Fisheries New Zealand
Tini a Tangaroa

Best management practice guidelines for salmon farms in the Marlborough Sounds

Part 1: Benthic environmental quality standards and monitoring protocol (Version 1.2 August 2022)

New Zealand Aquatic Environment and Biodiversity Report No. 294

Fletcher, L.; Bennett, H.; Elvines, D.; Preece, M.; Broekhuizen, N.; Ford, R.; Heath, P.; Murray, C.; Jorgensen, E.; Wade, O.; Ferguson, G.

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This report is an update of Keeley et al. (2019), prepared for Fisheries New Zealand by the Benthic Standards Working Group:

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EXECUTIVE SUMMARY

Fletcher, L.; Bennett, H.; Elvines, D.; Preece, M.; Broekhuizen, N.; Ford, R.; Heath, P.; Murray, C.; Jorgensen, E.; Wade, O.; Ferguson, G. (2022). Best management practice guidelines for salmon farms in the Marlborough Sounds: Part 1: Benthic environmental quality standards and monitoring protocol (Version 1.2 August 2022).

***New Zealand Aquatic Environment and Biodiversity Report No. 294*. 53 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: Cawthron Institute, New Zealand King Salmon, National Institute of Water and Atmospheric Research, the Ministry for Primary Industries/Fisheries New Zealand, Marlborough District Council (MDC), and the Sounds Advisory Group to MDC. 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. The guidance document was first published in 2015, revised in 2019, and further revised in this version after an out-of-cycle review in 2022.

The out-of-cycle review largely focused on the inclusion of environmental DNA (eDNA) monitoring as a faster and cheaper alternative to the traditional macrofauna monitoring used to assess benthic enrichment. A bacterial Metabarcoding Biotic Index (b-MBI) derived from eDNA sampling can be used to calculate an enrichment stage (ES) score against which compliance can be determined.

The out-of-cycle review provided an opportunity to assess other aspects of the guidelines which were also updated and are included in this version. It was decided that bacterial mats are not sufficiently reliable to act as sole indicators of benthic enrichment > ES5, and the definition of "patchy" as it relates to bacterial mats has been clarified. Further clarification has been included related to the definition of the ES5 threshold and a calculation for 'Overall ES' has been included. Commentary related to variable feed discharges at salmon farms has been provided, particularly related to the period of maximum feed discharge because it may influence the timing of benthic monitoring. A monitoring protocol has been developed for Type 1 monitoring in accordance with previous recommendations from the Benthic Standards Working Group and has been published separately from these guidelines.

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, developed in an integrated working group environment, was completed in 2014 (Keeley et al. 2015) and was revised in 2018 (Keeley et al. 2019). The working group conducted an out-of-cycle review in 2022 to incorporate new monitoring technologies and update certain aspects of the guidelines; this document includes these changes. The working group included representation from:

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

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. While it was determined that a centralised regional BMP be developed, and that ideally all salmon farm consents should include a standard condition that incorporates compliance with the BMP, in reality this has not been achievable. It remains a goal for future development.

The current application of this BMP is therefore to provide consistent and clear requirements for the management and the independently conducted annual benthic monitoring for a small number 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 1.2 of this document and is an update of version 1.1 (Keeley et al. 2019).

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 focused on contemporary farming practices.

The five key components that were identified for developing the benthic standards and monitoring protocols are given below.

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. 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.

Issues pertaining to water column environmental standards are addressed by separate best practice guidelines (Elvines et al. 2019). 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).

Out-of-cycle review August 2022

During the review cycle of the best management practice (BMP) in 2019, some new monitoring technologies were discussed that had potential for integration into the BMP guidelines. Additionally, after several years of implementation, some aspects of the guidelines have been identified that would also benefit from revision. In response to this, Fisheries New Zealand contracted the Cawthron Institute to undertake a desk-top assessment to determine the relative importance of these issues and whether they warrant an out-of-cycle review (Keeley et al. 2021).

Collectively, the review of issues suggested that an out-of-cycle revision of the BMP guidelines was warranted. The most pressing and important changes considered are presented in Table 1. Note: a glossary of terms is provided in Section 11.

Table 1: Issues identified as potential topics for the out-of-cycle BMP review. (Continued on next page)

	Issue	Notes from preliminary review	Included in this review
1	Use of b-MBI (eDNA) for deriving ES	This method has been validated and the proposed next step for this work is incorporating it into the BMP guidelines.	Yes Section 4.1 and Appendix D
2	Use UV method to measure total free sulphide	While the new method is more accurate and provides a more user-friendly, cost-effective option than the current ISE method, it requires further development of method-specific thresholds.	No
3	Clarify that bacterial mats are not used as sole indicators of ES5	Bacterial mats alone are not sufficiently reliable to act as sole indicators of enrichment >ES5.	Yes Section 4.2
4	Statistical comparisons at OLE stations	While recognising the need to move away from professional judgement, this is not easily resolved and will need to be looked at more thoroughly.	No

Table 1: continued.

	Issue	Notes from preliminary review	Included in this review
5	Clarify the definition of the ES5 threshold	Enrichment Stage 5 was originally defined to be the point of peak macrofaunal abundance but is better thought of as a region of near maximum abundance. This region on the enrichment spectrum is important because further enrichment leads to rapid decline in abundance. Concern that undue weight may be applied to single variables. Therefore, need to ensure that the threshold is estimated based on combination of variables.	Yes Section 4.2
6	Versatility of the ES metric	Use of the metric for sites outside of the Marlborough Sounds will likely be important for future situations (e.g., Open Ocean Aquaculture (OOA), climate change) but is beyond the scope of this review.	No
7	Site flow-specific standards	Site specific standards will benefit from further discussion and are important for re-consenting of sites. They will likely require extending the conversation as it involves societal aspects.	No
8	Provision of example resource consent conditions	While potentially useful this is beyond the scope of this review.	No
9	Variable feed discharges	There will be some challenges with adjusting existing consents where this may apply, but in principle it can be addressed here.	Yes Sections 3.1.3 and 6.1
10	Clarification on how ES is calculated (access and proprietary information)	Appropriate calculation is available and can be included in the BMP.	Yes Appendix B
11	Clarification on intent around the use of ES (i.e., ES as sole indicator vs. individual variables for determining compliance)	If the BMP review supported an approach either of individual variables or total ES this could support the applicant in their application for a variation to existing conditions to align with the BMP.	Yes Section 4.2
12	Inclusion of Type 1 monitoring strategy	A monitoring protocol has been developed for Type 1 monitoring in accordance with previous recommendations from the BSWG. It is timely that they should be considered for formal inclusion and implementation.	Yes Section 3
13	Definition of "patchy" in regard to bacterial mats	Clarification will benefit interpretation.	Yes

1.1 Objectives

The broad over-arching objectives that underpin this benthic monitoring protocol are given below.

- To develop a standardised and accepted protocol to assess environmental compliance.
- To comply with international best practices[1] at a minimum, and where appropriate[2].
- To support environmentally responsible and profitable aquaculture.
- To minimise impacts on the environment and thereby minimise risks to biodiversity and associated ecosystem processes.
- To ensure sustainable management[3].
- To provide a monitoring and reporting approach that is fit-for-purpose (user-friendly, focused, 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 (and subsections) should provide the geographical and physical setting of the research and describe survey design, sampling methods, and so on, depending on the nature of the project. It is important that any statistical techniques and analytical methods be fully explained (if new) or referenced. This section outlines the 'zones concept' and provides the rationale behind the five proposed monitoring locations (Table 2). 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).[4] 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 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

[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 BoI 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.

[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".

[4] Note 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.

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 close proximity and share physical (depth, flow) and substrate properties. Furthermore, Type 3 monitoring (Table 3) 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, VenOM). 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 2: Description of, and rationale for, proposed monitoring locations. Distances are indicative and will ultimately be determined by Type 3 monitoring.

Monitoring locations	Description and rationale	Position	
		Low flow	High flow
ZME	**Zone of maximum effects station:** Worst-case scenario. 'Checkpoint' for goal of maintaining functional/productive macrofauna, which is important for waste assimilation and sustainability.	Sampled beneath or at edge of pens.	Sampled at edge of pens, or nearby if area of 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 the 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 × 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 6 and associated Footnote 23.
[6] The footprint delineated by the OLE, outside the direct influence of farm derived particulates.

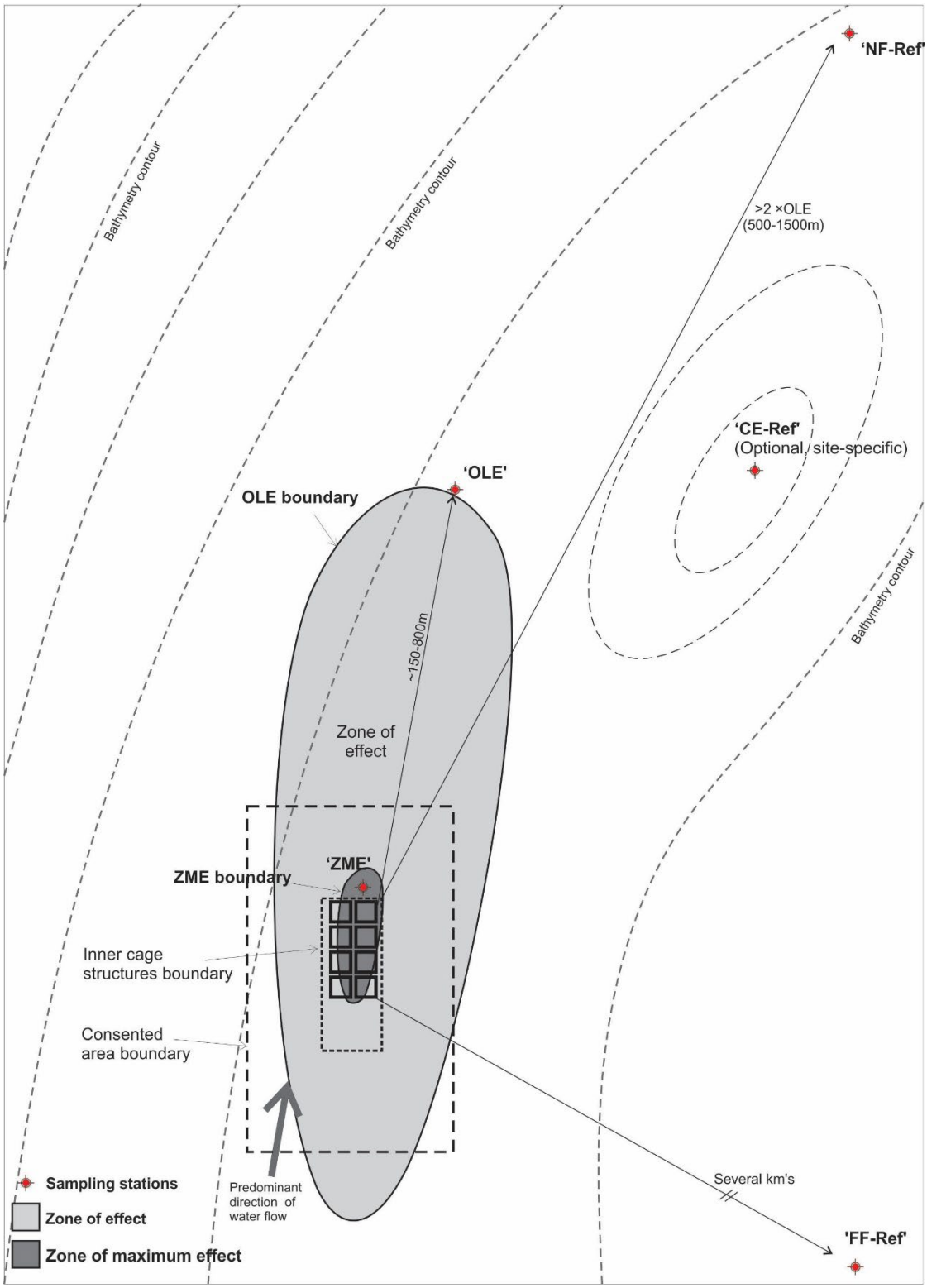

Figure 1: Zones concept with theoretical positions of sampling stations in relation to the farm and potential distortion of the footprint shape due to currents. ZME = zone of maximum effect, OLE = outer limit of effects, NF-Ref = near-field reference, FF-Ref = far-field reference (see Table 2 for further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

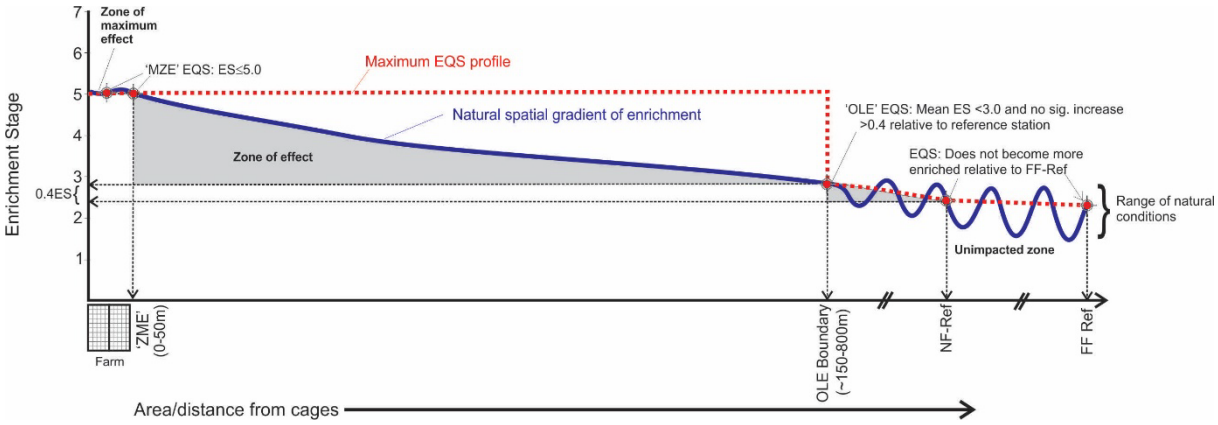

Figure 2: Stylised depiction of natural spatial enrichment gradient as permitted by the zones concept and associated environmental quality standards (EQS) in terms of overall enrichment stage (ES), along with 'maximum EQS profile' which represents the improbable, but maximum possible, EQS profile. ZME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

- Sampling regularly along radial transects to review whether the spatial arrangement of monitoring captures the zone of maximum effect.
- 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'[8], 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 are discussed in Section 6.

[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.

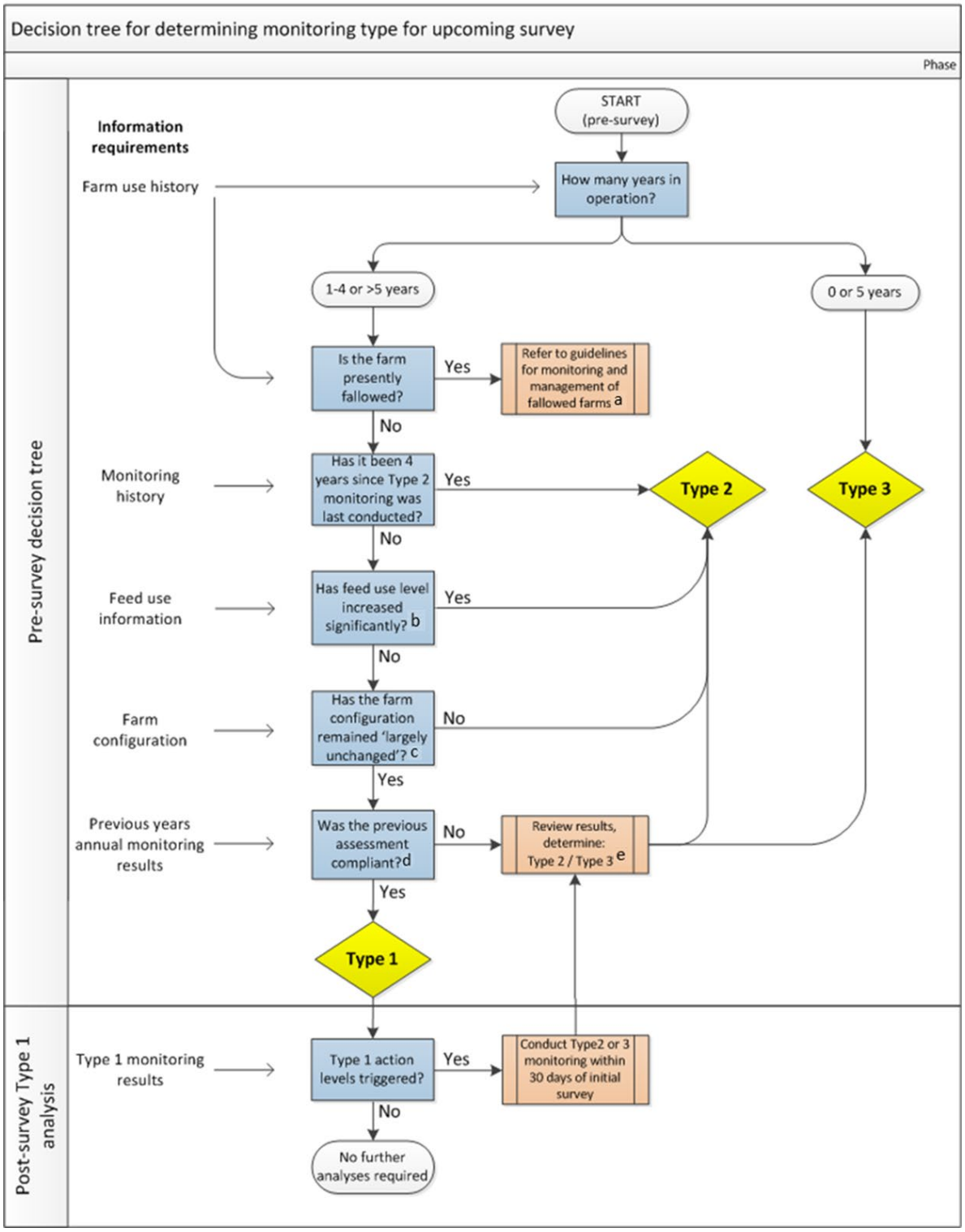

Figure 3: Decision tree for determining the type of annual benthic monitoring that is required. Superscripted characters: [(a)] Refer to guidelines in Section 3.1.2; [(b)] Refer to text above and definition in Footnote 7; [(c)] Compliance as determined with reference to EQS in Figure 5; and [(d)] Refer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

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

	Type 1: Indicator monitoring	Type 2: Full suite monitoring		Type 3: Spatial monitoring
Flow:	High and Low	Low	High	High and Low
Description:	Simplified semi-quantitative monitoring conducted when farm has been compliant and feed levels and effects are relatively stable.	Default form of quantitative monitoring conducted at prescribed zone boundaries at five-yearly intervals or when feed levels have increased7, if compliance was an issue in previous assessment, or if Type 1 EQS were triggered.		A more intensive form of monitoring with a flexible spatial design that aims to elucidate spatial patterns (e.g., footprint mapping), or address specific concerns.
Frequency:	Annual	Minimum five-yearly, more frequent if variable feed levels and effects.		Baseline and at year 5, then as necessary
Compliance monitoring stations[9]:	**Total = 4 (LF) or 5 (HF)** ZME ×2, OLE ×1 (LF) or ×2 (HF)	**Total = 5:** ZME ×2, OLE ×1 (down-current)	**Total = 7:** ZME ×3[10], OLE ×2 (opposing down-current)	Spatial sampling design. Varies according to situation.
Variables:	Qualitative assessment[11], TFS, redox[12], (Cu and Zn)[13]	Full macrofauna or bacterial eDNA, TFS, redox[11], (Cu and Zn)[13]		Dependent on sampling design. Initial survey includes sediment grain size[11]
Reference stations:	NF-Ref[14]	NF-Ref, FF-Ref, (CE-Ref[15])		Design dependent.
Replicates per variable:[16]	≥ 3[17]	3 (5)[18]		1–3
Qualitative variables:	As described in Table 7	As described in Table 7		Dependent on sampling design

[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. For 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 7 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.
[16] Replicate samples are to be collected over an area of approximately 15 m^2 in a semi-random manner that can be practically achieved by repeatedly deploying a sediment grab from a vessel.
[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/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.

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. Three potential modifications are where different pen configurations are proposed, if a farm site is to be fallowed, or if feed input at the farm is highly variable across a year. Guidance for these examples is provided below.

3.1.1 Different net pen arrangements

The monitoring approach outlined in Table 3 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 2). 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 if sampling in the down-current direction is not 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 fallowed[19], 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 of net pens. Therefore, monitoring undertaken prior to restocking may use a hybrid of the methods outlined in Table 3, and the intensity will be at the discretion of the consent holder.

3.1.3 Grow out strategies and feed discharges

New Zealand King Salmon farms are run using a variety of grow-out strategies, with farms typically being continuously stocked and often with multiple cohorts on one farm. Under this model, feed inputs generally peak during summer in accordance with elevated metabolic rates and therefore fish feeding and growth, but the farms are rarely without fish and therefore waste discharge.

There is potential to move to farming fish of a single year class (SYC) at some sites in the Marlborough Sounds. Farming SYC requires relatively low monthly feed loadings in the early stages of the

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

production cycle. In the later stages of production, high feed loadings are sustained before declining again as fish are harvested. Post-harvest, it is expected that the sites will be fallowed (i.e., with no feed discharged) for at least 4 weeks. Timing of monitoring may therefore need to vary to align with peak feed input and maximal predicted effect based on other variables such as seasonal water temperatures or other environmental inputs.

4. ENVIRONMENTAL QUALITY STANDARDS

Environmental quality standards (EQS) are a critical aspect of the benthic monitoring protocol because 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 1. The primary EQS that has been adopted for this BMP is overall enrichment stage (ES, Figure 4), which is a derivative of multiple physico-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).

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, Macleod et al. 2004, Kalantzi & Karakassis 2006) and are consistent with an organic enrichment response from other sources (Pearson & Rosenberg 1978, Glémarec & Hily 1981) (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 sulphide levels (TFS).

Note that, for reasons of consistency and evaluating compliance, redox and TFS must be assessed according to the methods outlined in Appendix G. A new method for measuring TFS has been identified (Cranford et al. 2020), but this requires further development for it to be used within the ES calculation. These methods may be incorporated in later reviews.

The relationships provided by Keeley et al. (2012a) are used to convert the values (in the native units) for each of the variables into an equivalent ES score. 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.

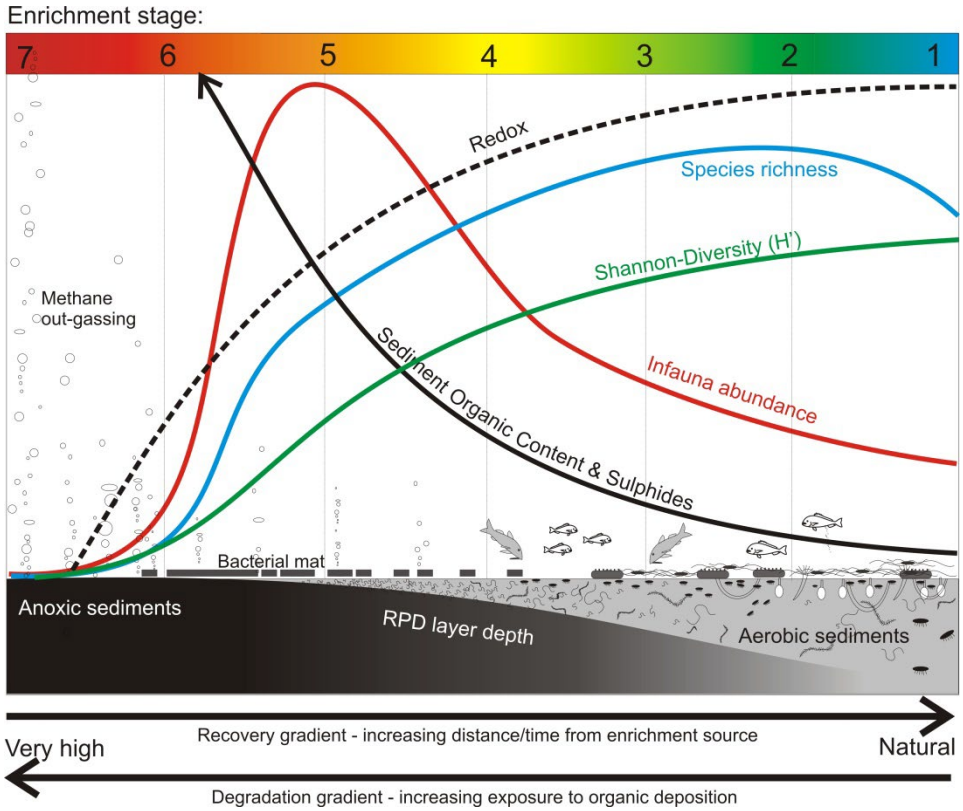

Figure 4: Stylised depiction of a typical enrichment gradient experienced at low flow sites (from Keeley 2013), showing generally understood responses in commonly measured environmental variables (species richness, infauna abundance, sediment organic content, and sulphides and redox). Apparent Redox Potential Discontinuity depth (aRPD) and prevalence of bacteria (*Beggiatoa* sp.) mats and methane/H_2S 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).

Seven enrichment stages (ES) are identified along the continuum (see Table 4 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 (ES5.0). 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 is impacted 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 early stages of enrichment by comparison with low flow sites. This has been accommodated in the EQS by developing flow regime specific empirical relationships between ES and the selected environmental variables (Keeley et al. 2012a). Detailed methods for calculating ES are in Appendix B.

4.1.1. Enrichment Stage based on the bacterial Metabarcoding Biotic Index (b-MBI)

Bacterial-based Metabarcoding Biotic Index (b-MBI) using environmental DNA (eDNA) has been trialled as a proxy for macrofaunal assessments on both low and high flow sites since 2012. This trial was conducted in parallel with the established ES index currently used in the Marlborough Sounds and has involved the elaboration of a standardised, quality-controlled protocol, with automatic computation of b-MBI (Keeley et al. 2018, Pochon et al. 2020a, b).

The refined product is a robust and defensible 'Molecular ES' index that operates on the same compliance threshold scale as the traditional macrofauna-based ES index for which it is a proxy. Essentially, b-MBI replaces the macrofauna ES component of the ES equation (see Appendix D). The method offers savings in time and cost over traditional macrofaunal analysis.

The molecular ES has a high accuracy benchmarked against the traditional ES method. It offers greater certainty in results due to narrower confidence intervals and is also a simpler means of calculating ES compared with complex polynomials currently required for macrofaunal ES calculations. The latter advantage is two-fold since it virtually eliminates the need for input of 'best professional judgement' (BPJ; see Appendix B) in calculating ES.

The b-MBI derived molecular ES can therefore reliably replace the macrofauna-based ES index within the existing Type 2 monitoring framework for determining seabed enrichment against compliance criteria. It is important that calculation of overall molecular ES follows the methods detailed by Pochon et al. (2021a). Detailed methods for calculating ES using the b-MBI are provided in Appendix D.

Table 4: 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 unenriched sediments. Natural state, but uncommon in many modified environments.	LF	Environmental variables comparable with an unpolluted/unenriched pristine reference station.
		HF	As for LF, but infauna richness and abundances naturally higher (about 2 × 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 sub-peak. 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 ES5.0 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 with reference stations. %OM can be very high (3–6 × 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 sulphide 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 × 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 5). 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 5.

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 3 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 interval (CI) 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 6. Benthic enrichment stages greater than ES5.0 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 ES5.0 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 time frames, then more drastic responses are required (Table 5 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 given below.

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

While these indicators provide evidence that excessive enrichment may be occurring, they should not be used in isolation. If any of these indicators are observed, they trigger analysis of additional macrofaunal samples to confirm ES scores (Figure 5).

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. An alert management response is required if the ES level at the OLE station increases significantly relative to appropriate reference stations (Figure 5 and Table 6). A minor management response is required if a significant increase is observed and the mean incremental increase is greater than ES0.4, 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 5: Action levels and associated management responses (refer Figure 5 and Table 6).

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 up to the monitoring survey at the end of the 24-month period.
Major	As for minor action response, but the 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 6).
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 latter[20]. 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 6).

[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.

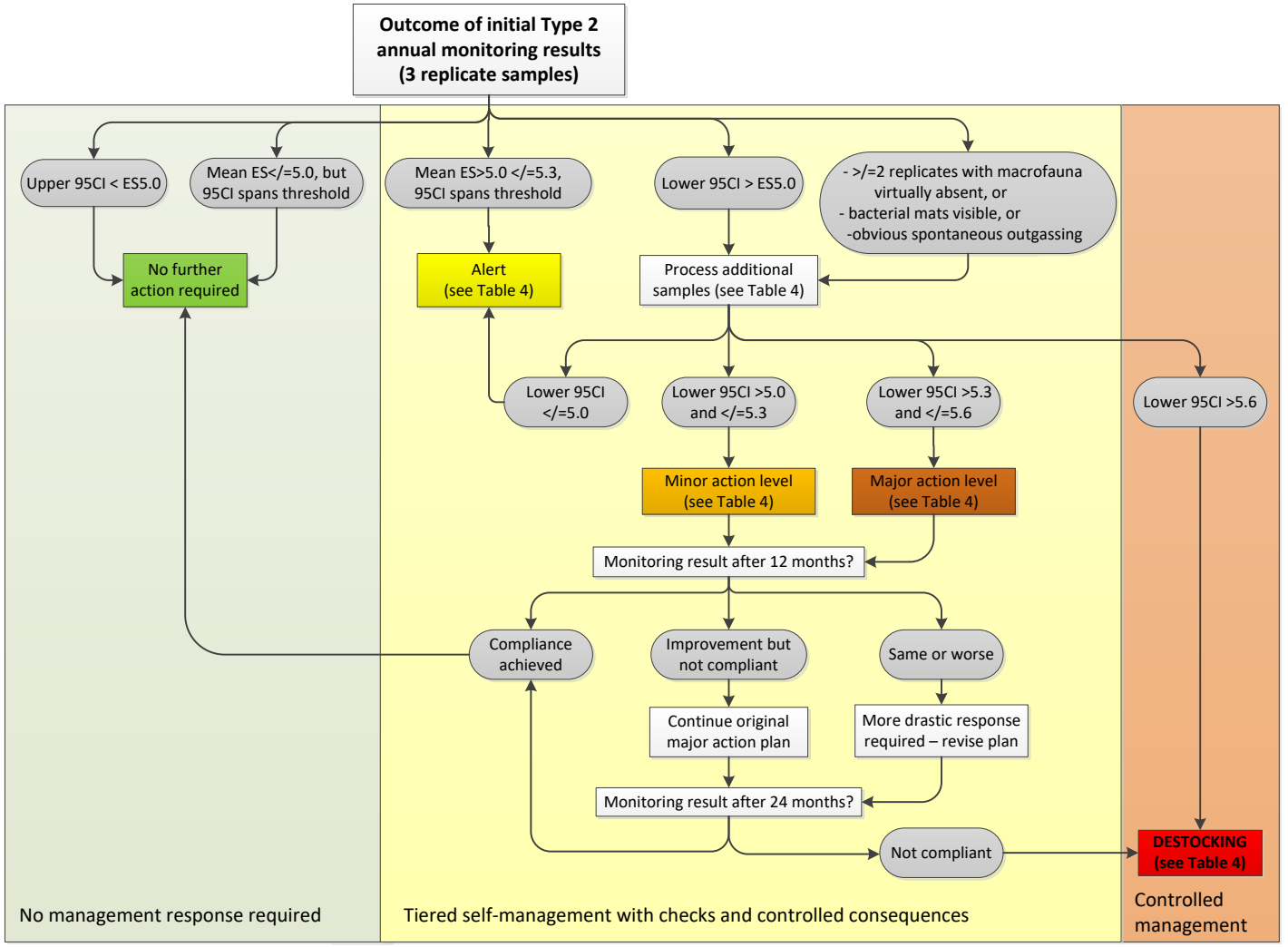

Figure 5: Decision tree for determining the level of management response required in relation to Type 2 (quantitative) annual benthic monitoring results. Diagram primarily relates to the ZME (see Tables 4 and 5), however the pathway below the 'Minor action level' box also pertains to the OLE. Refer to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

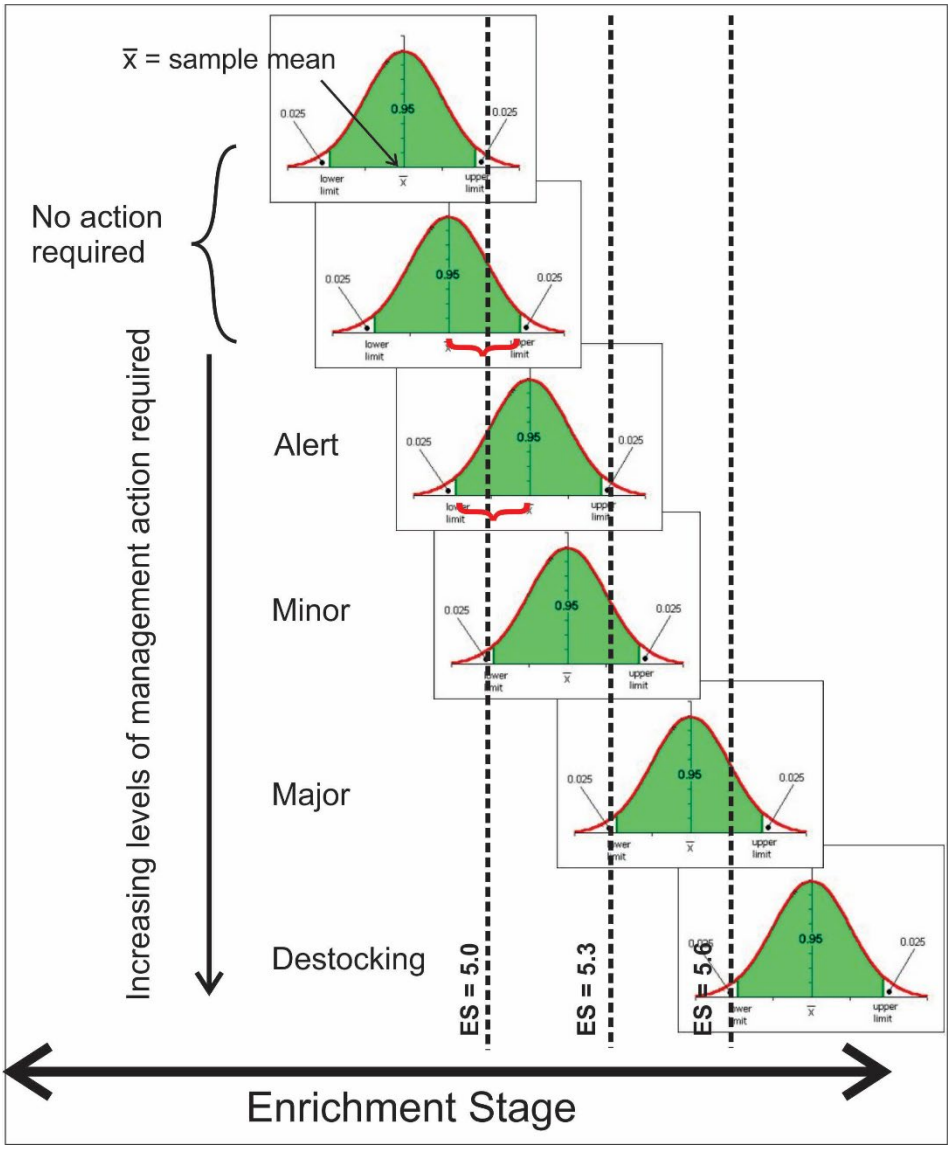

Figure 6: **Example of how 95% confidence intervals are utilised in relation to ES thresholds with the various levels of management responses. For further clarity, an alternative way of displaying the relationship between the mean ES value, the associated confidence interval, and the required action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

Table 6: 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 sulphides in sediments. CI = confidence interval.

Action level (Figure 5)	Sampling station: Zone of maximum effects (ZME)	Sampling station: Outer limit of effects (OLE)
Industry operational goal	-Overall ES ≤ 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 µM, High flow > 2400 µM -Total qualitative score > 6.0 and macrofauna score > 2.0 (refer Table 7)	-TFS > 390 µM[25] -Total qualitative score > 0 (refer Table 7)
EQS for Type 2 monitoring		
Alert	-Overall ES > 5.0 and ≤ 5.3[22] and 95% CI spans threshold	-A statistically significant increase in Overall ES relative to appropriate reference station(s)[26]
Process Additional Samples	-Two or more replicates with macrofauna virtually absent[27] -Bacterial mats > 50% cover (patchy-major)[28] -Obvious spontaneous outgassing[29]	
Minor	Lower 95% CI for Overall ES > 5.0 and ≤ 5.3[22] (including additional samples)	Overall ES ≥ 3.0 OR -Overall 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 ≤ 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. ES5.0 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 4 and Figure 4.

[22] These ES categories are consistent with those proposed by the Environmental Protection Authority at the conclusion of the NZ King Salmon Board of Inquiry in 2013.

[23] ES3.0 corresponds to discernible 'moderate enrichment' (Keeley et al. 2012a, Table 4 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 ES1.5 to 2.5 (but no greater than ES2.9). 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 Hargrave et al. (2008), Keeley et al. (2012a), 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 maximum effect (ZME).

[25] The 95% CIs associated with ES3.0 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. However, when bacterial eDNA sampling is used, this EQS will not be possible to determine and can be excluded as a trigger to process additional samples.

[28] Defined as: white bacterial mat (mainly *Beggiatoa* sp.) smothering sediment surface. Excludes patchy-minor coverage (i.e., < 50% of surface area, see Table 7) 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 7). 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. Thus, it is hoped that assessments may be voluntarily and more regularly conducted by the consent holder and, as a result, 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 6. 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 7). 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 3). The visual macrofauna assessment is considered to be a particularly important indicator because 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 during Type 1 monitoring).

A Type 1 monitoring protocol has been developed (Bennett & Elvines 2022) and 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.

Table 7: Qualitative assessment methods and criteria for Type 1 benthic monitoring.

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 x 1 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 (*Beggiatoa* sp.) within sediments or attached to edges of cobbles or shells.	1
Patchy-minor	Obvious patches of bacterial mat (*Beggiatoa* sp.) on sediment surface, occupying < 50% of surface area.	2
Patchy-major	Obvious patches of bacterial mat (*Beggiatoa* 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 sulphide 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.	0
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 > 7 (Outgassing + Bacteria coverage + Macrofauna + Sulphides), 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 timescales. 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[30] 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 8). 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 2).

[30] The ANZECC guidelines for sediment and water quality were updated in 2018; however, the thresholds for most metals (including copper and zinc) remain the same (ANZG 2018).

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 reflect 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 sulphide forms). However, this suppression may be reduced by consequent fallowing.

Table 8: 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 Type 1 monitoring). 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 μm) 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 sulphide 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 metal 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 by 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.

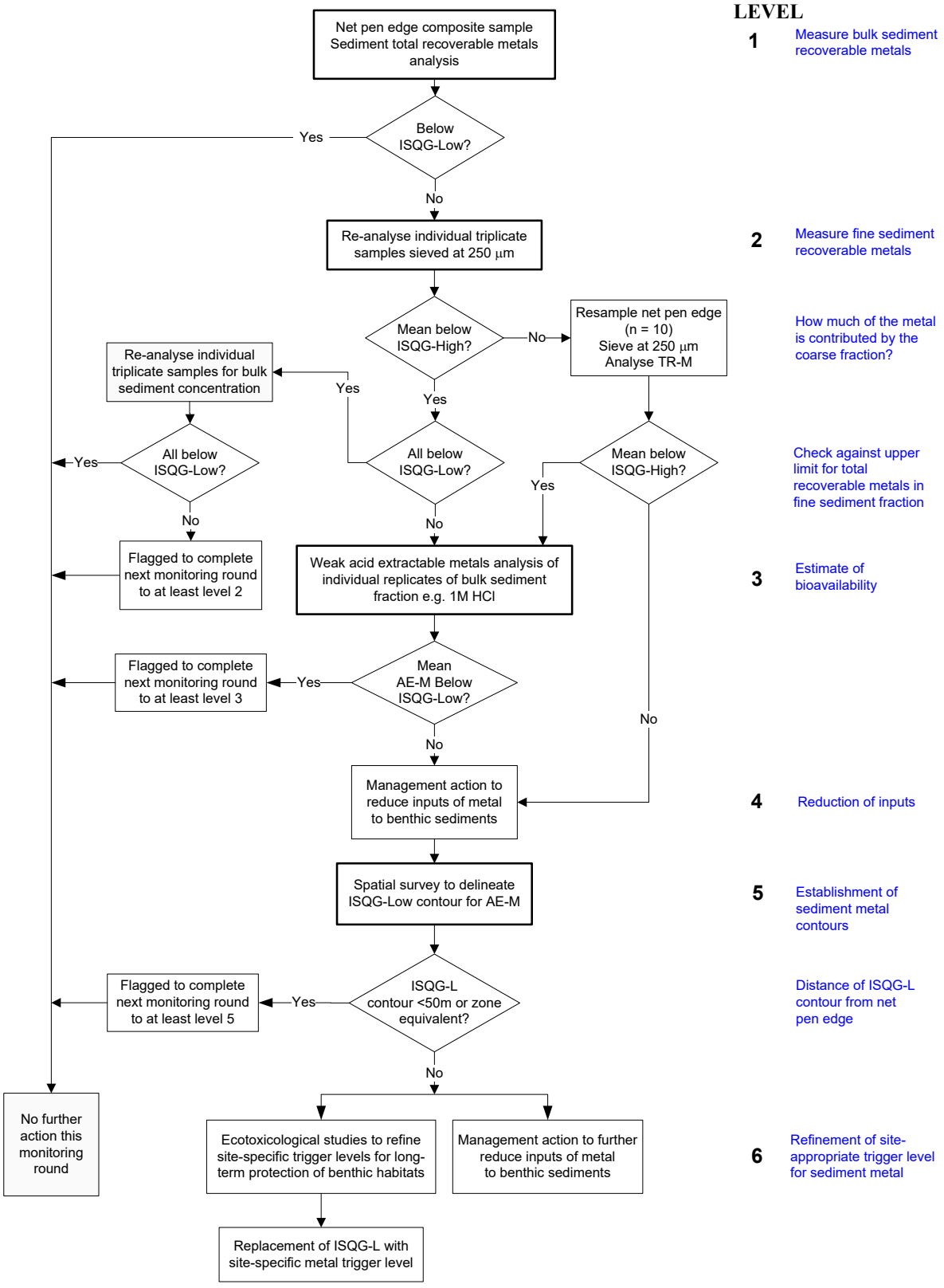

Figure 7: Decision tree for monitoring and operational responses to the accumulation of metals (copper and zinc) within sediments in the vicinity of salmon farms.

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, updated in 2019). For NZ King Salmon farming in the Marlborough Sounds, many of the farms contain multiple year classes and so there is often no single period that should be targeted for sampling. 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.

For farms containing a single year class of fish, or that employ other grow-out strategies where feed use may peak outside the mid-January to mid-March period, timing of monitoring may need to vary to align with peak feed input and maximal predicted effect based on other variables such as seasonal water temperatures or other environmental inputs.

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 NZ King Salmon 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 3), 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 PROCESS

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 group. 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. ACKNOWLEDGEMENTS

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11. GLOSSARY

Term / acronym	Definition
%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)
b-MBI	Bacterial Metabarcoding Biotic Index
BoI	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 by Cromey et al. (2002)
eDNA	Environmental DNA
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).
Molecular ES	Enrichment Stage score derived from an eDNA-derived bacterial Metabarcoding Biotic Index (b-MBI) and other environmental indicators. See details in Appendix D and Pochon et al. (2021a).
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
Station	Refers to an approx. 10 m^2 area of seabed within which replicate samples are collected for environmental monitoring purposes.
TFS	Total free sulphide concentrations in sediments (μM)
ZME	Zone of maximum effect – typically the area of seabed immediately beneath a farm which enrichment effects are maximal

12. 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 ES5.1.

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 7, 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 ES5.0 (Table 6), 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 Appendix F Table 13 of the guidelines.

The majority of working participants were in agreement to adopt a separate trigger for high flow farms in Table 6, 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.

13. APPENDIX B: CALCULATION OF ENRICHMENT STAGE USING MACROFAUNA-BASED INDICATORS

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) (Appendix B Figure 8, Figure 9 and Figure 10). 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 (Appendix B Table 9). 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 which can then be combined quantitatively (by averaging) to arrive at an 'overall ES' (value from 1.0 to 7.0) 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 and focuses 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 sulphides (sediment chemistry), and total abundance (N), richness (number of taxa, S), J′, d, H′, AMBI, M-AMBI, and BQI (infauna composition, for definitions see Appendix B Table 10). 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 95% confidence intervals.

Overall ES = (Organic loading ES × 0.1) + (Sediment chemistry ES × 0.2) + (Macrofauna ES × 0.7)

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), Appendix B 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: 2.0–3.4% = ES1.0; 3.5–3.9% = ES2.0; 4–6.4% = ES3.0; 6.5–7.9% = ES4.0; 8–11.9% = ES5.0; 12–15.9% = ES6.0; and >16% = ES7.0.

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 ES7.0 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 because they could be from cross-contamination, or transient surface-dwelling taxa, in which case the sample is still essentially 'azoic'. Because 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), and other macrofauna metrics excluded from the calculation of Overall ES.

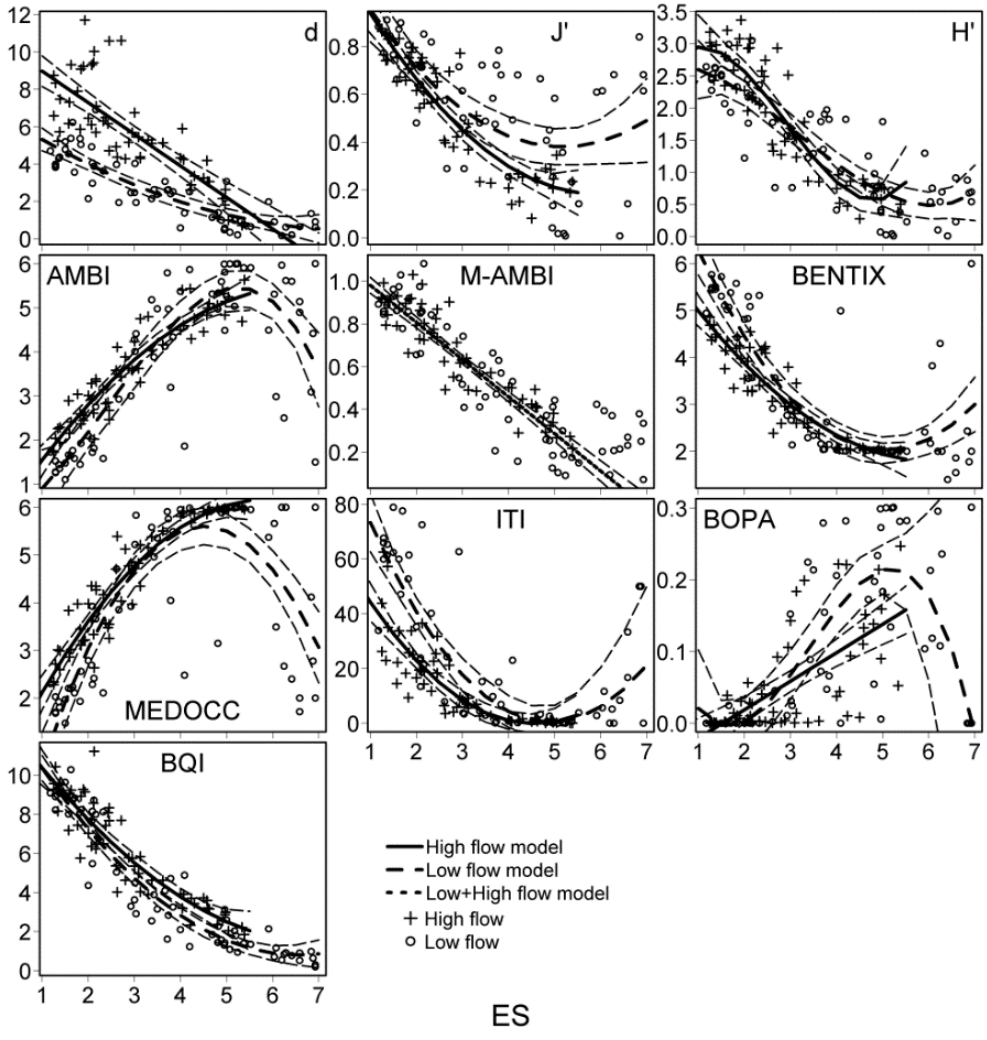


Figure 8: Scatterplots displaying optimum models with 95% confidence intervals for 10 biotic indices in relation to enrichment stage (ES) (from Keeley et al. 2012a).

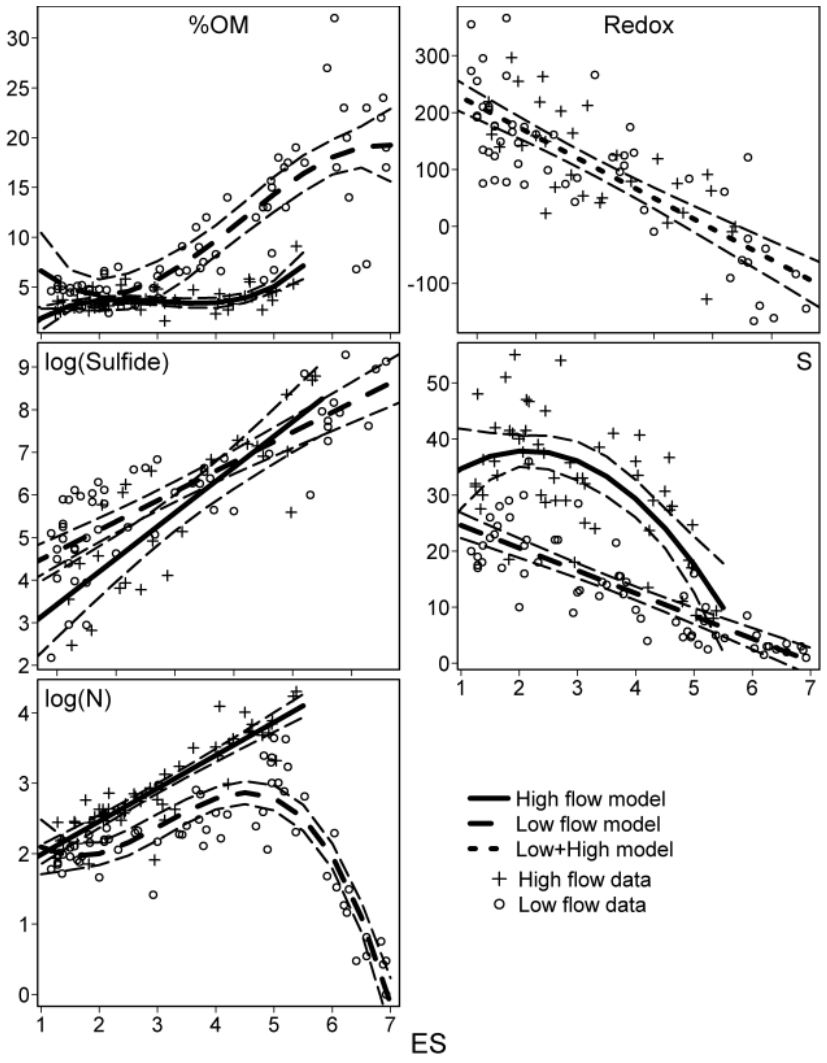


Figure 9: Scatterplots displaying optimum models with 95% confidence intervals for each of the physico-chemical and biological indicators in relation to enrichment stage (ES) (from Keeley et al. 2012a).

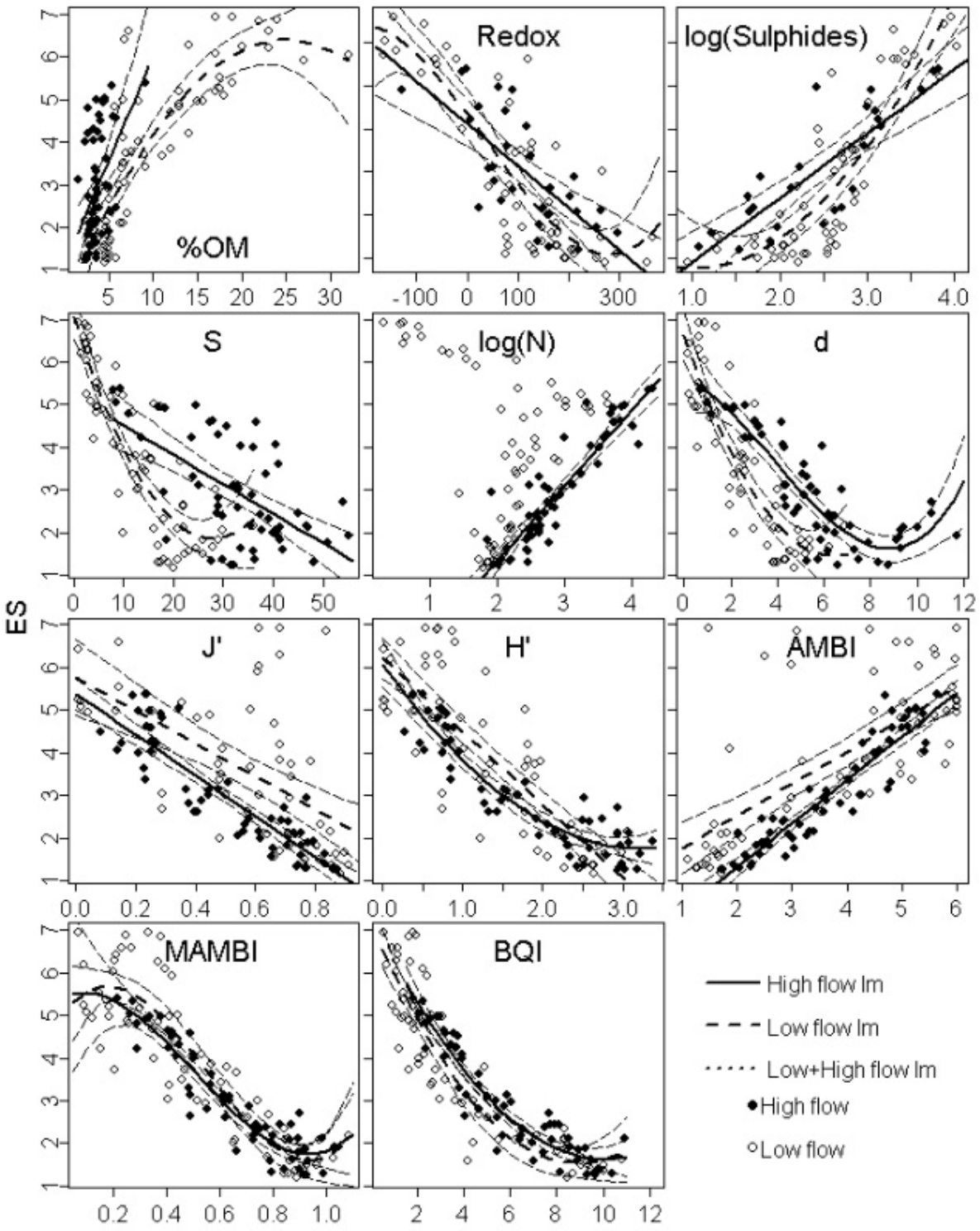


Figure 10: Inverted scatterplots displaying optimum models with 95% confidence intervals for the environmental indicators and biotic indices used to predict enrichment stage (ES). Corresponding polynomials and model fit statistics are provided in Appendix B Table 9. (From Keeley 2012)

Table 9: Polynomial relationships used to derive equivalent enrichment stages (ES) score from native values of individual variables.

HIGH FLOW

X Variable	Deg. Poly	x^3	x^2	x	Int	Res. SE	df	Mult. R^2	Adj. R^2	p-value
log(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
BQI	2		0.055	-1.123	7.321	0.4296	53	0.8861	0.8818	<2.2e-16
M-AMBI	3	12.631	-19.453	3.236	5.391	0.4363	52	0.8847	0.8781	<2.2e-16
No. Taxa (S)	1			-0.0691	5.2102	0.9984	54	0.3731	0.3615	5.76e-07
P. evenness (J′)	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
M. diversity (d)	3	0.0084	-0.0967	0.2654	5.67	0.588	52	0.7906	0.7785	<2.2e-16
log(Sulphides)	1			-1.4885	-0.5738	0.7925	23	0.6936	0.6803	2.4e-07
SWDI (H′)	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

X Variable	Deg. Poly	x^3	x^2	x	Int	Res. SE	df	Mult. R^2	Adj. R^2	p-value
log(Abundance)**	1			1.9641	-1.5924	0.9482	49	0.5459	0.5366	6.03e-10
AMBI***	1			0.7464	0.5517	0.6363	51	0.8123	0.8086	<2.2e-16
BQI	2		0.071	-1.278	7.177	0.7712	62	0.8293	0.82338	<2.2e-16
M-AMBI	1			-5.396	6.6615	0.8949	63	0.7665	0.7628	<2.2e-16
No. Taxa (S)	2		0.0067	-0.373	7.0575	0.8674	62	0.7841	0.7771	<2.2e-16
P. evenness (J′)***	1			-4.2216	5.5967	0.9601	50	0.5709	0.5623	9.54e-11
Redox	1			-0.009	4.129	0.923	28	0.513	0.4956	8.54e-06
M. diversity (d)	2		0.1251	-1.6091	6.6194	0.8734	57	0.7651	0.7569	<2.2e-16
log(Sulphides)	2		0.7095	-1.4397	1.5039	0.9429	51	0.7465	0.7366	6.32e-16
SWDI (H′)	1			-1.717	6.2135	1.024	63	0.6945	0.6897	<2.2e-17
TOM	2		-0.0098	0.492	0.2464	0.9867	62	0.7206	0.7116	2.2e-16

* relationship not used in the calculation of ES at high flow sites (see Appendix B 2b).
** relationship based on ES ≤ 5.5 data only.
*** relationship based on ES < 6 data only.

Table 10: Definitions of biological indicators used in the calculation of Enrichment Stage (ES).

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's diversity index. Ranges from 0 (very low diversity) to about 12 (very high diversity)	Margalef (1958)
J'	*H'* / log S 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.	Pielou (1966)
H'	$- \sum_i p_i \log(p_i)$ where *p* is the proportion of the total count arising from the *i*th 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 × %GI + 1.5 × %GII + 3 × %GIII + 4.5 × %GIV + 6 × %GV)]/100 where GI, GII, GIII, GIV, and GV are ecological groups and $\%G_i$ are percentages (of all assigned taxa) falling into ecological group *i*. AZTI's 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., *Capitella capitata*). Index values are between 1 (normal) and 6 (extremely disturbed).	Borja et al. (2000)
M-AMBI	Uses AMBI, S, and H′, combined with factor analysis and discriminant analysis (see source reference). 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 allow 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).	Muxika et al. (2007)
BQI	$= (\sum_{i=1}^{n}(\frac{A_i}{totA} \times \mathrm{ES50}_{0.05i})) \times {}^{10}\log(S+1)$ Where ES50 = expected number of species as per Hurlbert (1971) And, $ES50_{0.05}$ the species tolerance value, given here as the 5th 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).	Rosenberg et al. (2004)
b-MBI	b-MBI = 0.0169 × %EG I + 0.0307 × %EG II + 0.0250 × %EG III + 0.0389 × %EG IV + 0.0665 × %EG V + 0.085 × %EG VI where EG I, EG II, EG III, EG IV, EG V, and EG VI are ecological groups and $\%G_i$ are percentages (of all assigned ASVs) falling into ecological group *i*. Bacterial Metabarcoding Biotic Index. Uses bacterial environmental DNA (eDNA) for quantifying benthic organic enrichment. The b-MBI is calculated based on the presence of bacterial indicators, which have been assigned to an Eco-Group (EG) with an associated weighting. Results range from 1.69 (pristine) to 8.5 (capped at 7, severe enrichment).	Pochon et al. (2021a)

14. APPENDIX C: ALTERNATIVE EQS COMPLIANCE TABLE

Table 11: 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
0.0																
0.1																
0.2																
0.3																
0.4																
≥0.5																

	NO ACTION REQUIRED
	ALERT
	MINOR
	MAJOR
	DESTOCKING

15. APPENDIX D: CALCULATION OF ENRICHMENT STAGE USING BACTERIAL METABARCODING BIOTIC INDEX

As discussed in Section 4.1.1, research by the Cawthron Institute over the last ten years has shown bacterial environmental DNA (eDNA) can be used for quantifying benthic organic enrichment under New Zealand salmon farms. Documentation underpinning the development and validation of the b-MBI method are available on the MPI website[31]. As a first step, DNA is extracted from sediment samples following an accredited standard operating protocol (SOP). Samples are subsequently sequenced for bacterial eDNA and bioinformatically analysed using DNA sequence data as described by Pochon et al. (2020a). Key bacterial indicators, that consistently change in abundance as a function of organic enrichment gradients, are then identified through metabarcoding techniques and subsequently incorporated into the bacterial Metabarcoding Biotic Index (b-MBI, Appendix D Table 12) (Pochon et al. 2021a).

The b-MBI is calculated based on the presence of indicator bacterial amplicon sequence variants (ASVs), which have been assigned to an Eco-Group (EG) with an associated weighting. ASVs are assigned to Eco-Groups based on their affinity to stages of the traditional ES scale (as detailed by Pochon et al. 2020a and 2021a). Eco-Groups are defined as: EG I = very sensitive, EG II = sensitive, EG III = ubiquitous, EG IV = transitory, EG V = opportunistic, and VI = resistant. The b-MBI index is then calculated using the formula presented by Pochon et al. (2021a), as follows:

b-MBI = 0.0169 × %EG I + 0.0307 × %EG II + 0.0250 × %EG III + 0.0389 × %EG IV + 0.0665 × %EG V + 0.085 × %EG VI

The final derived weighting of 0.085 for EG VI implies that the theoretical maximum possible value for b-MBI would be 8.5 in the unlikely event that all ASVs in a given sample were allocated to EG VI. In such instances, the b-MBI would be capped to 7. Similarly, the weighting of 0.169 for EG I means the theoretical minimum possible value for b-MBI is 1.69. As such, potential results range from 1.69 (pristine) to 7 (severe enrichment) (Pochon et al. 2021a). The b-MBI variable can then be used in place of macrofauna to calculate an 'overall molecular ES' score that operates on the same compliance threshold scale as the traditional macrofauna-based ES index (Pochon et al. 2021a). The formula is as follows:

Overall molecular ES = (Organic loading ES × 0.1) + (Sediment chemistry ES × 0.2) + (b-MBI × 0.7)

As with the traditional method, the overall molecular ES for the sampling station is given by the average of the replicate samples (at least three) and the variability between samples is reflected in the associated 95% confidence intervals.

Validation of overall molecular ES based on 2020-2021 farm data

A validation exercise carried out using sediment samples from all nine farms in the Marlborough Sounds (collected between December 2020 and February 2021) confirmed the eDNA-derived method accurately determined the level of benthic enrichment as it relates to the ES scale (Pochon et al. 2021b). A larger proportion of transitory, opportunistic, and resistant bacteria (i.e., EG IV, V, and VI) were detected in pen and close proximity farm stations, compared with control stations which were dominated by sensitive or very sensitive bacteria (i.e., EG I and II). In addition, there was a highly significant relationship between the traditional and molecular ES indices (ANCOVA, $p < 0.001$), with no differences between the slopes for each flow regime, confirming the index performance consistency across flow regimes.

[31] https://www.mpi.govt.nz/fishing-aquaculture/aquaculture-fish-and-shellfish-farming/aquaculture-resources-and-research/

The validation exercise also confirmed the robustness of the overall molecular ES tool in determining benthic compliance, as currently outlined under the BMP framework. Analysis of station compliance was carried out for both methods based on the consented (or historically applied[32]) environmental quality standards (EQS). This analysis found 40 true positives (i.e., compliant under both methods), one false positive (i.e., compliant under the molecular method but non-compliant under the traditional approach), zero false negatives (i.e., non-compliant under the molecular method but compliant under the traditional approach), and three true negatives (i.e., non-compliant under both methods). The confusion matrix analysis indicated the molecular index correctly classified station compliance in 97.7% of the instances (i.e., true positives and true negatives; 43 of 44 stations). Specifically, there was 100% and 93.8% agreement between methods for the high flow and low flow farms, respectively (Pochon et al. 2021b).

In general, mean overall ES scores based on the traditional method tended to be slightly higher than the corresponding overall molecular ES score at the low flow farms, particularly where sediments were highly enriched (i.e., between ES5 and 6). Under severely enriched conditions, macrofauna become depauperate, which is problematic for calculating several of the diversity metrics and biotic indices. In these instances, equivalent ES scores for macrofauna are manually assigned using the best professional judgement (BPJ, see Section 10.2) process. This will generally involve making reference to the scores of other relevant indicators, as well as to historical trends, and as such requires sufficient expertise to undertake this process. In contrast, the b-MBI approach has illustrated that microbial communities not only change predictably throughout the enrichment spectrum, but also that they are prolific at all stages. As such, the molecular approach virtually eliminates the need for input of BPJ, which is a time-consuming step requiring specialist expertise in the determination of equivalent ES scores for the macrofauna-based indices.

Table 12: Definition of index used in the calculation of Enrichment Stage (ES) using molecular tools.

b-MBI	b-MBI = 0.0169 × %EG I + 0.0307 × %EG II + 0.0250 × %EG III + 0.0389 × %EG IV + 0.0665 × %EG V + 0.085 × %EG VI where EG I, EG II, EG III, EG IV, EG V, and EG VI are ecological groups and %G*i* are percentages (of all assigned ASVs) falling into ecological group *i*.	
	Bacterial Metabarcoding Biotic Index. Uses bacterial environmental DNA (eDNA) for quantifying benthic organic enrichment. The b-MBI is calculated based on the presence of bacterial indicators, which have been assigned to an Eco-Group (EG) with an associated weighting. Results range from 1.69 (pristine) to 8.5 (capped at 7, severe enrichment).	Pochon et al. (2021a)

[32] Cawthron has historically interpreted the existing description-based consent conditions of the low flow farms in a quantitative manner and has 'assumed' the equivalent ES (i.e., 'assumed ES') for each of the consented zones according to the description provided by the consent wording.

16. APPENDIX E: NOTES ON THE APPLICATION OF THE MOLECULAR-BASED SAMPLING METHOD FOR FARM MONITORING

Inclusion of a molecular approach to benthic monitoring now provides two sampling methods that can be used to assess ES under Type 2 monitoring: bacterial eDNA-based sampling or traditional macrofauna sampling. The consent holder may choose to use either (or both) of the methods for calculating ES. However, using both methods present a challenge for interpretation if differences are observed between macrofaunal and molecular ES scores. To avoid confusion, we therefore recommend that a single ES system is used.

While molecular ES comprises an accepted primary approach for Type 2 monitoring, it has been proposed that monitoring might also consider integrating macrofauna sampling into the management response levels (e.g., as an alert level response). However, this adds additional complication for interpretation of the results and potentially adds significant expense without necessarily providing clarity. This approach is not recommended.

If the consent holder chooses to use bacterial eDNA-based sampling, a minimum of three replicates will be required to calculate ES. The mean ES value of these three replicates will be used to calculate the overall ES as described in Appendix D above.

Where eDNA sampling is used, the macrofauna-related indicator of excessive enrichment (i.e., two or more replicates with macrofauna virtually absent) is not applicable and this indicator can be excluded in such cases. Where there is potential for exceeding compliance limits for ES scores (i.e., ZME and OLE stations), and to align with macrofaunal sampling requirements, five eDNA samples should be processed. Consideration of any appropriate additional eDNA-related management triggers will be included in a future review (Appendix F Table 13).

Whilst eDNA sampling may also be appropriate for Type 1 monitoring it offers no advantages in terms of time or cost implications. Type 1 macrofaunal-based monitoring does not require the levels of taxonomic expertise required for Type 2 monitoring. At this stage we have therefore not recommended including eDNA within the Type 1 monitoring protocol.

To identify the bacterial DNA present in a sample and calculate b-MBI, a reference database of known DNA sequences is required. A GenCodeID database has been developed by the Cawthron Institute. Third party access to this database is under discussion, with one option being the development of a web portal that allows any science provider to upload their sequence data and receive molecular ES values for each sample based on membership or a pay-per-use basis (yet to be defined). Links to this portal will be available from the MPI and Cawthron websites once it is developed.

As b-MBI based ES scores are affected by the bacterial DNA identified within the sample, it is recommended that no modifications are made to the bacterial GenCodeID database used to calculate b-MBI for a period of three years from the date of this review.

Reference material underpinning the development and validation of the b-MBI based ES scores is available on the MPI website.

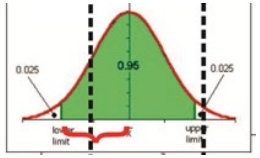
17. APPENDIX F: REGISTER OF ISSUES FOR FUTURE CONSIDERATION BY THE BENTHIC STANDARDS WORKING GROUP

Subsequent to external peer review and the public comment phase, several technical issues were identified by the Benthic Standards 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 Guidelines, 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 (Appendix F Table 13). The BMP Guidelines in their entirety are intended to be formally reviewed five-yearly after their finalisation.

Following the out-of-cycle review in 2022, this list has been updated to address issues identified but not addressed in the review.

Table 13: Register of issues for future consideration by the Benthic Standards Working Group (BSWG).

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.	It was generally agreed (one dissenting view) that a dual (high/low flow) trigger was appropriate, and consistent with the existing monitoring program (which already differentiates high and low flow sites. The level for the Type 1 ZME should be altered in accordance with report #2742 and the dissenting view be recorded. It was acknowledged that the method proposed by Cranford et al. 2017 may be an improvement, however there are also possible limitations to its application, and it should not be adopted and used in conjunction with the existing sulphide – ES regressions or trigger levels because the method may result in a scale-shift in concentrations.	The appropriateness of the triggers should be reanalysed with the inclusion of the last 3–4 years of data. The ES values against which sulphides are compared should not include sulphides in their calculation. This should be made available for review in the next BSWG meeting. This was not addressed in the out of cycle review.
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% CIs is that there may be less overlap with ES trigger levels for reducing feed or fallowing above ES5.0	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 determine its true appropriateness. To be reassessed at the next meeting. This was not addressed in the out of cycle review.

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 5 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 ES5.3 (see diagram below from Figure 6 of the guidelines) Alert 	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 *true* mean (as opposed to *sample* 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.	This was not addressed in the out of cycle review.
4.	Use UV method to measure total free sulphide	The current Total Free Sulphide analytical techniques are described in Appendix G. An alternative analytical method using UV analysis is now available (Cranford et al. 2020).	While the new method is more accurate and provides a more user-friendly, cost-effective option than the current ISE method, it requires further development of method-specific thresholds.	Include in future review
5.	Statistical comparisons at OLE stations	It has been identified that there are limitations to statistical comparisons and the use of hypothesis testing, in particular the focus on p-values and whether statistical significance equates to biological significance. Clarification and standardisation of optimum method is required. The guidelines recommend use of a BACI-type analysis; however, what constitutes a 'before' scenario is unclear in this context. There are also potential issues related to the level of replication and associated statistical power.	While recognising the need to move away from professional judgement, this is not easily resolved and will need to be looked at more thoroughly.	Include in future review

6.	The relevance of %OM measure	%OM is the measure used as the measure of organic loading within the ES score calculation.	%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.	Include in future review
7.	Conducting a full review	MDC suggested that the out-of-cycle review (2022) was an opportune time to review the entire BMP and its efficacy in terms of implementation and the consenting and compliance processes.	The rest of the working group did not support this and decided to wait until the next review cycle (2024) to adopt this approach.	Include in future review
8.	Bacterial-based trigger for processing additional samples	Bacterial eDNA sampling (when used in place of macrofaunal sampling) precludes the use of azoic macrofauna cores as a trigger for processing additional samples.	The loss of azoic macrofauna samples as a trigger is likely of low consequence considering that bacterial mats are also likely to be present as an indicator of enrichment. The additional bacterial-based trigger will be considered in a future review after the implementation of bacterial eDNA sampling. A threshold that incorporates a given proportion of resistant (EG VI) bacteria (e.g., > 20%) could be considered as a management trigger when eDNA sampling is used.	Include in future review
9.	Broader Application of these Guidelines	This document was initially developed to focus on the BoI consents.	There is potential to consider the BMP process application for other salmon farms both within and outside of the Marlborough Sounds.	Include in future review

18. APPENDIX G: REDOX AND TOTAL FREE SULPHIDES ANALYTICAL PROCEDURES

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.

18.1 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 by 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 gently with a 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 220 mV 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.*

18.2 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 45º 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 ($Na_2S \cdot 9\ H_20$) ~ 20 g/L or 86 000 µM:

a. Prepare a primary standard from clear, crystalline $Na_2S \cdot 9\ H_2O$
b. In a hood, place a small chunk (~5 g) of the $Na_2S \cdot 9\ H_2O$ 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.
d. Dilute the stock standard to 50 mL with previously degassed MilliQ water.
e. Mix to dissolve.
    - Sonicati on 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 10× 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)	S1	1	10	8 600	9
Standard 3 (S3)	S2	1	10	860	9
Standard 4 (S4)	S3	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

1.2.1 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.

1.2.2 Allow samples to thaw to room temperature if they have been frozen or cooled.

1.2.3 Dispense ~5 mL of the sample into a 50 mL Falcon tube.

1.3 Ion Specific Electrode Measurement

1.3.1 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).

e. 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.

f. 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.

1.3.2 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).

1.3.3 Suspend and centrifuge the sample tubes in groups of nine + one balance tube. (1500 relative centrifugal force (rcf) for 5 min at room temperature (15–20 °C).

1.3.4 Decant the supernatant into a 20 mL scintillation vial, add a small stirring bar, and place on a magnetic stirrer set at 200 rpm.

1.3.5 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.)

g. Each standard should have approximately 30 mV difference from the previous standard.

h. i.e., S1 = -941 mV, S2 = -914 mV, S3 = -882 mV.

1.3.6 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).

1.3.7 Rinse the electrode prior to use with a new sample.

i. While the sides of the probe may be wiped down, do not wipe the flat probe face on the bottom of the probe.

1.3.8 Using the spreadsheet, plot the concentration in µM (log) vs. mV (linear).

1.3.9 Enter each sample mV reading on the spreadsheet to calculate its corresponding sulphide concentration.

1.4 Electrode Storage

1.4.1 Store the sulphide probe in 4M potassium chloride solution for up to 1 week.

1.4.2 For storage longer than one week, drain the probe dry and rinse 3 times with MilliQ water before emptying completely.

j. Ensure cap is on the probe surface and store dry in the box.

k. When restoring the probe for use after long term storage, the day before intended use, rinse the probe 3 times with electrode filling solution.

l. Allow the probe to sit overnight in 4 M KCl solution.

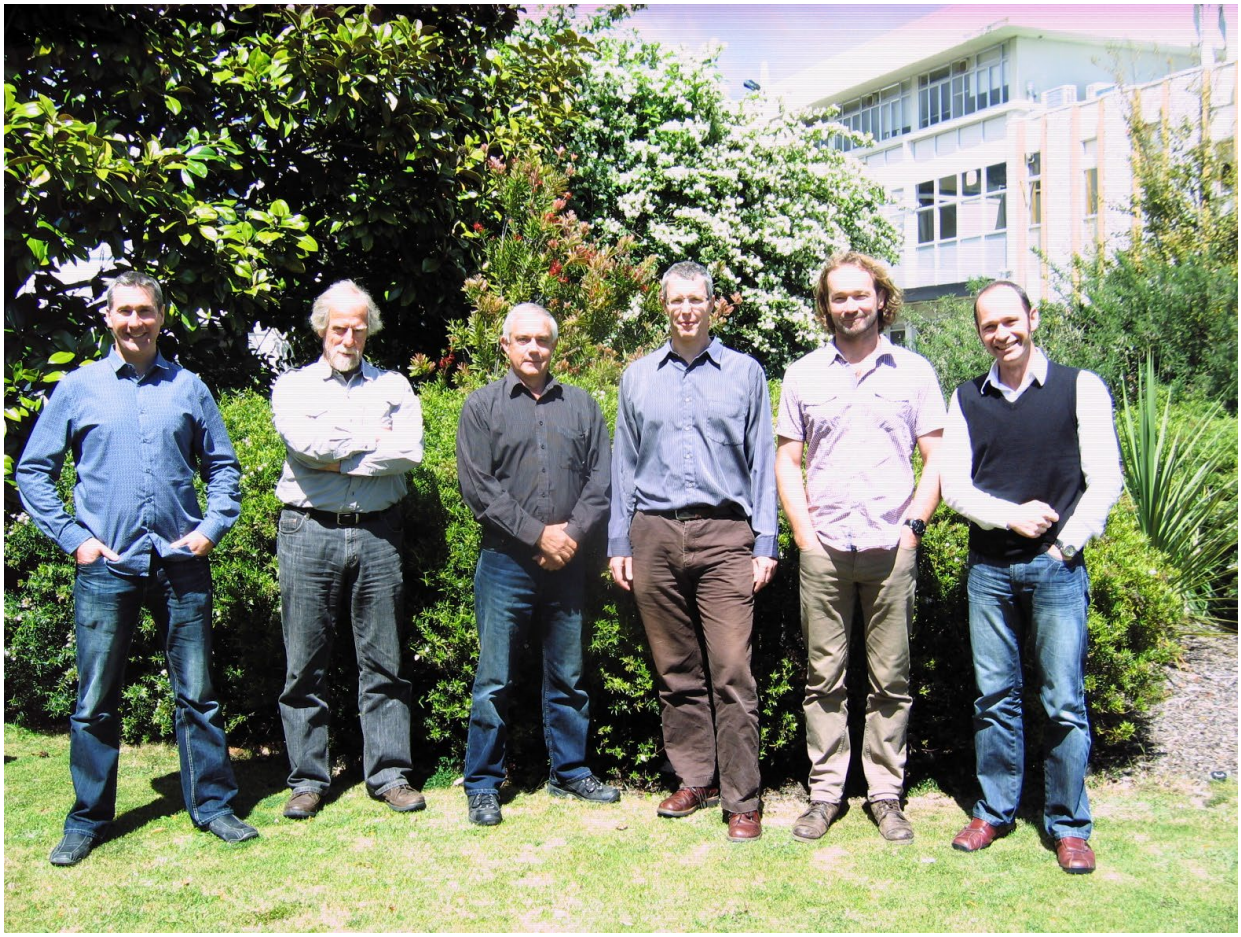
1.4.3 Fresh electrode filling solution should be placed in the probe each day of use. Probe was previously filled with 'Solution A', up to the filling hole. However, no difference was observed when only filled up the 'N' of 'Orion'.

QUALITY CONTROL CRITERIA

System Suitability Criteria

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

19. APPENDIX H: PANEL MEMBERS OF THE ORIGINAL BMP



From left to right: Stephen Urlich, Rob Schuckard, Mark Gillard, Niall Broekhuizen, Nigel Keeley, Richard Ford.