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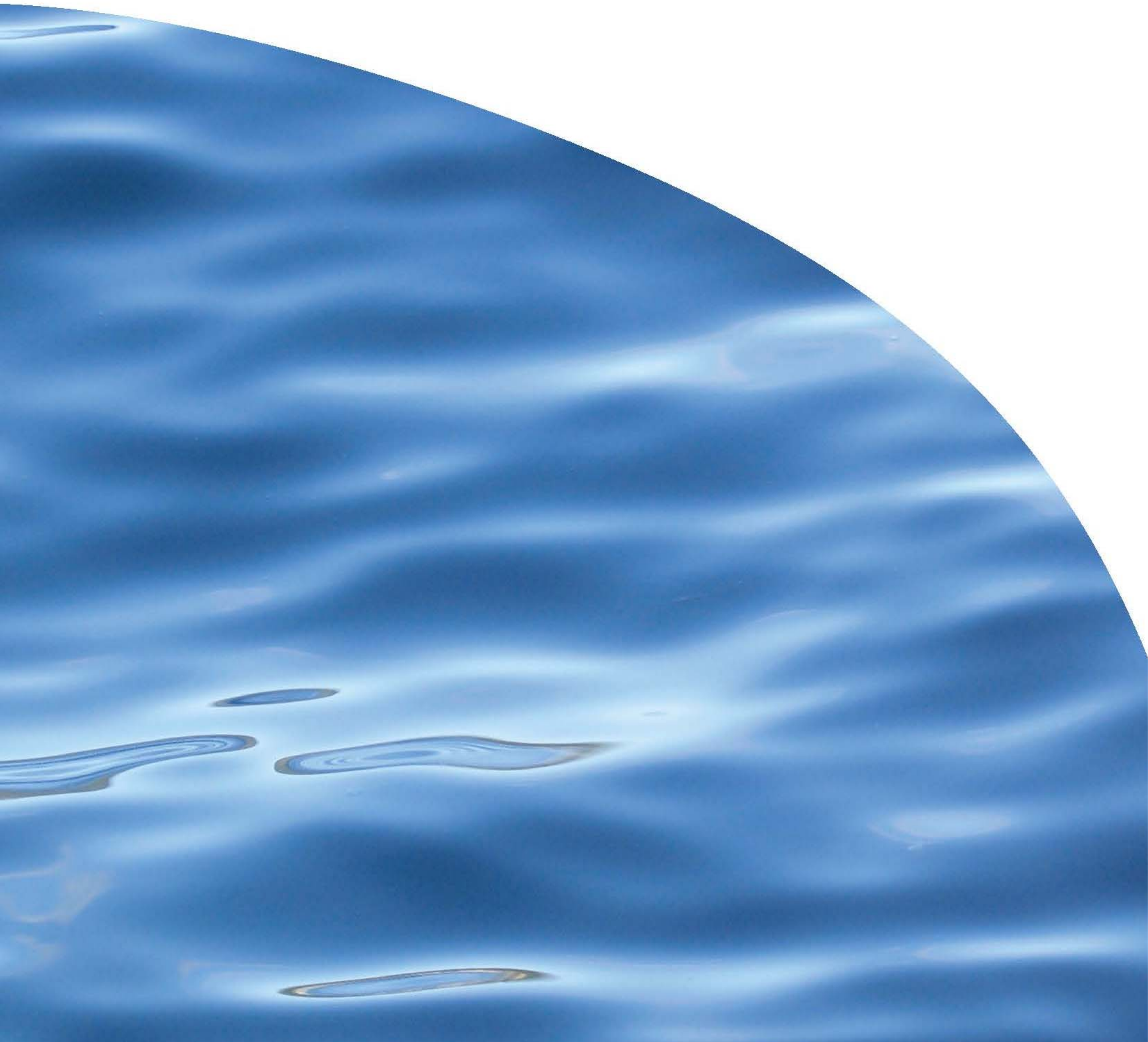

A REVIEW OF TOTAL FREE SULFIDE CONCENTRATIONS IN RELATION TO SALMON FARM MONITORING IN THE MARLBOROUGH SOUNDS

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Prepared for Marlborough District Council

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1. INTRODUCTION

In 2014, the Benthic Standards Working Group (BSWG; comprising a representative from each of Cawthron, NIWA, New Zealand King Salmon Co. Ltd, Marlborough District Council, Sounds Advisory Group, and Ministry for Primary Industries) developed some Best Management Practice guidelines (BMP) and standards for monitoring around salmon farms in the Marlborough Sounds, New Zealand (Keeley *et al*. 2015). Environmental quality standards (EQS) for managing resource consents are based on seabed enrichment stages (see table 5, Keeley *et al*. 2015). The EQS utilise sulfide thresholds as one of the triggers for transitioning from a minimal qualitative assessment (Type 1) to a more rigorous (and more expensive) quantitative assessment (Type 2) of Enrichment Stage or ES.

The existing total free sulfide (TFS) triggers for the zone of maximum effects and the outer limit of effects are 1700 and 390 μM respectively. These trigger levels are based on the relationship between TFS and macrofaunal responses from New Zealand (Keeley *et al*. 2012; Keeley *et al*. 2013) and overseas (Hargrave *et al*. 2008; NBDELG 2012a, 2012b). An additional consideration taken into account was that the TFS triggers were to be applied on a station-by-station basis (rather than a farm average), and that they were also to be coupled with other qualitative investigations[1].

The BSWG has identified a need to evaluate the appropriateness of TFS trigger values. There is no universally accepted TFS value for benthic compliance monitoring and its use in New Zealand is relatively new. This has meant that the previous assessment of the relationships between TFS and other biological variables was based on only three consecutive years' worth of regionally relevant monitoring data. Recently there have been three additional full rounds of annual benthic monitoring conducted around all salmon farms in the Marlborough Sounds. These additional data provide the opportunity to re-evaluate the relationship between TFS and biological function within the sediments, as well as the overall ES.

The main objective of this report was to carry out a desktop review on the use of TFS as one of the environmental monitoring compliance triggers. This study was funded by an MBIE Envirolink medium advice grant (CAWX1406 1530-MLDC98) and was conducted on behalf of the Marlborough District Council.

1.1. Background

1.1.1. Information used to set triggers

The existing trigger levels in the Benthic BMP Guidelines for determining monitoring intensity (*e.g*. Type 1 versus Type 2) take into account a variety of information

[1] Also incorporated are qualitative assessments of presence and degree of sediment outgassing along with bacterial mat formation and a qualitative visual evaluation of the macrofauna assemblage (table 6, Keeley *et al.* 2015).

sources relating to TFS thresholds. More specifically, the proposed levels in the Benthic BMP guidelines reflect:

- the upper 95% confidence intervals associated with ES5 conditions in the Marlborough Sounds for low- and high-flow sites[2] (estimated to be 1705 and 2409 µM, respectively; calculated values associated with figures 2 and 5 in Keeley *et al*. 2012)
- the proposed threshold between Oxic-A and Hypoxic-A status classifications of 1500 µM (Hargrave *et al*. 2008)
- the level of 3000 µM used in Canada as a trigger for more intensive monitoring and at which adverse environmental impacts on benthic sediments are likely to occur (NBDELG 2012a, 2012b)
- some evidence that suggests 1500 µM is a significant biological threshold in Marlborough Sounds sediments (Keeley *et al*. 2013).

In setting the existing triggers, the greatest emphasis was placed on the locally established relationships between TFS and benthic condition, as well as on taking a conservative approach where there was uncertainty. Although this rationale mainly applies to the zone of maximum effects, a similar approach was taken in determining the initial outer limits of effects trigger, where impacts on conditions are expected to be minimal.

1.1.2. Sulfide in sediments

The use of TFS as an indicator of benthic health is based on the role sulfides play on biogeochemical processes in the sediments, and the known relationship between sulfide concentrations and overall benthic condition. A detailed summary of these processes can be found in the literature (Hargrave, 2010; Hargrave *et al*. 2008; Wildish *et al*. 2001). Briefly, organic material deposited on the seabed is broken down and metabolised, and this process influences the chemical and biological composition of the sediments. The biogeochemical cycle involves chemical oxidation, which is aerobic at the sediment surface (utilising oxygen), but becomes hypoxic (oxygen-limited) and anoxic (without oxygen) in deeper sediments, where it is replaced by sulfate reduction (converting sulfate to sulfide). When sediments are anoxic and the sulfate is depleted, methanogenesis becomes the dominant metabolic process. Some sulfides are produced during sulfate reduction, and the dissolved component (total dissolved (or free) sulfides; $S^{2-} = H_2S + HS^{-} + S^{2-}$) is toxic to most animals. Similarly, methane is produced (as gas) during the methanogenesis process. These two gases are responsible for the 'outgassing' that can be observed when sediments become excessively enriched and highly anoxic. Biological effects beyond the sediments are

[2] Broadly categorised according to whether a site's mean current speed in mid-water is above or below 10 m s^{-1} (which corresponds to a generally accepted critical erosion velocity for waste particulates, and therefore scope for resuspension) (Keeley *et al.* 2013).

considered uncommon since TFS is oxidised very rapidly upon contact with oxygenated water.

Concentrations of TFS in the sediments are therefore thought to provide an indication of the chemical oxidative state of the sediments, and of the potential toxicity to biota. The state of this chemical process can also be measured in terms of 'redox potential' (redox, Eh_{NHE}) *in-situ* with the use of redox probes (Wildish *et al*. 1999; Wildish *et al*. 2001). Both redox and TFS are used in conjunction within salmon farm monitoring programs as a proxy for benthic condition (including biological activity within the sediments) because they are relatively cheap and easy to measure (*e.g*. NBDELG 2012a, 2012b).

2. RESULTS AND DISCUSSION

A desktop analysis was carried out using 595 observations collected between 2009 and 2014. Each observation involved paired biological and sediment chemistry samples that, in most instances, were drawn from within the same Van Veen grab sample (*i.e*. from very close spatial proximity, c.10-25 cm apart) which constituted one of three replicate samples for a given station.

Since 2009, some very high TFS concentrations of between 6,000 and 10,000 µM and a maximum of approximately 11,000 µM have been recorded (Figure 1). Most of the biological indicators consistently show highly degraded conditions (and a severely impoverished faunal assemblage) beyond TFS concentrations of approximately 4,000 µM (Figure 1). The only possible exception concerns macrofaunal total abundance (N) at high-flow sites (upper left panel of Figure 1), which remained highly elevated (*i.e*. at peak abundance) despite highly elevated TFS concentrations in some instances. For example, at the high-flow Clay Point farm in 2010, total abundance in one of the net pen samples was > 17,000 individuals core^{-1} despite a TFS concentration of 5,948 µM. Similarly, the net pen site from the high-flow Te Pangu farm in 2010 had a total abundance > 20,000 individuals core^{-1} with a corresponding TFS concentration of 6,535 µM. These results are contrary to the basic assumption that highly elevated TFS concentrations and the associated persistent anoxia should preclude the existence of macrofauna.

For the purposes of this exercise, it was considered most pertinent to focus on the range over which the majority of the degradation occurs in relation to the existing trigger thresholds. The same data has therefore been truncated (or ‘zoomed in’) to the 0 to 5,000 µM range and best-fit linear models have been fitted to the data from high-flow and low-flow sites (along with associated confidence intervals) to aid in visualising central tendencies (Figure 2).

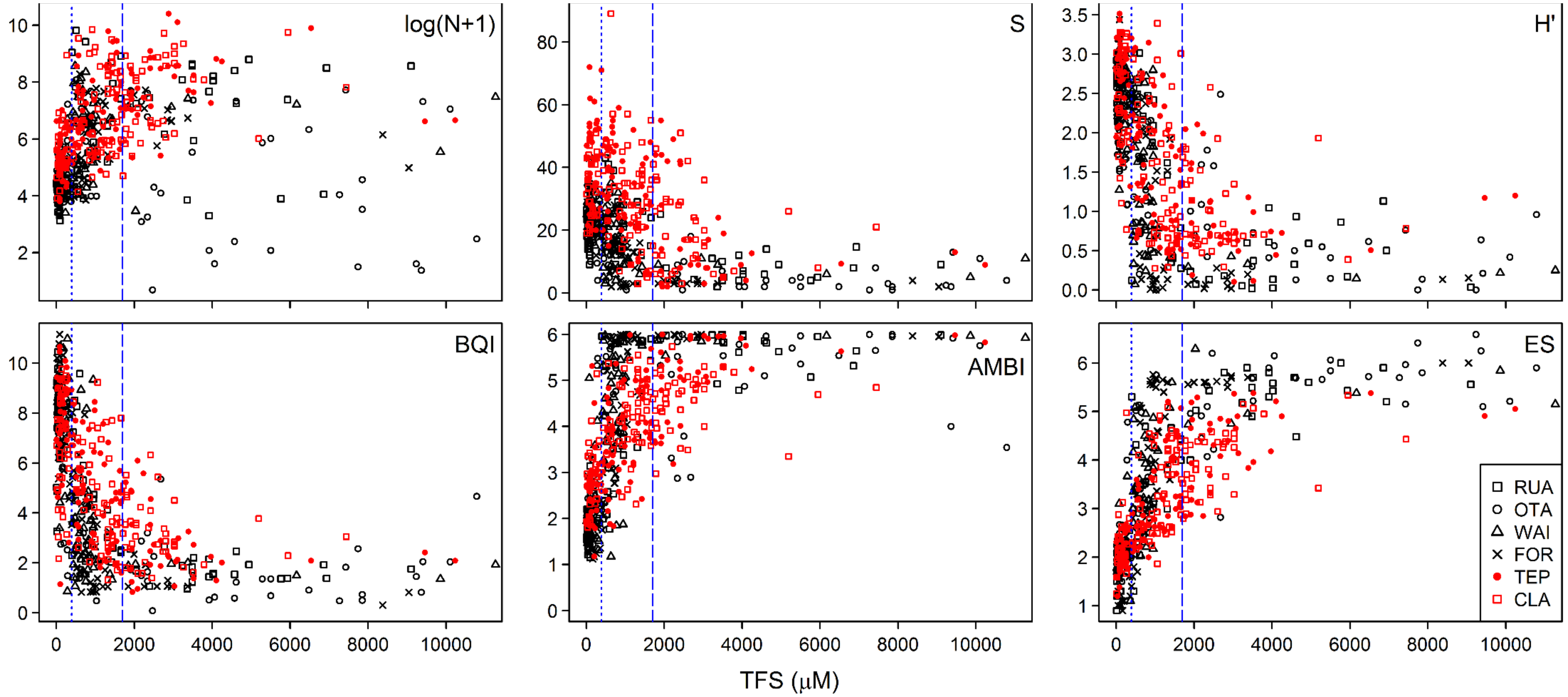

Figure 1. Compilation of all total free sulfide (TFS) data collected during routine annual monitoring between 2009 and 2014 plotted against biological variables (N = total abundance, S = total number of taxa, H' = Shannon Weiner, BQI and AMBI are biotic indices, and ES = overall Enrichment Stage). Black and red symbols represent data from low- and high-flow sites, respectively. Symbol shape represents individual salmon farms: RUA = Ruakaka, OTA = Otanerau, WAI = Waihinau, FOR = Forsyth, TEP = Te Pangu, CLA = Clay Point. Vertical dashed blue lines indicate existing trigger thresholds for Type 2 monitoring of outer limit of effects (390 μM) and maximum zone of effects (1,700 μM).

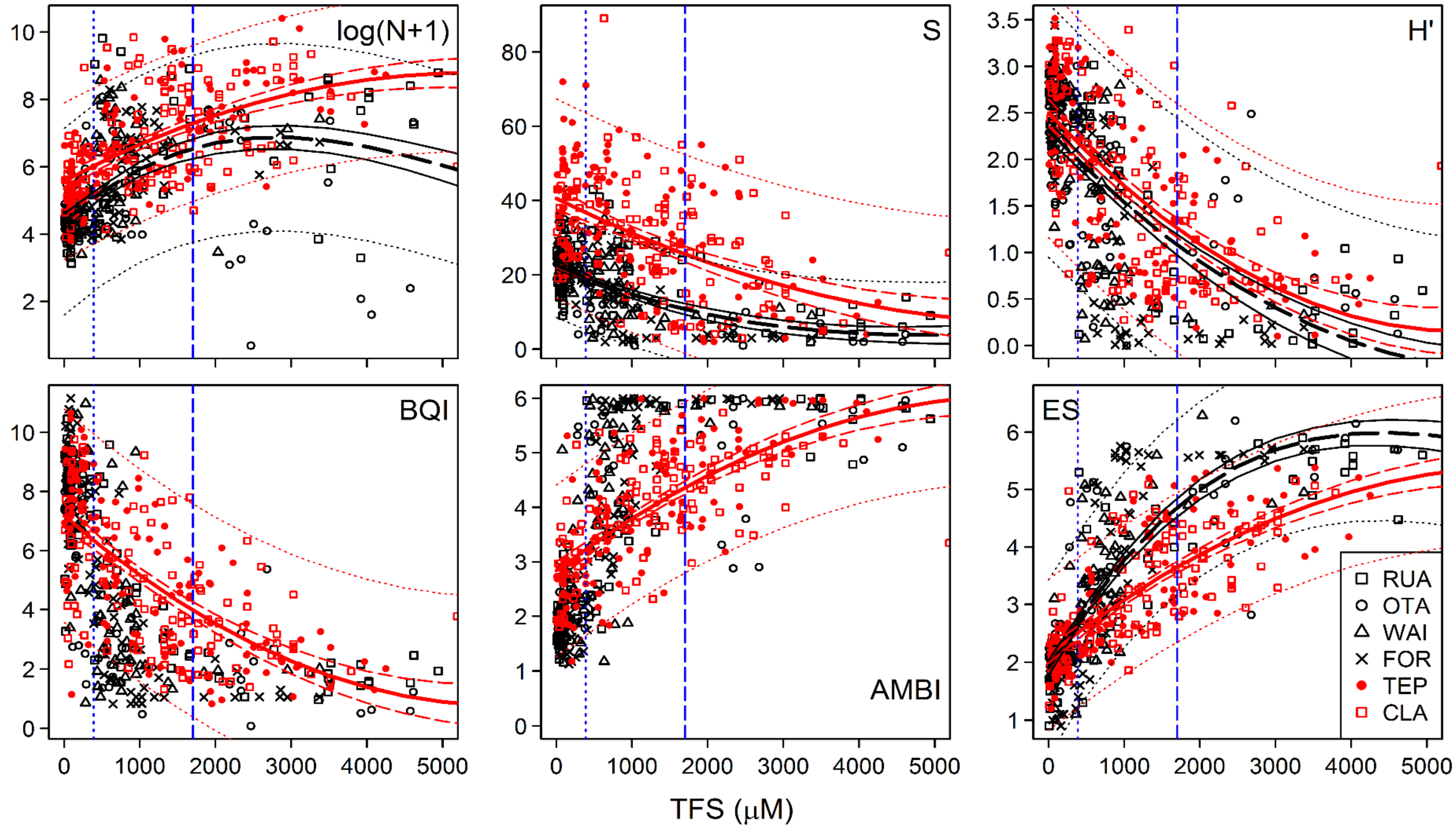

Figure 2. Compilation of all total free sulfide data collected during routine annual monitoring between 2009 and 2014 plotted against biological variables (N = total abundance, S = total number of taxa, H' = Shannon Weiner, BQI and AMBI = biotic indices, and ES = overall Enrichment Stage) truncated at 5,000 µM. Black and red symbols represent data from low- and high-flow sites, respectively. Symbol shape represents individual salmon farms: RUA = Ruakaka, OTA = Otanerau, WAI = Waihinau, FOR = Forsyth, TEP = Te Pangu, CLA = Clay Point. Thick red and black lines represent best-fit linear models for high-flow and low-flow datasets, respectively, and thin dashed lines indicate associated prediction and confidence intervals. Vertical dashed blue lines indicate existing trigger thresholds for Type 2 monitoring of outer limit of effects (390 µM) and maximum zone of effects (1,700 µM).

It is important to recognise that there is considerable scatter in the data, with a relatively wide range of biological responses occurring between TFS concentrations of 500 and 3,000 µM. This is not unusual or unexpected and occurs for a variety of reasons, including small-scale spatial 'patchiness' (with paired samples being slightly spatially separated), varying vertical gradients within sediments for both biology and chemistry, or lags (temporal delays and off-set oscillations) between chemical and biological parameters. TFS concentration should be (and generally is) treated as an indicator of 'likely' biological state, as these factors are not always tightly coupled.

This, in effect, contributes to the rationale behind the development and implementation of the Enrichment Stage index, which incorporates both chemical and biological information to arrive at an overall assessment of benthic condition. Importantly, the 'overall ES' places emphasis on the biological indicators, which are known to provide a very good time-integrated picture of recent (previous few weeks to months) environmental challenges or oxidative stress.

2.1.1. Farm or flow-specific relationships

As a preliminary part of the analysis, ANOVAs were constructed to test for differences in the relationships between TFS and biology that could be attributed to either specific farm attributes ('Farm') or flow conditions (high or low = 'FlowCat'). This was conducted in R using 'Farm' as a fixed factor nested within 'FlowCat' (also a fixed factor), and with TFS as the main explanatory factor. TFS and N (total organism abundance) were log-transformed to increase normality in the data, which are naturally right-skewed.

The results indicate that 'FlowCat' as a factor was highly significant ($P < 0.001$) for all variables except AMBI, which was significant at $P < 0.05$ (Table 1). 'Farm' as a factor across low- and high-flow sites was less important, being non-significant ($P > 0.05$) for the Shannon-Weiner index (H'), the Biological Quality Index (BQI) and the AZTI's Marine Biotic Index (AMBI), and only significant at $P < 0.05$ for the total number of taxa (S) and ES. Log(N) had highly significant ($P < 0.001$) differences associated with both 'Farm' and 'FlowCat'.

Closer analysis of variance partitioning in the models (using the 'summary()' function in R) indicated that the 'Farm'-related differences in abundance (log(N)) were largely due to the Otanerau Bay farm being different from the other low-flow farms. This difference was also evident (more weakly) for ES, which is derived from all variables. As such, there was strong evidence to suggest flow-specific analysis was appropriate, but only weak evidence that farm-specific analysis was necessary—and only in one main variable. Hence, the following analysis focusses on differences that were due to the previously established flow categories.

Table 1 Summary of Pr (> F) values for two-way ANOVAs testing effect of specific farm attributes ('Farm') and flow conditions (high or low = 'FlowCat') on TFS–biological variable relationships. N = total abundance, S = total number of taxa, H' = Shannon Weiner, BQI and AMBI are biotic indices, ES = overall Enrichment Stage. * P < 0.05, ** P < 0.01, *** P < 0.001, Not significant (NS) P > 0.05.

Factor	**log(N)**		**S**		**H'**	
log(TFS)	<2.2e-16	***	< 2e-16	***	<2.2e-16	***
as.factor (FlowCat)	<2.2e-16	***	< 2e-16	***	1.57E-05	***
as.factor (Farm)	0.000322	***	0.01235	NS	0.1196	NS
	BQI		**AMBI**		**ES**	
log(TFS)	<2.2e-16	***	<2e-16	**	<2.2e-16	***
as.factor (FlowCat)	2.01E-06	***	0.04888	*	2.64E-12	***
as.factor (Farm)	0.09716	NS	0.21279	NS	0.03383	*

2.1.2. Flow-specific analysis of relationships between biological variables and TFS

Close analysis of Figure 2 revealed some general trends in the data that were helpful for the purpose of determining meaningful trigger thresholds. These are as follows:

- Peaks in total macrofauna abundance generally occurred at TFS concentrations between 1,500 µM and 2,500 µM, and as low as 500 µM, especially at low-flow sites (Figure 2). Therefore, the use of 1,700 µM as a trigger for Type 2 monitoring in the zone of maximum effects seems appropriate for low-flow farms as it generally indicates early stages of peak abundance (which typifies ES5 conditions). The lower trigger of 390 µM also seems appropriate for the outer limit of effects. It appears to be a minimum concentration below which elevated abundances (N) were not encountered.
- At low-flow sites the total number of taxa was noticeably diminished beyond approximately TFS concentrations of 1,200 µM (*i.e.* consistently reduced by c. 50%), whereas at high-flow sites, relatively high taxa richness persisted at TFS concentrations between 2,000 and 2,500 µM. There were no obvious trends in the total number of taxa at TFS concentrations between 0 and 500 µM. Appreciable reductions in S were not apparent until TFS concentrations exceeded 500 to 1,000 µM.
- Good indicators of enrichment effects (H', BQI and AMBI) all showed a strong (almost) linear deterioration between TFS concentrations of approximately 300 and 1,000 µM (Figure 2). At TFS concentrations > 2,000 µM, these indicators consistently reflected a highly impacted macrofaunal assemblage, although there were still some exceptions, especially with H' and AMBI. In general, highly degraded conditions (*i.e.* H' < 1, BQI < 3 and AMBI > 5) were first encountered just beyond the outer limit of effects trigger of 390 µM. TFS concentrations < 390 µM were therefore not indicative of obviously impacted conditions.

- ES5 conditions at low-flow sites were most commonly encountered at TFS concentrations of approximately 2,000 µM, whereas ES5 conditions at high-flow sites were generally less common and associated with considerably higher TFS concentrations (in the order of 3,000 to 4,000 µM). ES5–type conditions occurred as early as 1,000 µM at low-flow sites, whereas at high-flow sites ES5 tended to be associated with much higher concentrations. The apparent 'resilience' to enrichment despite high TFS concentrations at high-flow sites was most evident in the mid-stages of enrichment, from ES3-ES5.
- The relationship between sulfides and ES identified for low-flow sites during this analysis was very comparable to that initially described from the smaller three-year dataset, with similar predicted mean TFS concentrations bounding the ES categories (according to the polynomial that was constructed using the same statistical process, Table 2). The TFS concentrations bounding ES5 (ES4.5 and 5.5) for example were previously 872 and 1,753 µM, and the values obtained from this assessment were 747 and 1,840 µM.
- Conversely, the relationship between sulfides and ES identified during the analysis of high-flow sites was noticeably higher than was described previously, particularly for the mid ranges of enrichment (Table 2). The differences are primarily due to some exceptionally high TFS concentrations that were recorded during the past two years monitoring (> 6,000 µM), and a small cluster of relatively high values (c. 1,000-1,500 µM) that occurred at a site containing a relatively unimpacted macrofaunal population.

Table 2. Excerpt of nomogram from Keeley *et al*. (2012) showing sulfide concentrations (µM) in relation to ES 1 to 7 from initial assessment based on A: the initial three year dataset and B: the extended six year (2009-2014) dataset.

A: Reproduced from Keeley et al. (2012) and based on 3 years' worth of monitoring results

ES	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Sulfides **LF**	51	107	216	434	872	1 753	3 523
Enrichment zones:	Oxic A				Oxic B	Hypoxic A	Hypoxic B / Anoxic
HF	7	31	90	263	770	2 251	*6,582**
Enrichment zones:	Oxic A				Oxic B / Hypoxic A	Hypoxic B	

B: Same analysis as for Keeley et al. 2012, but utilising six years of monitoring results.

ES	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Sulfides **LF**	26	50	123	303	747	1 840	4 535
Enrichment zones:	Oxic A				Oxic B / Hypoxic A	Hypoxic B	Anoxic
HF	2	40	451	1405	2165	2 965	*6 483*
Enrichment zones:	Oxic A		Oxic B	Hypoxic A		Hypoxic B	Anoxic

3. CONCLUSIONS AND RECOMMENDATIONS

This study examined the biological response in the sediments, according to established macrofauna-derived enrichment indicators, to changes in TFS concentrations. The primary question under consideration was whether the presently adopted thresholds of 390 and 1,700 μM are appropriate for triggering a more comprehensive impact assessment at the outer limit of effects and at the zone of maximum effects, respectively. The main conclusions are as follows.

- Significant flow-specific differences between biological variables meant that it was appropriate to consider the data in terms of previously established flow categories Some farm specificity was evident, but this was limited principally to the relationship between TFS concentration and total organism abundance (and to a lesser extent S—and ES by association), where the Otanerau Bay farm was considered different from the other low-flow farms. This difference was not evident in the other biological variables and hence, was not considered sufficient to warrant a detailed farm-level analysis of relationships.
- The majority of the biological changes occurred between TFS concentrations of 0 and 3,000 μM. Changes in diversity and ecological status were most pronounced and nearly linear between 300 and 1,500 μM, which is consistent with previous observations (Keeley *et al*. 2013). The phase of abrupt change from natural to highly enriched conditions appropriately spans the outer limit of effects and zone of maximum effects triggers, which were intended to indicate when conditions were significantly more enriched than background levels, and when conditions may be approaching (or at) ES5 or 'peak-abundance' – a state that precedes complete collapse of the macrofauna population. The biological condition at TFS > 4,000 μM were consistently highly degraded for both low- and high-flow situations.
- There are characteristic differences between the way high- and low-flow sites respond to organic enrichment (Keeley *et al.* 2012, 2013), and this was evident in the macrofauna–sediment chemistry relationships. Biota at the low-flow sites tended to be more impacted at lower TFS concentrations than at high-flow sites. Conversely, relatively high concentrations of TFS were encountered at high-flow sites when the impacts to the macrofauna were not so apparent. This is most likely related to greater flushing and the propensity for waste to be resuspended (rather than to settle out), and a greater flux of oxygen to the sediments and fauna therein from stronger currents.
- As well as being elevated relative to the low-flow relationship, the TFS-ES regression for high-flow sites was substantially elevated (*i.e.* higher TFS for equivalent ES values) relative to the earlier assessment, which was based on a smaller dataset. This was attributable to some very high TFS concentrations recorded during the last two years' monitoring and a small cluster of relatively high TFS concentrations that occurred in conjunction with a relatively unimpacted macrofaunal population. Therefore, it is apparent that in several instances the existing relationship between TFS concentration and ES may overestimate the

impact to the macrofauna in a relative sense. This is an important consideration, especially if TFS is used as a single proxy for overall effects.

- Therefore, it may be appropriate to develop flow-specific TFS trigger thresholds for the zone of maximum effects areas. In this case, the low-flow TFS concentration seems appropriate and should remain unchanged, but we recommend that the high-flow TFS concentration be increased (c. from 1,700 to c. 2,500-3,000 µM). This would reduce the risk of having Type 2 monitoring required unnecessarily at high-flow sites. This situation may be viewed as conservative and precautionary, and therefore low risk until such point as a new trigger can be adopted. The exact process driving this seeming disconnect between sediment chemistry and biology is unclear at present and probably warrants a focused study. As stated in Keeley *et al*. (2013), it is most likely related to (i) resuspension and the associated lack of organic deposition and accumulation in dispersive locations, and (ii) the high delivery rates of oxygen, which allows the fauna near the sediment surface to be maintained and in some cases to proliferate and reach extreme abundances.
- Obvious impacts to the macrofauna at TFS concentrations < 390 µM were uncommon and this proved to be a meaningful trigger beyond which obvious impacts could be anticipated. This was true for both low- and high-flow sites. Therefore, the existing 390 µM trigger seems fairly conservative and appropriate for the outer limit of effects, where the fundamental requirement is that conditions should not become obviously impacted.

In summary, the analysis has confirmed that a TFS trigger of 390 µM for outer limit of effects is appropriate for low- and high-flow sites, and that a TFS trigger of 1,700 µM for zone of maximum effects is appropriate for low-flow sites. The analysis suggests that the trigger for zone of maximum effects may need to be increased in order for it to work effectively at high-flow sites as indicated above. The association between biogeochemical processes and benthic ecology at high-flow sites warrants further targeted investigation and is a recommended topic for discussion at the next Benthic Standards Working Group meeting.

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