (2012b).

FF-Ref Far-field reference stations – Reference station that is unlikely to be influenced by far-field effects

Footprint Used here to refer to the area of seabed that is influenced by the deposition of farm-derived wastes.

'Primary footprint' refers to the area initially affected by the primary deposition of waste, ignoring any

subsequent resuspension and redistribution of waste particles.

H' Shannon-Weiner diversity index (or SWDI).

HF High flow sites – define by sites where mean mid-water current speeds ≥10 cm s⁻¹

J' Pielou's evenness.

ISQG ANZECC Interim sediment quality guidelines. ISQG-Low representing a 10% probability that a significant

toxicity measure will occur in sensitive species, while ISQG-High represents a 50% probability of the

same.

LF Low flow sites – define by sites where mean mid-water current speeds <10 cm s⁻¹

Macrofauna Fauna living predominantly within the sediments which are in this case retained on a 1 mm sieve

(conventionally 0.5 mm).

MOM Modelling-Ongrowing fish farms-Monitoring (refer Ervik et al. 1997)

MDC Marlborough District Council

N Total infauna abundance = number of individuals per 13 cm diameter core

NF-Ref Near-field reference stations - Reference station situated near to farm but outside of primary depositional

footprint

NIWA New Zealand National Institute of Water and Atmospheric Research

OLE Outer limit of effects - Delineates outer extent of obvious and measurable effects.

Redox Redox potential — a measurement of the oxic status of sediments (Eh_{NHE} , mV).

S Taxa richness = number of taxa per 13 cm diameter core

SEPA Scottish Environmental Protection Agency

Site Refers to the area within which farming can take placed

Refers to an approx. $10\,\text{m}^2$ area of seabed within which replicate samples are collected for environmental monitoring purposes. Station

TFS Total free sulfide concentrations in sediments (µM)

ZME Zone of maximum effect – typically the area of seabed immediately beneath a farm which enrichment

effects are maximal

Executive Summary

Keeley, N.; Gillard, M.; Broekhuizen, N.; Ford, R.; Schuckard, R.; Urlich, S.C. (2019).

Best Management Practice guidelines for salmon farms in the Marlborough Sounds: Part 1: Benthic environmental quality standards and monitoring protocol (Version 1.1 January 2018).

New Zealand Aquatic Environment and Biodiversity Report No. 219. 48 p.

This is a guidance document to inform the development and implementation of benthic monitoring of salmon farms in the Marlborough Sounds, New Zealand. The standards were developed in an integrated working group environment, with representation from: Marlborough District Council (MDC), New Zealand King Salmon, the Sounds Advisory Group to MDC, Cawthron Institute, National Institute of Water and Atmospheric Research and the Ministry for Primary Industries/Fisheries New Zealand. The aim is to provide consistent and clear requirements for the benthic monitoring and management of existing farms, based around an agreed set of environmental quality standards with accompanying rationale. Details are provided about how and when to conduct the surveys, along with consequences in the event of non-compliance. It is intended to be a living document that will be reviewed, updated and amended as required. This is the first revision of the guidance document published in 2015 (Keeley et al. 2015).

1 Introduction

This is intended as a guidance document to inform the development and implementation of benthic monitoring programmes for salmon farms in the Marlborough Sounds. The review of management practices and with it, the benthic standards and monitoring protocol, was initiated in 2013 by The New Zealand King Salmon Co. Limited (NZ King Salmon) and the Marlborough District Council (MDC). The review was completed in 2014 (Keeley et al. 2015) and was developed in an integrated working group environment, including representation from:

- Council (MDC),
- Industry (NZ King Salmon),
- Sounds Advisory Group (Community stakeholder),
- Science providers (Cawthron Institute and NIWA),
- Ministry for Primary Industries (MPI).

The need for the document arose because the industry has developed to a stage where clear articulation of Best Management Practice (BMP) is needed to enable a common understanding of how the industry is managed, both from an operational perspective and in respect to environmental performance expectations and regulations. The existing salmon farm consents span three decades and because of this, they have a variety of conditions, standards and requirements that have come about because of constantly evolving knowledge and technologies. Additionally, some of the existing environmental quality standards (EQS) have proven ambiguous and therefore difficult to implement. Advances have been made over this thirty year period in both the knowledge about (e.g. MPI 2013), and the degree of certainty surrounding, seabed effects as a result of the monitoring and management responses to date, so it is appropriate at this stage to move from an adaptive management-type framework to a BMP-type framework (Allen & Gunderson, 2011).

Amendments have been made to some of the consent conditions in recent years (via Section 127, Resource Management Act 1991 [RMA] applications), but doing so is a time-consuming, challenging and expensive process; and one that has the potential to introduce further complications and inconsistencies among consents. It was therefore determined that a centralized regional BMP be developed and that ideally all salmon farm consents should include a standard condition, incorporating compliance with the BMP.

The primary purpose of this BMP is therefore to provide consistent and clear requirements for the management and the independently conducted annual benthic monitoring of existing farms. Central to this is a set of agreed EQS with accompanying transparent rationale for their selection and use. This document therefore provides details about what should be measured, where, and how often, and specifies consequences in the event of non-compliance. It is intended to be a living document that will be reviewed, updated and amended to accommodate evolution in knowledge and technologies. This is version 1.1 of this document (Keeley et al. 2015) incorporating feedback from a review of the standard initiated in 2017.

There was also an up-front intention to align these standards and protocols with the consent conditions resulting from the NZ King Salmon Board of Inquiry (BoI) process where appropriate. As a consequence the standards and monitoring protocols outlined reflect the

substantial body of knowledge that was assembled through that process and are focussed on contemporary farming practices. The BMP are being considered by MDC to inform the setting benthic activity standards for salmon farms in the aquaculture chapter of the proposed Marlborough Environment Plan.

The five key components that were identified for developing the benthic standards and monitoring protocols are as follows:

- 1. The optimum placement of spatial boundaries for delineating effects (effect zones).
- 2. The level of effort that is necessary to identify an effect (a tiered sampling design).
- 3. Clear and testable environmental quality standards, with associated consequences for non-compliance.
- 4. The appropriate timing and frequency of sampling.
- 5. A mechanism for reviewing the process in the future to ensure that the protocols and standards remain optimal.

Note that the standards and protocols associated with operational farming practices are under development and expected to be published separately in 2019. Issues pertaining to water column environmental standards and monitoring are still under consideration, as they will be informed by monitoring and modelling of discharges from the proposed new farms over several years, as required by the Board of Inquiry (BoI). It is anticipated that an analogous set of standards covering these issues will be developed and incorporated into this document once that phase is complete (notably the water quality standards development process which started in 2018). Also note that while the primary concern in this BMP is organic enrichment of the seabed, the potential contributing effects of other possible contaminants are also addressed (e.g. copper and zinc, Section 5). This is because their potential to persist in sediment differs from that of organic enrichment *per se*, and therefore may at times require a different approach to monitoring and management. There are also established environmental quality guidelines for copper and zinc that need to be considered (ISQG-Low and –High, ANZECC, 2000).

1.1 OBJECTIVES

The broad over-arching objectives that underpin this benthic monitoring protocol are as follows:

- To develop a standardised and accepted protocol to assess environmental compliance.
- To comply with international best practices at a minimum, and where appropriate².
- To support environmentally responsible and profitable aquaculture.
- To minimise impacts on the environment and thereby minimise risks to biodiversity and associated ecosystem processes.

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¹ For this purpose, 'international best practice' was determined with reference to the following documents: SEPA Annex A (2005), NBDELG (2012a,b), ASC (2012), Wilson et al. (2009), Macleod et al. (2004), Macleod & Forbes (2004), Management Controls specified in the Marine Farm Development Plan for the D'Entrecastreaux Channel farm, Tasmania and the final Bol NZ King Salmon Conditions of Consent.

² The 'Where appropriate' caveat is necessary because some 'international' standards may have limited national / regional transferability and therefore, may not be appropriate. Additionally, external standards should be viewed as a minimum requirement as it may be possible and appropriate to achieve higher standards.

- To ensure sustainable management³.
- To provide a monitoring and reporting approach that is fit-for-purpose (user-friendly, focussed, relevant, efficient and cost-effective).
- To promote openness and transparency with respect to monitoring and reporting.
- To account for environmental differences between sites where those might influence impact levels or monitoring (e.g. flow regimes).
- To establish a process to regularly review these guidelines.

2 Spatial boundaries and placement of sampling stations

This section outlines the 'zones concept' and provides the rationale behind the five proposed monitoring locations (Table 1). This approach focuses on the area of maximum likely impact (worst-case scenario), and on the outer extent of effects in relation to local (near-field) and distant (far-field) reference stations (i.e. NF-Ref and FF-Ref; see Figures 1 and 2). Understanding and monitoring the area where the greatest impacts occur (Zone of maximum effect; ZME) is important for farm management in relation to potential benthic assimilation capacity and therefore also long-term sustainability. Monitoring the 'outer limit of effects' (OLE) provides a checkpoint for the total spatial extent of the measurable 'footprint', and reassurance that the effects have not expanded beyond the agreed distance (Figure 1). It is assumed that the level effects between ZME and OLE will follow a natural and reasonably predictable gradient in accordance with distance from the farm (e.g. Figure 2).

The NF-Ref station is situated outside of the primary footprint, but in the same proximity (i.e. about 300–1000 m) and with comparable depth and substrate. NF-Ref constitutes a conventional reference station, situated in a position that is unlikely to be directly impacted by farm discharges. The FF-Ref station is situated further away (i.e. more than 1000 m), in a location where it is very unlikely to be exposed to any secondary or cumulative farm-related effects. The FF-Ref station therefore provides a comparison point for natural or broader system changes. A third type of reference station is provided for a cumulative effects reference site (CE-Ref), which is optional (and site-specific) and targets areas potentially susceptible to cumulative effects, e.g. a nearby depression or naturally depositional area.

All reference stations will form part of a wider regional reference station monitoring network that may also be used for State of the Environment (SOE) monitoring, where comparisons can be made across space and time to identify any trends that are not attributable to fish farms. Accordingly, farms may share reference stations within the network where appropriate, i.e. in

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³ 'Sustainable management' as defined in Section 5 of the RMA (1991): "managing the use, development and protection of natural and physical resources in a way, or at a rate which enables people and communities to provide for their social, economic, and cultural well-being and for their health and safety while: (a) Sustaining the potential of natural and physical resources (excluding minerals) to meet the reasonably foreseeable needs of future generations; and (b) Safeguarding the life-supporting capacity of air, water, soil, and ecosystem; and (c) Avoiding, remedying or mitigating any adverse effects of activities on the environment."

⁴Note that the previous NZ King Salmon zones concept included an 'intermediate' zone at 50 m–100 m from the net pens (previously called the Zone 2-3 boundary). This intermediate zone has been omitted from the current design on the basis that, if there are controls in place on both the inner (maximum) extent and outer (minimum) extent, then a natural gradient will exist between the two and it is therefore unnecessary to regulate the transitional zones.

close proximity and share physical (depth, flow) and substrate properties. Furthermore, Type 3 monitoring (Table 2) can be invoked if more information is required about the spatial gradient of effects away from the pens (see Section 3).

The positioning of the ZME and OLE is to be determined on a site-specific basis. For new sites, the initial distances should be set based on the benthic footprint that is predicted using an established depositional model (e.g., DEPOMOD). This is done by relating the predicted depositional flux levels to the associated levels of ecological effects (e.g. Keeley et al. 2013b) and then referencing those effects to the relevant EQS to identify appropriate spatial boundaries. Once the farm has been established, the ZME and OLE station positioning may be further refined to ensure that they are appropriate subsequent to the Type 3 (see Section 3) monitoring conducted after five years of operation. Distances from the farm can be specific to transect directions or orientations due to the potential for deformity caused by currents.

Table 1: Description of, and rationale for, proposed monitoring locations. Distances are indicative and will ultimately be determined by Type 3 monitoring.

Monitoring			Position
locations ZME	Description and rationale Zone of maximum effects station: Worst-case scenario. Check point for goal of	Low flow Sampled beneath or at edge of pens.	High flow Sampled at edge of pens, or nearby if area of
	maintaining functional / productive macrofauna, which is important for waste assimilation and sustainability.		greatest deposition is offset due to currents
OLE	Outer limit of effects station: Delineates outer extent of obvious and measurable effects. 'Natural' conditions ⁵ expected (measured at outer boundary). Assumes a 'zone of reasonable mixing' as provided for in the RMA (1991).	150 m from edge of net pen	200–800 m from edge of net pen (site specific)
NF-Ref	Near-field reference station: Reference station situated near to farm but outside of primary depositional footprint ⁶ . Must be situated in location with comparable depth, substrate and flow regime.	300–1000 m away (>2 × OLE)	500–1500 m away (>2 x OLE)
FF-Ref	Far-field reference station: Reference station that is unlikely to be influenced by far-field effects — geographically or hydrodynamically removed. There may be more than one relevant far-field station, and similarly, farms may share references stations if applicable.	> 1000 m away	>1500 m away
CE-Ref	Potential accumulative effects reference station: An optional additional monitoring station situated in an area that is potentially predisposed to organic accumulation or is otherwise of concern, e.g. a nearby depression or an area close to habitats of ecological significance.	Variable, <1000 m	Variable, <2000 m

⁵ As defined in Table 5 and associated Footnote 23.

⁶The footprint delineated by the OLE, outside of the direct influence of farm derived particulates.

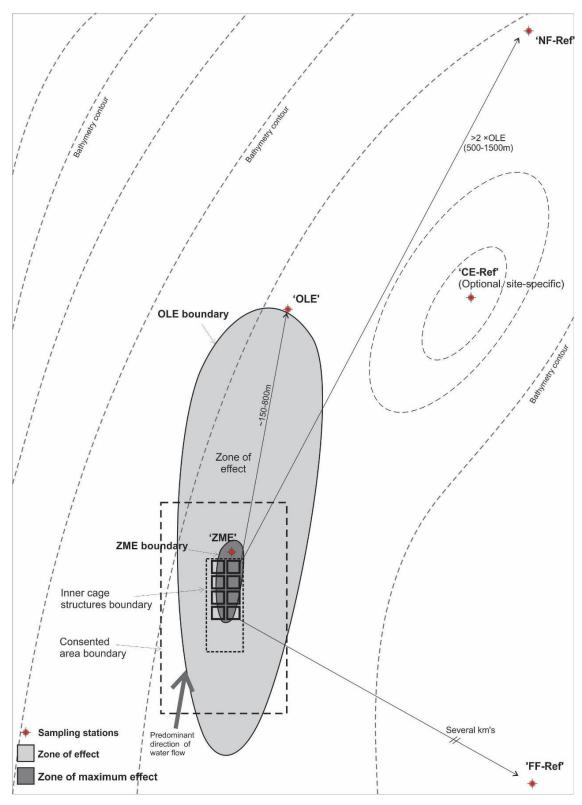


Figure 1: Zones concept with theoretical positions of sampling stations in relation to the farm and potential distortion of the footprint shape due to currents. ZME = zone of maximum effect, OLE = outer limit of effects, NF-Ref = near-field reference, FF-Ref = far-field reference (see Table 1 for further definitions). Also see corresponding profile view of zones in Figure 2.

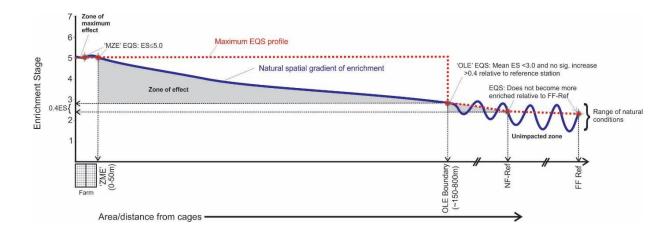


Figure 2: Stylised depiction of natural spatial enrichment gradient as permitted by the zones concept and associated environmental quality standards (EQS) in terms of overall enrichment stage (ES), along with 'maximum EQS profile' which represents the improbable, but maximum possible EQS profile. ZME = zone of maximum effect, OLE = outer limit of effects, NF-Ref = near-field reference, 'FF-Ref' = far-field reference (see Table 1 for further definitions). Also see corresponding aerial view in Figure 1.

3 Determining monitoring effort – a tiered design

A three-tier monitoring design has been proposed to provide incentive to manage farms in a stable, consistent and environmentally sustainable manner. Increasing feed levels and/or managing at the upper limits of environmental thresholds attracts a higher intensity of monitoring that provides greater precision and confidence in the results. Matching monitoring intensity to production intensity and background environmental conditions in this manner is also consistent with approaches adopted elsewhere in the world, e.g. the 'MOM' system in Norway (Ervik et al. 1997; Hansen et al. 2001), and other approaches in Canada, Chile, Ireland, and the United Kingdom (Wilson et al. 2009).

There are three approaches for annual monitoring; the different types reflect the different operational risk levels:

- Type 1 monitoring is the least intense form of monitoring. This approach places greater emphasis on qualitative indicator variables that can be rapidly evaluated enabling feedback to be provided quickly (in about two weeks). It focuses on assessment at two ZME stations, one OLE station (for low flow sites) or two OLE stations (for high flow sites), and the NF-Ref station.
- Type 2 monitoring is the default level of monitoring at all farm sites. Type 2 monitoring is more rigorous than Type 1 and will be conducted at two or three ZME stations, one or two OLE stations (flow dependent), and the NF-Ref and FF-Ref stations. Five replicate samples of the full suite of quantitative variables are collected from each station. Three of the samples are processed initially; the remaining two samples will be processed if greater certainty is required (e.g. in the event that the standard error exceeds the maximum permitted EQS).
- Type 3 monitoring: Type 3 is the most intensive type of monitoring with a flexible spatial design that aims to elucidate spatial patterns (e.g. footprint mapping), or address specific concerns. It is conducted at year 0 (baseline) and after five years of operation at full capacity, and then as necessary (Figure 3). The methods used to conduct these surveys are unspecified as they are likely to evolve with time. In effect, this is an avenue for gaining a better understanding of the causal factors (farm-based and otherwise) and a meaningful plan to avoid non-compliance an adaptive management response. However, two anticipated forms of Type 3 sampling design are:
 - Sampling regularly along radial transects to review whether the spatial arrangement of monitoring captures the zone of maximum effect.
 - O Sampling over a grid pattern to map the distribution and extent of the habitats and resulting footprint, e.g. a pre-farm baseline or after five years to cross-check actual against predicted footprint.

Type 2 is the default level of monitoring at all farm sites and forms the basis for determining the level of management response required should the EQS be exceeded (see Section 4.2). Progression to less intensive monitoring (i.e. from Type 2 to Type 1) is contingent on:

1. how long the farm has been operational,

- 2. whether feed levels have increased 'significantly'⁷, and
- 3. whether the results of the previous year's annual monitoring survey were compliant with the EQS (Section 4).

Type 1 monitoring may continue as long as these conditions continue to be met, the farm configuration remains 'largely unchanged' and there are no other reasons to suspect that more intensive monitoring is warranted (e.g. where the sampling design is missing the ZME or the qualitative assessment is inadequate). In the event of non-compliance, the monitoring results are reviewed to determine whether routine Type 2 monitoring is appropriate (e.g. for a beneath net pen issue), or if a higher level approach might be required, i.e. Type 3 (e.g. in the case of an outer zones issue, or suspicion that the ZME is not being properly targeted, or is bigger or smaller than anticipated). Where Type 1 monitoring was conducted and the EQS are triggered, then Type 2 monitoring must be conducted within 30 days of the initial Type 1 survey. The consent holder can opt to collect the broader suite of Type 2 samples in conjunction with the initial Type 1 survey to minimise costs. Samples can be archived and retrieved in the situation that higher level monitoring is deemed necessary.

Frequency and timing of monitoring is dealt with in Section 6.

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⁷ In this context 'significantly' is defined as more than a 15% increase in feed use over the preceding 12 months (relative to the previous year).

⁸ For this purpose 'largely unchanged' means that the farm has not been shifted or reoriented substantially within the site (by more than 20 m in any direction) and the type of net pens and fish species being used are the same.

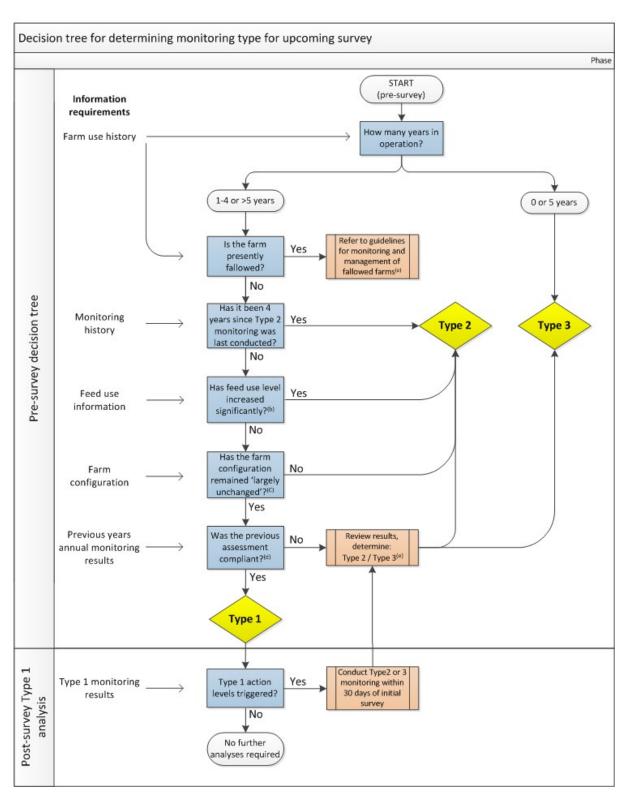


Figure 3: Decision tree for determining the type of annual benthic monitoring that is required. Superscripted characters: (a) Refer to guidelines in Section 3.1.2; (b) Refer to text above and definition in Footnote 7; (c) Compliance as determined with reference to EQS in Figure 5; and (d) Refer to text above for example situations.

Table 2: Summary of sampling methods and target variables associated with the three types of monitoring: Type 1–Type 3. TFS = total free sulfides.

	Type 1: Indicator monitoring	Type 2: Full si	uite monitoring	Type 3: Spatial monitoring
FLOW:	High and Low	Low	High	High and Low
Description:	Simplified semi-quantitative	Default form of qua	antitative monitoring	A more intensive form of
	monitoring conducted when	conducted at p	orescribed zone	monitoring with a flexible
	farm has been compliant and	boundaries at five	yearly intervals or	spatial design that aims to
	feed levels and effects are	when feed levels	have increased ⁷ , if	elucidate spatial patterns (e.g.
	relatively stable	compliance was a	n issue in previous	footprint mapping), or address
		assessment, or if	Type 1 EQS were	specific concerns.
		trigg	jered.	
Frequency:	Annual	An	nual	Baseline and at year 5, then
				as necessary
Compliance	Total = 4 (LF) or 5 (HF)	Total = 5:	Total = 7:	Spatial sampling design.
monitoring	ZME ×2,	ZME x2, OLE x1	ZME $\times 3^{10}$, OLE $\times 2$	Varies according to situation.
stations9:	OLE ×1 (LF) or ×2 (HF)	(down-current)	(opposing down-	
			current)	
Variables:	Qualitative assessment ¹¹ , TFS,	Full macrofauna, TI	FS, redox ¹¹ , (Cu and	Dependent on sampling
	redox ¹² , (Cu and Zn) ¹³	Zr	า) ¹³	design. Initial survey includes
				sediment grain size ¹¹
Reference	NF-Ref ¹⁴	NF-Ref, FF-R	Ref, (CE-Ref ¹⁵)	Design dependent.
stations				
Replicates per	≥ 3 ¹⁷	3 ((5) ¹⁸	1–3
variable:16				
Qualitative	As described in Table 6	As describe	ed in Table 6	Dependent on sampling
variables:				design

3.1 MODIFICATIONS

Any deviations from the agreed monitoring protocol are to be considered by a review panel to be agreed upon by MDC and the consent holder. Two obvious potential modifications are where

⁹ Based on the traditional single block of net pens farm configuration used by NZ King Salmon to date. For multiple individual circular pen arrangements see Section 3.1.

¹⁰ ZME stations at high flow sites are positioned to target the zone of maximum impact. In the case of high flow sites, this may not always be directly beneath the net pens; therefore, these stations may be shifted to other locations near to the farm (e.g. 20–60 m away) dependent on the results of Type 3 monitoring.

¹¹ Includes visual assessment of seabed (bacterial mat and outgassing) and qualitative evaluation of macrofauna samples. See Table 6 for details.

¹² Sampled to provide supporting information only, no associated EQS. For sediment grain size, this is to be sampled in the initial survey unless otherwise required.

¹³ As required according to the copper and zinc monitoring decision tree (see Section 5).

¹⁴ Sampling of FF-Ref is not required for Type 1 monitoring on the assumption that the scope for effects at the NF-Ref is negligible. However, the FF-Ref stations should still be routinely monitored as part a regional monitoring network programme that is presently under development.

¹⁵ Cumulative effects-Reference. As required - optional and site-specific, see Section 2.

¹⁷ Normally conducted in triplicate sampling, however, indicators such as redox may be measured twice from each sample, i.e. triplicate pairs of samples.

¹⁸ Five replicate samples are to be collected during Type 2 monitoring, but only the first three samples will be analysed in the first instance. The remaining two samples will be analysed if the 95% confidence intervals spans the relevant threshold/

different pen configurations are proposed, or if a farm site is to be fallowed. Guidance for these examples is provided below.

3.1.1 Different net pen arrangements

The monitoring approach outlined in Table 2 was designed based on the predominant current farming practices; a single, continuous block of net pens in the centre of the site. However, an alternative approach exists, which utilises multiple single circular pens that can be spread out across a site and more readily moved, thereby facilitating potential fallowing strategies. The monitoring protocols and the underpinning EQS would, for the most part, be appropriate to this other form of fish grow-out, with the tiered monitoring strategy, the basic zones concept and the EQS remaining applicable. The main point-of-difference concerns the number and arrangement of monitoring stations that would be required to capture a representative impression of the state of the seabed across the site.

For the circular pen arrangements, **one ZME station** and one OLE station must be monitored **for every three circular net pens** at the farm site, with a minimum of two ZME stations per farm. The ZME stations should be oriented at the down-current edge of the pens, focusing on those that are known to have had the most feed use in the previous 12 months. Outer zone effect monitoring should be conducted at a distance that is appropriate to the site (refer Table 1). The orientation of the OLE stations should originate from net pen(s) that have been most intensively used in recent months and are nearest to the down-current boundary of the farm; or in an alternative optimum direction should sampling in the down-current direction not be possible (e.g. due to the presence of neighbouring mussel farms). Each site of multiple net pens would still only have one NF-Ref and FF-Ref.

3.1.2 Monitoring required during fallowing

Where farms are fallowed¹⁹, alternative monitoring and sampling arrangements may be necessary and appropriate. It is envisaged that these arrangements will be tailored to the proposed farm layout.

Farms that have been destocked do not generally require annual monitoring as they are assumed to be in a state of recovery, and a farm may remain unstocked for many years. However, the regulatory body may request that a site is monitored for a specified period subsequent to fallowing where the destocking has been as a result of a non-compliance with previous environmental assessments. Benthic monitoring may also be necessary prior to the reinstatement of a farm to determine appropriate restocking levels. The onus is on the consent holder to ensure that the amount of fish restocked is consistent with the farm meeting the required EQS (Section 4) in the following year. Any monitoring prior to reinstatement should therefore logically focus on the ZME, and not necessarily the OLE or the reference stations, as this will best inform the assessment of reinstatement capacity and the optimum placement

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standard, unless the consent holder opts to take the conservative response regardless. The extra two samples are to be held in archive by the analytical service provider for six months following survey.

¹⁸ Five replicate samples are to be collected during Type 2 monitoring, but only the first three samples will be analysed in the first instance. The remaining two samples will be analysed if the 95% confidence intervals spans the relevant threshold/standard, unless the consent holder opts to take the conservative response regardless. The extra two samples are to be held in archive by the analytical service provider for six months following survey.

¹⁹ i.e. Shifting of net pens or temporary retirement of farming lease area.

net pens. Therefore, monitoring undertaken prior to restocking may use a hybrid of the methods outlined in Table 2, and the intensity will be at the discretion of the consent holder.

4 Environmental Quality Standards

Environmental Quality Standards (EQS) are a critical aspect of the benthic monitoring protocol as they provide the quantitative (and qualitative) criteria, or environmental 'bottom lines', against which effects will be assessed. Importantly, these criteria have been designed with the intention of achieving the aims and objectives that are outlined in Section 0. The primary EQS that has been adopted for this BMP is overall Enrichment Stage (ES, Figure 4), which is a derivative of multiple physio-chemical and biological variables, as described below in Section 4.1.

The standards are to be used in relation to spatial zones (Section 2), whereby the level of acceptable impact reduces with distance from the net pens. As discussed in Section 2, the primary compliance locations are at the net pens (the ZME) and at the OLE, some hundreds of metres away (site dependent). The EQS are also designed to accommodate a tiered monitoring design, where there are two main types of monitoring (Type 2 is the default, and intensive, and Type 1 is less intensive). As discussed previously, the type of monitoring used is dependent on factors relating to the pre-existing state of the farm (Section 3; Figure 3).

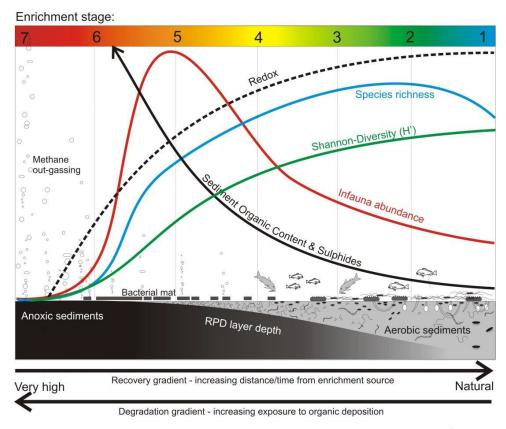


Figure 4: Stylised depiction of a typical enrichment gradient experienced at low flow sites (from Keeley, 2013), showing generally understood responses in commonly measured environmental variables (species richness, infauna abundance, sediment organic content and sulfides and redox). Apparent Redox Potential Discontinuity depth (aRPD) and prevalence of bacteria (*Beggiatoa* sp.) mats and methane / H₂S out-gassing are also indicated. The gradient spans from natural or pristine conditions on the right (ES = 1.0) to highly enriched azoic conditions on the left (ES = 7.0).

4.1 CALCULATING ENRICHMENT STAGE

The expected changes in macrofaunal community composition and abundance associated with salmon farm enrichment are well-documented (Brown et al. 1987; Kalantzi & Karakassis, 2006; Macleod et al. 2004), and are consistent with organic enrichment response from other sources (Glémarec & Hily, 1981; Pearson & Rosenberg, 1978), Figure 4. The fundamental principles have also been used to underpin ecological models (e.g. Grall & Glémarec, 1997) and benthic health indices (e.g. the AZTI's Marine Biotic Index, Borja et al. 2000; Borja & Muxika, 2005).

These changes along the enrichment gradient have been numerically defined for a suite of widely-used benthic environmental indicators and biotic indices based on a meta-analysis of historical data from beneath fish farms in the Marlborough Sounds (Keeley et al. 2012a). Through this process, the relationships between ES and the following enrichment-indicating variables have been numerically described: number of taxa, abundance, evenness, Shannon diversity H', AMBI, Multivariate-AMBI (Muxika et al. 2007), BQI (Rosenberg et al. 2004), sediment organic content, redox, and total free sulfide levels (TFS). Note that, for reasons of consistency and evaluating compliance, redox and TFS must be assessed according to the methods outlined in Appendix E. The relationships provided in Keeley et al. (2012a) are used to convert the values (in the native units) for each of the variables into an equivalent ES score (value from 1.0 to 7.0). The scores for the different variables can then be combined quantitatively (by weighted averaging) to arrive at an 'overall ES' that has an associated statistical variance and as such provides an assessment of the environmental condition and the level of certainty associated with that assessment. Hence, it is a multi-variable, 'weight-of-evidence' type approach.

Seven enrichment stages (ES) are identified along the continuum (see Table 3 for full descriptions), encompassing the full range of possible effects – from pristine unenriched conditions (ES = 1.0) to extremely enriched conditions (ES = 7.0). An important feature along the gradient is the stage at which seabed productivity is greatly enhanced (ES 5.0). Under these conditions one, or a few, enrichment-tolerant 'opportunistic' species (e.g. Capitellid worms and nematodes) tend to proliferate. At this stage the benthos is still considered biologically functional and is often associated with the greatest benthic biomass (Keeley et al. 2013a) and therefore has the greatest waste assimilation capacity. Enrichment stages over 5.0 are characterised by very highly enriched sediments, becoming excessively enriched at ES6.0, and it is at these stages that the infaunal communities tend to collapse, with waste metabolism declining abruptly and organic accumulation exacerbated. For these reasons, ES5.0 is recommended as the upper level of acceptable seabed effects beneath salmon farms in the Marlborough Sounds. It is important to recognise, that although ES1.0 represents the pristine, natural end of the spectrum, in many situations, the seabed can be naturally enriched and/or disturbed; for example in the Marlborough Sounds much of the seabed is ES2–2.5.

Some variables are better predictors of ES than others (i.e. exhibit a tighter statistical relationship) and this has been used to guide variable selection and to weight groups of variables in the overall calculation. For example, %OM is considered to be a poor indicator of enrichment at high flow sites as it is highly variable and does not tend to increase until enrichment levels are relatively high. As such, its inclusion in the calculation of overall ES is something that will be reviewed in the near future. Furthermore, recent analyses of the environmental data from the existing NZ King Salmon sites in the Marlborough Sounds has highlighted other characteristic differences in the way the seabed impacts at high and low flow sites (Keeley et al. 2013a). For example, taxa richness tends to be higher at high flow sites and tends not to be reduced in the



Table 3: General descriptions and primary environmental characteristics for the seven enrichment stages (see Keeley et al. 2012 a,b). HF = High Flow sites (mean mid-water current speeds ≥ 10 cm.s⁻¹), LF = Low Flow sites (< 10 cm.s⁻¹).

ES General description

Environmental characteristics

- 1.0 Pristine end of spectrum. Clean LF unenriched sediments. Natural state, but uncommon in many modified environments
 - LF Environmental variables comparable to an unpolluted / un-enriched pristine reference station.
 - HF As for LF, but infauna richness and abundances naturally higher (about $2 \times LF$) and %organic matter (OM) slightly lower.
- 2.0 *Minor enrichment.* Low-level enrichment. Can occur naturally or from other diffuse anthropogenic sources. 'Enhanced zone.'
- LF Richness usually greater than for reference conditions. Zone of 'enhancement' minor increases in abundance possible. Mainly a compositional change. Sediment chemistry unaffected or with only very minor effects.

HF As for LF

- **3.0** *Moderate enrichment*. Clearly enriched and impacted. Significant community change evident.
- LF Notable abundance increase; richness and diversity usually lower than reference station. Opportunistic species (i.e. Capitellid worms) begin to dominate.

HF As for LF

- 4.0 *High enrichment.* Transitional stage between moderate effects and peak macrofauna abundance. Major community change.
- LF Diversity further reduced; abundances usually quite high, but clearly subpeak. Opportunistic species dominate, but other taxa may still persist. Major sediment chemistry changes (approaching hypoxia).
- HF As above, but abundance can be very high while richness and diversity are not necessarily reduced.
- **5.0 Very high enrichment.** State of peak macrofauna abundance.
- LF Very high numbers of one or two opportunistic species (i.e. Capitellid worms, nematodes). Richness very low. Major sediment chemistry changes (hypoxia, moderate oxygen stress). Bacterial mat usually evident. Out-gassing occurs on disturbance of sediments.
- HF Abundances of opportunistic species can be extreme (10 × LF ES 5.0 densities). Diversity usually significantly reduced, but moderate richness can be maintained. Sediment organic content usually slightly elevated. Bacterial mat formation and out-gassing possible.
- 6.0 Excessive enrichment. Transitional stage between peak abundance and azoic (devoid of any organisms).
- LF Richness and diversity very low. Abundances of opportunistic species severely reduced from peak, but not azoic. Total abundance low but can be comparable to reference stations. %OM can be very high (3–6 \times reference).
- HF Opportunistic species strongly dominate, with taxa richness and diversity substantially reduced. Total infauna abundance less than at stations further away from the farm. Elevated %OM and sulfide levels. Formation of bacterial mats and out-gassing likely.
- 7.0 Severe enrichment. Anoxic and azoic; sediments no longer capable of supporting macrofauna with organics accumulating.
- LF None, or only trace numbers of infauna remain; some samples with no taxa. Spontaneous out-gassing; bacterial mats usually present but can be suppressed. %OM can be very high $(3-6 \times \text{reference})$.
- HF Not previously observed but assumed similar to LF sites.

4.2 TYPE 2 MONITORING - STANDARDS AND TIERED MANAGEMENT RESPONSES

There are four levels of response, dependent on the assessment of the overall enrichment stage (ES, described in Section 4.1) as the result of Type 2, quantitative monitoring (Section 3). These are termed: 'Alert', 'minor action level', 'major action level', and 'destocking' (Table 4). The severity of the required management response increases in response to the assessed level of overall enrichment stage as outlined in Figure 5 and Table 4.

The standards are based on station-averaged (mean) results, i.e. on the average of replicate samples collected from within a single station (three replicates by default, or five under some circumstances, see Table 2 and Figure 5), and therefore assessed on a station-by-station basis. Inevitably there will be variability about the estimates, and this has been accommodated by also utilising the 95% Confidence Intervals (CI's) in relation to the proposed standards, thereby setting the boundaries for action at a point where there is some certainty that the standard has been breached, and in doing so giving the consent holder the benefit of the doubt (Figure 6 and Appendix C).

The EQS for monitoring of salmon farms in the Marlborough Sounds are provided in Table 5. Benthic enrichment stages greater than ES 5.0 are considered unacceptable anywhere within the lease area for reasons of waste assimilation, minimising waste accumulation and long-term sustainability. Therefore, maintaining seabed conditions at or lower than ES 5.0 has been adopted as the main compliance goal within the ZME (i.e. at the pen edge, Figure 1). A minor exceedance of this EQS (i.e. the lower CI is greater than ES5.0) requires a management response appropriate to reduce the enrichment levels to within the required EQS within 24 months (Figure 5). A larger exceedance of the standard (i.e. lower CI > ES5.3) requires a more substantive management response. The compliance goals are 'effects based' and the management responses are at the discretion of the consent holder, however, their effectiveness will be checked at 12 and 24 months. If they have not been effective within those timeframes, then more drastic responses are required (Table 4 and Figure 5). If after 24 months (from the survey where the EQS was initially exceeded) no improvements are evident, or if the lower CI exceeds 5.6 at any point, the farm must be destocked (or 'fallowed').

In addition to the overall ES criteria, three readily assessable and widely established indicators of excessive enrichment and anaerobic conditions were also adopted. These associated EQS are as follows:

- 1. Two or more replicates with macrofauna virtually absent.
- 2. Bacteria mat (*Beggiatoa* sp.) coverage must be no more than localised / patchy in distribution.
- 3. No obvious spontaneous out-gassing (of H_2S or methane).

At the OLE (a set distance 150–600 m away) and beyond, the level of enrichment is required to be indicative of natural or background conditions. A minor management response is required if the ES level at the OLE station increases significantly relative to appropriate reference stations (Figure 5 and Table 5). A management response is required if a significant increase is observed and the mean incremental increase is greater than 0.4ES, or if ES is greater than 2.9. This overarching ES cap is intended to prohibit a series of small incremental increases amounting to a large increase long-term.

Timelines for monitoring and reporting are discussed further in Section 6.

Table 4: Action levels and associated management responses (refer Figure 5 and Table 5).

Action level

Management response

Type 2 monitoring

Type 2 is the default form of monitoring, but less intensive Type 1 monitoring is conducted when certain conditions are met (Figure 3). Resumption of Type 2 monitoring is triggered in response to Type 1 monitoring results, see Figure 3 and Section 4.3. This represents a shift from a qualitative to a quantitative assessment.

Alert

The consent holder must provide a written management response plan intended to reduce the level of seabed enrichment. The response plan must be made available to Council within 20 working days of having received the final annual monitoring reports.

Process additional samples

The two additional samples are to be processed and the results incorporated into the overall assessment of enrichment to improve the confidence and accuracy of the assessment. The results are to be reported on and made available to Council within 20 working days of having received the final annual monitoring reports.

Minor

The consent holder must plan and undertake management response(s) appropriate to reduce the enrichment levels to within the required EQS within 24 months from the initial survey that exceeded the permitted EQS. A written planned response must be made available to Council within 20 working days of having received the final annual monitoring reports.

If an improvement in seabed conditions is not achieved within 12 months (i.e. defined as a statistically significant improvement in the ES score relative to the initial survey or achievement of mean ES≤5.0) then a more drastic response is required to bring the ES level into compliance by 24 months from the initial breach.

and

Type 1 monitoring should be regularly undertaken prior to the next major restocking to inform the stocking level for the 12 month period leading in to the monitoring survey at the end of the 24 month period.

Major

As for minor action response, but consent holder must undertake a more significant management response appropriate to the level by which the EQS has been exceeded (e.g. substantial feed reduction).

In the event that a feed reduction was the chosen management response, the amount of feed discharged may be increased again once it has been demonstrated that the site is clearly within the relevant EQS. The increase must be at a level that will allow the site to continue to meet the required EQS (Table 5).

Destocking The consent holder must:

- remove stock and fallow the site until the farm is within the relevant EQS. Destocking must occur
 within four months from the date that the consent holder was officially deemed non-compliant, or at
 the end of the production cycle, whichever is the latter²⁰. An additional one month (from the date the
 non-compliance notice was issued) is allowed for re-testing.
- ensure at the time of restocking, that the stocking plan is appropriate to allow the site to meet the required EQS in future surveys (Table 5).

²⁰ The second part of this condition deviates from the BoI consent conditions, and was considered necessary because there may be situations where the four month requirement is difficult to meet without farm-wide culling of stock.

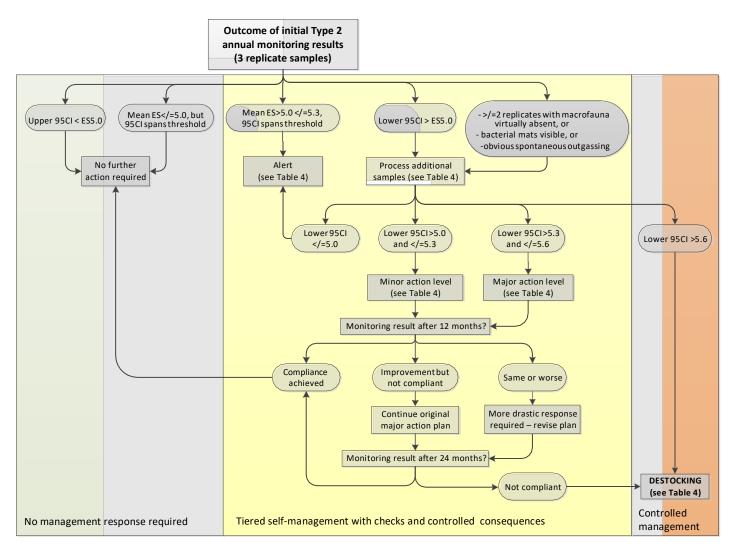


Figure 5: Decision tree for determining the level of management response required in relation to Type 2 (quantitative) annual benthic monitoring results. Diagram primarily relates to the ZME (see Tables 4 and 5), however the pathway below the 'Minor action level' box also pertains to the OLE. Refer to Figure 6 and Appendix C for a diagrammatic example of how sample variability relates to the various thresholds.

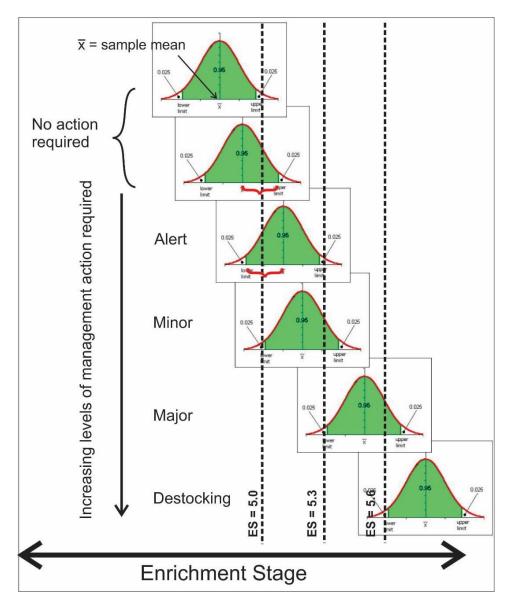


Figure 6: Example of how 95% confidence intervals are utilised in relation to ES thresholds with the various levels of management responses. For further clarity, an alternative way of displaying the relationship between the mean ES value, the associated confidence interval and the required action response levels

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Table 5: Industry operation goals and benthic environmental quality standards (EQS, or 'triggers') to be applied based on station-averaged result, indicating action levels for non-compliance. TFS = total free sulfides in sediments. CI = Confidence Interval.

	Action level		Sampling station
	(Figure 5)	Zone of maximum effects (ZME)	Outer limit of effects (OLE)
Industry operational goal		-Overall ES $\leq 5.0^{21},^{22}$	-Overall ES< 3.0 ²³ (i.e. maintain natural conditions)
EQS for Type 1 monitoring	Type 2 monitoring	-TFS: 24 Low flow $>1700~\mu M,~High~flow >~2400~\mu M -Total qualitative score >6.0 and macrofauna score >2.0 (refer Table 6)$	-TFS $>$ 390 μM^{25} -Total qualitative score $>$ 0 (refer Table 6)
EQS for Type 2 monitoring	Alert	-Mean Overall ES > 5.0 and $\le 5.3^{22}$ and 95% CI spans threshold	-A statistically significant increase relative to appropriate reference station(s) ²⁶
		-Lower 95% CI for Overall ES > 5.0 and $\leq 5.3^{22}$	-Overall ES ≥ 3.0
	Minor	-Two or more replicates with macrofauna virtually absent ²⁷ -Bacterial mats visible ²⁸ -Obvious spontaneous outgassing ²⁹	AND -Mean ES 0.4 higher than previous year, and increase is significant relative to appropriate reference station(s) ²⁶
	Major	-Lower 95% CI for Overall ES > 5.3 and $\le 5.6^{22}$	-
	Destocking	-Lower 95% CI for Overall ES $> 5.6^{22}$	-

²¹ 'Upper limit' corresponds to the point of peak (maximal) abundance, where the less impacted side of the curve is acceptable, but the more impacted, declining (post-peak) side is unacceptable. ES 5.0 corresponds to a seabed that is very highly enriched and where opportunistic taxa (e.g. capitellids and nematodes) are most prolific and waste assimilation is theoretically maximal (Keeley et al. 2012a, 2013a). Bacterial mats and obvious spontaneous outgassing are not permitted. A description of the general conditions can be found in Table 3 and Figure 4.

²² These ES categories are consistent with those proposed by the EPA at the conclusion of the NZ King Salmon Board of Inquiry in 2013.

²³ES 3.0 corresponds to discernible 'moderate enrichment' (Keeley et al. 2012a, Table 3 and Figure 4) and is a state that is unlikely to be found naturally. 'Natural' (i.e. non-farm impacted) seabed in the Marlborough Sounds varies from about ES 1.5 to 2.5 (but no greater than ES 2.9). Careful reference station selection is therefore critical.

²⁴ Suggested initial threshold based on a balance of the evidence relating to the relationship between TFS and macrofaunal responses: the supporting information includes: Keeley et al. (2012a); Hargrave et al. (2008); NBDELG (2012a,b); Keeley et al. (2013b) and the prediction intervals given in a recent review (Keeley & Taylor, 2015). Additional considerations are that it is applied here on a station by station basis (rather than a farm average), the trigger is coupled with other qualitative investigations, and it is applied at the zone of <a href="maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-maintain-main

 $^{^{25}}$ The 95% CI's associated with ES 3.0 conditions in the Marlborough Sounds are 390 and 244 μM for low and high flow sites, respectively (Keeley et al. 2012a, 2013). The transition between Oxic-A and Oxic-B status in Canada is 750 μM (Hargrave et al. 2008). Hence, a relatively conservative trigger has been adopted that will be reviewed in the near future.

²⁶ Statistically significant increase relative to appropriate reference station(s) implies the use of a BACI-type analysis to test for a significant Station:Survey interaction term. More than one reference station may be included in the analysis.

²⁷ Intent consistent with SEPA 'action level *within* allowable zone of effects' (SEPA 2005). Words 'virtually absent' used in lieu of 'absent' or 'azoic' because of the likelihood of chance inclusions of one or a few (drift) individuals regardless of state. Defined as fewer than 3 taxa and fewer than 6 individuals.

²⁸ Defined as: white bacterial mat (mainly *Beggiatoa* sp.) smothering sediment surface. Excludes patchy presence and where *Beggiatoa* is only observed on hard substrates, such as shells and other debris.

²⁹ Defined as: Clear outgassing occurring freely without disturbance. Bubbles obvious on surface around net pens.

4.3 QUALITATIVE ASSESSMENTS

Type 1 benthic monitoring (the qualifications for which are described in Section 3) is based on qualitative assessment criteria (Table 6). Qualitative assessments using visual indicators (Macleod et al. 2004; Macleod & Forbes, 2004; SEPA, 2005; Wilson et al. 2009) are internationally recognised as a means to provide a simple assessment of sediment conditions, and can provide a standardised and cost-effective means of checking for seabed impacts. In doing so, it is hoped that assessments may be voluntarily and more regularly conducted by the consent holder, and in doing so reduce the risk of adverse ecological conditions and non-compliance at the time of annual monitoring.

The circumstances that determine when this type of monitoring can be conducted are provided in Figure 3 and the associated EQS are described in Table 5. Each qualitative variable has a suggested 'acceptable level' (category) which can be scored, and these scores added together give a cumulative score, which must be less than or equal to the sum of the suggested acceptable levels (i.e. no more than 6). Scores higher than this by any combination will be considered to be non-compliant for Type 1 monitoring, and more intensive investigations will be triggered (see Table 2). The visual macrofauna assessment is considered to be a particularly important indicator as it relates directly to the ecological state of the benthos, and it therefore also has a stand-alone trigger (in that it must not be more than 2).

A 'Qualitative Assessment of Enrichment Guide' methods booklet is currently in development, and once completed, can be formally implemented. The qualitative information that is used to make the Type 1 assessment (i.e. video footage, macrofauna photos) will be presented in the annual report (where feasible) and/or archived for future reference.

Qualitative outgassing classifications (suggested acceptable level: ≤ 2)

Method: Assessment made from observations at surface and from real-time video footage of seabed. Requires repeated physical contact with seabed to assess disturbance, e.g. with camera or frame.		Score
None	No outgassing observed	0
Minor	Minor or suspected outgassing. Not obvious.	1
On disturbance	Clear outgassing on disturbance of seabed	2
Spontaneous	Clear outgassing occurring freely without disturbance. Bubbles obvious on surface around net pens (evident in calm conditions).	3

Qualitative bacterial coverage classifications (suggested acceptable level: ≤ 2)

Method: Visual assessment from video or drop-camera. Assessment to be made from at least $2 \times 1 \text{ m}^2$ of seabed with reference to catalogue of images.

None-natural	No bacterial matter observed, sediment appear natural / healthy	0
Trace	Traces of bacterial mat (<i>Beggiatoa</i> sp.) within sediments or attached to edges of cobbles or shells.	1
Patchy-minor	Obvious patches of bacterial mat (<i>Beggiatoa</i> sp.) on sediment surface, occupying <50% of surface area	2
Patchy-major	Obvious patches of bacterial mat (<i>Beggiatoa</i> sp.) on sediment surface, occupying >50% of surface area	3
Mat	White mat of bacterial mat ($Beggiatoa$ sp.) smothering sediment surface (>90% coverage over area >1 m ²)	3
None	Bacterial mat absent, but sediments black and highly anaerobic and probably anoxic (redox very low, e.g. <-150 mV). Very strong sulfide odours	3

Macrofauna visual inspection classifications (suggested acceptable level: ≤2)

Methods: Washed and sieved (0.5 mm mesh) macrofauna sample spread over white tray and inspected by dissecting scope or equivalent by appropriately trained personnel (i.e. with necessary taxonomic skills). Qualitative categorical assessments made with reference to catalogue images. Full macrofauna samples are to be archived for six months in case they are needed for full taxonomic analysis.

Healthy	Healthy array of taxa. Enrichment sensitive organisms such as small bivalves, ophiuroids echinocardium present.	
Diverse but enriched (ES3-4)	Seemingly healthy array of taxa, but capitellids, nematodes and/or other opportunistic polychaetes noticeably more abundant.	1
Heavily enriched (ES≈5)	Clearly dominated by capitellids and/or nematodes, with few other taxa. Total abundance very high.	2
Post-peak	Capitellids and/or nematodes present in low to moderate abundances but no other taxa observed.	3
Azoic?	No macrofauna present; i.e. fewer than 5 individuals	4

Compliance trigger for Type 2 monitoring:

- Cumulative score >6 (Outgassing + Bacteria coverage + Macrofauna), or
- Macrofauna inspection classification > 2

5 Copper and zinc monitoring

Copper and zinc are ubiquitous metals that occur naturally in the environment. They are both essential trace nutrients required at low concentrations by nearly all organisms. However, toxic effects can occur where these metals are concentrated in biologically available (bioavailable) forms above threshold concentrations. Copper is the principal active agent in antifouling paints that may be applied to underwater structures. It is released into the environment through leaching to the water and by physical abrasion during use or via *in situ* cleaning operations. Some paint formulations also contain zinc. Salmon feed contains zinc as an additive for fish health, leading to its discharge in faecal matter and uneaten feed. Consequently both metals are associated with finfish farming operations, and can accumulate in sediments beneath and adjacent to farms over time. The potential for accumulation of these metals will be mediated by settlement processes and as a result both metals are expected to follow the pattern predicted for organic enrichment.

The principal difference between organic enrichment of the seabed and accumulation of metals within sediments relates to the likely recovery rates, and stems from the conservative nature of metal contaminants. As elements, metals do not break down over time; nor are they utilised by biota at rates which would see attenuation over fallowing time-scales. The main mechanisms by which local concentrations of metals may reduce in sediments over time are resuspension and dispersion, and dilution as a result of ongoing deposition. Deposition of clean non-metal affected sediments can result in the burial of metal contaminated sediments in deeper strata below the biotic zone (about 150 mm) and this process is likely to be accelerated beneath operational farms (MacLeod et al. 2014). The normal operational approach to manage organic enrichment would be to fallow the sediments; however, due to the uncertainty over site-specific rates of resuspension / dispersion, the effectiveness of fallowing as an approach to control sediment metal concentrations cannot be assumed. Furthermore, resuspension and consequent lateral dispersion may also contribute to an expanding and ultimately spatially more extensive metals footprint.

Monitoring of copper and zinc can be incorporated into the general approach proposed for organic enrichment effects (Type 1 and Type 2 monitoring schedules). However, it must be recognised that there is potentially a legacy aspect to metals accumulation, which may persist after operations and inputs have ceased. Hence both standards and operational responses must reflect the fact that action should be taken well before concentrations reach a level at which significant ecological effects might ensue. In situations where historical accumulation is an issue (i.e. for older farms), it may be necessary to take a longer-term view of remediation targets and associated management responses. Due to the potential for trends in sediment metals to be independent of those for organic enrichment, it is appropriate for a Type 1, 2 or 3 monitoring regime to be specific to either component (i.e. heavy metal accumulation or organic enrichment only).

5.1 STANDARDS FOR COPPER AND ZINC

The ANZECC (2000) sediment guidelines are considered appropriate to apply to the monitoring of benthic conditions in the vicinity of salmon farms. These are risk-based criteria developed from a wide range of international toxicity data. For a range of contaminants, the guidelines specify an ISQG-Low (Interim Sediment Quality Guideline-Low) concentration, representing a 10% probability that a significant toxicity measure will occur in sensitive species, and ISQG-High concentration, representing a 50% probability (Table 7). These guidelines are applicable

anywhere in the vicinity of the farm, and therefore should logically be monitored in the worst affected area, which is consistent with the goal of the ZME stations (Table 1).

Consistent with the approach outlined by the ANZECC (2000) guidelines, the ISQG-Low values should be adopted as triggers in an adaptive, decision tree framework which addresses the following requirements:

- 1. The need to be protective of ecological values.
- 2. The need to collect meaningful monitoring data which can be compiled over time to adequately show trends and increase the understanding of risks.

Such trigger values, applied to the total recoverable fraction of metals, makes them inherently conservative since it is only the bioavailable fraction to which the guideline values strictly apply. The weak acid extractable metals fraction is an appropriate analytical proxy for bioavailability, which is supported by recent ecotoxicological testing of copper-enriched salmon farm sediments (MacLeod et al. 2014). However, it is important that monitoring data reflects the total accumulation of metals in the first instance. Application to weak acid extractable metals is recommended only for lower tiers of the monitoring framework for the following reasons:

- Inputs from sources such as paint particulates may have limited immediate bioavailability despite a larger fraction being ultimately bioavailable.
- Bioavailability may be suppressed beneath farms by reducing conditions maintained by organic inputs (especially where metals are precipitated in effectively insoluble sulfide forms). However, this suppression may be reduced by consequent fallowing.

Table 7: ANZECC interim sediment quality guidelines for copper and zinc.

	ISQG-Low	ISQG-High
Copper (mg/kg)	65	270
Zinc (mg/kg)	200	410

5.2 A COPPER AND ZINC MONITORING PROTOCOL

The monitoring record for both copper and zinc from beneath established NZ King Salmon farms has proven to be extremely variable — to the extent that true bulk sediment concentrations of copper and zinc beneath farms have been uncertain, and the reliable analysis of temporal trends has not been possible. This situation can be most efficiently addressed using a tiered monitoring approach where effort is minimised when it can be demonstrated that sediments beneath farms are maintained below appropriate trigger levels for each metal (as in the Type 1 monitoring schedule). Upon exceedance of these triggers, monitoring effort intensifies progressively to maximise the collection of useful data and to remove uncertainty. Where it becomes clear that sediment trigger levels are exceeded by copper or zinc in potentially bioavailable forms, management action is precipitated to curb inputs to the system and/or research is instigated to examine the actual bioavailability and toxicity of the contamination and potentially replace the trigger levels in the monitoring protocol with site-

specific criteria. Conversely, should the consent holder be able to demonstrate that future inputs (of either contaminant) will be negligible and that the concentrations in the sediments have been compliant with the trigger levels for the last 3 consecutive years, then monitoring of that contaminant may be discontinued.

Figure 7 shows the recommended form which the decision tree framework should take for the monitoring of sediment copper and zinc. The requirement to analyse the finer sediment fraction ($<250~\mu m$) recognises the potential for the chance inclusion of discrete paint flake material to produce outlier results in the testing of samples of the bulk sediment. More intensive replication at the lower tiers reflects the need to generate an accurate estimate of potential bioavailability and ultimately the spatial extent of contamination.

The ISQG-High criterion is recommended as a limit for the total recoverable metal fraction. This is in recognition of the fact that, while the future potential release of metals in bioavailable form may occur through oxidative dissolution of sulfide minerals, such processes will occur at rates limited by decreases in organic enrichment and to an extent limited by the long-term retention of natural hypoxic conditions close to the sediment surface.

The option to comprehensively research the metals concentrations at which longer-term toxicity manifests (Level 6, Figure 7), and thereby derive site-specific standards to replace ISQG-Low, is in line with the approach outlined in the ANZECC (2000) guidelines. However, recent investigations of sediments from salmon farms in Tasmania have indicated that the ANZECC trigger value applied to weak acid extractable copper is a realistic limit for protection against chronic toxicity to sediment organisms (MacLeod et al. 2014).

Lastly, this decision tree framework is oriented around compliance with the ANZECC guidelines, monitoring in the worst affected areas (i.e. the ZME stations) and discerning the ISQG-Low boundary; however, there may also be occasions when it is appropriate to investigate the overall spatial extent of the copper and zinc footprints. As discussed previously, this may be particularly pertinent at dispersive sites. In this situation the ISQG criteria are less relevant and it is more appropriate to conduct spatial and temporal analysis of the results (with reference to background conditions), which may then inform a range of possible management responses.

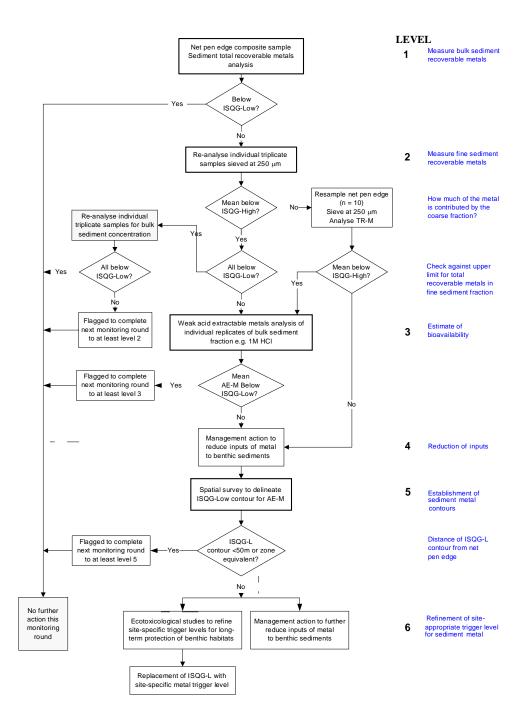


Figure 7: Decision tree for monitoring and operational responses to the accumulation of metals (copper and zinc) within sediments in the vicinity of salmonfarms.

6 Timing and reporting

6.1 TIMING OF MONITORING

Annual monitoring surveys are to coincide with the period of maximum biological impact, in accordance with international best practice (ASC 2012). In the case of NZ King Salmon farming in the Marlborough Sounds, many of the farms contain multiple year-classes and so there is often no single sampling period. Fish stocking and harvesting strategies also vary considerably between farms, but historically the summer months have been associated with the highest feed use. Mid to late summer also generally coincides with highest water temperatures and hence highest benthic mineralisation rates and oxygen consumption, and therefore benthic impacts.

It is therefore proposed that in the future, annual monitoring will be conducted between the middle of January and the middle of March in each calendar year. In the event that Type 1 monitoring is conducted and the EQS are triggered and Type 2 monitoring is required, this will be conducted as soon as practically possible within 30 days of the initial monitoring. In the event that a minor or major management response is triggered (Figure 5) a written planned response must be made available to Council within 20 working days of having received the final annual monitoring reports.

6.2 REPORTING

The overall aim is to ensure that the Annual Monitoring Reports (AMRs) are a succinct summary of the general monitoring approach used (leaving many of the details to this BMP document), the sampling locations, the monitoring results and an assessment of compliance with the existing standards. In addition to the AMRs, an **Annual Monitoring Plan** (AMP) is to be produced prior to conducting the monitoring, for approval by MDC and NZKS Ltd. The AMP shall include:

- a site-specific account of any recommendations or management responses from the previous year,
- the proposed site-specific monitoring (in accordance with Figure 3 and Table 2), and
- detailed sampling methods.

The AMR requirements will vary depending on the Type of monitoring that is conducted.

Type 1 monitoring requires a short report that includes:

- 1. a summary of annual feed use,
- 2. a figure displaying the locations of the monitoring station,
- 3. results tables,
- 4. a brief summary about compliance,
- 5. recommendations for future monitoring or management (including the need for Type 2 monitoring).

Type 1 reports are to be produced within one month of the date that the survey was conducted.

Type 2 monitoring requires a more detailed report. In addition to the requirement for Type 1 monitoring reports, the Type 2 report will include: quantitative analysis, graphs of results, raw data (in Appendices), replicate and mean overall enrichment stage calculations, ES weighting scores and information that enables readers to compare current results and feed levels with

previous years, i.e. temporal comparisons. Type 2 reports are to be produced within three calendar months of the date that the survey was conducted. Both the AMP and the AMRs are to be produced by an appropriately qualified and experienced research provider.

7 The review processes

This BMP is intended to be a living document. As such it will be updated at regular intervals to take account of any new knowledge, improvements in monitoring technology, or relevant modifications to farming practices. This will ensure that we have the best possible understanding of the environmental conditions associated with current farming practices. It is important that the monitoring programme is scientifically valid and reliable, and as cost effective as possible; consequently any potential for improvements in these areas will be carefully considered at each review. The review process will be undertaken every five years unless otherwise requested by any member of the working party. The need for a review must be approved by both the consent holder and the regulatory body, and care should be taken so that the review does not unnecessarily hold up the monitoring process, which is a requirement of the consent conditions.

8 Communication and dissemination of information

The annual reports presenting the results are to be made available as soon as practically possible on the MDC and consent holder's websites along with a copy of the BMP, the 'qualitative assessment booklet' (Section 4.3), and the proposed farm and year-specific detailed annual monitoring plan (AMP, detailing the type and arrangement of the proposed sampling) for the current year.

9 Acknowledgments

The working group would like to thank Professor Kenneth Black, formerly of the Scottish Association of Marine Sciences, and Associate Professor Catriona MacLeod of the Institute of Antarctic and Marine Studies, University of Tasmania for their valuable guidance and review in formulating the first version of this document. The assistance of Dr Ross Sneddon, Cawthron Institute, Nelson, is also greatly appreciated in the development of the copper and zinc standards. Deanna Elvines, Cawthron Institute, provided an experienced benthic ecology perspective and review on the revision of the guidelines. Finally the working group would like to thank Marianne Vignaux for her patient editorial assistance in bringing this revision to print.

References

- Allen, C.R.; Gunderson, L.H. (2011). Pathology and failure in the design and implementation of adaptive management. *Journal of Environmental Management 92*:1379–1384.
- ANZECC (2000). Australian and New Zealand Guidelines for Fresh and Marine Water Quality 2000. Australian and New Zealand Environment and Conservation Council.
- ASC (2012), ASC Salmon Standard. Version 1.0 June 2012. Salmon Aquaculture Dialogue. 103 p.
- Borja, A.; Franco, J.; Perez, V. (2000). A marine biotic index to establish the ecological quality of soft-bottom benthos within European estuarine and coastal environments. *Marine Pollution Bulletin 40*: 1100–1114.
- Borja, A.; Muxika, H. (2005). Guidelines for the use of AMBI (AZTI's Marine Biotic Index) in the assessment of the benthic ecological quality. *Marine Pollution Bulletin 50*: 787–789.
- Brown, J.R.; Gowen, R.J.; McLusky, D.S. (1987). The effect of salmon farming on the benthos of a Scotish sea loch. *Journal of Experimental Marine Biology and Ecology* 109: 39–51.
- Cranford, P.J.; Brager, L.; Wong, D. (2017). A dual indicator approach for monitoring benthic impacts from organic enrichment with test application near Atlantic salmon farms. *Marine Pollution Bulletin 124*, 258–265.
- Cromey, C.J.; Nickell, T.D.; Black, K.D. (2002). DEPOMOD modelling the deposition and biological effects of waste solids from marine cage farms. *Aquaculture 214*, 211–239.
- Ervik, A.; Hansen, P.K.; Aure, J.; Stigebrandt, A.; Johannessen, P.; Jahnsen, T. (1997). Regulating the local environmental impact of intensive marine fish farming I. the concept of the MOM system (modelling ongrowing fish farms monitoring). *Aquaculture 158*: 85–94.
- Glémarec, M.; Hily, C. (1981). Perturbations apportees a la macrofaune benthique de la baie de Concatneau par les effluents urbains et portuaires. *Acta Ecologica* 2, 139–150.
- Grall, J.; Glémarec, M. (1997). Using biotic indices to estimate macrobenthic community perturbations in the Bay of Brest. *Estuarine, Coastal and Shelf Science* 44, 43–53.
- Hansen, P.K.; Ervik, A.; Schaanning, M.; Johannessen, P.; Aure, J.; Jahnsen, T.; Stigebrandt, A. (2001). Regulating the local environmental impact of intensive, marine fish farming II. The monitoring programme of the MOM system (Modelling-Ongrowing fish farms-Monitoring). *Aquaculture 194*: 75–92.
- Hargrave, B.T.; Holmer, M.; Newcombe, C.P. (2008). Towards a classification of organic enrichment in marine sediments based on biogeochemical indicators. *Marine Pollution Bulletin 56*: 810–824.
- Hurlbert, S.H. (1971) The Nonconcept of Species Diversity: A Critique and Alternative Parameters. *Ecology* 52: 577–586.
- Kalantzi, I.; Karakassis, I. (2006). Benthic impacts of fish farming: meta-analysis of community and geochemical data. *Marine Pollution Bulletin 52*: 484–493.

- Keeley, N.; Forrest, B.; Crawford, C.; Macleod, C. (2012a). Exploiting salmon farm benthic enrichment gradients to evaluate the regional performance of biotic indices and environmental indicators. *Ecological Indicators* 23: 453–466.
- Keeley, N.; Forrest, B.; MacLeod, C. (2013a). Novel observations of benthic enrichment in contrasting flow regimes with implications marine farm management. *Marine Pollution Bulletin 66*: 105–116.
- Keeley, N.; MacLeod, C.; Forrest, B. (2012b). Combining best professional judgement and quantile regression splines to improve characterisation of macrofaunal responses to enrichment. *Ecological Indicators* 12: 154–166.
- Keeley, N.B. (2013). Quantifying and predicting benthic enrichment: lessons learnt from southern temperate aquaculture systems, Quantitative Marine Science Program, Institute of Marine and Antarctic Sciences. University of Tasmania, Tasmania, Australia, 257 p.
- Keeley, N.B.; Cromey, C.J.; Goodwin, E.O.; Gibbs, M.T.; Macleod, C.M. (2013b). Predictive depositional modelling (DEPOMOD) of the interactive effect of current flow and resuspension on ecological impacts beneath salmon farms. *Aquaculture Environment Interactions* 3: 275–291.
- Keeley, N.; Gillard, M.; Broekhuizen, N.; Ford, R.; Schuckard, R.; Urlich, S.C. (2015). Best management practice guidelines for salmon farms in the Marlborough Sounds: Part 1: Benthic environmental quality standards and monitoring protocol (Version 1.0 January 2015). MPI Technical Paper No: 2015/01. Prepared for the Ministry for Primary Industries by the Benthic Standards Working Group.
- Keeley, N.; Taylor, D. (2015). A review of total free sulfide concentrations in relation to salmon farm monitoring in the Marlborough Sounds. Prepared for Marlborough District Council. Cawthron Report No. 2742. 13 p.
- Keeley, N.; Wood, S.A.; Pochon, X. (2018). Development and preliminary validation of a multi-trophic metabarcoding biotic index for monitoring benthic organic enrichment. *Ecological Indicators* 85: 1044–1057.
- Macleod, C.; Bisset, A.; Burke, C.; Forbes, S.; Holdsworth, D.; Nichols, P.; Revill, A.; Volkman, J. (2004). Development of novel methods for the assessment of sediment condition and determination of management protocols for sustainable finfish cage aquaculture operations, Aquafin CRC Project 4.1 Final Report. Tasmanian Aquaculture and Fisheries Institute, 228 p.
- Macleod, C.; Eriksen, R.; Simpson, S.; Davey, A.; Ross, J. (2014). Assessment of the environmental impacts and sediment remediation potential associated with copper contamination from antifouling paint (and associated recommendations for management). FRDC Project No. 2011-041. 91 p.
- Macleod, C.; Forbes, S. (2004). Guide to the assessment of sediment condition at marine fish farms in Tasmania., Aquafin CRC Project 4.1. Tasmanian Fisheries & Aquaculture Institute, Australia, 63 p.
- Margalef, R. (1958). Information theory in ecology. *General Systems 3*: 36–71.
- MPI (2013). Literature Review of Ecological Effects of Aquaculture. A collaboration between Ministry for Primary Industries, Cawthron Institute & National Institute for Water and

- Atmospheric Research Ltd. August 2013. Ministry for Primary Industries, Wellington, New Zealand. ISBN: 978-0-478-38817-6.
- Muxika, I.; Borja, A.; Bald, J. (2007). Using historical data, expert judgement and multivariate analysis in assessing reference conditions and benthic ecological status according to the European Water Framework Directive. *Marine Pollution Bulletin* 55: 16–29.
- NBDELG (2012a) The Environmental Management Program for the Marine finfish Cage Aquaculture Industry in New Brunswick, version 3.0. New Brunswick Department of Environment and Local Government, NBDELG, Fredericton, NB, Canada.
- NBDELG (2012b) Standard Operating Practices for the Environmental Monitoring of the Marine Finfish Cage Aquaculture Industry in New Brunswick. New Brunswick Department of Environment and Local Government, NBDELG, Fredericton, NB, Canada.
- Pearson, T.H.; Rosenberg, R. (1978). Macrobenthic succession in relation to organic enrichment and pollution of the marine environment. *Oceanography and Marine Biology*. *An Annual Review 16*: 229–311.
- Pielou, E.C. (1966). The measurement of diversity in different types of biological collections. *Journal of Theoretical Biology* 13: 131–144.
 - Pochon, X.; Wood, S.A.; Keeley, N.B.; Lejzerowicz, F.; Esling, P.; Drew, J.; Pawlowski, J.; (2015). Accurate assessment of the impact of salmon farming on benthic sediment enrichment using foraminiferal metabarcoding. *Marine Pollution Bulletin* 100: 370–382.
- Rosenberg, R.; Blomqvist, M.C.; Nilsson, H.; Cederwall, H.; Dimming, A. (2004). Marine quality assessment by use of benthic species-abundance distributions: a proposed new protocol within the European Union Water Framework Directive. *Marine Pollution Bulletin* 49: 728–739.
- SEPA (2005) Regulation and monitoring of marine cage fish farming in Scotland Annex A: Standards. Scottich Environmental Protection Agency.
- Wildish, D.J.; Akagi, H.M.; Hamilton, N.; Hargrave, B.T. (1999). A recommended method for monitoring sediments to detect organic enrichment from mariculture in the Bay of Funday., Canadian Technical Report of Fisheries & Aquatic Sciences No. 2286. DFO, Canada.
- Wilson, A.; Magill, S.; Black, K.D. (2009). Review of environmental impact assessment and monitoring in salmon aquaculture. Environmental impact assessment and monitoring in aquaculture. FAO, Rome, Italy, pp. 455–535.

10Appendices

10.1 APPENDIX A: RECORD OF DISSENTING VIEW

January 2014:

All of the content of this document has been contributed to, reviewed and approved by the Benthic Standards Working Group (listed authors) who represent the six different agencies or groups. However, there was one issue on which unanimous consensus was not met.

Rob Schuckard representing Sounds Advisory Group would like to have it recorded that, while he is in general agreement with the approach that is being taken for benthic effects monitoring, he holds a different view on the consequences of exceeding permitted Environmental Quality Standards (EQS) for the zone of maximum effect (ZME) under the net pens.

He noted that the development of the Standards was constrained by the consent conditions set by the Board of Inquiry (BoI). In his opinion, the BoI's finding that fallowing of the farm should occur when the enrichment stage (ES) underneath the net pens exceeds 5.6, was too high. Accordingly, he recommended that de-stocking and fallowing of the farm should occur at ES 5.1.

This view was based on a desire to adopt a generally better environmental outcome with a more conservative approach to farm management, by implementing an action level prior to achieving the point of peak worm (polychaete) abundance in the seabed sediments. He emphasised the importance of monitoring of the seabed by farm managers, using the qualitative tools set out in Table 6, to prevent permitted enrichment levels from being exceeded.

In light of the recent BoI determination that there be a gradation of consequences for exceedance of ES 5.0 (Table 5), which the other Working Group participants were in accord with, it was decided that Rob Schuckard's view be recorded as a dissenting view.

January 2018:

In discussions on the revision of the guidelines in 2018, the issue of sulphide trigger levels for Type 1 and Type 2 monitoring was revisited. This issue had been recorded in the Register of issues for future consideration in Table 11 of the guidelines.

The majority of working participants were in agreement to adopt a separate trigger for high flow farms in Table 5 with sulphide trigger levels informed by quantitative analysis of six years of farm data.

Rob Schuckard preferred to retain the existing trigger for both low flow and high flow farms. He pointed to a potential problem between sulphide levels used for the ES calculation and cited recent research which suggested existing sulphide measurement techniques are prone to estimation errors. Accordingly, he wished to have this recorded as a dissenting view.

10.2 APPENDIX B: CALCULATION OF ENRICHMENT STAGE

As stated in Section 4.1 the relationships between ES and the primary environmental indicators (as well as for some lesser known indicators) were described by Keeley et al. (2012a) (Figure 8 and Figure 9). Flow-specific relationships (i.e. for low and high flow sites) are provided for each variable, unless the analysis determined that there was no significant difference (Table 8). The initial criteria proposed for classification is whether the mid-water current speeds are above or below 10 cm s⁻¹. Using these relationships, the native values for each of these variables can be converted to an equivalent ES score (value from 1.0 to 7.0) which can then be combined quantitatively (by averaging) to arrive at an 'overall ES' that has an associated statistical variance. Hence, it is a multi-variable, 'weight-of-evidence' type approach.

The average overall ES score is calculated from a subset of the variables, focussing on those that best discern the enrichment gradient, are the most versatile (low and high flow situations) and provide complementary information (i.e. organic loading, sediment chemistry and infauna composition) (Keeley 2013). Accordingly, the selection of variables includes %OM (for organic loading), redox and sulfides (sediment chemistry), and total abundance, richness (number of taxa), H', AMBI and BQI (infauna composition, for definitions see Table 9). The 'overall ES' for a sample is given by a weighted average of those three groups of variables, where the greatest emphasis is placed on the biological indicators (infauna composition). The present weighting arrangement is: organic loading = 0.1, sediment chemistry = 0.2 and infauna composition = 0.7). Finally, the overall ES for the sampling station is given by the average of the (three) replicate samples and the variability between samples is reflected in the associated standard error.

The role of best professional judgment

While the quantitative method of determining ES described above works well for results that are within the 'normal' or expected range at NZ King Salmon sites, and hence removes much of the subjectivity in the assessment, there are still situations where professional judgement is required. For example, ES scores greater than 5.5 are poorly accommodated by most biotic indices (Keeley et al. 2012a). Additionally, some variables have a 'C-shaped' relationship with ES, meaning that a single Y-value can have two X-values (i.e. ES scores, e.g. log(N), Figure 9). Therefore, there remains a role for best professional judgment to correct or override potentially erroneous or misleading ES scores for individual variables.

The following are general rules that will be implemented to accommodate some of the more common issues:

- 1. Numerical bounds for the range of responses that were well described (i.e. the relationship between ES and each variable is considered reliable) have been determined from each plot. These bounds are referenced such that a 'best professional judgment' (BPJ) warning is triggered if the value is outside of the reliable range. This forces a manual allocation of the equivalent ES. In this case BPJ involves making reference to the values of other indicators for the sample, as well as making reference to historical trends.
- 2. Total number of taxa and %OM are both poor predictors of ES at low to moderate levels of enrichment at high flow sites. As stated previously, the use of this variable in the calculation of ES is going to be reviewed in the near future. In the meantime, the following rules are to be applied:

- a. The influence of the %OM result (i.e. 'organic loading' score) in the calculation of overall ES is down-weighted to 10% or 0.1.
- b. For %OM a look-up table has been created with the following categorical equivalencies for %OM to ES: 2% = ES 1.0, 3.5% = ES 2.0, 4% = ES 3.0, 6.5% = ES 4.0, 8% = ES 5.0, 12% = ES 6.0 and 16% = ES 7.0.
- c. For number of taxa (S), the equivalent ES score will not be utilised in the calculation of overall ES for samples where S > 20 (corresponding to the range over which S was an unreliable predictor of ES at HF sites).
- 3. The 'azoic' state that typifies ES 7.0 is virtually impossible to achieve in the strictest sense because the samples will almost always contain one or two individuals. The significance of these individuals with regard to ES is questionable as they could be from cross-contamination, or transient surface dwelling taxa, in which case the sample is still essentially 'azoic'. As this region of enrichment is poorly dealt with by most of the diversity measures it is manually assessed when abundance (N) < 800 and No. taxa (S) < 5 (true infauna). In this case the equivalent ES score is to be manually assigned for variables total abundance (N) and no. taxa (S).

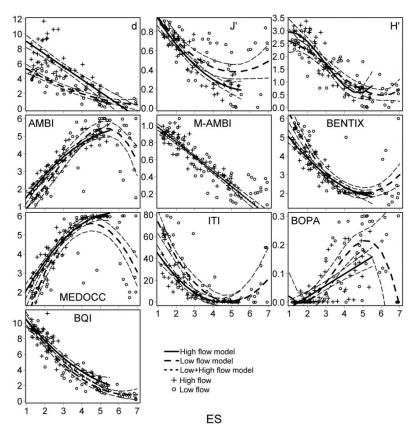


Figure 8: Scatterplots displaying optimum models with 95% confidence intervals for 10 biotic indices in relation to enrichment stage (ES) from Keeley et al. (2012a).

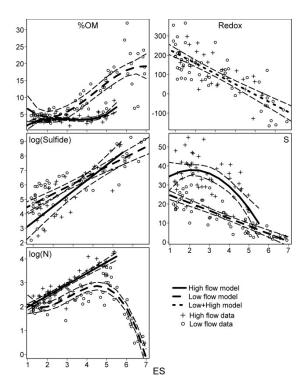


Figure 9: Scatterplots displaying optimum models with 95% confidence intervals for each of the physicochemical and biological indicators in relation to enrichment stage (ES) from Keeley et al. (2012a).

Table 8: Polynomial relationships used to derive equivalent enrichment stages (ES) score from native values of individual variables.

	ъ					D				
X Variable	Deg. Poly	x^3	\mathbf{x}^2	x	Int	Res. SE	df	Mult. R ²	Adj. R ²	p-value
Abundance	1			1.7636	-2.1658	0.2698	54	0.828	0.824	<2.2e-16
AMBI	1			1.0221	-0.727	0.4667	54	0.863	0.8605	<2.2e-16
BENTIX	3	-0.4664	5.0795	-18.7551	25.7266	0.395	52	0.9055	0.9	< 2.2e-16
BOPA	2			11.415	1.705	0.932	54	0.0453	0.4433	0.1298
BQI	2		0.055	-1.123	7.321	0.4296	53	0.8861	0.8818	<2.2e-16
ITI	3	-7.9E-05	0.008428	-0.2766	4.652	0.4685	52	0.867	0.8594	<2.2e-16
M-AMBI	3	12.631	-19.453	3.236	5.391	0.4363	52	0.8847	0.8781	<2.2e-16
MEDOCC	3	0.1798	-2.0459	8.0918	-8.8829	0.3583	52	0.9222	0.9178	<2.2e-16
No. Taxa	1			-0.0691	5.2102	0.9984	54	0.3731	0.3615	5.76E-07
P. evenness	1			-4.7849	5.3782	0.5597	54	0.803	0.7993	<2.2e-16
Redox	1			-0.009	4.129	0.923	28	0.513	0.4956	8.54E-06
Richness	3	0.0084	-0.0967	-0.2654	5.67	0.588	52	0.7906	0.7785	<2.2e-16
log(Sulfides)	1			1.4885	-0.5738	0.7925	23	0.6936	0.6803	2.4E-07
SWDI	2		0.4113	-2.6661	6.0768	0.4821	53	0.8565	0.8511	<2.2e-16
TOM	1			0.4854	1.1525	1.123	54	0.2075	0.1928	0.0004
LOW FLOW										
LOWFLOW						_				
X Variable		\mathbf{x}^3	\mathbf{x}^2	X	Int	Res. SE	df	Mult. R ²	Adj. R ²	p-value
	1	x ³	\mathbf{X}^2	x 1.9641	Int -1.5924		df 49		Adj. R² 0.5366	p-value 6.03E-10
X Variable	1	x ³	\mathbf{x}^2			SE		Mult. R ² 0.5459 0.8123		•
X Variable Abundance		x³ -0.191	x ² 2.4441	1.9641	-1.5924	SE 0.9482	49	0.5459	0.5366	6.03E-10
X Variable Abundance AMBI	1			1.9641 0.7464	-1.5924 0.5517	SE 0.9482 0.6363	49 51	0.5459 0.8123	0.5366 0.8086	6.03E-10 <2.2e-16
X Variable Abundance AMBI BENTIX	1	-0.191	2.4441	1.9641 0.7464 -10.59	-1.5924 0.5517 17.707	SE 0.9482 0.6363 0.6299	49 51 49	0.5459 0.8123 0.8233	0.5366 0.8086 0.8124	6.03E-10 <2.2e-16 < 2.2e-16
X Variable Abundance AMBI BENTIX BOPA	1 3 3	-0.191	2.4441 -309.707	1.9641 0.7464 -10.59 49.043	-1.5924 0.5517 17.707 1.931	SE 0.9482 0.6363 0.6299 0.716	49 51 49 49	0.5459 0.8123 0.8233 0.7162	0.5366 0.8086 0.8124 0.6988	6.03E-10 <2.2e-16 < 2.2e-16 1.04E-08
X Variable Abundance AMBI BENTIX BOPA BQI	1 3 3 2	-0.191	2.4441 -309.707 0.071	1.9641 0.7464 -10.59 49.043 -1.278	-1.5924 0.5517 17.707 1.931 7.177	SE 0.9482 0.6363 0.6299 0.716 0.7712	49 51 49 49 62	0.5459 0.8123 0.8233 0.7162 0.8293	0.5366 0.8086 0.8124 0.6988 0.82338	6.03E-10 <2.2e-16 <2.2e-16 1.04E-08 <2.2e-16
X Variable Abundance AMBI BENTIX BOPA BQI ITI	1 3 3 2 2	-0.191	2.4441 -309.707 0.071	1.9641 0.7464 -10.59 49.043 -1.278 -0.1149	-1.5924 0.5517 17.707 1.931 7.177 4.6425	SE 0.9482 0.6363 0.6299 0.716 0.7712 0.7036	49 51 49 49 62 50	0.5459 0.8123 0.8233 0.7162 0.8293 0.775	0.5366 0.8086 0.8124 0.6988 0.82338 0.766	6.03E-10 <2.2e-16 <2.2e-16 1.04E-08 <2.2e-16 <2.2e-16
X Variable Abundance AMBI BENTIX BOPA BQI ITI M-AMBI	1 3 3 2 2 2 3	-0.191 606.81	2.4441 -309.707 0.071 0.001	1.9641 0.7464 -10.59 49.043 -1.278 -0.1149 -5.396	-1.5924 0.5517 17.707 1.931 7.177 4.6425 6.6615	SE 0.9482 0.6363 0.6299 0.716 0.7712 0.7036 0.8949	49 51 49 49 62 50 63	0.5459 0.8123 0.8233 0.7162 0.8293 0.775 0.7665	0.5366 0.8086 0.8124 0.6988 0.82338 0.766 0.7628	6.03E-10 <2.2e-16 <2.2e-16 1.04E-08 <2.2e-16 <2.2e-16 <2.2e-16
X Variable Abundance AMBI BENTIX BOPA BQI ITI M-AMBI MEDOCC	1 3 3 2 2 2 3 3	-0.191 606.81	2.4441 -309.707 0.071 0.001 -1.4981	1.9641 0.7464 -10.59 49.043 -1.278 -0.1149 -5.396 5.7823	-1.5924 0.5517 17.707 1.931 7.177 4.6425 6.6615 -4.9129	SE 0.9482 0.6363 0.6299 0.716 0.7712 0.7036 0.8949 0.7098	49 51 49 49 62 50 63 49	0.5459 0.8123 0.8233 0.7162 0.8293 0.775 0.7665	0.5366 0.8086 0.8124 0.6988 0.82338 0.766 0.7628 0.7618	6.03E-10 <2.2e-16 <2.2e-16 1.04E-08 <2.2e-16 <2.2e-16 <2.2e-16 6.35E-16
X Variable Abundance AMBI BENTIX BOPA BQI ITI M-AMBI MEDOCC No. Taxa	1 3 3 2 2 3 3 2	-0.191 606.81	2.4441 -309.707 0.071 0.001 -1.4981	1.9641 0.7464 -10.59 49.043 -1.278 -0.1149 -5.396 5.7823 -0.373	-1.5924 0.5517 17.707 1.931 7.177 4.6425 6.6615 -4.9129 7.0575	0.9482 0.6363 0.6299 0.716 0.7712 0.7036 0.8949 0.7098 0.8674	49 51 49 49 62 50 63 49 62	0.5459 0.8123 0.8233 0.7162 0.8293 0.775 0.7665 0.7756	0.5366 0.8086 0.8124 0.6988 0.82338 0.766 0.7628 0.7618	6.03E-10 <2.2e-16 <2.2e-16 1.04E-08 <2.2e-16 <2.2e-16 <2.2e-16 6.35E-16 <2.2e-16
X Variable Abundance AMBI BENTIX BOPA BQI ITI M-AMBI MEDOCC No. Taxa P. evenness	1 3 3 2 2 2 3 3 3 2	-0.191 606.81	2.4441 -309.707 0.071 0.001 -1.4981	1.9641 0.7464 -10.59 49.043 -1.278 -0.1149 -5.396 5.7823 -0.373 -4.2216	-1.5924 0.5517 17.707 1.931 7.177 4.6425 6.6615 -4.9129 7.0575 5.5967	0.9482 0.6363 0.6299 0.716 0.7712 0.7036 0.8949 0.7098 0.8674 0.9601	49 51 49 49 62 50 63 49 62 50	0.5459 0.8123 0.8233 0.7162 0.8293 0.775 0.7665 0.7756 0.7841 0.5709	0.5366 0.8086 0.8124 0.6988 0.82338 0.766 0.7628 0.7618 0.7771	6.03E-10 <2.2e-16 < 2.2e-16 1.04E-08 <2.2e-16 <2.2e-16 <2.2e-16 6.35E-16 <2.2e-16 9.54E-11
X Variable Abundance AMBI BENTIX BOPA BQI ITI M-AMBI MEDOCC No. Taxa P. evenness Redox	1 3 3 2 2 3 3 3 2 1 3	-0.191 606.81	2.4441 -309.707 0.071 0.001 -1.4981 0.0067	1.9641 0.7464 -10.59 49.043 -1.278 -0.1149 -5.396 5.7823 -0.373 -4.2216 -0.009	-1.5924 0.5517 17.707 1.931 7.177 4.6425 6.6615 -4.9129 7.0575 5.5967 4.129	SE 0.9482 0.6363 0.6299 0.716 0.7712 0.7036 0.8949 0.7098 0.8674 0.9601 0.923	49 51 49 62 50 63 49 62 50 28	0.5459 0.8123 0.8233 0.7162 0.8293 0.775 0.7665 0.7756 0.7841 0.5709 0.513	0.5366 0.8086 0.8124 0.6988 0.82338 0.766 0.7628 0.7618 0.7771 0.5623 0.4956	6.03E-10 <2.2e-16 < 2.2e-16 1.04E-08 <2.2e-16 <2.2e-16 <2.2e-16 6.35E-16 <2.2e-16 9.54E-11 8.54E-06
X Variable Abundance AMBI BENTIX BOPA BQI ITI M-AMBI MEDOCC No. Taxa P. evenness Redox Richness	1 3 3 2 2 2 3 3 2 1 3 2	-0.191 606.81	2.4441 -309.707 0.071 0.001 -1.4981 0.0067	1.9641 0.7464 -10.59 49.043 -1.278 -0.1149 -5.396 5.7823 -0.373 -4.2216 -0.009 -1.6091	-1.5924 0.5517 17.707 1.931 7.177 4.6425 6.6615 -4.9129 7.0575 5.5967 4.129 6.6194	SE 0.9482 0.6363 0.6299 0.716 0.7712 0.7036 0.8949 0.7098 0.8674 0.9601 0.923 0.8734	49 51 49 62 50 63 49 62 50 28	0.5459 0.8123 0.8233 0.7162 0.8293 0.775 0.7665 0.7756 0.7841 0.5709 0.513 0.7651	0.5366 0.8086 0.8124 0.6988 0.82338 0.766 0.7628 0.7618 0.7771 0.5623 0.4956 0.7569	6.03E-10 <2.2e-16 <2.2e-16 1.04E-08 <2.2e-16 <2.2e-16 <2.2e-16 <2.2e-16 <2.2e-16 <2.2e-16 <2.2e-16 <2.2e-16 <2.2e-16

Table 9:	Definitions of selected biological indicators.
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Indicator	Calculation and description	Source reference
N	Sum (n)	-
	Total infauna abundance = number of individuals per 13 cm diameter core	
S	Count (taxa)	-
	Taxa richness = number of taxa per 13 cm diameter core	
d	(S-1) /log N	Margalef (1958)
	Margalef's diversity index. Ranges from 0 (very low diversity) to about 12 (very high diversity)	
J'	H'/log S	Pielou (1966)
	Pielou's evenness. A measure of equitability, or how evenly the individuals are distributed among the different species. Values can range from 0.00 to 1.00, a high value indicates an even distribution and a low value indicates an uneven distribution or dominance by a few taxa.	
H'	$-\sum_i p_i \log(p_i)$	-
	where p is the proportion of the total count arising from the ith species	
	Shannon-Weiner diversity index (SWDI). A diversity index that describes, in a single number, the different types and amounts of animals present in a collection. Varies with both the number of species and the relative distribution of individual organisms among the species. The index ranges from 0 for communities containing a single species to high values for communities containing many species with each represented by a small number of individuals.	
AMBI	= $[(0 \times \%GI + 1.5 \times \%GII + 3 \times \%GIII + 4.5 \times \%GIV + 6 \times \%GV)]/100$	Borja et al. (2000)
	where GI, GII, GIV and GV are ecological groups.	
	Azites Marine Biotic Index: relies on the distribution of individual abundances of soft-bottom communities according to five Ecological Groups (GI-GV). GI being species sensitive to organic pollution and present under unpolluted conditions, whereas, at the other end of the spectrum, GV species are first order opportunists adapted to pronounced unbalanced situations (i.e. <i>Capitella capitata</i>). Index values are between 1 (normal) and 6 (extremely disturbed)	
M-AMBI	Uses AMBI, S and H', combined with factor analysis and discriminant analysis (see source reference).	Muxika et al. 2007)
	Multivariate-AMBI. Integrates the AMBI with measures of species richness and SWDI using discriminant analysis (DA) and factorial analysis (FA) techniques. Utilises reference conditions for each parameter (based on 'pristine conditions') that allows the index to be tailored to accommodate environments with different base ecological characteristics. Scores are from 1 (high ecological quality) to 0 (low ecological quality).	
BQI	$= \left(\sum_{i=1}^{n} \left(\frac{A_i}{totA} \times \text{ES50}_{0.05i}\right)\right) \times {}^{10}\text{log}(S+1)$	Rosenberg et al. (2004)
	Where ES50 = expected number of species as per Hurlbert (1971)	
	And, ES50 _{0.05} the species tolerance value, given here as the 5 th percentile of the ES50 scores for the given taxa as per Rosenberg et al. (2004).	
	Benthic quality index: uses species specific tolerance scores (ES50 _{0.05}), abundance and diversity factors. Results can range from 0 (being highly impacted) and 20 (reference conditions).	

10.3 APPENDIX C: ALTERNATIVE EQS COMPLIANCE TABLE

Table 10: Alternative way of displaying the relationship between mean ES value, the size of the lower bound of the associated 95% Confidence Interval (relative to the mean) and the required management action level of response (refer Section 4.2).

95%CI\Mean	4.5	4.6	4.7	4.8	4.9	5.0	5.1	5.2	5.3	5.4	5.5	5.6	5.7	5.8	5.9	6.0	116	
0.0																		NO ACTION REQUIRED
0.1																		ALERT
0.2																		MINOR
0.3																		MAJOR
0.4																		DESTOCKING
>0.5																		

10.4 APPENDIX D: REGISTER OF ISSUES FOR FUTURE CONSIDERATION BY THE BENTHIC STANDARDS WORKING GROUP

Subsequent to external peer review and the public comment phase, several technical issues were identified by the Benthic Working Group. These areas are not critical for the functioning of the document as it stands. Resolution of the issues may potentially improve the BMP, although this is by no means certain. Therefore, as the document has already been subjected to external peer review and undergone public commentary, it was determined that these issues should be recorded on a register of issues for future consideration (Table 11). The BMP in their entirety are intended to be formally reviewed five-yearly after their finalisation.

Table 11: Register of issues for future consideration by the benthic standards working group

No.	Description	Current situation	Analysis	Outcome Improvement
1.	Sulphide trigger levels for Type 1 and Type 2 monitoring	2015: Envirolink medium advice grant MLDC97 (Cawthron – Dr Nigel Keeley) involves analysing 7 years of sulphide data with ES scores at existing farms. 2018: Report produced from the Envirolink recommended separate triggers for the ZME for high and low flow farms (2400 and 1700 respectively). Not doing so would mean that it would be unlikely that Type 1 monitoring would ever be possible at high flow sites, and this was not the intention of the tiered strategy. The working group reviewed and discussed findings of Cranford et al. (2017) with regards to the trigger level, which outlines a new (improved) approach for measuring TFS.		The appropriateness of the triggers should be reanalysed with the inclusion of the last 3-4 years' worth of data. The ES values against which sulphides is compared should not include sulphides in their calculation. This should be made available for review in the next BWG meeting.
2.	Revisit the confidence intervals and the acceptable degree of accuracy	The Enrichment Stage (ES) data are presented with 95% confidence intervals. 95% confidence intervals are acceptable scientific practice for determining the distribution of a population (or ES level in this case).	The question is whether a lower level of accuracy (i.e., 80% confidence intervals) are acceptable for determining compliance. The advantage of 80% CI's is that there may be less overlap with ES trigger levels for reducing feed or fallowing above ES 5.0	Discussed in the 2018 meeting. It was concluded that the current situation is probably adequate, however there was not really enough information with respect to compliance on hand to

3.	Adjust the Alert level to require action after 12 months and full compliance after 24 months	Currently if the Alert level is triggered (Table 4 of the benthic guidelines), "the consent holder must provide a written management response intended to reduce the level of seabed enrichment. The response plan must be made available to Council with 20 working days of having received the final annual monitoring reports." This is when the 95% CI span ES5.0 but do not exceed ES 5.3 (see diagram below from Fig 6 of the guidelines)	A larger CI means that the mean ES can be a bit higher whilst remaining in the 'Alert' status. There is a perspective that taken to the extreme a site can be repeatedly be in a state where Mean ES > 5. This may risk a situation where the <i>true</i> mean (as opposed to <i>sample</i> -mean) could quite readily be 5.5 or more. In addition, the absence of any penalty should the 'alert status' be repeatedly triggered (without triggering the 'minor action status') could be a potential inconsistency between the stated goals of the system (aim to keep mean ES <=5.0), and the incentives created by the regulation regime.	determine its true appropriateness. To be reassessed at the next meeting.
4.	Potential implementation of new eDNA (metabarcoding) monitoring technologies (Pochon et al. 2015; Keeley et al. 2018)	eDNA metabarcoding of bacteria and eukaryotes looks promising for characterise benthic organic enrichment gradients around salmon farms. Dr's Pochon and Keeley demonstrated that it has the potential to be a faster, cost effective substitute for the macro-invertebrate indicator in calculating the ES at the ZME (Zone of Maximal Effect) and OLE (Outer Limit of Effect). It was concluded that it was appropriate to reflect the potential of eDNA in the BMP.	Pochon and Keeley recommended that further testing would be necessary to ensure that it was robustly validated against the conventional method. Cawthron scientists to advance testing and validation for review in subsequent BWG meeting.	
5.	Methods development for Type 1 monitoring	A small Envirolink has been obtained to develop the methods for Type 1 (qualitative) monitoring. A protocol was tested in 2018, and should be available by end-2019.	Currently, very few of the farms meet the criteria that would allow them to be monitoring using Type 1 methods but it is relevant at new and retired sites. A protocol was tested in 2018, and should be available by end-2019.	

10.5 APPENDIX E: REDOX AND TOTAL FREE SULPHIDES ANALYTICAL PROCEDURES

In order for the sediment geochemistry results to be used in conjunction with the established variable – ES relationships and for them to continue to be comparable in space and time, redox and TFS should be assessed using the following methods.

10.5.1 Redox

SAMPLE COLLECTION (specific to salmon farm routine monitoring)

At each sampling station, samples are typically collected in triplicate, using a van Veen grab operated from a boat. Each successful (full) grab constitutes a replicate. Redox potential is measured directly from the grab at the sediment/water interface <1 cm depth using a probe (see next section). When the meter reads the same whole number for three or more readings, the result is assumed to be stabilised. Between samples, the probe is rinsed and stored in freshwater.

PRINCIPLE OF METHOD

The method is based on that detailed in Wildish et al. (1999), and uses a redox probe coupled with a millivolt (mV) meter. Undisturbed sediment is measured directly using the probe from the sampler, immediately after sample retrieval onto the boat.

Measurements are recorded in relative mV, whereby the temperature is set either with a probe that integrates the temperature, or prior to sampling with a separate temperature probe.

After use, the probe is rinsed in Milli-Q, cleaned with a gently non- abrasive tissue or brush, and stored in KCl solution. Calibration of the probe is done prior to long field trips, or if the instrument has been in storage for more than 1–2 months.

INSTRUMENT STORAGE AND CALIBRATION

Calibration is usually conducted using a 220mV solution (solution and method as per manufacturer's guidelines).

QUALITY CONTROL CRITERIA

If drift is observed in the results, the probe is cleaned using a bottle brush or other gently abrasive material. If readings are still not consistent with the sample qualitative observations, the instrument is re-calibrated.

REFERENCES

Wildish, D.; Akagi, H.; Hamilton, N.; Hargrave, B. (1999). A recommended method for monitoring sediments to detect organic enrichment from mariculture in the Bay of Fundy. *Canadian Technical Report of Fisheries and Aquatic Sciences No.* 2286.

10.5.2 Total free sulphides

SAMPLE COLLECTION (specific to salmon farm routine monitoring)

At each sampling station, samples are collected in triplicate, using a van Veen grab operated from a boat. Each successful (full) grab constitutes a replicate that is sub-sampled for sulphides using a 5 ml syringe inserted into the surface sediment at a 45° angle. The syringe is sealed using a rubber bung, ensuring no visible air bubbles are trapped within the sample. Samples are chilled for transportation and short-term storage until they can be analysed (within 24 hours).

PRINCIPLE OF METHOD

A method using a sulphide probe coupled with a millivolt (mV) meter has been implemented at Cawthron for testing of sediment samples collected by the Coastal and Freshwater group. Sediment samples are buffered 1:1 with a high pH (~12) buffer containing complexing reagent (EDTA) and anti-oxidant (ascorbic acid). This buffer is termed sulphide antioxidant buffer with ascorbic acid (SAOB:AA). The response of a sulphide specific electrode is measured in mV. This response is changed to a concentration, by referring to a spreadsheet relating mV of standard to concentration of standard. The standards are prepared in the same way as samples.

1. ANALYTICAL PROCEDURE

1.1 Standard Preparation

- 1.1.1. Sulphide (Na₂S· 9 H₂0) ~ 20 g/L or 86,000 μ M:
 - a. Prepare a primary standard from clear, crystalline Na₂S· 9 H₂O
 - b. In a hood, place a small chunk (~5 g) of the Na₂S· 9 H₂O in a small mortar and break with the pestle to a size that will pass through the neck of a 50 mL volumetric flask.
 - c. Weigh ~ 1 g into a 50 mL volumetric flask (in hood if possible) and record the weight to 4 decimal places. See 8.2
 - d. Dilute the stock standard to 50 mL with previously degassed MilliQ water.
 - e. Mix to dissolve.
 - Sonication may be used to speed up dissolution, but allow solution to cool to room temperature prior to use. Standardise using the assay procedure in USP sodium sulphide monograph, 20 mL aliquot. (See GMP for reagents).
 - f. Expiry: 3 days at 2-8°C

1.1.2. Working Mixed Standards (8-86,000 µM)

- a. Shortly before analysis of the samples, make 10x dilutions for a series of five standard concentrations.
- b. Standards to be labelled and dilutions to be prepared as:

Standard name	Aliquot Solution ID	Volume Aliquot (mL)	Final Volume (mL)	Concentration (µM)	Volume remaining for reaction with SAOB:AA (mL)
Standard 1 (S1)	Stock	10	10	86,000	10
Standard 2 (S2)	S 1	1	10	8,600	9
Standard 3 (S3)	S2	1	10	860	9
Standard 4 (S4)	S 3	1	10	86	9
Standard 5 (S5)	S4	1	10	9	10

- c. All standard solutions are to be diluted to volume with degassed MilliQ water.
- d. For each working standard, transfer the volume remaining for reaction with reagents (SAOB:AA) into separate small containers such as clear 50 mL Falcon tubes or 20 mL scintillation vials.

1.2 Sample Preparation

- 1.1.3. Note the sample volume of the sediment sample as indicated on the sampling syringe- likely ~5 mL. This is important in determining the volume of SAOB:AA to add to the sample.
- 1.1.4. Allow samples to thaw to room temperature if they have been frozen or cooled.
- 1.1.5. Dispense ~5 mL of the sample into a 50 mL Falcon tube.

1.3 Ion Specific Electrode Measurement

- 1.1.6. In the 50 mL Falcon tube containing standard or sample, add an equal volume of SAOB:AA solution to the sample (dilute the sample or standard 1:1 with SAOB-AA solution).
 - a. For standards, prepared at the same time as the samples, add the following volumes of SAOB-AA to each solution using a 10 mL adjustable pipettor:
 - 10 mL SAOB-AA to S1 (Final volume ~20 mL)
 - 9 mL SAOB-AA to S2 (Final volume ~18 mL)
 - 9 mL SAOB-AA to S3 (Final Volume ~18 mL)
 - 9 mL SAOB-AA to S4 (Final Volume ~18 mL)
 - 10 mL SAOB-AA to S5 (Final Volume ~20 mL)
 - Mix and dispense the standard-SAOB-AA into 20 mL scintillation vials. Place a small magnetic stir bar in the vial before measurement with the electrode, stirring at 200 rpm.
 - Record mV of the standards after the reading has stabilised.
 - b. For samples, add an equal volume of SAOB:AA solution (5 mL) to the 5 mL of sample for a total volume of 10 mL.
- 1.1.7. Cap the 50 mL tube, and shake vigorously to mix or to break the sample up and create a slurry. Complete dissolution can be assessed by a change in the sound when shaking the sample (e.g. a change from a tapping noise for a large solid sample when shaken to a swishing noise).
- 1.1.8. Suspend and centrifuge the sample tubes in groups of nine + one balance tube. (1500 rcf for 5 min at room temperature (15-20°C).

- 1.1.9. Decant the supernatant into a 20 mL scintillation vial, add a small stirring bar, place on a magnetic stirrer set at 200 rpm.
- 1.1.10. Place the electrode in the solution and record the mV reading at equilibrium, such that no drift is observed in the mV measurement. (Ensure the meter is reading mV).
 - a. Each standard should have approximately 30 mV difference from the previous standard.
 - b. i.e S1 = -941 mV, S2 = -914 mV, S3 = -882 mV
- 1.1.11. Once samples are mixed 1:1 with SAOB:AA, they have a short term stability for sulphide analysis. After approximately 1 hr, samples should be discarded as they are no longer useful. (The colour of the solution changes over this time to a pinkish tinge).
- 1.1.12. Rinse the electrode prior to use with a new sample.
 - a. While the sides of the probe may be wiped down, do not wipe the flat probe face on the bottom of the probe.
- 1.1.13. Using the spreadsheet, plot the concentration in μM (log) vs. mV (linear).
- 1.1.14. Enter each sample mV reading on the spreadsheet to calculate its corresponding sulphide concentration.

1.4 Electrode Storage

- 1.1.15. Store the sulphide probe in 4M potassium chloride solution for up to 1 week.
- 1.1.16. For storage longer than one week, drain the probe dry and rinse 3 times with MilliQ water before emptying completely.
 - a. Ensure cap is on the probe surface and store dry in the box.
 - b. When restoring the probe for use after long term storage, the day before intended use, rinse the probe 3 times with electrode filling solution.
 - c. Allow the probe to sit overnight in 4 M KCl solution
- 1.1.17. Fresh electrode filling solution should be placed in the probe each day of use. Probe was previously filled with "Solution A", up to the filling hole. However, no difference was observed when only filled up the the "N" of "Orion".

QUALITY CONTROL CRITERIA

System Suitability Criteria

a. Ensure the mV readings for the standards are consistent with historical and that there is a \sim 30 mV difference for each 10 fold dilution of standard. ($R^2 > 0.9$).

10.6 APPENDIX F: PANEL MEMBERS

