Evaluation of the variability, applicability, and suitability of the redox potential and total sulfide measurement methodologies (Wildish *et al.*, 1999) for determining benthic organic enrichment state and their use as regulatory compliance standards in Maine

Prepared for

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Table of Contents

List of Figures

List of Figures (Continued)

List of Tables

Executive Summary

Data collected between the fall of 2003 and the fall of 2006 as part of the Maine Department of Environmental Protection's General Permit for Atlantic Salmon Aquaculture in Maine monitoring requirements were analyzed to determine whether the methods and standards for redox and total sulfide, as set forth in the permit, are appropriate for use in monitoring effects of salmon aquaculture on the sea floor in Maine

A comparison of redox and total sulfide data collected in Maine with similar data collected in New Brunswick, Canada show similar trends, but the Maine data show a higher variability. This variability in redox and total sulfide results is attributed to several factors but principally to differences in methods used to collect samples and the wider spectrum of sediment types encountered at Maine salmon sites compared to New Brunswick where the analyses were exclusively done in soft, fine-grain sediments.

An analysis of redox and total sulfide results by sediment type shows similar redox median values across the spectrum of sediment types with a slight trend upward in the coarsest sediments; total sulfide median values trend upward with sediment coarseness, a result which is counterintuitive in view of the stronger currents and consequent increased oxygen flux characteristic of areas with coarse sediments.

The biological data developed from samples collected simultaneously with the geochemical samples indicate that although total taxa (an index of the biological community) declines with decreasing redox and increasing sulfide, the numbers of taxa present at the established regulatory levels are not necessarily indicative of the level of degradation implied by the regulatory levels.

We believe these results are due to the confounding effect caused by Eh and total sulfide values obtained from non-representative material, particularly in coarse sediment. In progressively coarser sediments fine material must be selected and removed from coarser material in order to prepare a slurry for measurement; however, this fine material becomes progressively less representative of the general sediment composition and bottom condition as sediment coarseness increases. This problem becomes particularly pronounced in coarse sand-pebble and gravel-rock sediments where soft material can only be obtained from the interstitial spaces between coarser materials. In such cases the soft material represents but a small fraction of the overall sediment profile and is composed primarily of organic material discharged from the cages which is subject to decomposition, thus yielding low Eh and elevated total sulfide values. So, while the measurement results may be accurate for the material being tested, the material being tested is not representative either of the composition or conditions of the general bottom, thus possibly leading to erroneous conclusions on the extent and degree of organic enrichment.

This conclusion leads to several recommendations for consideration as these methods and standards undergo review and reevaluation as monitoring and enforcement tools, respectively:

1. Eliminate redox (Eh) as a metric and standard for determining the level of impact and organic enrichment associated with net pen aquaculture;
2. Eliminate total sulfide ($S^{=}$) as a metric and standard for determining the level of impact and organic enrichment associated with net pen aquaculture in all but soft sediments (silt-clay) similar to those in which the method was developed;

3. Reevaluate the total sulfide standard of 1,300µM Warning Level and >6,000µM and consideration of moving to a site average rather individual station averages when determining site-related impacts;

4. Pursue research to determine the sediment types (granulometry) in which total sulfide measurement is appropriate and valid prior to application of total sulfide as a standard in any sediment other than soft sediments (silt-clay) similar to those in which the method was developed; and

5. Increase reliance on the semi-annual video recordings to assist in the interpretation of total sulfide results and undertake additional work to develop semi-quantitative and quantitative methods for the analysis of epibenthic communities as indicators of organic enrichment.

Introduction

Monitoring of the aquaculture industry in Maine began in the early 1980s in response to requirements established by the U.S. Army Corps of Engineers as part of their navigable waterways projects application process. The monitoring requirements during the early stage of development of the salmon and steelhead trout marine farming industry were initially restricted simply to a diver survey in the general vicinity and brief reporting of direct observations during the dive; the first salmon farming sites were located in Cobscook Bay, a macrotidal embayment with a mean tidal amplitude of 5.6m (18.35ft) and a spring amplitude 6.5m (21.18ft). As development of the industry progressed with increased production and farm siting outside of Cobscook Bay in lower tidal amplitude areas, the possible impacts associated with the deposition of net pen wastes on the bottom raised concerns within the Maine departments of Environmental Protection (DEP) and Marine Resources (DMR) that led to increased monitoring requirements focused primarily on dissolved oxygen content of the water column and benthic community structure; however, a uniform monitoring method and program had not yet been developed and monitoring plans were developed on a case-by case, site-by-site basis.

In 1991 the Maine State Legislature requested that a study be undertaken to investigate net pen aquaculture monitoring programs elsewhere in the United States and abroad to ultimately lead to the development of a uniform and standardized monitoring program for the State of Maine. The study was completed in early 1992 and the resulting unified site application and monitoring program, named the Finfish Aquaculture Monitoring Program (FAMP), was completed and first implemented in the fall of 1992. The FAMP included water column monitoring of dissolved oxygen and benthic monitoring based on diver observations and video recordings, and sediment sampling for total organic carbon, granulometric, and infauna community analyses. Over the course of the following ten years the program remained in effect with periodic slight modifications to methods.

In February 2002, in response to continued concern over the possible environmental effects and impacts to the endangered Atlantic salmon, the US Environmental Protection Agency (EPA) completed development and issuance of a Clean Water Act (CWA) National Pollutant Discharge Elimination System (NPDES) permit for net pen aquaculture for Acadia Aquaculture in Blue Hill Bay, Maine. Also in 2002, EPA delegated authority to Maine for the development and issuance of NPDES permits, including those for the remaining existing aquaculture sites (http://www.epa.gov/NE/pr/2002/feb/020213.html). After an extensive public hearing process the Maine DEP completed development of a General Permit for Atlantic salmon aquaculture in Maine (General Permit) (http://www.maine.gov/dep/blwq/docstand/aquaculture/MEG130000.pdf) the final version of which was released in June 2003.

Similar to the FAMP, the MEG130000 includes environmental monitoring requirements for both water column dissolved oxygen and benthic conditions. As in the FAMP, benthic monitoring includes video recording of the bottom conditions beneath and adjacent to the net pen structures for evidence of hypoxic or anoxic conditions and to determine the percent coverage by the sulfur-reducing bacteria *Beggiatoa* sp. Also as in the FAMP, benthic sediment sampling is required for measurement of granulometry, total organic carbon, and infauna community structure for the determination of taxa, absolute relative abundance, and diversity (Shannon-Wiener Diversity Index).

Unlike the FAMP, MEG130000 requires geochemical analyses of redox, total sulfide, metals (copper and zinc), and medication residue; the first two are the subject of this study. The inclusion of redox and total sulfide measurements in the General Permit is based on the work of Hargrave, *et al*., (1995) and Wildish, *et al.* (1999); the latter work intended to provide the methods for use of redox and sulfide measurement as a surrogate for the much more time-consuming, and therefore costly, benthic infauna analysis in determining the general magnitude of effect related to organic enrichment. This work consisted of measurements from 65 sites in soft, fine-grain sediments.

The results of redox and total sulfide measurements presented in Wildish *et al.* (1999) yield the regression equation

$$y = -65.949\mathrm{Ln}(x) + 473.36$$

with a relatively high correlation coefficient (R^2) value of 0.6722, indicating a relatively strong correlation between total sulfide and redox.

The above regression equation can be used to predict an Eh value ($\hat{E}$) (y in the above equation) from a given total sulfide value ($S^{=}$) (x in the above equation), using the general equation $\hat{E} = b\mathrm{Ln}(x) + a$, where $a = 473.36$ and $b = -65.949$. Similarly, a predicted total sulfide value ($\hat{S}^{=}$) can be obtained from a given Eh value (E) using the equation $\hat{S}^{=} = \exp^{(E-a)}/b$. Accordingly, Wildish *et al.* calculated the respective hypoxic and anoxic limits as shown n Table 1.

Table 1. Prediction of Eh ($\hat{E}$) and total sulfide ($\hat{S}^{=}$) values from the regression equation presented in Wildish *et al.* (1999).

Data source	Regulatory level	$S^{=}$	$\hat{E}$	E	$\hat{S}^{=}$
Wildish *et al.*	Warning	1300	***0.50***	0	***1310***
	Impact	6000	***-100.36***	-100	***5967***

Based on these results, Wildish *et al.* recommended "hypoxic" limits of Eh = 0 and $S^{=} = 1300$ and "anoxic" limits of Eh = -100 and $S^{=} = 6000$.

The redox and total sulfide sampling and measurement methods of Wildish, *et al.* (1999) were developed for soft sediments and at the time of the issuance of the General Permit had not been proven applicable in other, coarser sediments such as those often found around Maine salmon farming sites. Questions were therefore raised regarding the applicability of the methods across the broad spectrum of sediments found at Maine sites and the use of the associated organic enrichment gradient zone limits as regulatory standards.

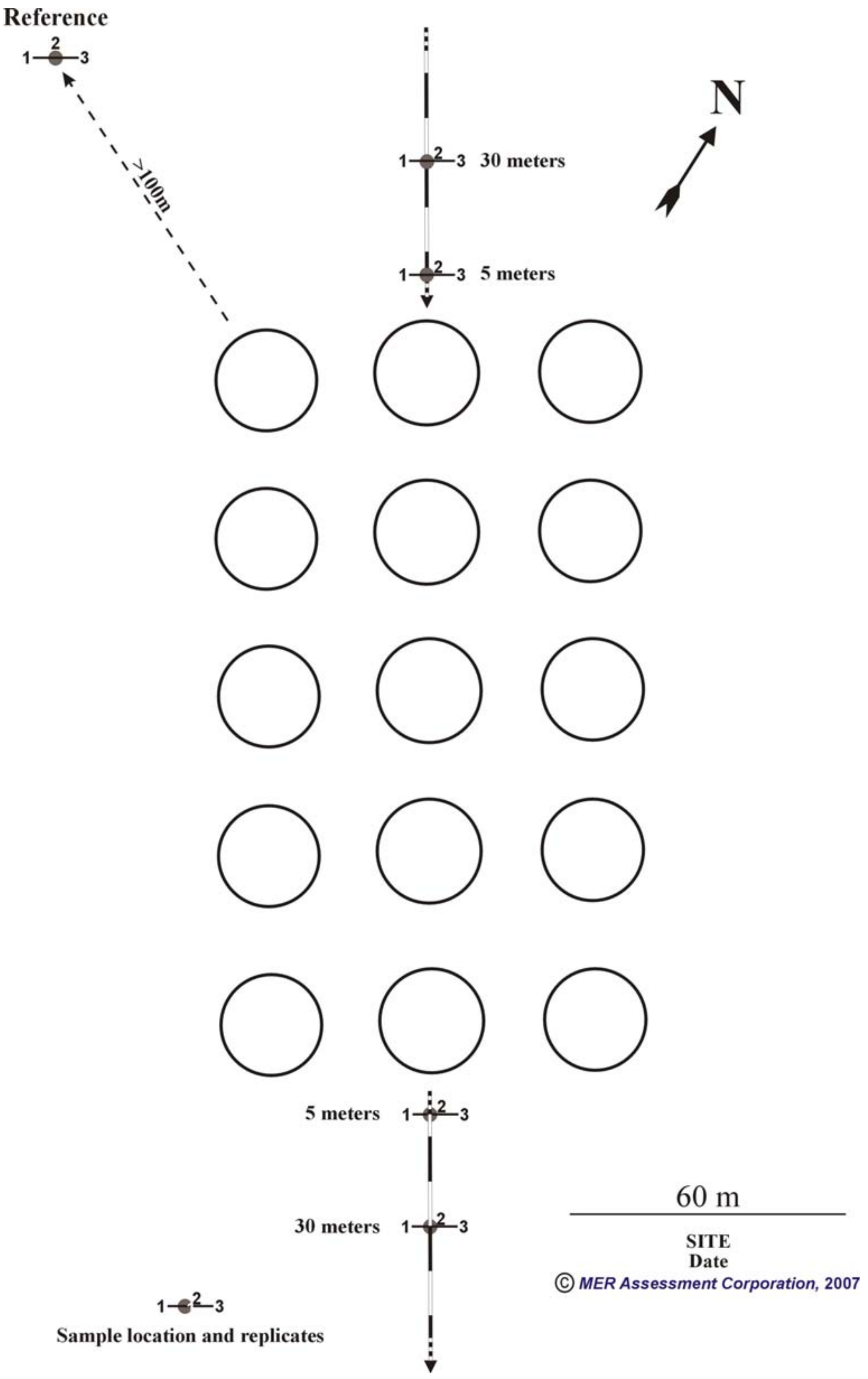
After three years of implementation of the General Permit monitoring requirements and measurement of redox and total sulfide at all of the active salmon farming sites in Maine as well as at non-affected sites, the study presented here was proposed to analyze the results of redox and total sulfide testing to evaluate whether the methods of Wildish *et al.* are, indeed, applicable and suitable for the monitoring and regulation of Maine farming sites.

Methods

Redox and total sulfide sampling

According to the General Permit, redox and total sulfide sampling is required at 0-5 meters and 30 meters from the net pen system off both ends of the system along the predominant current axis, usually the longer axis of a net pen array, usually designated by a distance and a compass direction, *e.g.* 5mN indicating 5 meters from the north side of system. Three samples are taken at each of the distances, designated 1, 2, and 3, as shown in Figure 1, thus full sample identifier appear as 5mN1, 5mN2, etc.

Figure 1 Benthic sediment sampling locations as required by the General Permit shown on a generic site layout.



Where water depth is <80 feet, sampling is done by a diver; where water depth is >80 feet, sediment samples are taken as subsamples of benthic grabs taken by a 9-inch square Ponar® sampler. In either case, a sediment core is taken at each sampling location using a 4” diameter PVC pipe coring device. The open end of the corer is pushed into the sediment to a depth of 10cm or resistance; the closed end has 1/8” holes drilled in the cap to allow water to escape from the corer as the corer is driven into the sediment. Once fully inserted, the bottom end of the corer is covered by hand, the corer is withdrawn from the bottom, and a PVC plastic cap placed over the open end of the corer. The corer is maintained in the upright position until delivered to the point of sediment removal for analysis. It should be noted that the sampling corers used for General Permit sampling are different from those used by Wildish *et al.*, 1999. Additionally, the measurement methods used by MER for General Permit sampling are those recommended by Wildish and Hargrave (2003) in their Interim Recommendations, described below and attached hereto as Appendix I, which are different from those used by Wildish *et al.* in 1999.

Redox and total sulfide measurements

One half of the core surface material is removed down to a depth of 2 cm and the sediment placed in a small 125 ml plastic container and thoroughly mixed with a plastic spoon for approximately 1-2 minutes. Following mixing, the redox potential is measured using an Accumet® AP63 pH/mV/Ion meter equipped with a Thermo Orion model 9678BN Combination Redox electrode (replaced annually) filled with Thermo Orion Ag/AgCl Reference Electrode Filling Solution (900011) by immersing the electrode (standardized against Zobell’s solutions per Wildish *et. al*, 1999; see Appendix II) into the mixed sediment and waiting for the reading to stabilize while gently mixing the sediment with the electrode. Meter mV values are corrected by applying a correction factor for temperature (Thermo Orion Platinum Redox Electrode Instruction Manual, Model 96-78-00, 2001, p. 5).

After redox measurement, a 5 ml portion of the mixed sediment is removed with a modified 5 ml plastic syringe with the needle attachment end removed to form an open cylinder; the open end is immersed into the mixed sediment slurry and the sample extracted by pulling back on the plunger, thus obtaining a sample containing no bubbles. Immediately after obtaining the sample, the open end of the syringe is covered with plastic wrap insuring no air is trapped beneath the wrap. Aluminum foil is then placed over the end of the syringe to secure the plastic wrap in place. The syringe is then placed in a cooler with ice to maintain a temperature of <5^{O}C during transport to the laboratory for total sulfide ($S^{=}$) analysis within <72 hrs. of sample collection.

Once at the lab, all total sulfide sample syringes are allowed to warm to room temperature (≈20^{O}C). Analysis is carried out with the Accumet® AP63 pH/mV/Ion meter equipped with a Thermo Orion model 9616BN Combination Silver/Total sulfide electrode filled with Thermo Orion Ionplus B Optimum Results™ Reference Electrode Filling Solution (900062) with standards prepared according to Wildish *et al.,* 1999. The meter is standardized at 1.00 (100μM), 10.0 (1,000μM), and 100 (10,000μM). All samples are analyzed within a maximum of 3 hrs. Following analysis of all samples, measurements of the three standards are retaken and recorded on the calibration sheets. Actual S_2 μM values are calculated by multiplying the meter readings by 100. The MER Assessment Corporation (MER) standard operating protocols for redox and total sulfide sampling and measurements are attached hereto as Appendix II.

Sediment type

Sediment samples for granulometric analysis are simultaneously collected when samples for benthic infauna analysis are collected; granulometric analysis is not performed when no benthic infauna samples are collected. According to the General Permit requirements benthic infauna analysis is required to be performed once every five years in a year toward the end of the production cycle, that is, when market fish are on-site. Due to fallowing requirements and the fact that the General Permit monitoring had only been in effect for three years as of the 2005 data, a large number of sites where redox and total sulfide sampling was done were not simultaneously sampled for granulometric analysis, thus there are few grain size measurement data associated with the redox and total sulfide values. However, based partly on historic granulometry results and almost 20 years of experience with the various salmon farming sites in Maine sediments at the sites can be categorized by general coarseness. Accordingly, for the purposed of this analysis we have developed six sediment type categories: 1- soft, silt/clay, 2- sandy silt, 3- sand, 4- coarse sand/pebbles, 5- gravel, 6- rocks.

Benthic infauna data

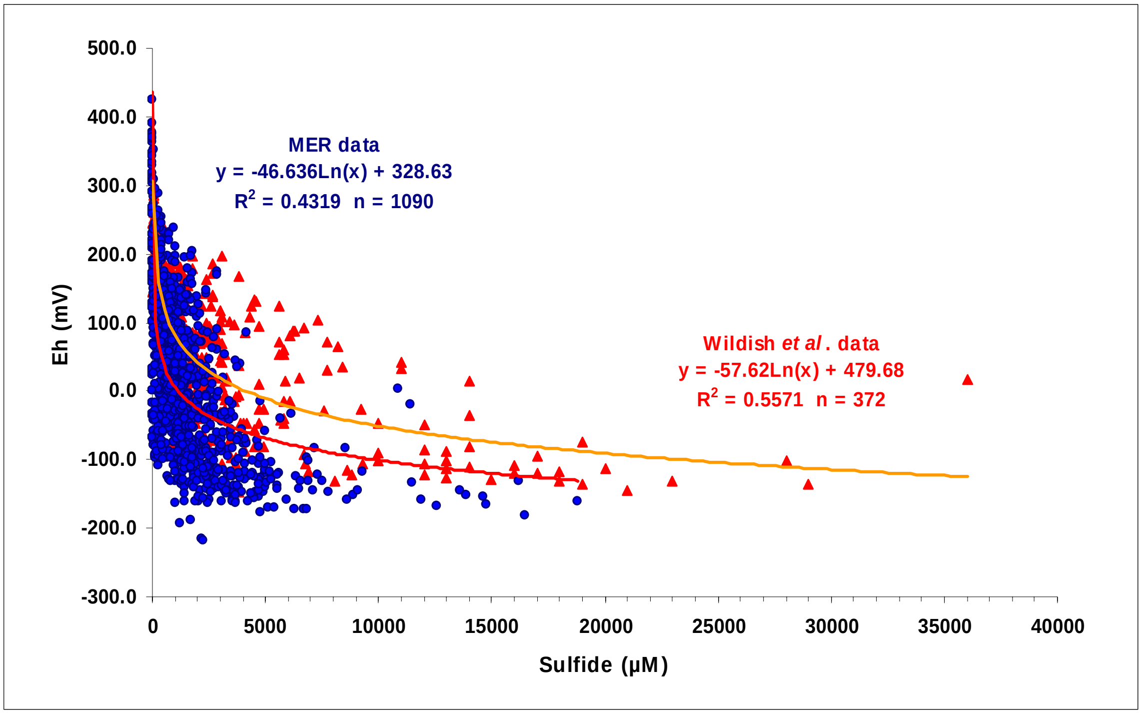
Benthic infauna data, specifically species richness and abundance, were developed through standard benthic sorting and identification procedures and the data analyzed using Excel spreadsheets developed by MER Assessment Corporation (MER) as part of the annual routine salmon farm site monitoring.

Results

Redox, total sulfide, and sediments type

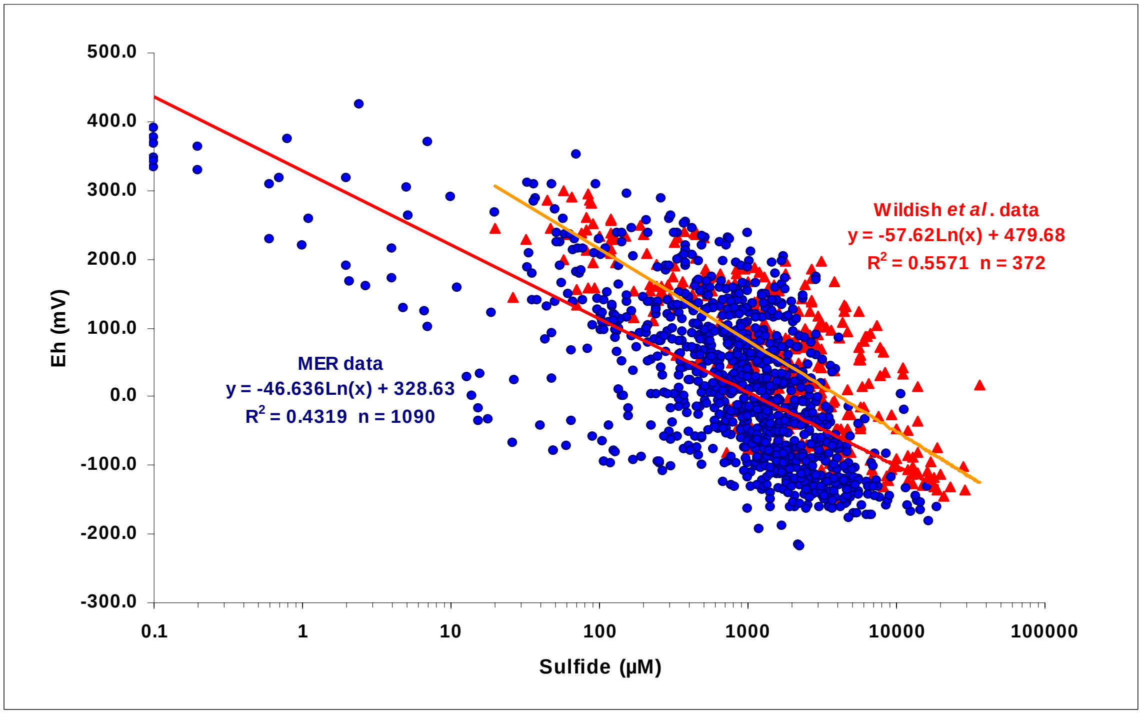
A total of 1090 redox and total sulfide measurements have been collected since the implementation of the General Permit. The results of the measurements used in this analysis represent, for the most part, conditions at active farm sites (n = 823); however, some measurements have also been made at non-farm sites, either during the collection of baseline data for purposes of site lease applications or at reference stations for active farm site comparisons (n = 190) or in proximity to the net pens but at distances >30 meters from the pens (n = 77). The full set of data used in the analysis is included here as Appendix III. The full set of redox and total sulfide data developed by MER between 2003 and 2006 are shown in Figure 2 plotted against the Wildish *et al.* 1998 Bay of Fundy salmon mariculture environmental monitoring results only (Wildish *et al*. 1999, Appendix I), included here as Appendix IV, using a standard arithmetic scale for total sulfide.

Figure 2 Redox-total sulfide relationship comparison between MER 2003-06 sediment data (blue circles) and Wildish *et al.* 1998 Bay of Fundy salmon mariculture monitoring data (red triangles)



As Figure 2 shows, the relationship between redox and total sulfide is logarithmic with respect to total sulfide and the same data are therefore plotted on a logarithmic total sulfide scale in Figure 3, a similar plot as used by Wildish *et al*.

Figure 3. Redox-total sulfide relationship comparison between MER 2003-06 sediment data (blue circles) and Wildish *et al*. 1998 Bay of Fundy salmon mariculture monitoring data (red triangles) on a logarithmic total sulfide scale.



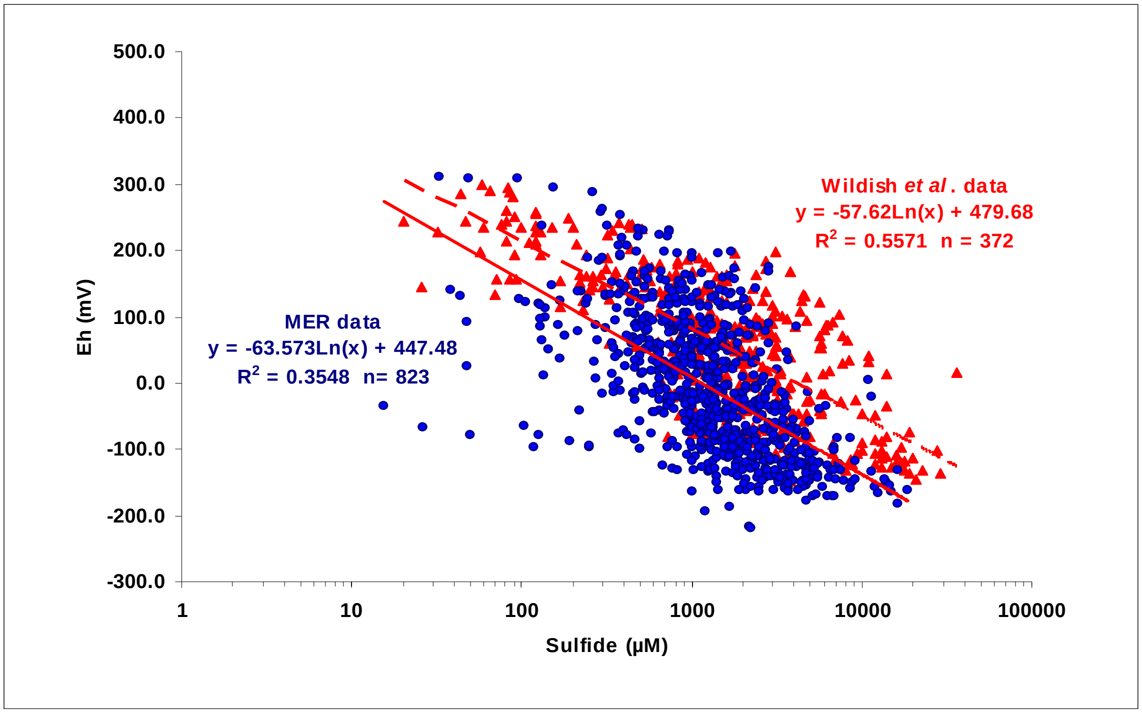
The linear equation for the Wildish *et al.* salmon farm-only data is different from that of the combined salmon farm and Fundy Isles 1994-95 with the salmon farm-only data having a slightly shallower slope, but similar y-intercept, and a lower $R^2 = 0.557$. The MER data yield an even shallower slope, a lower y-intercept, and a lower $R^2 = 0.432$; in both cases $p<0.001$, thus we reject the null hypothesis H_0 that the values are random and no relationship exists between Eh and total sulfide. The MER Maine data generally cluster and trend similarly to the Wildish *et al.* data, although the spread is greater and shifted to the left, thus yielding lower Eh values for given total sulfide values and lower total sulfide values for given Eh values compared to the Wildish *et al*. data distribution, as shown in Table 2.

Table 2. Prediction of Eh ($\hat{E}$) and total sulfide ($\hat{S}^{=}$) values from regression equation for Wildish *et al.* 1998 salmon mariculture monitoring only and all MER redox and total sulfide data collected between 2003 and 2006.

Data source	**Regulatory level**	$S^{=}$	$\hat{E}$	E	$\hat{S}^{=}$
Wildish *et al.*	Warning	1300	*67.1*	0	*4186*
	Impact	6000	*-20.7*	-100	*23932*
MER	Warning	1300	*-5.76*	0	*1149*
	Impact	6000	*-77.1*	-100	*9808*

The MER regression analysis in Table 2 is based on the entire 2003-06 MER data set corresponding to the full spectrum of sediment types sampled, and both unimpacted and impacted conditions. Since the Wildish et al. (1999) data used are strictly from environmental monitoring of salmon farm sites, we therefore eliminated all measurements representing non-net pen affected conditions and restricted data to MER 2003-2006 salmon farm site monitoring, as shown in Figure 4.

Figure 4. Redox-total sulfide relationship for all MER data measurements representing net pen-affected conditions only (blue circles) compared to Wildish *et al*. 1998 Bay of Fundy salmon mariculture monitoring data (red triangles).



Again, the MER measurements cluster similarly to the Wildish *et al.* data, but with a greater spread resulting in a lower $R^2 = 0.355$ indicating that a large portion of the variability in the relationship between Eh and total sulfide remains *unexplained*.

Table 3. Predicted Eh ($\hat{E}$) and total sulfide ($\hat{S}^=$) values from the regression equation for Wildish *et al.* 1998 salmon mariculture monitoring only and MER 2003-2006 salmon farm site monitoring.

Data source	Regulatory level	$S^=$	$\hat{E}$	E	$\hat{S}^=$
Wildish *et al.*	Warning	1300	*67.1*	0	*4186*
	Impact	6000	*-20.7*	-100	*23932*
MER	Warning	1300	***-8.4***	0	***1140***
	Impact	6000	***-105.6***	-100	***5496***

Based on this analysis, the MER results are close to the Wildish *et al.* recommended hypoxic (warning) and anoxic (impact) limits, particularly for the impact level. We then reanalyzed the redox and total sulfide measurements used to generate equations in Figure 4 according to sediment type, as shown in Figures 5-8, to determine if the predicted values would hold for individual sediment types. It should be noted that Categories 3 and 4 (sand; coarse sand-pebbles) have been grouped, as have Categories 5 and 6 (gravel; rock), because these sediment types tend to occur together and are difficult to separate.

Figure 5 Redox-total sulfide relationship for MER Category 1 (soft silt-clay) sediment data.

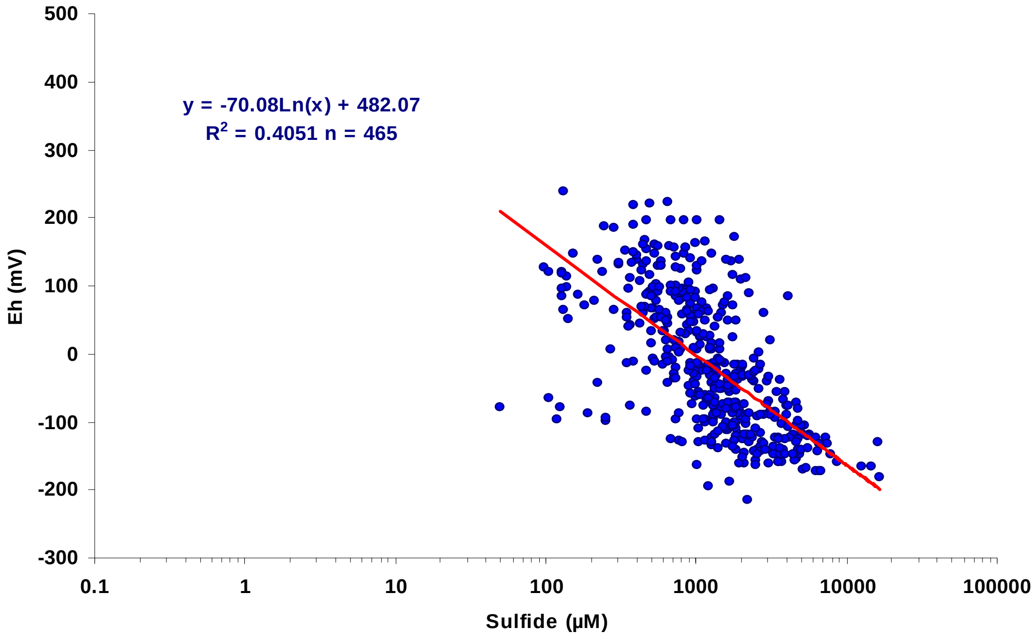


Figure 6 Redox-total sulfide relationship for MER Category 2 (sandy silt) sediment data.

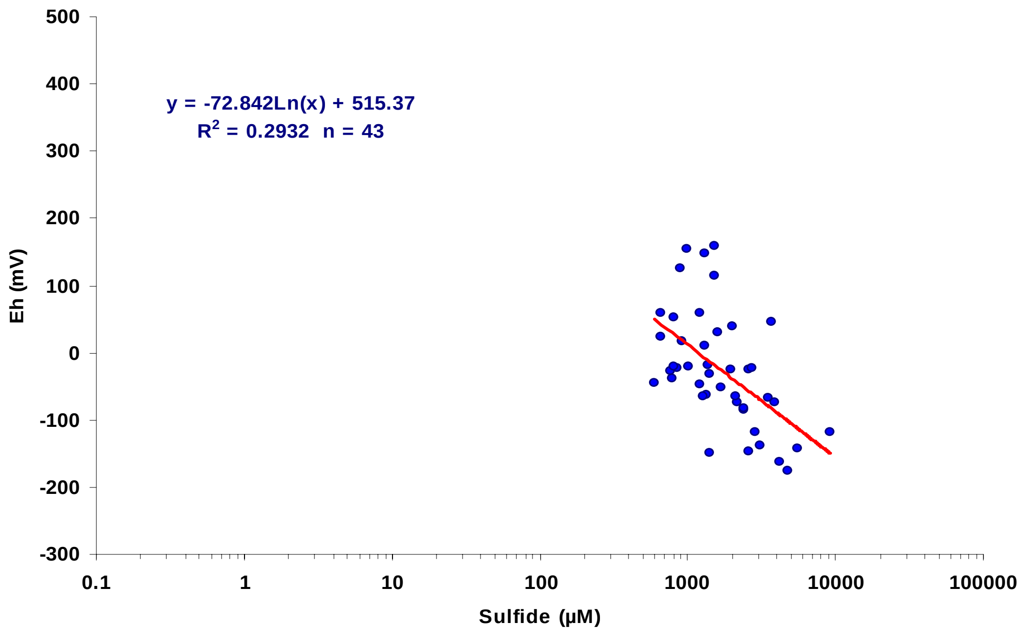


Figure 7 Redox-total sulfide relationship for MER Category 3 and 4 (sand and coarse sand-pebbles) sediment data.

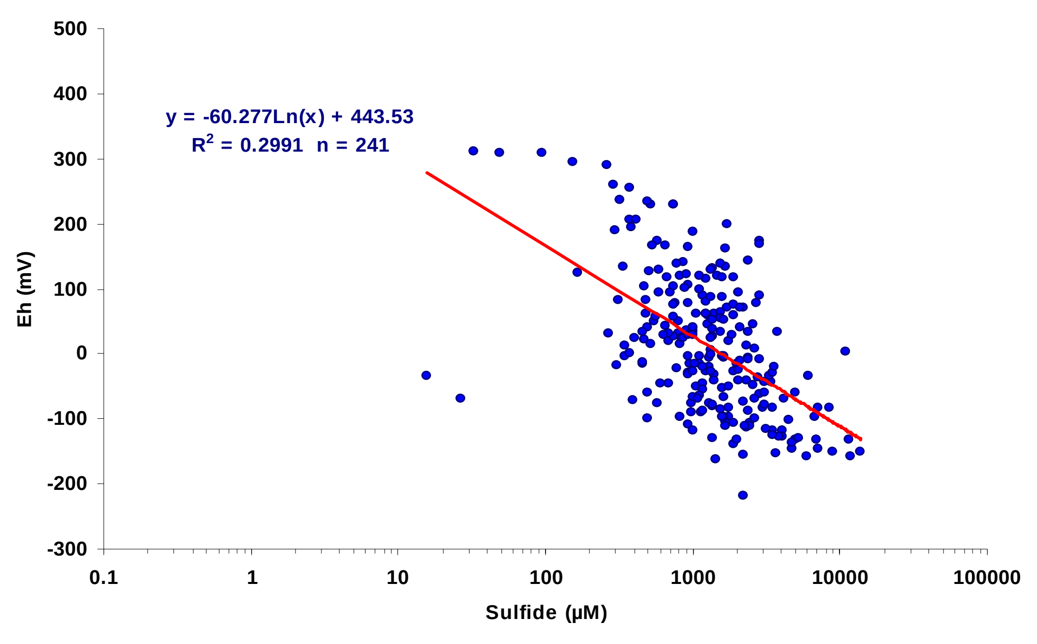
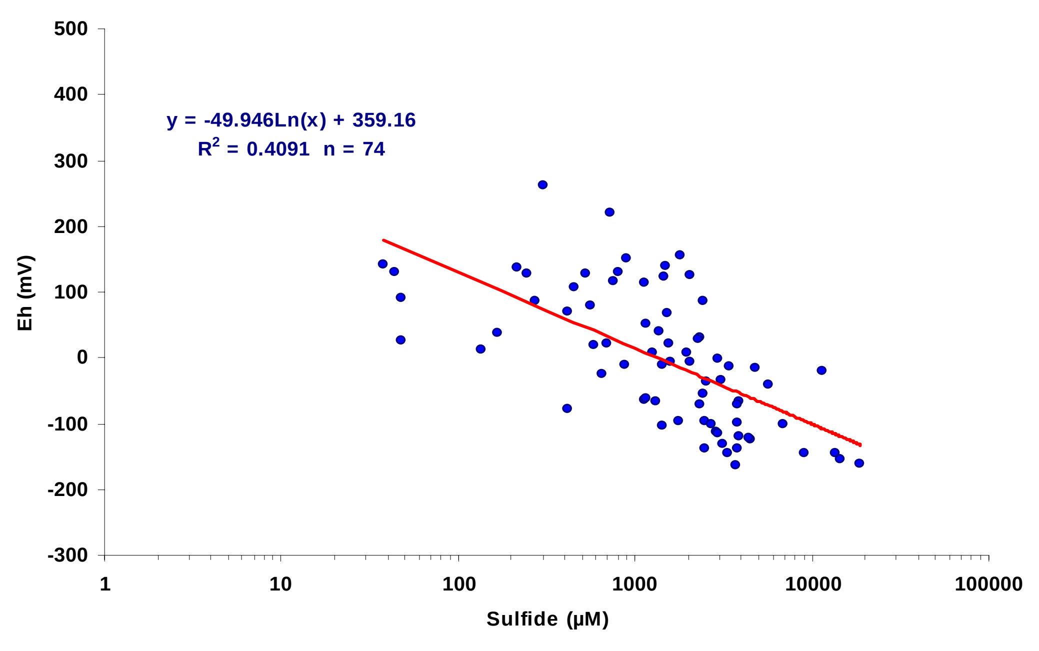


Figure 8 Redox-total sulfide relationship for MER Category 5 and 6 (gravel and rock) sediment data.



The regression coefficients for all four cases are moderately low to low due to the spread of the data around the line, especially in the cases of Category 2 and combined Categories 3 and 4.

Using the resulting linear equations developed by the sediment type regression analyses and solving the equations for predicted Eh values given the recommended warning and impact level total sulfide value and predicted total sulfide values given the recommended warning and impact level Eh values we obtain the values shown in Table 4.

Table 4. Prediction of Eh ($\hat{E}$) and total sulfide ($\hat{S}^{=}$) values from regression equation for MER individual sediment type categories.

Sediment type	Regulatory level	$S^{=}$	$\hat{E}$	E	$\hat{S}^{=}$
MER Cat. 1 soft silt	Warning	1300	-20.4	0	972
	Impact	6000	-127.6	-100	4047
MER Cat. 2 sandy silt	Warning	1300	-6.9	0	1182
	Impact	6000	-118.3	-100	4666
MER Cat. 3-4 sand and pebbles	Warning	1300	11.3	0	1569
	Impact	6000	-80.9	-100	8244
MER Cat. 5-6 gravel and rock	Warning	1300	1.0	0	1327
	Impact	6000	-75.4	-100	9829
Mean predicted values	***Warning***		***-3.75***		***1263***
	Impact		***-100.6***		***6697***

Although the means of the predicted values across all sediment types for both Eh and total sulfide for both the warning and impact levels are very close to the Wildish *et al.* recommended limits, the individual sediment category predicted values vary considerably, particularly with respect to total sulfide which nearly consistently increases with increased coarseness. The variability in these predicted MER values reflects the variability seen in actual measurements despite similar clustering patterns between the Wildish *et al.* (1999) and MER data sets which accounts for the closeness in the mean values with the recommended limits of Wildish *et al.* (1999).

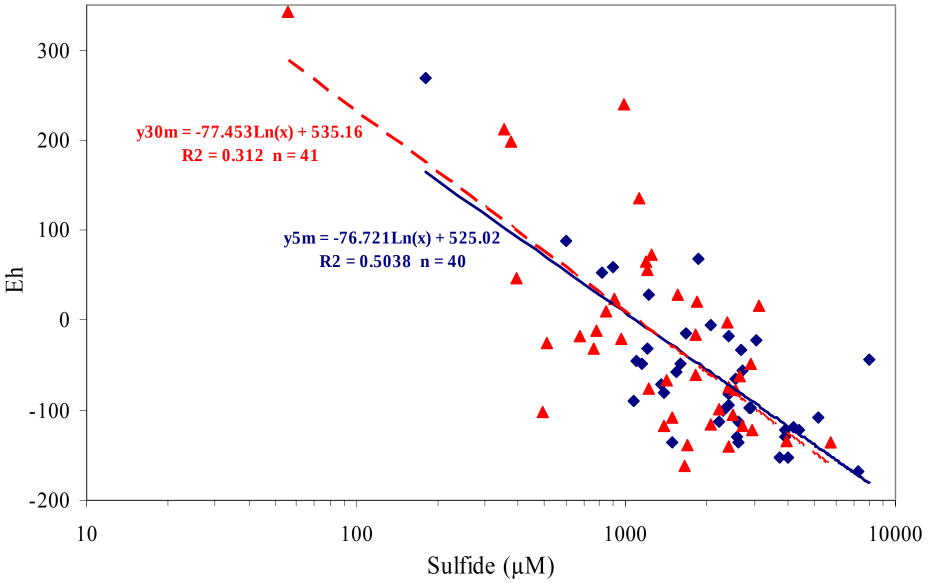
Redox, total sulfide, and usefulness in predicting benthic infauna

The DEP *Standards for classification of estuarine and marine waters* (MRS Title 38 §465-B.) refer to receiving waters as having to be "…of sufficient quality to support all estuarine and marine species indigenous to the receiving water without detrimental changes in the resident biological community." To investigate the effects of Eh and total sulfide on the resident biological community we selected total taxa as the metric to describe the benthic community condition.

A total of 81 sampling stations have been simultaneously sampled for benthic infauna and redox and total sulfide between the implementation of General Permit monitoring and fall 2006. The total taxa values represent all of the species found in the three replicates taken at each station; the redox and total sulfide values for each station represent means for the three replicates from which the total taxa values are taken. The original taxa identification data set used for General Permit monitoring analysis is very large and cannot be practically included here. The redox, total sulfide and total taxa data used for analyzing the relationship between total taxa and Eh and total sulfide are included here as Appendix V.

The distribution of the paired redox and total sulfide values for the 81 samples for which benthic data were taken are shown in Figure 9, grouped by distance from the net pens at 5 and 30 meters.

Figure 9 Redox-total sulfide relationship comparison for MER sediment data associated with biological data collected at 5 meters (blue circles, solid line) and 30 meters (red triangles, dashed line).



Clearly, the trends of the two data sets are very similar as indicated by the similarity of their respective linear equation slopes and y-intercepts; however, the variance of values at the 30 meter distance is greater than at the 5 meter distance resulting in a lower $R^2 = 0.312$ compared to the 5 meter $R^2 = 0.504$, a result that is not surprising considering the proximity of the 5 meter samples to the discharge (net pens) compared to the 30 meter samples where greater dispersion of waste is expected. Figures 10 and 11 present the total taxa values plotted against their associated Eh and total sulfide values, respectively.

Figure 10 Total taxa-Eh relationship comparison for all MER data collected at 5 meters (blue diamonds, solid line) and 30 meters (red triangles, dashed line).

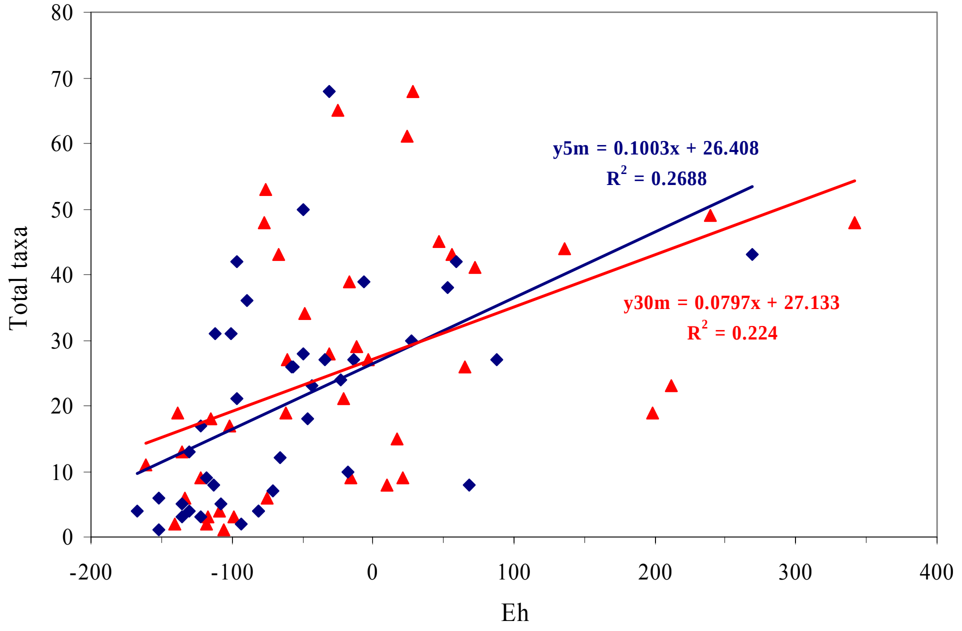
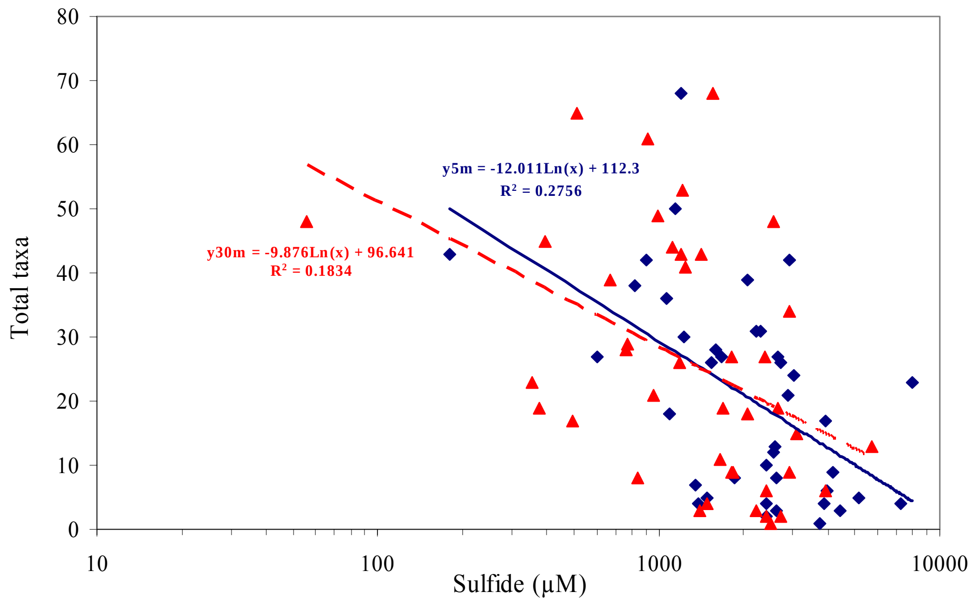


Figure 11 Total taxa-total sulfide relationship comparison for MER data collected at 5 meters (blue diamonds, solid line) and 30 meters (red triangles, dashed line).



Similar to the results reported by Wildish and Poole (2001), total taxa generally decrease with decreasing Eh and increasing total sulfide, although there appears to be evidence of biostimulation at the start of enrichment, discussed further below. Consequently, the change in total taxa with increasing level of enrichment is not strictly linear but curved, similar to the model proposed by Pearson and Rosenberg (1978). However, for ease of calculations, a linear relationship is used below to approximate the predicted total taxa at the regulatory levels. It should be noted that extrapolation beyond the range of the data set is inappropriate since in reality as Eh increases and total sulfides decrease total taxa will level off.

As before, using the respective equations for Eh and total sulfide for the 5-meter and 30-meter distances, we predict the total taxa by substituting the respective limits for the warning and impact regulatory levels as shown in Table 5.

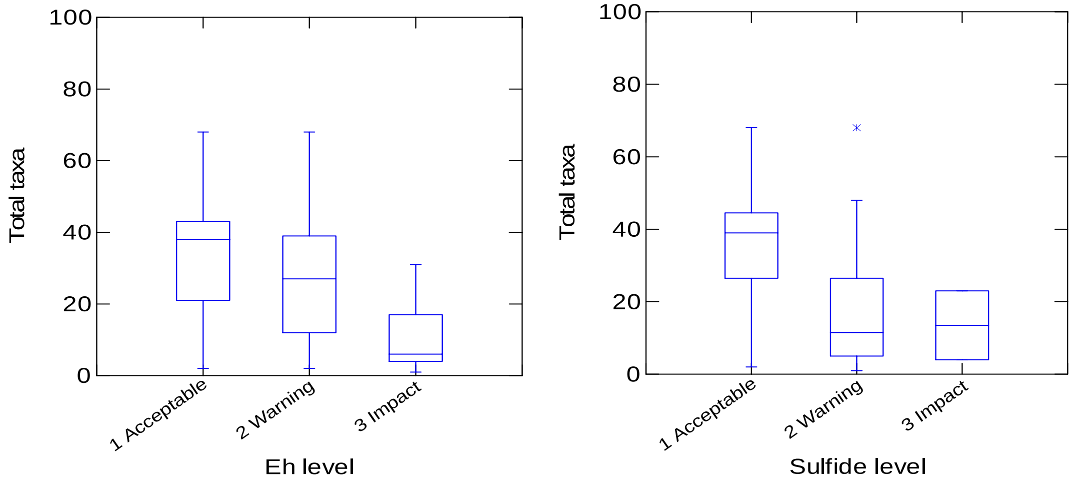
Table 5. Predicted total taxa (*T*) from regression equation for MER total taxa and Eh and total sulfide values.

Metric	Distance	Regulatory level	Metric value	*T*
Eh	5 meters	Warning	0	30.2
		Impact	-100	15.7
	30 meters	Warning	0	27.0
		Impact	-100	19.2
Total sulfide	5 meters	Warning	1300	28.4
		Impact	6000	6.1
	30 meters	Warning	1300	25.5
		Impact	6000	13.6

Although the predicted total taxa values decline with increasing level of impact, total taxa remain high at the regulatory Warning level and azoic conditions are not predicted for either metric at the Impact level. Based on these predicted values, *T* appears to be more sensitive to total sulfide than Eh, but the current regulatory limits for Eh appear too high and too low for total sulfide.

A box plot comparison of the actual total taxa found by regulatory level is shown in Figure 12 where the box represents the range for the middle 50% of the values, the upper and lower ranges of which are the 25th and 75th percentile, and the line in box represent the median value. Clearly there is a trend towards reduced total taxa with increasing level of impact and total sulfide appears to show greater sensitivity than Eh. However, although the median value at the impact level for Eh is low, the median for sulfide is rather high, and in both cases the upper range ≥20.

Figure 12 Box plots showing relationship between total taxa at the three regulatory levels for Eh and total sulfide, respectively.



PRIMER (Plymouth Routines in Multivariate Ecological Research, version 6.1.5) (Clarke and Warwick, 2001; Clarke and Gorley, 2006) was used to analyze benthic data on a species basis. Environmental data are routinely analyzed using MS Excel spreadsheets developed and designed by MER specifically for the purposes of determining mean, variance, and standard deviations for specific sampling stations. These data sets were reformatted to insure compatibility and importability into PRIMER. Organic enrichment, or impact, levels were categorized as 1-unimpacted, 2-warning, and 3-impacted (limit) per the respective Eh and total sulfide levels in the General Permit. The data were then separated into two distance categories, 5 meters and 30 meters, respectively. Environmental data were normalized, analyzed using Euclidian distance, and plotted using multi-dimensional scaling (MDS) as described in Clarke and Gorley (2006).

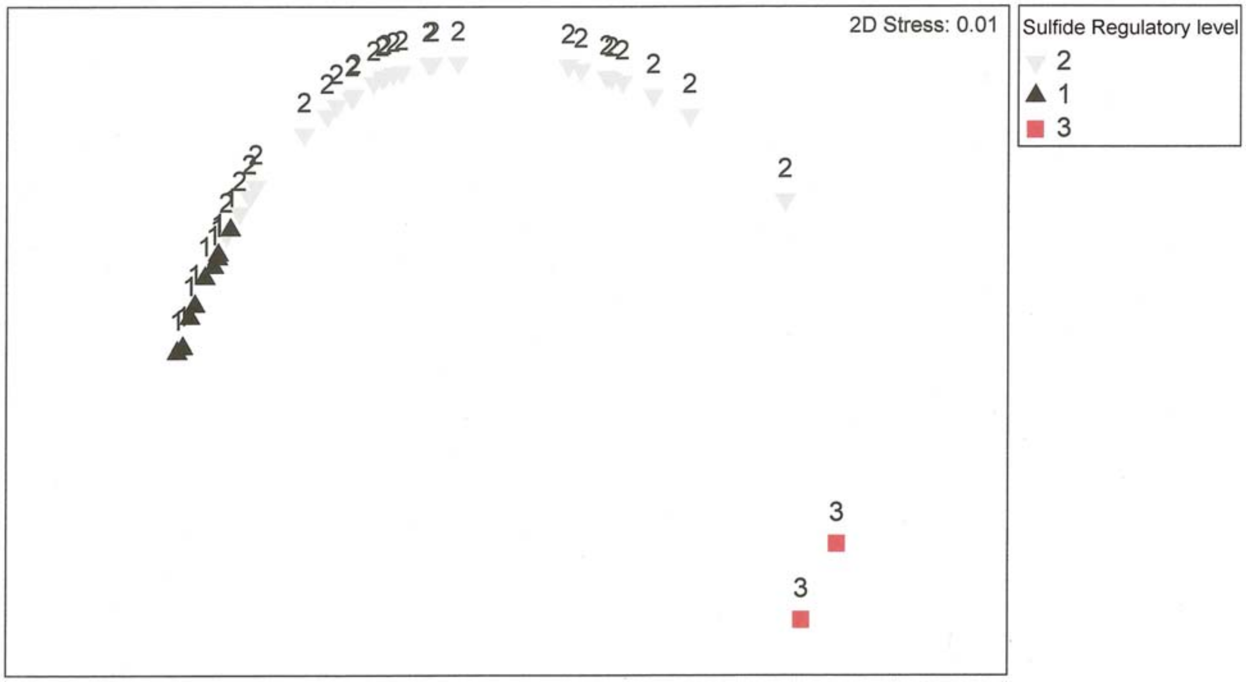
Similar to the environmental data, benthic infauna data collected under the General Permit environmental monitoring requirements are routinely analyzed using MS Excel spreadsheets developed and designed by MER specifically for the purposes of determining standard indices including total abundance, total taxa, total species, total families, relative diversity (Shannon-Wierner *H'*) and percent *Capitella capitata* on both a replicate and station basis. The benthic infauna data were square root transformed, analyzed using the Bray-Curtis formula (1957), and plotted using MDS bubble plots for both the 5 meter and 30 meters distances.

Once individually plotted, the environmental and benthic infauna data were then combined to produce plots relating individual species to the environmental data using bubble plots. The full set of benthic species is large and presentation of the relationships between each species to both Eh and total sulfide is well beyond the scope of this project. Based on experience with the occurrence of species under various levels of organic enrichment, the total list was reduced to five species that occur over the spectrum of organic enrichment stages, *i.e.* oxic through severely hypoxic. The species selected are: *Mediomastus ambiseta* and *Ophelina acuminata* representing low pollution tolerance; *Nereis sp*. and *Eteone sp*. exhibiting a wider range of tolerance to pollution; and *Capitella capitata*, known for its opportunism and dominance under highly organically enriched conditions.

Figure 13 shows the MDS plot for samples grouped according to total sulfide level at 5 meters (a) and 30 meters (b) sampling distances. It should be noted that MDS plots are based on multidimensional axis, therefore spatial distribution of samples in 3-dimensional space will vary depending on the data being plotted, thus in Figure 13a at the 5 meter distance total sulfide increases along a strongly arched curve from left to right while in Figure 13b at 30 meter distance the total sulfides increase from right to left. Figures 14-18 show individual MDS bubble plots (size of bubble increases with abundance) for abundance of the five selected species in relation to total sulfide level superimposed over Figure 13. Note that no level 3 total sulfides were measured at the 30 meter distance.

Figure 13 MDS plot of sample groupings based on total sulfide levels, a. at 5 meter, b. at 30 meters.

a. 5 meters



b. 30 meters

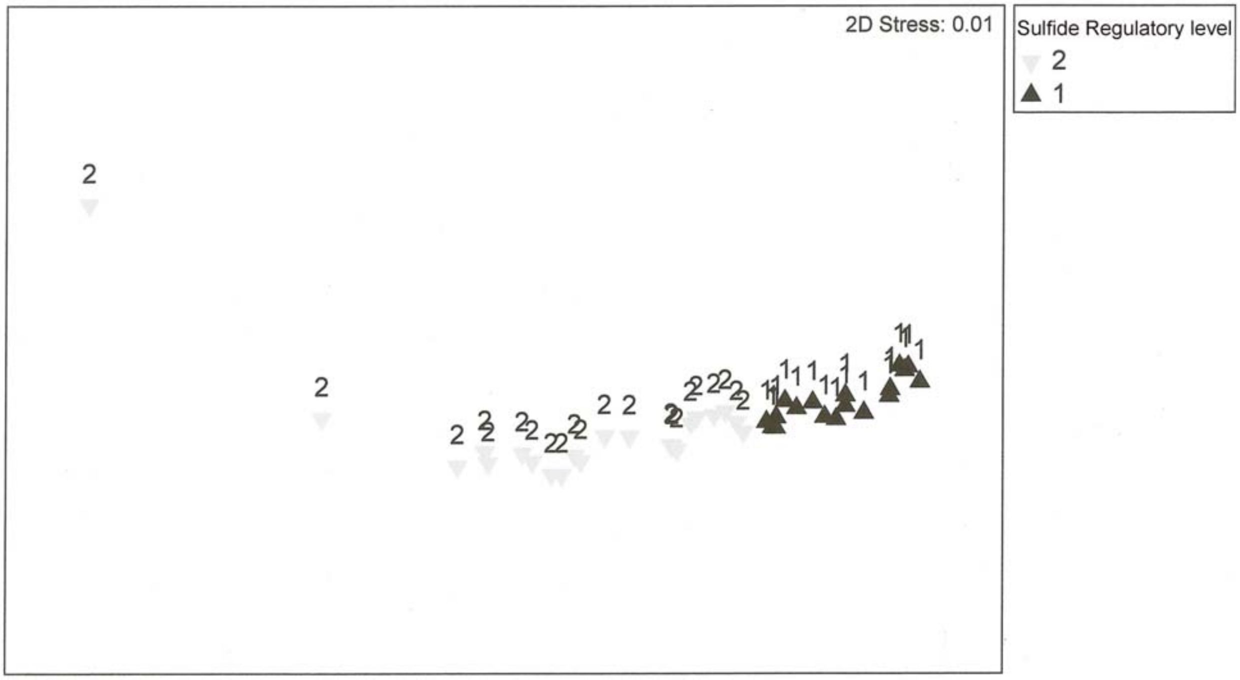
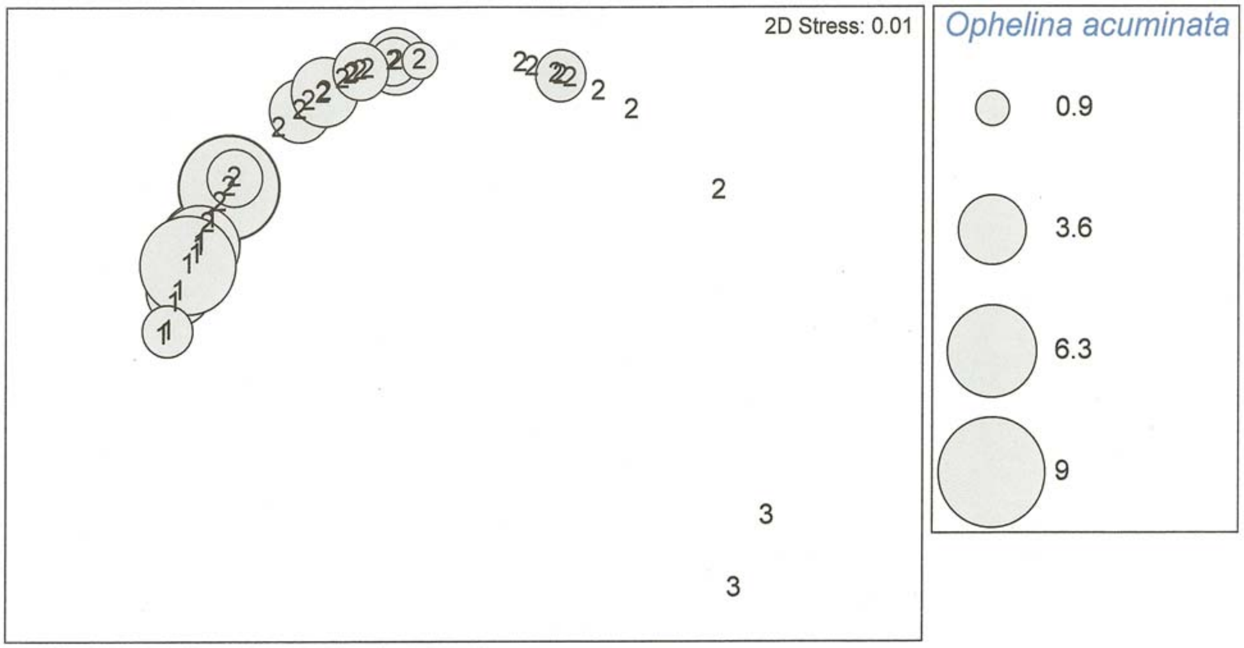


Figure 14 MDS bubble plot of the number of *Ophelina acuminata* based on total sulfide levels, a. at 5 meter, b. at 30 meters.

a. 5 meters



b. 30 meters

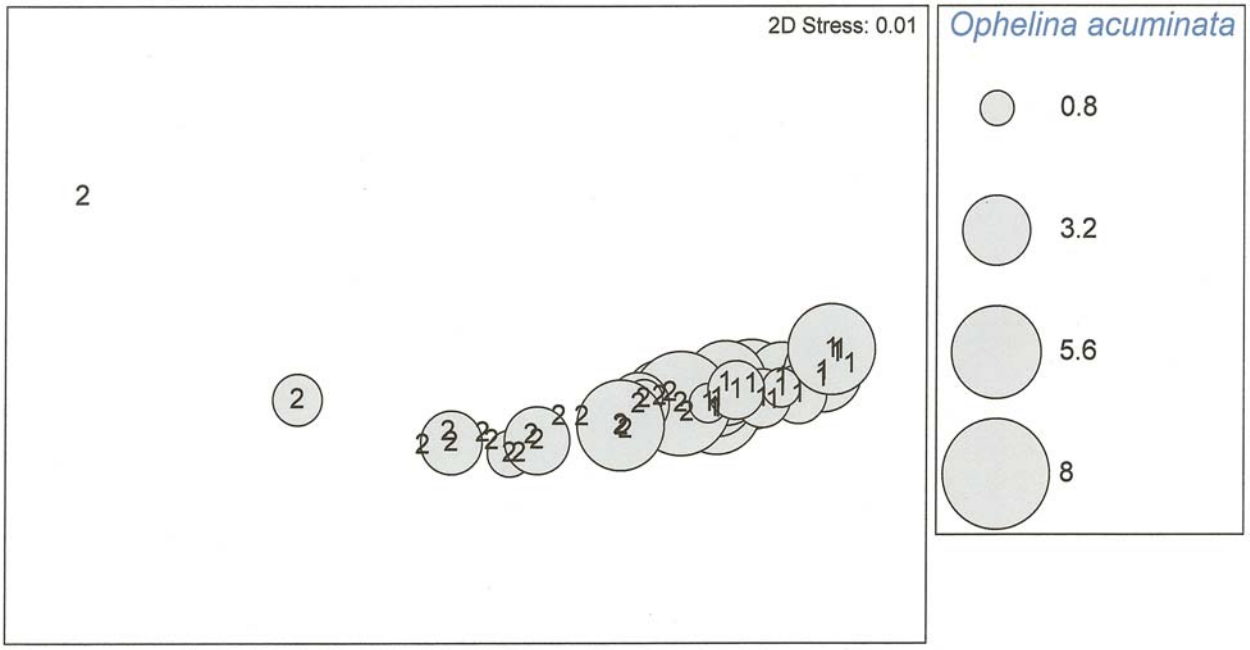
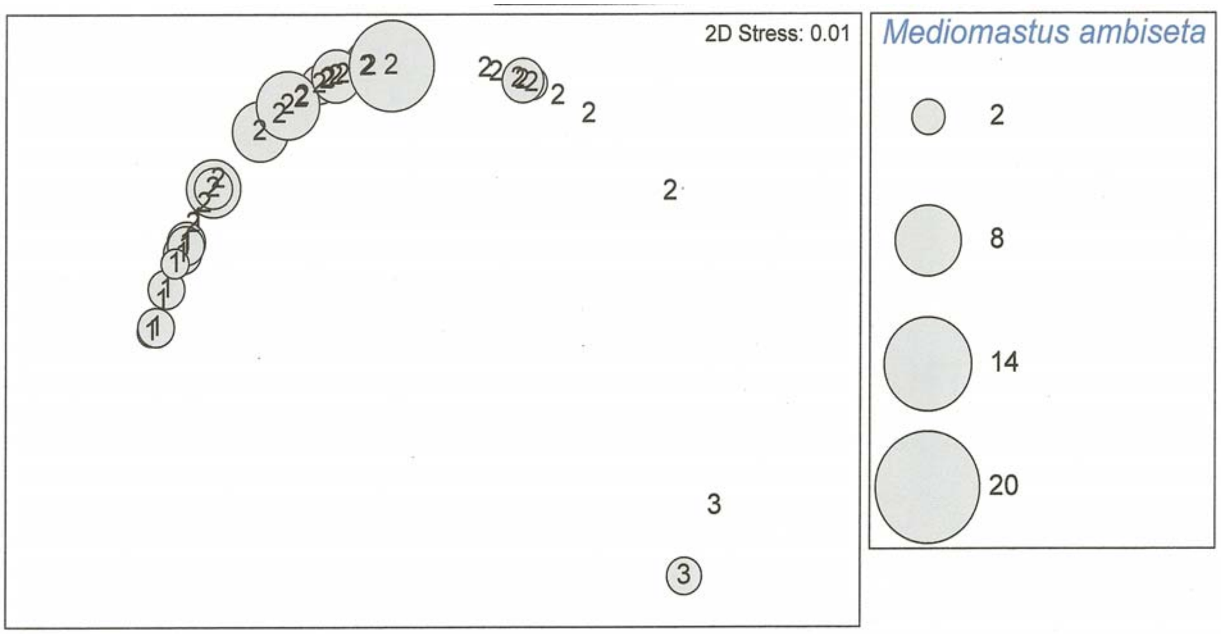


Figure 15 MDS bubble plot of the number of *Mediomastus ambiseta* based on total sulfide levels, a. at 5 meter, b. at 30 meters.

a. 5 meters



b. 30 meters

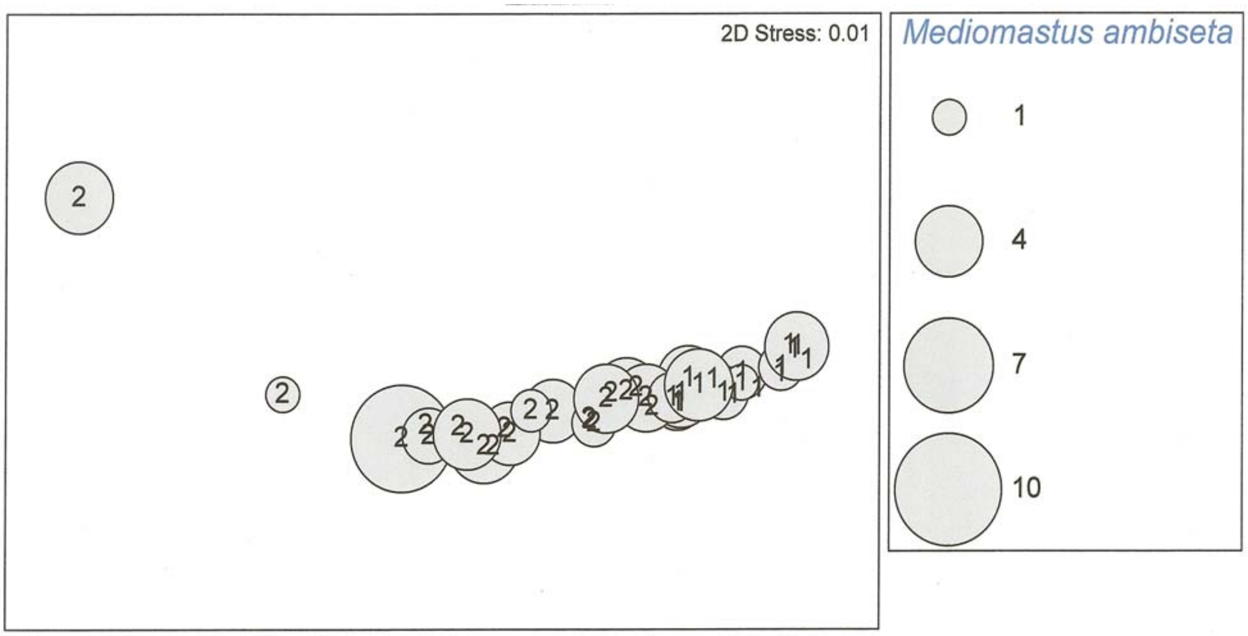
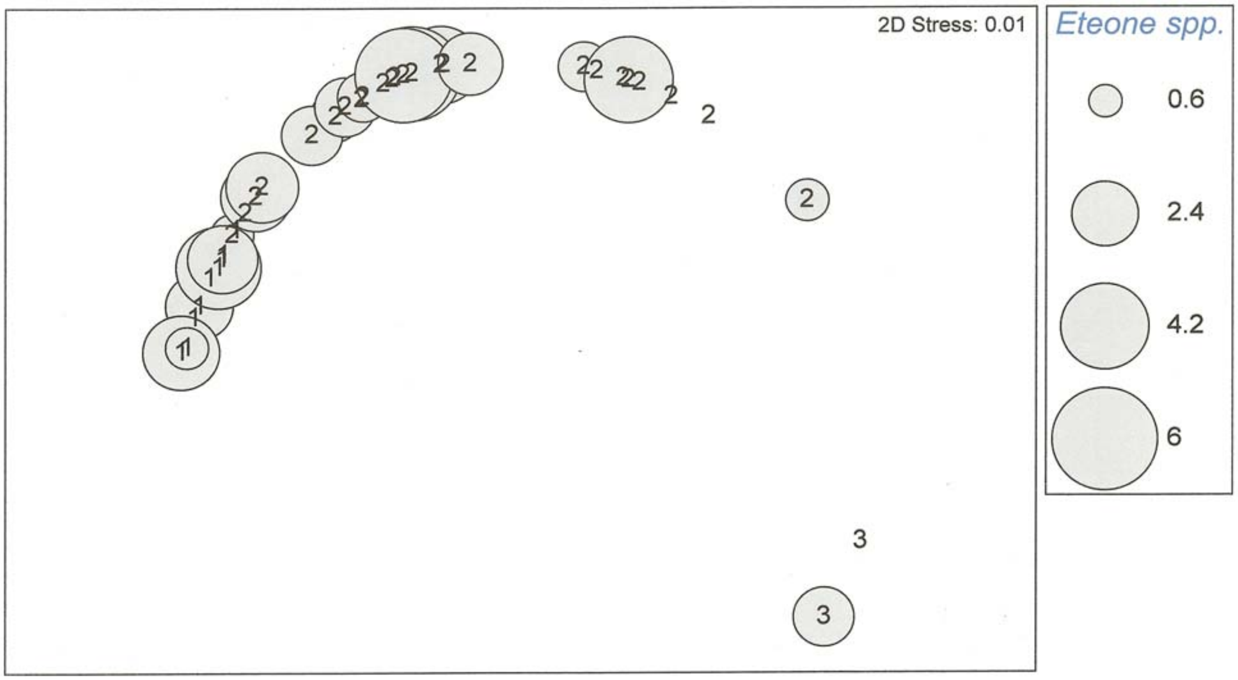


Figure 16 MDS bubble plot of the number of *Eteone* spp. based on total sulfide levels, a. at 5 meter, b. at 30 meters.

a. 5 meters



b. 30 meters

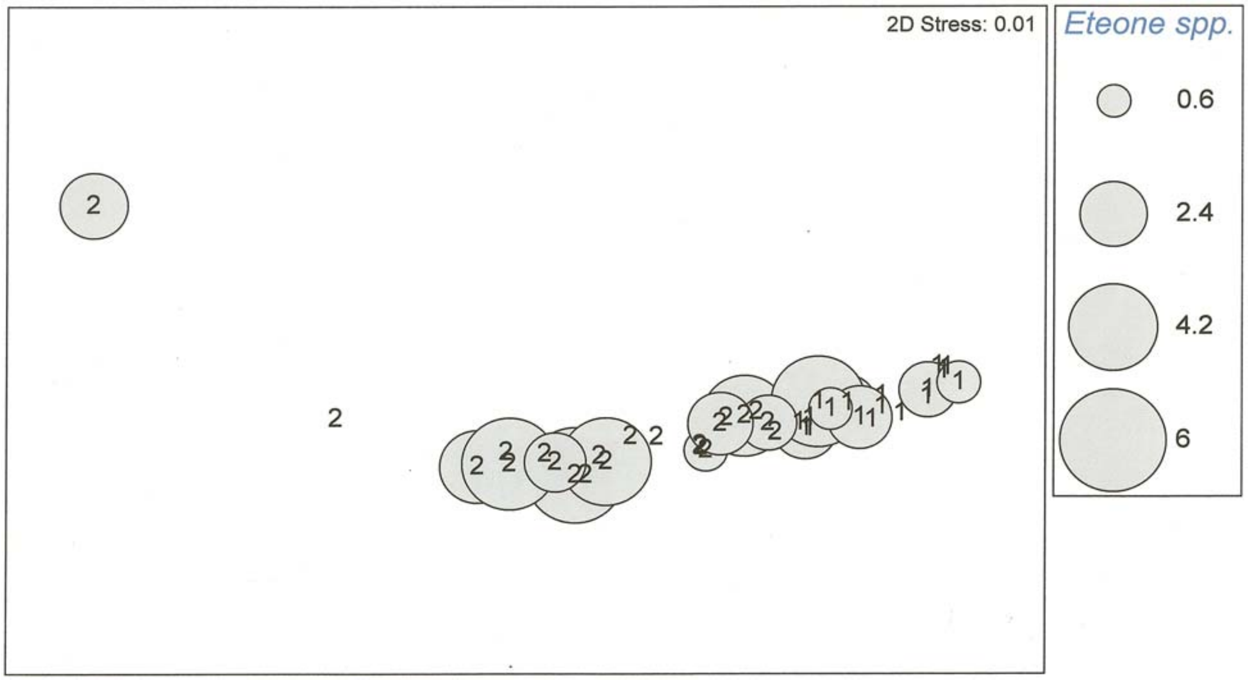
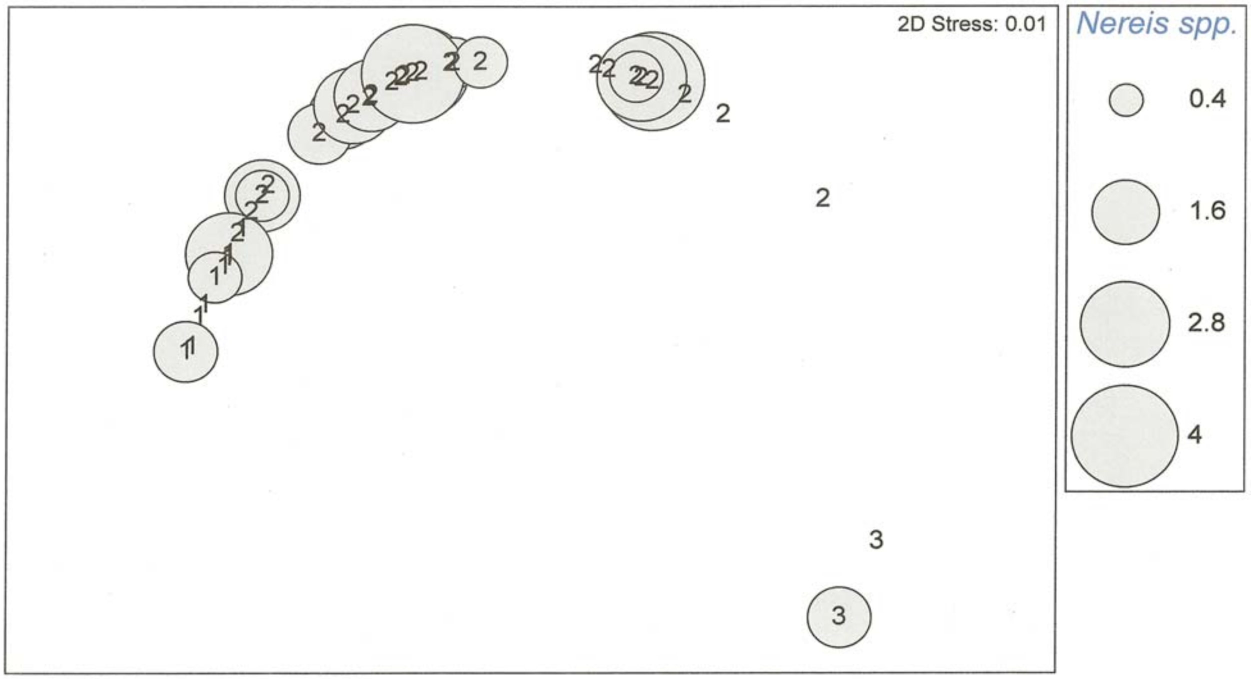


Figure 17 MDS bubble plot of the number of *Nereis* spp. based on total sulfide levels, a. at 5 meter, b. at 30 meters.

a. 5 meters



b. 30 meters

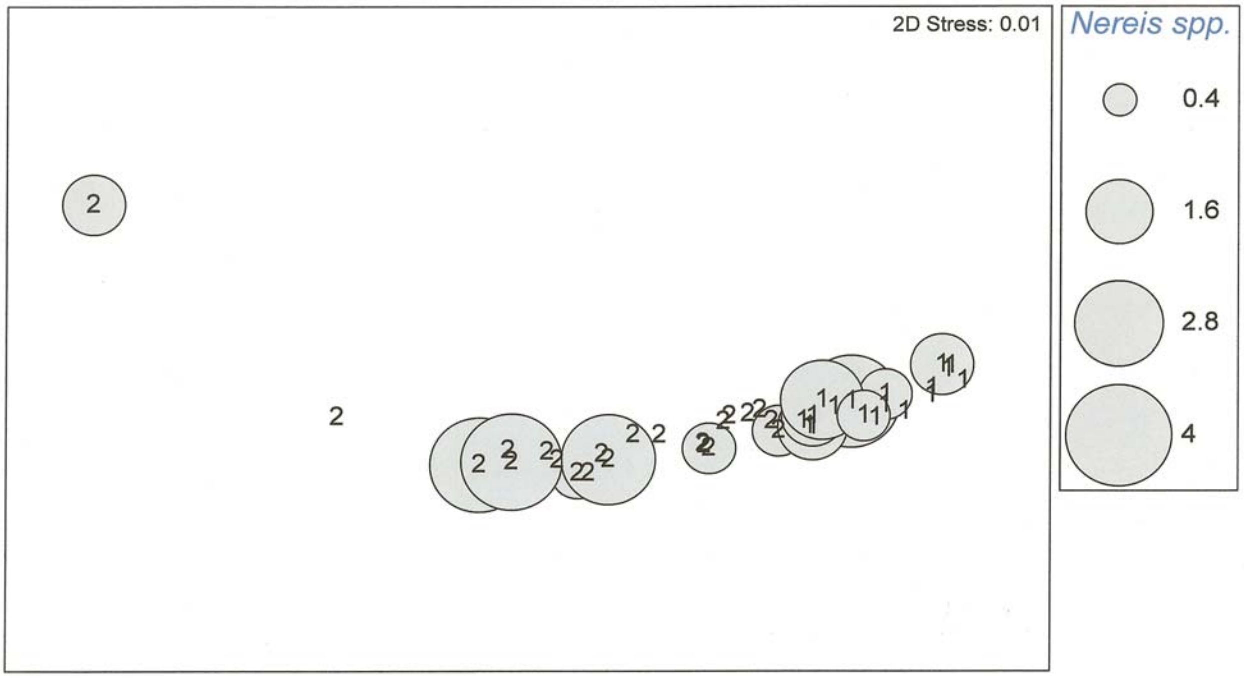
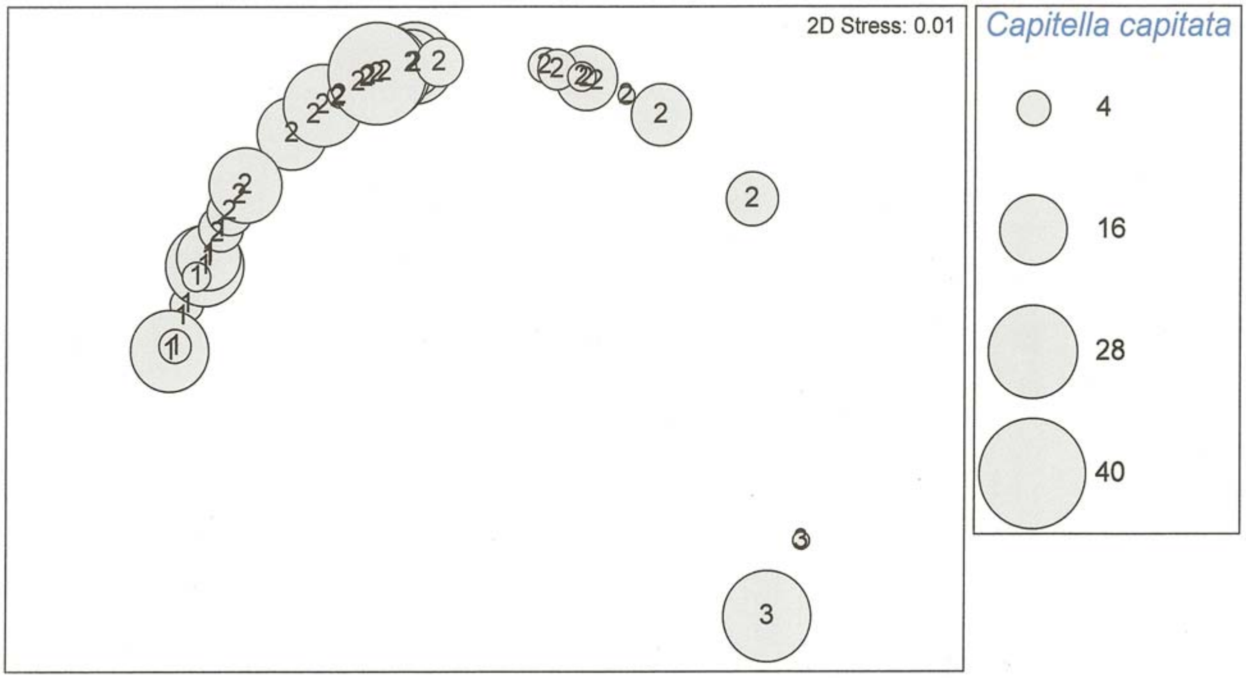
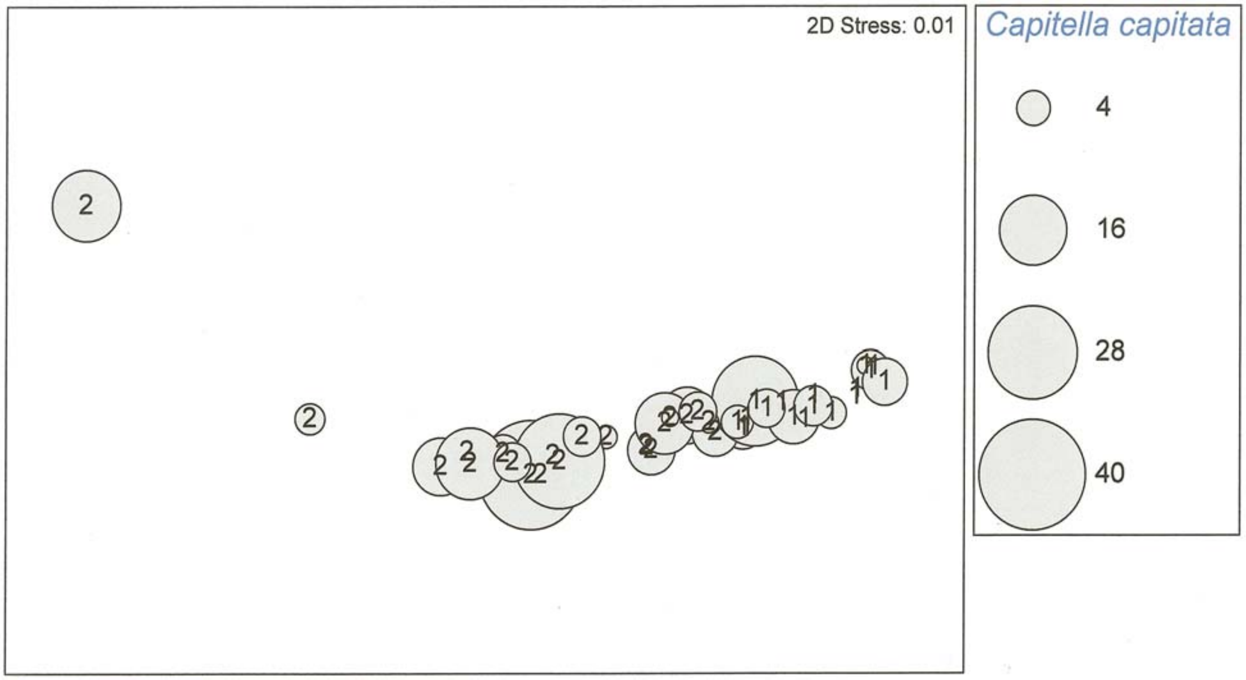


Figure 18 MDS bubble plot of the number of *Capitella capitata* based on total sulfide levels, a. at 5 meter, b. at 30 meters.

a. 5 meters



b. 30 meters



The pattern of abundance of the five selected species is generally consistent with what would be expected given their varying tolerance to organic enrichment, that is, pollution intolerant species occurring predominantly in association with total sulfide level 1 (unimpacted) samples, species with a wider tolerance occurring predominantly in association with total sulfide level 2 (warning level) samples, and pollution-tolerant *C. capitata* occurring in association with total sulfide level 2 (warning level) and level 3 (impact level) samples, but at an order of magnitude greater abundance. It is also noteworthy that, with the exception of pollution-intolerant *O. acuminata* at the 5 meter distance, in all other cases and species the peak in abundance is seen within total sulfide level 2, that is, a moderate level of organic enrichment, a finding that is consistent with the Pearson and Rosenberg (1978) model. Also, with the exception of pollution-intolerant *O. acuminata*, all other species are found at total sulfide level 3, albeit at small numbers with the exception of *C. capitata*.

Discussion

The full redox and total sulfide data for all samples collected in Maine since the implementation of the General Permit monitoring requirements and presented here (Figure 3) show similar overall patterns and trends to the mariculture environmental monitoring data presented in Wildish *et al*., 1999. However, the spread, or variability, in the MER data is greater than that of the 1999 Wildish *et al.* data, consequently resulting in a lower confidence coefficient; the regression line is also shifted to the left, *i.e.* toward lower Eh for a given total sulfide value, and has a lower y-intercept (+329) compared to Wildish *et al.* (+480). Interestingly, the equation for the MER data set yields predicted values for Eh and total sulfide which are closer to the original limits recommended by Wildish *et al.* (1999) than their own salmon environmental monitoring data when taken alone. It should be noted that when reassessing the Wildish *et al.* 1998 environmental monitoring data (Appendix I in Wildish *et al.*, 1999) we were unable to exactly duplicate the linear regression equation they obtained, although the equation we obtained and theirs are quite close.

The full MER data set includes measurements from non-salmon farm-affected sites and these are therefore eliminated to allow a more appropriate comparison between the two data sets. The resulting MER regression line is more similar to that of the Wildish *et al.* salmon environmental monitoring data, although still shifted slightly to the left and a lower, although closer, y-intercept (+448). Surprisingly, the resulting MER equation ($y = -63.573\text{Ln}(x) + 447.48$) is remarkably close to the original Wildish *et al.* (1999) equation ($y = -65.949\text{Ln}(x) + 473.36$) and consequently yields predicted Eh and total sulfide values that are very close to those predicted by the original Wildish *et al.* equation, particularly at the impact level, *i.e.* warning Eh = -8.4 and $S^{=} = 1140$; impact Eh = -105.6 and $S^{=} = 5496$. However, there is a substantially greater spread within the MER data set resulting in a moderately low $R^2 = 0.355$, compared to the rather high $R^2 = 0.672$ of Wildish *et al*., indicating that much of the variability in the MER data remains unexplained by the regression.

We believe the unexplained variability in the MER data and discrepancy with the Wildish *et al*. data may result from several sources of error: sampling error, difference in sampling method, technical problems (Eh probe “poisoning”), difficulties with total sulfide standard instability, and the heterogeneous nature of impacts and sediments.

Despite every effort being made to reduce sampling error, some degree of error is inevitable. Occasionally, when sample corers are opened the sediment surface is clearly not intact or an oblique sample will slump, resulting in a mixing of the top 2cm of sediment with deeper sediment. When such mixing appears sufficiently extensive to preclude acceptable measurement, the sample is discarded; if a measurement is taken on such a sample, a notation is made on the data reporting sheet indicating “mixed sample”, since such measurements can result in some biasing. In firm, compacted sediments such as sand and gravel, vertical sampling is often impossible and sampling must be done obliquely, thereby increasing the possibility of sample mixing. Similarly, subsampling of soft sediments (silt/clay) can be accomplished as described in Wildish *et al.* (1999), when the sediment is in the form of a slurry, *i.e.* by simply withdrawing a sample into a 5cc syringe by gently pulling back on the plunger. However, in sediments coarser than fine sand, collection of subsamples for total sulfide determination by simple withdrawal using the syringe plunger of the modified 5cc syringes is difficult, if not impossible. In some cases syringes must be "packed" by spooning sediment into the syringe barrel, often, if not usually, resulting in introduction of air; in cases where sediment coarseness precludes sample collection or would result in excessive air-filled interstitial spaces, samples are not taken and are reported as Unable to Sample, or UTS.

Sample treatment for the measurement of redox and total sulfide for samples collected by MER is different from the methods used by Wildish *et al.*, 1999. In August 2003, prior to the first redox and total sulfide sample collection and measurement, MER received the Interim Recommendations for the N.B. EMP (Appendix I) as a personal communication from David Wildish. Accordingly, the recommended method was adopted and continues to be used to-date. This method, however, is substantially different from the previous method and calls for the homogenization of the upper 2cm of sediment rather than direct sampling of the undisturbed sediment core, therefore some of the shift seen in the MER data relative to the Wildish *et al.* data may be attributable to this difference in sample processing.

The interim recommendation calls for Eh to be measured as the samples are prepared for total sulfide measurement, *i.e.* just prior to addition of SAOB. However, the time required to reach a stable Eh value coupled with the number of samples requiring processing and the 3-hour stability interval for prepared SAOB makes Eh measurement in the laboratory at the time of total sulfide measurement completely impractical; consequently, all Eh measurements reported here are field measurements taken at the time of initial sampling, corrected to ambient sample temperature.

Stable Eh measurement readings are generally quickly obtained for severely hypoxic and anoxic samples yielding very low values (≈ -100 mv); however, during measurement we have experienced high variability in Eh values where sediments are fully oxic or only mildly hypoxic, a phenomenon also reported by others (Brooks and Mahnken, 2003; Parker and Mallory, 2003; Wildish *et al.*, 2004) in what are described as "poorly poised, oxic sediments". Wildish *et al.* (2004) recommend a minimum of 5, and as many as 7 (D. Wildish, pers. comm.), replicates per sample to achieve an acceptable level of statistical significance, particularly where high variability is encountered. From a practical field sampling point of view, under high variability conditions, each paired Eh sample measurement and total sulfide sample collection can require between 5-10 minutes, depending on Eh variability. If 5 replicate Eh measurements and total sulfide samples were collected per station sample, this would result in 15 measurements and subsamples per station (based on the current requirement of 3 replicate sample locations, or cores, per distance station), that is, 60 Eh measurements and total sulfide samples per farm site. The minimum time required to process this number of samples on-site would be 5-10 hours and does not include laboratory time for the total sulfide measurements; the alternative method of Eh measurement in the laboratory just prior to total sulfide measurement is also impractical, as stated above.

Part of the variability observed in Eh values may also be attributable to Eh probe "poisoning", a phenomenon well documented by Wildish *et al.* (2004). According to Wildish *et al.* (2004) the poisoning is the result of formation of coatings of total sulfide or oxide on the platinum probe surface which alter electrical response. This phenomenon is usually seen in "old" probes, defined by Wildish *et al.* as having been used prior to testing in their calibration experiments, in some cases "exhaustively", in both anoxic and oxic sediments. Although MER purchases new Eh probes annually, they nevertheless meet the definition of "old" by being repeatedly exposed to a wide spectrum of conditions ranging from oxic to anoxic. Consequently, the probes may begin reporting progressively more negative values over the course of a year, possibly accounting for at least some of the shift towards lower Eh values in the MER data set compared to the Wildish *et al.* data in Figure 3.

In addition to the difficulties experienced with Eh, we have also occasionally experienced difficulties with the stability of the total sulfide standard during the 3-hour measurement period, a problem identified by Wildish *et al.* (2005). In the interest of insuring the accuracy and reliability of the standard solutions used for meter calibration, MER has opted to purchase prepared NIST-traceable 3% $Na_2S * 9H_2O$ stock solution (Ricca Chemical Company Cat. No. 7570-4) over in-house preparation of the stock solution. The stock solution is replaced at least annually, but usually every 6 months just prior to a monitoring event. At the time standards are prepared prior to each set of measurements, the stock solution bottle is opened only long enough to withdraw 4 ml by volumetric pipette after which the bottle is immediately tightly resealed and the cap taped to avoid potential loss of strength. MER uses a 3-point standardization curve; standards are prepared immediately prior to initiating a measurement series and the meter standardized as in Wildish *et al.*, 1999. Meter standardization is periodically checked during the course of a 2- to 3-hour measurement process to insure the meter is reading correctly; the meter is again checked against the standards at the end of a measurement run and the values obtained are recorded on the sediment chemistry data sheet provided in the site monitoring report. Slight drifts are often seen, these are usually <5%, however, drifts as great as 14-15% have been observed; these usually occur across all three standards simultaneously indicating that such drifts may be due to changes in room temperature during the course of the measurement period rather than actual changes in concentration of the standards, in which case the samples being tested would be similarly affected; nevertheless, such drifts will affect the results and may contribute to the cumulative error.

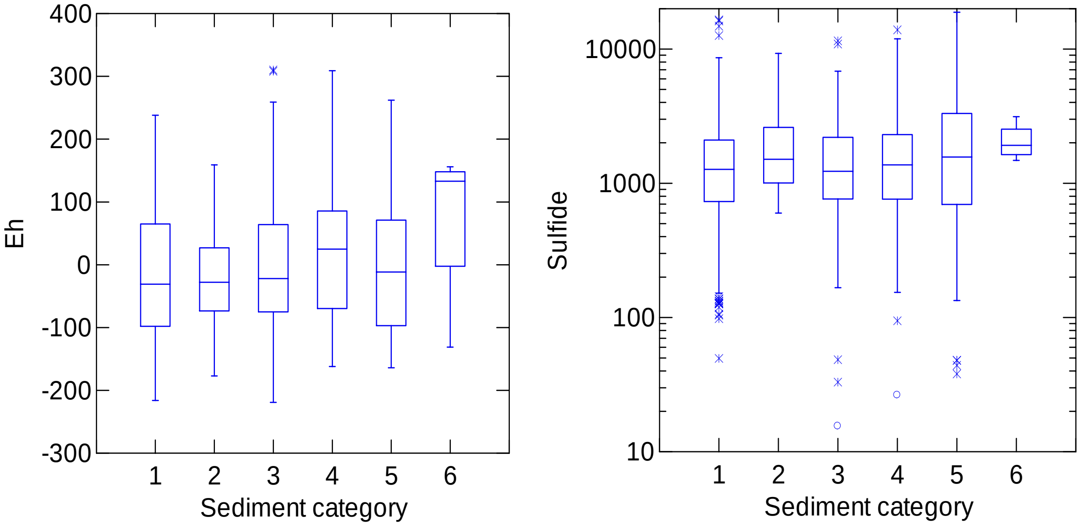
We believe that the cumulative effect of the sampling and instrument errors contribute substantially to the variability in results. However, we also believe the heterogeneous nature of the spatial distribution of discharges from net pens and the heterogeneous composition of sediments around farm sites may account for an even greater portion of the variability.

Monitoring of the salmon industry in Maine over the past 20 years has shown that the distribution of waste, as feed and feces, discharged from net pens varies considerably from site-to-site, but also within a single site; Hargrave *et al.* (1997) have reported similar heterogeneity in discharge distribution at New Brunswick, Canada salmon sites. In areas of slow current velocity, bottom sediments are usually primarily soft and deposition immediately adjacent to the net pens is often uniform; however, only a short distance away from the net pens the distribution is much less uniform, often seen as pockets or patches rather than contiguous areas of deposition. Where sediments are firmer and currents stronger, deposition tends to be scoured from high spots and deposited in depressions along the bottom, resulting in a highly variable pattern across the bottom. This variability is seen at multiple spatial levels down to the level where patchiness can be found on the surface of a 4 in. diameter core. This variability at multiple spatial levels leads to variability amongst samples taken at a given site, at a given distance, and likely even within a given core, hence the recommendation by Wildish *et al.* (2004) of 5 or more replicates to achieve statistical significance.

The result of separating the MER salmon site monitoring redox and total sulfide data by sediment type (Figures 5-8), shows the changing slope for each category, the equations for which yield the predicted values in Table 4 for the warning and impact levels for Eh ($\hat{E}$) and total sulfide ($\hat{S}^{=}$). Although the predicted Eh values for the softer Category 1 and 2 sediments are lower than predicted by Wildish *et al.* for the respective regulatory levels, the values steadily rise with increasing sediment coarseness, a trend which is expected given that coarser sediments are associated with higher current velocities which in turn increase the oxygen flux to the bottom. Surprisingly, however, the predicted total sulfide values for the respective regulatory levels *increase* with increased sediment coarseness, a trend that is intuitively contradictory given the current regimes just described above. Furthermore, review of the video recordings made at some of the coarsest sediment sites in the vicinity of where sediment sample cores are taken show no indication of hypoxic, much less anoxic, conditions despite exceptionally high total sulfide measurements and in many cases reveal a robust epibenthic community.

A comparison of the Eh and total sulfide data by individual sediment category shows little statistical difference between the various sediment types. In the box plot shown in Figure 19 the box represents the range for the middle 50% of the values, the upper and lower ranges of which are the 25th and 75th percentile, with the horizontal line representing the median values; the vertical lines, or "whiskers", at either end represent the full range; asterisks and circles represent outliers.

Figure 19 Box plot distributions of Eh and total sulfide values by sediment category



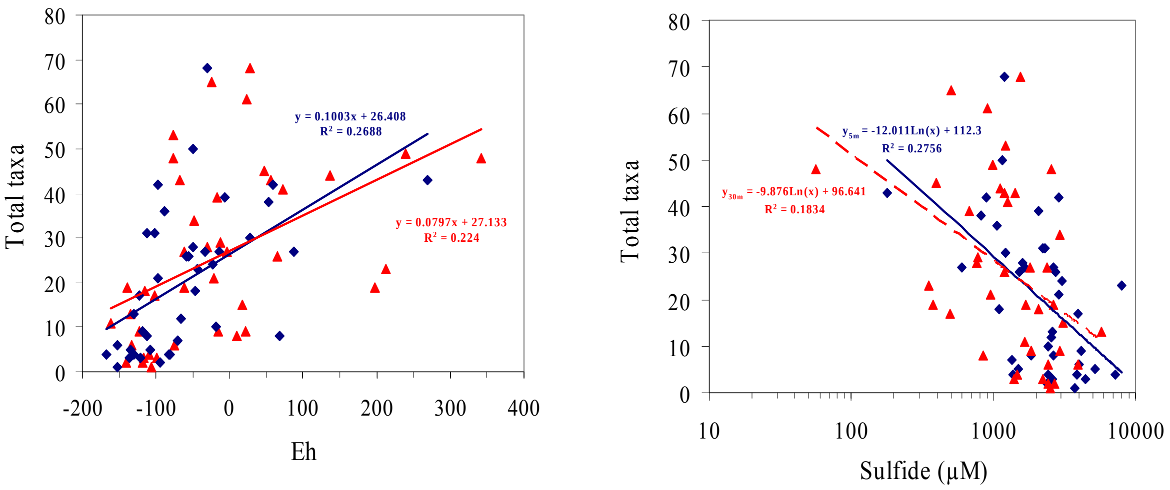
The trend toward higher median Eh and total sulfide values with increasing coarseness is discernable from these plots, but the full ranges of data, as well as the central 50% of values, for each sediment category generally overlap across the entire spectrum of sediment types. These results imply that there is no difference in the range of impacts observed across the range of sediments tested and appear to confirm the results of Table 4 that impacts, measured as total sulfide, increase with sediment coarseness. We believe these unusual trends are artifacts of sampling rather than indicative of the general organic enrichment state of the bottom.

Our experience with sampling for measurement of redox and total sulfide over the past 5 years has shown that the sediment subsampling technique developed by Wildish *et al.* is very effective in soft, silt-clay sediments and to a large extent in silt-fine sand compositions where a sediment slurry can be prepared that is truly representative of the sediment composition and surrounding environment. However, in progressively coarser sediments fine material must be selected and removed from coarser material in order to prepare a slurry, material which becomes progressively less representative of the general sediment composition and bottom condition as sediment coarseness increases. This problem becomes particularly pronounced in coarse sand-pebble and gravel-rock sediments where soft material can only be obtained from the interstitial spaces between coarser material; this soft material, however, represents but a small fraction of the overall sediment profile and is composed primarily of organic material discharged from the cages which is subject to decomposition, thus yielding low Eh and elevated total sulfide values. So, while the measurement results may be accurate for the material being tested, the material being tested is not representative either of the composition or conditions of the sediment, thus possibly leading to erroneous conclusions on the degree of organic enrichment.

Indeed, the biological data presented here supports that possibility. Wildish *et al.* (2001) showed that organic enrichment at salmon farms resulted in a decline in total taxa similar to the effects of organic enrichment described in the classic work by Pearson and Rosenberg (1978). The general trends in the

MER data are similar showing a downward trend in total taxa with decreasing Eh and increasing total sulfide, as shown in Figure 10 and 11 (duplicated below for convenience in Figure 20), and consequently also generally decline with increase level of impact for both Eh and total sulfide, as shown in Figure 12. However, although the general trends are apparent, the variability in total taxa for any given value of Eh or total sulfide is high.

Figure 20 Scatter plots of total taxa distributions compared to Eh and total sulfide values

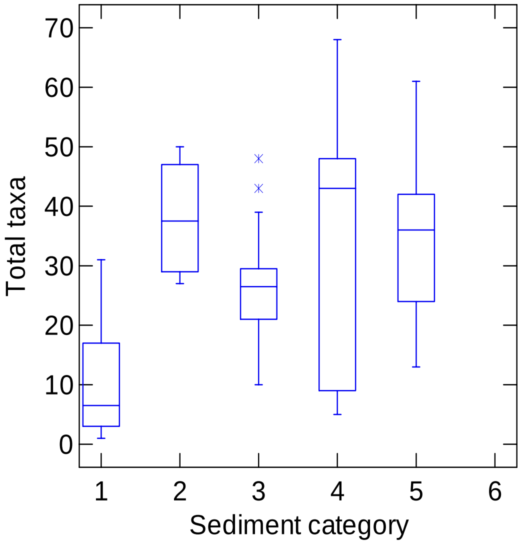


Based on the organic enrichment gradient grouping and associated geochemical measurement limits presented in Wildish *et al.* (1999; 2001), the Anoxic group (Poole *et al.*, 1978; Wildish *et al.*, 2001), defined as Eh <-100 and $S^{=}$ >6000, corresponds to a grossly polluted condition characterized as azoic, *i.e.* no macrofauna (Pearson and Rosenberg, 1978), and also corresponds to the impact level in the General Permit. However, as shown in Figure 14, the maximum total taxa for both Eh and total sulfide is reached at or close to the warning level of Eh = 0 and $S^{=}$ = 1300; at or near the impact level of Eh = -100 the total number of taxa can range above 40; similarly, at the impact level of $S^{=}$ >6000 the total number of taxa can reach 25. Clearly then, at the warning level biological function, as measured by total taxa, is not only continuing, it is reaching a peak, and continues well through the impact level when anoxic and azoic conditions are predicted. Based on these results, the current Warning and Impact standard appear to be overly protective.

We believe these results are due to the confounding effect caused by Eh and total sulfide values obtained from non-representative material. Indeed, a review of the total taxa by sediment category reveals that the lowest total taxa values, with a median of approximately 6, occur in soft, silty sediment (Category 1) as shown in Figure 21. This is not surprising since this is where organic enrichment is expected to be highest since soft sediment is characteristic of naturally depositional areas with slow current velocities. By comparison, in the coarser sediments (Category 4 and 5) the total taxa median is 6 to 7 times higher (Figure 21), despite the fact that the median total sulfide of these sediments is similar to that of the softer sediments (refer to Figure 13). It is important to note that conditions at the time of benthic infauna sampling represent the maximum (worst-case) discharge conditions since infauna sampling is required to be done at the end of the production cycle, that is, when fish held in the net pens are reaching or are at market size and are being fed, and therefore defecating, at the highest rate.

Additionally, the MDS results show that all five of the species selected for analysis, covering a wide range of tolerance to organic enrichment, persist through the warning level that begins at a total sulfide level of 1,300µM and the three moderately to highly tolerant species persist through the impact level with total sulfides <6,000µM.

Figure 21 Box plots of total taxa by sediment category



The combination of high variability of Eh and total sulfide results and the inconsistencies shown between them and sediment coarseness and total taxa raise serious questions regarding the reliability and the appropriateness of universal application of these metrics to all Maine salmon aquaculture sites. The Maine DEP has, in fact, acknowledged the possibility that these metrics may be inappropriate in certain cases by exempting certain sites where the bottom sediment is extremely coarse, *i.e.* our Category 5-6, from Eh and total sulfide monitoring; we believe, however, based on the data presented here, that environmental regulation based on Eh and total sulfide may be inappropriate in other, less coarse conditions.

Conclusions

The data presented here support the general conclusion that Eh and total sulfides can serve as general indicators of declining environmental conditions associated with organic enrichment. Furthermore, the remarkable similarity between the regression equation used by Wildish *et al.* (1999) to develop their recommended limits for Eh and total sulfide for specific organic enrichment groups and the equation resulting from the MER 2003-06 monitoring data suggests that fundamental processes causing changes in Eh and total sulfide around New Brunswick and Maine salmon sites are similar. However, despite the similarities in trends and general predictability, the data presented here indicate sufficient variability and uncertainty in results to question the appropriateness of relying on these metrics and their established limits as regulatory standards in Maine. We believe that the higher variability seen in the MER data, taken from a wider spectrum of sediment types compared to the Wildish *et al.* 1999 data taken from soft sediments, indicate that Eh and total sulfide testing in coarser sediments is inappropriate and could ultimately lead to erroneous conclusions regarding the degree of organic enrichment occurring at the site.

The technical problems associated with potential "poisoning" of the probes used to measure Eh can result in excessively negative readings which render Eh unreliable and limit its usefulness as an indicator of organic enrichment (Wildish *et al.*, 2004). Indeed, in a recent publication on benthic monitoring methods, Wildish *et al.* (2005) specifically exclude Eh as a geochemical method for defining organic enrichment stages.

Total sulfide appears to be more sensitive than Eh as an indicator of organic enrichment stage. However, due to the need for collection of soft sediment material to carry out total sulfide measurements, in coarse sediments such material and the resulting measurements are not representative of the overall sediments or conditions from which they are taken; in cases where total sulfide measurements indicate azoic conditions, not only are taxa present, in some cases total taxa is quite high.

The decision by DEP to exempt certain sites with very coarse sediments from the redox and total sulfide sampling requirement indicates an acknowledgement of a potential problem with the use of these metrics as regulatory tools and standards, a problem that, based on the data presented here, may extend beyond the sites exempted to-date.

Recommendations

Based on our field experience over the past 5 years, the data, results, and conclusions presented here, and our review of recent work related to benthic monitoring methods we make the following recommendations that are intended to be taken together:

1. Elimination of redox (Eh) as a metric and standard for determining the level of impact and organic enrichment associated with net pen aquaculture; Eh could be retained as a general indicator to inform interpretation of total sulfide;

2. Elimination of total sulfide ($S^{=}$) as a metric and standard for determining the level of impact and organic enrichment associated with net pen aquaculture in all but soft sediments (silt-clay) similar to those in which the method was developed;

3. In light of the total taxa data presented here, reevaluation of the total sulfide standard of 1,300µM Warning Level and >6,000µM and consideration of moving to a site average rather individual station averages when determining site-related impacts (New Brunswick, Canada uses a level of 3,000µM not as a standard but as a trigger for elevation to Tier 2 monitoring from Tier 1 and 4,500µM for elevation to Tier 3 monitoring from Tier 2) (Anon. 2006).

4. Additional work be undertaken to determine the sediment types (granulometry) in which total sulfide measurement is appropriate and valid prior to application of total sulfide as a standard in any sediment other than soft sediments (silt-clay) similar to those in which the method was developed; and

5. Increase reliance on the semi-annual video recordings to assist in the interpretation of total sulfide results and undertake additional work to develop semi-quantitative and quantitative methods for the analysis of epibenthic communities as indicators of organic enrichment.

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Appendix I
MER Assessment Corporation Standard Operation Protocol for redox and total sulfide sampling and measurement

Interim Recommendations for the N.B. EMP

By

D.J.Wildish & B.T.Hargrave

As a result of the recent intercalibration experiment on 7th August 2003 we make the following suggestions designed to improve the practicability and reproducibility of measures of redox potential (Eh) and total total sulfide ($S^{=}$) in surface (= interfacial) sediments:

- sampling corers and grabs: scrape off the top 2 cm and mix for 2-3 minutes with a spatula in a clean bucket
- take 5cc sub-samples in a 5cc cut-off syringe, taking care to exclude air, and cap. The syringe samples should be placed on crushed ice in a cooler immediately. On return to the lab the samples can be stored for up to 72 hours before analysis
- allow the sediment samples to come to lab temperature before analysis by placing in a beaker
- Measure Eh on the sample followed by adding SAOB to the same sample, in preparation for the total sulfide determination.
- We suggest a minimum of 5 replicates per core for Eh and $S^{=}$ to give adequate statistical power for each determination.
- We do not recommend taking profile samples at depths below 0-2cm for Eh and $S^{=}$ for routine monitoring purposes.

Comments

The changes suggested above will mean that meters and probes do not have to be taken in the field and the analyses can be completed in the lab. The new protocol, if followed carefully, will improve the reproducibility of the method and the inverse relationship between redox potential (x) and the log. of total total sulfide. Mixing does destroy the sharp gradients and spatial heterogeneity known for these variables in sediments.

Appendix II
MER Assessment Corporation Standard Operation Protocol for redox and total sulfide sampling and measurement

III. Field sampling procedures

A. Describe required collection, preparation, and preservation techniques, proper containers, correct sample container cleaning procedures, sample holding times from collection to analysis, and sample shipping and storage conditions for:

1. Reduction oxidation potential testing

Redox oxidation potential is measure on-site immediately after sample collection in order to obtain the most reliable measure possible given the potential for change with time, even under cooled conditions. Additionally, if redox were taken on samples at the time the total sulfide analysis was conducted, the amount of time required to obtain a stable, reliable redox mV reading would exceed the 3 hour time window within which the total sulfide samples analysis must be run from the time initial warm-up begins from 4ºC to room temperature.

Method: Wildish, D.J., H.M. Akagi, N. Hamilton and B.T. Hargrave, 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. September, 1999. 31 pp. as modified by the Interim Recommendations for the N.B. EMP, D.J.Wildish & B.T.Hargrave, received 22 August 2003.

Sample collection: One half of the core surface material from each core is removed down to a depth of 2 cm and the sediment placed in a small 125 ml plastic container and thoroughly mixed with a plastic spoon for approximately 1-2 minutes.

Measurement method: Following mixing, the redox potential is measured using an Accumet® AP63 pH/mV/Ion meter equipped with a Thermo Orion model 9678BN Combination Redox electrode filled with Thermo Orion Ag/AgCl Reference Electrode Filling Solution (900011) by immersing the electrode into the mixed sediment and waiting for the reading to stabilize while gently mixing the sediment with the electrode. Meter mV values are corrected by applying a correction factor for temperature, *e.g.* +214 at 10^{O}C (Thermo Orion Platinum Redox Electrode Instruction Manual, Model 96-78-00, 2001, p. 5).

2. Total sulfide testing

Syringes used for total sulfide sample extraction are 5cc plastic, plunger-style syringes with the tapered needle-accepting end cut off to leave a clear circular opening; syringes are pre-labeled for site, station and replicate number on the plunger stem using indelible marker prior to departure from the lab. All syringes pertaining to a station are banded together with rubber bands and kept in station-specific re-closeable Zip-Loc® bags bearing the site, date, and sampling location along with all other sample bags. When benthic infauna sampling is being done, all syringes, Whirl-Paks®, and Zip-Loc® bags pertaining to the station replicated are place together in the station replicate Nalgene® containers.

Sample collection: After redox measurement, a 5 ml portion of the mixed sediment from each core is removed with a modified 5 ml plastic syringe with the needle attachment end removed to form an open cylinder; the open end is immersed into the mixed sediment slurry and the sample extracted by pulling back on the plunger, thus obtaining a sample containing no bubbles. Immediately after obtaining the sample, the open end of the syringe is covered with plastic wrap insuring no air is trapped beneath the wrap. Aluminum foil is then placed over the end of the syringe to secure the plastic wrap in place. The syringe is then placed in a cooler with ice to maintain a temperature of $>5^{O}$C during transport to the laboratory for total sulfide (S_2) analysis within 72 hrs. of sample collection.

Once the total sulfide syringes have been discharged, the plungers and syringe bodies are separated and allowed to soak in a plastic tub of warm freshwater containing a small amount of detergent. After soaking for up to 24 hours the detergent water is poured out and the syringes and plungers are rinsed several times with fresh tap water; if necessary, small brushes are used for mechanical cleaning; this is followed by a final rinse with distilled water, followed in turn by air drying over paper towels.

Once dried, the syringe plunger labeling is removed with 95% ethanol and the plungers relabeled and the syringes reassembled for storage in dry re-closeable plastic bags.

Instrument Calibration

Total sulfides: The Accumet® AP63 pH/mV/Ion meter equipped with a Thermo Orion model 9616BN Combination Silver/Total sulfide electrode is filled with Thermo Orion Ionplus B Optimum Results™ Reference Electrode Filling Solution (900062). The meter is standardized at 1.00 (100µM), 10.0 (1,000µM), and 100 (10,000µM) using standards prepared according to Wildish *et al.,* (1999). All samples are analyzed within a maximum of 3 hrs. Following analysis of all samples, measurements of the three standards are retaken and recorded on the calibration sheets. Actual S_2 µM values are calculated by multiplying the meter readings by 100 in an Excel spreadsheet prepared for this purpose. MER generated redox/total sulfide data are periodically graphed over Wildish, *et al.* graphs to establish proper comparison between the two data sets to confirm that MER data is within expected ranges. Refer to Appendices I-IV for details on TOC/TON, metals, granulometry, and oxytetracycline calibration procedures.

MER Assessment Corporation
Standard Operating Procedure 002
Redox Potentials/Total sulfides,
Revision 0, 9 January 2006

Redox Electrode Standardization

Prior to use in the field and again on return to the lab, the Thermo Orion Redox reference electrode is checked to ensure that it is operating correctly. This is carried out by using the Thermo Orion Platinum Redox Electrode Instruction Manual. The electrode is first filled with manufacturer's recommended solution which is 4M KCL (potassium chloride) saturated with Ag/AgCl (silver/silver chloride). Two reference Zobell's solutions are then made for a two point calibration:

1. Start by weighing out 2.11g of reagent grade $K_4Fe(CN)_6 \cdot 3H_2O$ (0.1M potassium ferrocyanide)* and 0.825g of reagent grade $K_3Fe(CN)_6$ (0.05M potassium ferricyanide). Place in a 50ml volumetric flask, add approximately 30ml of distilled water and swirl until all solids are dissolved and dilute to 50ml volume with distilled water. Transfer solution to 60ml sealable plastic bottle, place the electrode into the solution and wait for reading to stabilize. This solution is designated **Solution A** and the potential reading should be approximately 234mV± 9mV; mark 60ml sealable plastic bottle with solution preparation date.
2. Next weigh out 0.21g of reagent grade $K_4Fe(CN)_6 \cdot 3H_2O$ (0.01M potassium ferrocyanide), 0.825g reagent grade $K_3Fe(CN)_6$ (potassium ferricyanide) and 1.695g of reagent grade $KF \cdot 2H_2O$ (0.36M potassium fluoride). Place in 50ml volumetric flask, add approximately 30ml of distilled water, swirl until dissolved and dilute to volume with distilled water. Transfer solution to 60ml sealable plastic bottle, place the electrode into the solution and wait for reading to stabilize. This solution is designated **Solution B** and the potential reading should be approximately 66mV greater than the first solution reading or 300mV± 9mV; mark 60ml sealable plastic bottle with solution preparation date;
3. If leaving for the field immediately, leave the Ag/AgCl solution in electrode, otherwise the probe should be drained of reference solution, rinsed inside and out with distilled water and placed in storage.

Redox measurement

1. Measure the redox, as mV, using the AP63 pH/mV/ion Meter (pre-standardized as per above), by immersing the Platinum Redox Electrode into the homogenized sediment mixture ensuring not to rub the end of the electrode on the bottom of the Nalgene® container;
2. Use a stirring motion sufficient to maintain the electrode in constant motion, but not enough to create an air pocket in the mixture; continue to stir until reading stabilizes or continues to fluctuate within a specific range.
3. If the reading is stable, record the meter value on the data sheet; if the value remains unstable, record an approximate mean value and indicate the approximate range around the mean. *e.g.* -285mV ± 15mV;
4. Rinse electrode with clean seawater and wipe clean with a paper towel before taking next measurement;
5. Meter mV values are corrected by applying a correction factor for temperature, *e.g.* +214 at $10^{O}C$ (Thermo Orion Platinum Redox Electrode Instruction Manual, Model 96-78-00, 2001, p. 5).

Total sulfide Electrode Standardization

Due to the short shelf life of the calibration solutions, the electrode will have a three point calibrated just prior to and immediately after measurements. The calibration follows the procedure used Dr. Wildish and the Thermo Orion Instruction Manual.

1. The Thermo Orion model 9616BN Combination Silver/Total sulfide electrode is filled with Thermo Orion *Ionplus* B Optimum Results (Product No. 900062) filling solution.
2. a. Start by bulb-pipetting 4ml of Certified NIST traceable 3% Na2S*9H2O (sodium total sulfide solution) (Ricca Chemical Company Cat. No. 7570-4) into a 50ml volumetric flask and dilute to volume with deionized water to give a concentration of 0.01M.
   b. Pipette 5ml 0.01M solution into a 50ml volumetric flask and dilute to volume with deionized water to give concentration 0.001M.
   c. Repeat step b. using 0.001M solution for a concentration 0.0001M
3. Starting with the least concentrated solution, place 15ml in a 60ml sealable plastic bottle and add/mix 15ml of SAOB Part A + Part B solution (Thermo Orion Cat. No. 941609.
4. Turn on the Accumet AP63 pH/mV/ion meter, set mode for ion, press std then follow prompts to enter concentration of standard solutions, 1.00 for 100μM; repeat for remaining two standards, *i.e.* 10.0 for 1,000μM, and 100 for 10,000μM, thoroughly rinsing electrode with deionized water and blotting dry between measurements; this process may have to be repeated several times before reading reach acceptable stability.

Total sulfide measurement

1. Remove syringes from refrigerator and place in fiberglass tray on paper towels arranged in site-station-replicate order to allow them to warm to room temperature, *i.e.* 20º-25ºC.;
2. Once room temperature has been reached (approximately 1 hr), beginning in sequential order, remove the aluminum foil and plastic wrap from the syringe opening;
3. Depress the syringe plunger so that exactly 5cc of sediment remain in the syringe barrel and remove excess sediment cleanly from the end of the barrel;
4. Eject the remaining 5cc sediment core directly into a wide mouth 60 ml Nalgene® bottle and add 5 ml of SAOB solution using the pre-measured dispensing bottle;
5. Cap and swirl the Nalgene® bottle such that the sediment and SAOB solution become thoroughly mixed;
6. Immerse the Thermo Orion model 9616BN Combination Silver/Total sulfide electrode into the sediment-SAOB mixture and swirl such as not to rub the electrode against the bottom of the Nalgene® bottle; once the value on the meter stabilizes, record the value on the data sheet;
7. Rinse probe with deionized water until meter value returns to 0.00 mV and blot dry before proceeding with next sample;
8. All samples must be analyzed within a maximum of 3 hrs;
9. Once all samples are analyzed, measurements of the three standards are retaken and recorded on the calibration sheets and percent deviation from pre-measurement calibration calculated;
10. Actual S_2 μM values are calculated by multiplying the meter readings by 100.

Revision 0
Date: 9 January 2006

Appendix III
MER redox and total sulfide data set used for statistical analyses

Eh	$S^{=}$ µM	Rel. gran.	Eh	$S^{=}$ µM	Rel. gran.	Eh	$S^{=}$ µM	Rel. gran.
239	52	1	75	1100	1	-111	1050	1
225	54	1	54	1400	1	-113	1640	1
213	67	1	195	1450	1	-64	989	1
202	352	1	71	1500	1	-100	1860	1
-59	340	1	165	1140	1	-17	1890	1
-80	49	1	148	1270	1	-6	668	1
-73	60	1	-36	1680	1	87	476	1
-96	107	1	125	798	1	90	556	1
24	1080	1	84	735	1	-93	2550	1
95	360	1	119	127	1	-7	1410	1
106	430	1	102	543	1	-31	1360	1
66	525	1	93	857	1	-56	1670	1
41	870	1	122	1020	1	-32	1230	1
89	527	1	67	1190	1	-72	1850	1
-83	1950	1	30	1020	1	78	543	1
-155	4690	1	-29	1300	1	64	568	1
-33	1260	1	-120	4990	1	45	426	1
-148	3510	1	139	400	1	153	475	1
-123	3370	1	188	246	1	90	993	1
-117	2660	1	157	658	1	145	406	1
160	531	1	61	1100	1	92	822	1
95	819	1	74	912	1	128	565	1
132	448	1	79	798	1	57	977	1
67	1010	1	72	1770	1	131	311	1
47	977	1	86	809	1	89	476	1
7	1010	1	127	735	1	97	522	1
-73	1840	1	137	224	1	13	1070	1
-123	3510	1	32	893	1	-75	948	1
-157	4520	1	66	504	1	-21	1210	1
-68	3850	1	115	1770	1	-25	1090	1
-41	2440	1	162	984	1	29	851	1
-39	2380	1	129	1010	1	-20	730	1
-161	3050	1	-73	4640	1	352	71	1
-144	3200	1	-76	4010	1	224	143	1
-158	2520	1	25	1170	1	216	71.1	1
-15	1550	1	-50	548	1	235	59.3	1
-75	2100	1	-42	40	1	206	103	1
-129	2240	1	-58	448	1	228	101	1
182	70.5	1	29	12.8	1	110	136	1
143	98.6	1	-17	15.2	1	138	66.8	1
152	113	1	-34	17.9	1	225	52	1
87	264	1	32	15.8	1	55	931	1
81	264	1	-74	410	1	31	793	1
83	239	1	-64	308	1	8	1230	1
93	186	1	123	435	1	-86	1330	1
54	225	1	88	832	1	-96	1290	1
109	116	1	6	643	1	-111	1850	1
90	491	1	-71	1690	1	-82	1860	1

Eh	S$^{=}$ µM	Rel. gran.	Eh	S$^{=}$ µM	Rel. gran.	Eh	S$^{=}$ µM	Rel. gran.
-49	1660	1	-81	130	1	124	6.7	1
-96	1290	1	-59	91	1	-38	1910	1
15	1270	1	-101	304	1	-86	3350	1
-40	962	1	-163	1010	1	-80	1470	1
-67	1170	1	-131	1040	1	-73	1180	1
155	853	1	-42	222	1	-139	2860	1
-24	1980	1	-65	104	1	-42	1760	1
95	764	1	-97	119	1	59	448	1
-30	720	1	-92	168	1	-53	1650	1
-41	2950	1	-94	256	1	-102	1610	1
-53	1490	1	-109	270	1	6	796	1
-62	1150	1	224	643	1	-66	1330	1
64	282	1	150	384	1	7	1440	1
-7	520	1	-96	730	1	71	392	1
-42	1280	1	-96	1150	1	94	292	1
-82	1630	1	135	593	1	45	650	1
50	526	1	195	832	1	115	496	1
-15	1310	1	168	458	1	82	883	1
-141	2540	1	148	838	1	71	392	1
-137	3250	1	-129	773	1	94	292	1
-9	703	1	32	618	1	99	311	1
19	641	1	155	716	1	-13	929	1
-17	598	1	157	555	1	69	458	1
-48	1350	1	133	376	1	53	644	1
-20	1180	1	81	366	1	-29	1600	1
-46	1360	1	138	152	1	64	133	1
-11	1470	1	94	133	1	-96	1990	1
-112	1590	1	50	143	1	-33	2020	1
-40	1620	1	119	242	1	140	925	1
104	211	1	-42	653	1	98	572	1
91	206	1	-17	1220	1	100	728	1
99	206	1	-12	385	1	135	1090	1
119	102	1	-36	724	1	-30	1830	1
115	102	1	-22	953	1	33	500	1
131	123	1	6	273	1	-15	348	1
139	108	1	-5	630	1	-34	3060	1
139	35.7	1	-51	399	1	-172	6680	1
97	103	1	104	91	1	-167	12600	1
288	37	1	97	109	1	-76	4150	1
283	36	1	58	553	1	-141	4800	1
303	5	1	8	573	1	-120	5630	1
-56	1030	1	43	264	1	-105	5250	1
-98	252	1	49	1160	1	-90	4050	1
-76	370	1	87	164	1	-85	3110	1
-125	681	1	98	137	1	-33	2340	1
-79	126	1	78	213	1	-20	1330	1
-95	251	1	161	2.7	1	-23	2640	1
-78	49.6	1	128	4.8	1	126	98	1

Eh	$S^{=}$ µM	Rel. gran.	Eh	$S^{=}$ µM	Rel. gran.	Eh	$S^{=}$ µM	Rel. gran.
113	138	1	135	1700	1	-118	5180	1
121	106	1	-32	2030	1	-143	6490	1
17	778	1	7	1260	1	-147	4920	1
87	464	1	59	641	1	-124	4260	1
-16	2710	1	-140	1420	1	-56	3950	1
60	2860	1	9	743	1	57	820	1
-56	3460	1	-87	776	1	48	1630	1
-120	2170	1	-97	1010	1	91	678	1
-90	2090	1	39	355	1	9	968	1
-118	1930	1	53	585	1	-26	1180	1
-135	1270	1	-182	16500	1	69	433	1
-122	1830	1	-120	4460	1	-32	2270	1
-72	1300	1	59	1480	1	39	1340	1
61	1210	1	-132	6550	1	-18	2020	1
-54	1870	1	26	1250	1	-103	2150	1
-92	1990	1	196	1020	1	15	1450	1
-104	1750	1	28	1130	1	-28	2410	1
-26	984	1	151	343	1	53	1420	1
-61	1040	1	147	152	1	96	129	1
-24	1380	1	94	1250	1	112	364	1
27	1080	1	133	311	1	76	1550	1
65	865	1	-104	3720	1	118	128	1
7	1230	1	-170	5140	1	71	180	1
-8	2460	1	-35	1010	1	-53	1430	1
-75	1520	1	-68	1230	1	-125	2050	1
-39	1640	1	-30	977	1	-16	2020	1
-106	4690	1	52	351	1	85	128	1
-131	16200	1	-130	2740	1	84	1630	1
-123	7350	1	23	1750	1	91	743	1
-124	6350	1	-77	1320	1	129	966	1
-82	4730	1	-159	3530	1	117	1390	1
-116	4960	1	-51	1340	1	-119	2210	1
78	766	1	-88	1920	1	141	286	1
101	681	1	48	634	1	115	152	1
58	1040	1	-35	1800	1	80	344	1
60	344	1	143	735	1	73	569	1
-105	1800	1	219	386	1	-94	3330	1
52	546	1	190	380	1	-89	3100	1
43	370	1	197	474	1	-165	14800	1
-102	1140	1	161	443	1	-126	4810	1
96	1290	1	238	132	1	-132	7500	1
19	715	1	184	285	1	-122	5520	1
109	2010	1	138	1580	1	-111	1560	1
82	995	1	85	4150	1	-123	1270	1
197	690	1	88	2250	1	-118	1350	1
155	845	1	105	908	1	-25	2470	1
20	3160	1	47	918	1	-188	1680	1
220	489	1	-106	4990	1	-69	3060	1

Eh	$S^=$ µM	Rel. gran.	Eh	$S^=$ µM	Rel. gran.	Eh	$S^=$ µM	Rel. gran.
-61	1250	1	-133	1610	1	-87	2250	1
-73	1350	1	-61	489	1	-17	1800	1
-108	1530	1	44	406	1	-91	2810	1
-145	2100	1	-98	693	1	61	867	1
-143	2420	1	-9	1270	1	-45	1440	1
-136	2720	1	-16	1170	1	112	2150	1
12	565	1	-56	785	1	138	1960	1
81	252	1	-161	1930	1	-98	4780	1
53	585	1	-194	1200	1	172	1810	1
-33	3080	1	-127	1820	1	1	2620	1
-216	2200	1	-128	1150	1	-51	2630	1
-162	2070	1	-131	1280	1	146	532	1
12	565	1	-153	2040	1	-119	2310	1
81	252	1	-91	1290	1	-148	2590	1
53	585	1	-19	931	1	-141	2920	1
-66	1200	1	-102	1820	1	-164	2480	1
-97	1130	1	-88	191	1	-114	1410	1
-10	1430	1	-131	819	1	-39	3620	1
-125	2020	1	-86	468	1	-147	3950	1
-149	3260	1	-44	652	1	-169	5380	1
-77	1130	1	-64	1020	1	-141	1860	1
34	1020	1	-74	457	1	-89	1470	1
-143	2720	1	2	771	1	-48	1630	1
-141	5000	1	-59	923	1	-160	3540	1
-17	1050	1	-61	904	1	147	541	1
9	1230	1	-49	893	1	131	422	1
-26	908	1	57	248	1	73	762	1
-72	1810	1	-106	1780	1	129	540	1
-14	1020	1	-124	2400	1	170	471	1
-87	1770	1	-148	4580	1	-80	1590	1
-47	908	1	-148	3640	1	-91	2690	1
-36	728	1	-160	3720	1	-74	2940	1
-11	650	1	-125	3950	1	136	470	1
-73	1630	1	-141	3820	1	-46	993	1
-132	2810	1	-110	2510	1	-60	1060	1
-130	2250	1	-125	3560	1	-139	3610	1
-36	610	1	-120	2070	1	-147	7800	1
-46	638	1	147	542	1	-79	1830	1
-16	485	1	131	653	1	-87	1370	1
-46	726	1	73	964	1	-102	1320	1
-6	373	1	-172	6310	1	-137	1760	1
-104	1220	1	-172	6840	1	96	861	1
99	311	1	-159	8610	1	131	1380	1
-11	537	1	-81	1800	1	86	490	1
-26	465	1	-36	1350	1	139	692	1
34	598	1	-107	4070	1	108	1010	1
-96	2160	1	-138	5560	1	49	1830	1
14	511	1	-130	4700	1	129	590	1

Eh	$S^=$ µM	Rel. gran.	Eh	$S^=$ µM	Rel. gran.	Eh	$S^=$ µM	Rel. gran.
-149	4400	1	23	659	2	334	0.1	3
84	504	1	52	810	2	347	0.1	3
93	927	1	154	1001	2	342	0.1	3
9.23	923	1	159	1540	2	378	0.1	3
7.5	747	1	-20	1010	2	309	36	3
4.88	488	1	-74	2150	2	179	35	3
15.5	1550	1	10	1300	2	259	57	3
38.8	3880	1	-31	1400	2	164	56	3
14.3	1430	1	-63	1350	2	209	34	3
9.71	971	1	-65	2100	2	-51	1520	3
17	1700	1	-86	2380	2	190	280	3
12.4	1240	1	-26	2580	2	93	418	3
14.5	1450	1	-68	3480	2	119	286	3
23.9	2390	1	-73	3930	2	212	378	3
18.6	1860	1	58	1230	2	229	68	3
19.8	1980	1	-48	1200	2	237	144	3
26.8	2680	1	-118	9270	2	271	50	3
14.9	1490	1	126	900	2	267	20	3
11.5	1150	1	-51	1700	2	244	167	3
18.0	1800	1	-28	760	2	124	167	3
21.8	2180	1	45	3660	2	-29	156	3
26.5	2650	1	39	2010	2	191	135	3
9.53	953	1	-83	2380	2	190	136	3
13.2	1320	1	-39	798	2	214	112	3
17.1	1710	1	31	1590	2	56	555	3
20.7	2070	1	-147	2630	2	178	74.5	3
17.5	1750	1	-138	3110	2	203	168	3
8.20	820	1	-21	815	2	-39	314	3
8.87	887	1	-66	1270	2	-54	720	3
9.14	914	1	-24	2730	2	-42	115	3
5.85	585	1	-119	2900	2	-108	940	3
7.52	752	1	148	1320	2	-75	982	3
3.35	335	1	114	1510	2	-35	15.5	3
5.53	553	1	263	5.2	3	310	33	3
6.01	601	1	159	11.2	3	259	294	3
121	346	2	70	83	3	230	515	3
-22	843	2	23	27	3	72	2200	3
-45	600	2	187	33	3	115	1250	3
-52	300	2	163	135	3	140	857	3
-59	277	2	13	621	3	-29	923	3
-149	1410	2	-25	396	3	233	490	3
58	659	2	62	352	3	-140	1880	3
-19	1370	2	290	10	3	-7	1290	3
-26	1950	2	317	2	3	375	0.8	3
17	916	2	215	78	3	308	0.6	3
-177	4760	2	424	2.4	3	364	0.2	3
-162	4210	2	329	0.2	3	-17	302	3
-143	5520	2	318	0.7	3	13	344	3

Eh	$S^{=}$ µM	Rel. gran.	Eh	$S^{=}$ µM	Rel. gran.	Eh	$S^{=}$ µM	Rel. gran.
-22	780	3	-133	11500	3	-71	398	4
15	813	3	-156	2240	3	31	266	4
32	811	3	-98	6840	3	-18	158	4
-32	933	3	-34	6120	3	1	656	4
24	836	3	4	10900	3	66	64.4	4
308	48.5	3	82	315	3	101	7	4
41	493	3	139	77.5	3	216	4	4
-114	2330	3	52	212	3	-162	1440	4
207	372	3	30	348	3	-68	26.4	4
62	1400	3	79	764	3	20	697	4
5	1340	3	51	542	3	37	906	4
-41	2060	3	-60	3120	3	27	745	4
-146	4720	3	-42	2310	3	-25	2020	4
-84	3530	3	-15	2010	3	289	262	4
-96	1770	3	-99	2620	3	309	94.6	4
189	296	3	-83	3030	3	25	877	4
194	384	3	51	796	3	-83	1760	4
254	378	3	64	1540	3	34	1020	4
134	1690	3	81	1230	3	31	686	4
-43	3440	3	-44	3110	3	61	483	4
-1	1320	3	1.4	144	3	-28	1010	4
-106	1920	3	1.5	145	3	94	597	4
-35	3300	3	2.24	224	3	43	652	4
-36	2790	3	149	62.5	4	20	1760	4
-61	2880	3	138	50.6	4	46	1270	4
59	1280	3	259	1.1	4	30	933	4
-3	1600	3	167	2.1	4	60	1890	4
90	1180	3	219	1	4	30	1850	4
164	927	3	190	2	4	-70	4130	4
-52	1610	3	228	0.6	4	22	466	4
-219	2240	3	121	18.8	4	167	662	4
15	516	3	-35	64.5	4	105	942	4
-46	690	3	82	43.3	4	239	340	4
94	705	3	183	75.7	4	179	1540	4
-105	1690	3	367	0.1	4	159	798	4
-97	1630	3	391	0.1	4	211	1050	4
-100	492	3	144	200	4	204	1760	4
-51	1070	3	24	409	4	189	908	4
-46	1160	3	199	337	4	-81	1380	4
-16	964	3	137	250	4	-32	1420	4
-76	1290	3	208	92	4	36	1010	4
-56	1160	3	128	244	4	-101	4460	4
-16	1130	3	134	122	4	-26	1910	4
-66	1000	3	370	7	4	-4	1120	4
-106	2440	3	-4	348	4	-117	4110	4
-86	1560	3	-77	584	4	57	566	4
-64	1120	3	-59	494	4	294	154	4
-117	3520	3	1	372	4	-20	1290	4

Eh	$S^{=}$ µM	Rel. gran.	Eh	$S^{=}$ µM	Rel. gran.	Eh	$S^{=}$ µM	Rel. gran.
76	743	4	-9	2860	4	38	1370	4
-90	1150	4	-84	7200	4	-20	1170	4
-151	13900	4	52	1620	4	-91	982	4
-151	8860	4	123	918	4	-97	830	4
-145	7110	4	90	2880	4	-79	1380	4
-158	11900	4	101	897	4	24	405	4
-132	6940	4	83	478	4	34	462	4
88	1580	4	62	1060	4	-116	3130	4
71	1710	4	-74	2240	4	-110	2460	4
54	1550	4	129	589	4	-112	2280	4
-78	3060	4	33	1540	4	-133	5050	4
-30	3520	4	46	2550	4	-137	4740	4
-158	5930	4	99	1130	4	-131	2000	4
75	1920	4	40	2090	4	-111	1680	4
120	1490	4	61	1230	4	-129	5270	4
34	2390	4	-66	1630	4	-84	8550	4
119	1130	4	-87	2390	4	-88	1160	4
118	1610	4	27	1370	4	-152	3720	4
198	1710	4	-3	940	4	-127	3920	4
117	1880	4	117	678	4	-3	1630	4
174	2860	4	127	514	4	12	2330	4
142	2370	4	78	933	4	104	743	4
139	1560	4	103	470	4	-125	3480	4
207	418	4	134	335	4	-9	2390	4
72	2120	4	115	301	4	2.41	241	4
9	2640	4	28	1010	4	3.24	324	4
-6	2390	4	128	1320	4	14.2	1420	4
-19	3560	4	-118	1010	4	6.87	687	4
79	2710	4	-130	1360	4	2.9	290	4
61	1250	4	-70	2660	4	-9	1440	5
88	1320	4	40	1010	4	73	871	5
-49	2590	4	-51	1750	4	236	64	5
162	1680	4	57	748	4	239	214	5
131	1380	4	138	778	4	-79	414	5
-6	1640	4	25	1340	4	191	54	5
120	826	4	52	1370	4	109	104	5
173	574	4	-28	1250	4	110	124	5
167	535	4	-45	603	4	26	48	5
169	2880	4	-16	460	4	141	38	5
230	741	4	-14	462	4	91	48	5
237	321	4	-128	4110	4	11	134	5
41	1010	4	-25	2050	4	37	168	5
229	748	4	30	635	4	131	44	5
188	1000	4	-16	1030	4	171	4	5
-59	4960	4	-26	1330	4	8	1980	5
34	3750	4	-40	1410	4	30	2340	5
-10	2090	4	-96	1580	4	80	557	5
95	2030	4	-69	1090	4	127	531	5

Eh	$S^{=}$ μM	Rel. gran.	Eh	$S^{=}$ μM	Rel. gran.	Eh	$S^{=}$ μM	Rel. gran.
127	243	5	52	1160	5			
220	718	5	-26	655	5			
262	302	5	-21	1370	5			
251	375	5	2	783	5			
255	209	5	20	678	5			
245	425	5	114	1130	5			
-164	3720	5	123	1450	5			
239	989	5	-6	2070	5			
-96	2500	5	-103	1420	5			
-72	2320	5	-67	1310	5			
137	215	5	33	1720	5			
87	274	5	63	2690	5			
-35	3080	5	146	2360	5			
-138	3780	5	147	1260	5			
-161	18800	5	-32	3210	5			
-64	1130	5	54	3190	5			
-40	5650	5	7	1270	5			
-101	6940	5	-56	2420	5			
-15	4810	5	86	2440	5			
-14	3390	5	-101	2720	5			
-20	11400	5	29	2280	5			
-67	3860	5	-72	3760	5			
129	809	5	-119	3860	5			
71	420	5	-122	4400	5			
76	1420	5	-99	3830	5			
136	1300	5	-1	2960	5			
120	879	5	-115	2930	5			
106	454	5	156	*1800*	6			
-154	14600	5	126	*2040*	6			
-146	9120	5	140	*1480*	6			
-146	13600	5	-131	3140	6			
-12	869	5	0.14	14	6			
150	891	5	10.2	1020	6			
21	695	5	7.87	787	6			
21	1560	5						
-6	1580	5						
68	1510	5						
117	748	5						
40	1360	5						
19	585	5						
-137	2480	5						
-124	4500	5						
-97	1750	5						
-11	1430	5						
-112	2850	5						
-36	2550	5						
-144	3310	5						
-62	1150	5						

Appendix IV
Wildish *et al*., 1999 mariculture environmental monitoring data

$S^=$	Eh	$S^=$	Eh	$S^=$	Eh	$S^=$	Eh	$S^=$	Eh	$S^=$	Eh	$S^=$	Eh
14000	14.4	1200	139.9	3000	44.0	3800	-6.0	1800	86.0	6900	-118.0	1300	22.0
11000	41.3	1300	127.8	2000	29.0	5800	-16.0	1100	84.0	13000	-126.0	1400	28.0
36000	15.8	690	133.9	2600	124.0	4700	9.0	1000	96.0	12000	-122.0	1400	16.0
5600	52.7	3700	-97.0	3100	54.0	2500	94.0	91	251.9	10000	-91.0	990	4.0
5600	71.2	3000	-82.0	2800	19.0	1500	74.0	82	243.1	23000	-131.0	830	14.0
5800	60.7	6800	-106.0	2400	48.9	1300	108.0	120	256.0	29000	-136.0	1100	38.0
1100	138.0	17000	-96.0	1400	14.0	26	144.0	2400	84.0	6100	80.0	1100	-58.0
2700	172.0	16000	-108.0	2000	-6.0	230	124.0	1900	84.0	6200	88.0	1000	-54.0
1600	163.0	13000	-88.0	1700	24.0	170	154.0	2600	89.0	4100	86.0	1300	-27.0
5900	14.6	740	162.0	830	178.9	530	146.0	320	224.0	3100	197.2	740	-15.0
6500	18.0	1000	167.0	510	173.4	670	122.0	510	232.0	7300	103.0	780	-22.0
7700	72.1	1300	174.0	540	171.2	650	109.0	430	239.0	6700	93.0	680	-2.0
3700	-105.6	2100	69.0	830	185.1	120	214.8	2200	9.0	19000	-74.0		
13000	-101.0	1100	63.9	940	185.4	81	213.0	2400	4.0	16000	-120.0		
6700	-92.0	1100	84.0	440	202.1	210	208.4	2600	14.3	20000	-113.0		
2700	139.0	1100	60.0	1100	93.0	260	154.0	840	-48.0	18000	-117.0		
3100	70.0	1100	54.0	1200	182.0	220	163.0	1000	-56.0	8800	-122.0		
3100	108.0	1200	69.0	520	174.0	220	153.0	1300	-16.0	17000	-121.0		
4600	-77.0	5800	-46.0	240	192.4	650	179.0	1300	-37.0	3800	-147.0		
3800	-68.0	4500	-57.0	320	189.9	520	186.0	1300	-36.0	4300	108.0		
4000	-47.0	3100	-31.0	580	173.8	250	140.0	790	-16.0	5600	123.0		
1800	64.0	1300	30.0	4900	-81.0	310	173.0	630	61.0	1100	39.0		
2600	78.0	1100	44.0	4100	-89.0	360	168.0	750	62.0	1300	-13.0		
2300	74.0	1100	62.0	2200	-116.0	350	158.0	620	49.0	2100	-4.0		
3400	-31.0	4500	-81.0	1700	58.0	76	239.0	1000	-36.0	750	143.0		
4700	-46.0	3500	-97.0	1900	54.0	47	244.0	1300	-34.0	2200	-90.0		
2900	-35.0	2800	-72.0	1600	12.0	32	229.0	650	139.0	2100	-77.0		
3300	18.0	2500	-87.0	4100	-66.0	370	241.0	1900	-46.0	1100	98.0		
3100	5.0	1300	-92.0	1800	-96.0	450	240.0	2100	-41.0	3200	-26.0		
2600	24.0	3400	-72.0	1200	-81.0	100	234.0	4200	-47.0	2100	3.0		
4700	-27.0	1500	67.7	1500	162.0	190	249.0	3700	-8.0	1700	-36.0		
6100	-16.0	2100	74.0	870	159.0	120	229.0	1600	-2.0	1600	-56.0		
5700	-42.0	1800	196.1	810	165.0	200	236.0	3000	-32.0	1500	-48.0		
2100	34.0	4600	130.0	1600	154.0	60	235.0	2500	-70.0	1600	-23.0		
1900	42.0	4400	124.0	1400	160.0	120	238.0	2300	-67.0	2500	-21.0		
3000	90.0	3000	117.0	430	164.0	81	260.0	720	-82.0	1800	-16.0		
1100	188.0	13000	-114.0	1500	89.0	1100	24.0	1600	122.0	1500	-72.0		
2300	142.0	28000	-101.0	1500	84.0	2100	29.0	2200	124.0	1800	-46.0		
1100	134.0	9300	-106.0	1400	84.0	1200	14.0	2700	138.0	1400	-49.0		
3400	101.0	3200	54.0	70	134.0	330	59.0	1000	129.0	2500	-99.0		
2200	89.0	3100	42.0	57	199.0	470	54.0	790	133.0	1000	-76.0		
2900	76.0	1700	25.0	91	194.0	770	49.0	1500	125.0	2400	-75.0		
1800	84.0	3000	101.2	120	209.0	420	149.0	10000	-47.0	3600	-16.0		
2000	78.0	2400	99.4	110	211.0	570	154.0	12000	-49.0	1200	18.0		
1600	62.0	1800	177.5	130	194.0	490	154.0	5800	-41.0	1100	-4.0		
1500	154.0	3800	167.0	10000	-101.0	44	286.0	3600	97.0	3500	-56.0		
1300	141.0	2400	163.0	12000	-106.0	89	282.0	4700	94.0	4900	-26.0		
1200	144.0	2700	185.0	1900	-78.0	58	299.2	4500	133.0	3900	-47.0		
1300	83.0	14000	-110.0	3300	-136.0	65	290.3	9200	-26.0	3200	-6.0		
1200	72.0	8600	-116.0	8100	-131.0	83	294.6	14000	-36.0	3300	14.0		
1400	94.0	3100	-106.0	15000	-128.0	85	286.8	7600	-29.0	3200	-12.0		
950	104.0	21000	-146.0	71	155.4	440	236.0	2200	33.0	3300	-11.0		
890	78.0	19000	-136.0	93	157.0	340	230.0	2200	42.0	2900	-7.0		
960	86.0	18000	-131.0	790	147.0	150	234.1	3800	38.0	2000	18.0		
1200	74.0	2300	72.0	290	163.0	170	114.0	6300	88.0	1900	-2.0		
2100	82.0	2200	74.0	260	161.0	330	126.0	8200	64.0	1500	9.0		
2200	49.0	1800	49.0	360	158.0	230	111.0	5800	53.0	1000	4.0		
12000	-86.0	1100	94.0	300	145.0	84	157.3	11000	32.0	120	259.0		
14000	-81.0	990	102.0	260	144.0	240	161.0	8400	34.0	130	229.0		
2700	74.0	1900	119.0	380	144.0	510	165.0	7700	30.0	20	244.0		

Appendix V
MER redox, total sulfide and total taxa data

Eh	Eh impact level	$S^=$	$S^=$ impact level	SPR	FR	% Cap	Abund.	Sed cat
28	1	1550	2	68	47	23.6	1983	4
-74.7	2	2407	2	6	5	0	74	1
-122.3	3	2933	2	9	9	38.3	156	4
239	1	989	1	49	42	17.9	3461	5
16.7	1	3107	2	15	12	47.3	1037	1
-21	2	963	1	21	18	0	317	1
-31	2	762	1	28	25	0	280	1
-77	2	2560	2	48	32	85.6	7337	3
-76	2	1218	1	53	39	19.5	815	5
-135	3	5753	2	13	11	81	1320	3
-105.7	3	2503	2	1	1	100	66	1
-117	3	1393	2	3	3	97.5	300	1
-115	3	2070	2	18	11	11.2	259	1
-25	2	508	1	65	46	10.3	4745	4
10	1	841	1	8	6	41.1	132	1
-102	3	493	1	17	13	0	502	1
198	1	377	1	19	17	16.9	560	3
55.7	1	1205	1	43	35	0	922	4
63	1	567	1	23	18	0.4	798	3
65.3	1	1189	1	26	22	4.4	444	3
-3	2	2379	2	27	23	59.6	4160	5
59	1	898	1	42	32	12.4	486	4
-167.7	3	7253	3	4	5	16.7	17	1
-108.0	3	5167	2	5	5	75.2	436	4
68.3	2	1850	2	8	11	69.6	671	1
-46	2	1097	2	18	12	5.1	543	1
-58	3	1533	2	26	20	5.1	189	3
-97	2	2903	2	42	30	68.8	2765	5
-49	2	1150	1	50	36	59.7	3099	2
-43	2	7953	3	23	19	89.8	3485	3
-136	3	2607	2	3	3	44.4	37	1
-94	2	2403	2	2	2	98.6	243	1
-152	3	3980	2	6	3	57.9	230	1
53	1	818	1	38	28	49.5	2461	5
-81.7	2	2407	2	4	2	97.5	514	1
-71	2	1347	2	7	4	11.1	70	1
88	1	604	1	27	20	52.2	3572	3
27.7	1	1221	1	30	24	8.2	576	3
-130	2	2593	2	13	10	40.5	156	5
-65.7	2	2553	2	12	9	36.8	214	3
-122.0	3	3913	2	17	11	68.6	1086	5
-97	2	2880	2	21	16	56.7	1547	1
-118.3	3	4170	2	9	9	8.3	152	1
-112.7	3	2623	2	8	7	35.1	313	4
-56.3	2	2717	2	26	21	81	4197	1

Eh	Eh impact level	S2	S2 impact level	SPR	FR	% Cap	Abund.	Sed cat
-49	2	1600	2	28	21	2.5	827	3
-112	3	2230	2	31	26	0	255	1
-31	2	1203	1	68	47	42.6	2004	4
-6	2	2070	2	39	29	48.7	2317	5
-89	2	1064	1	36	32	1.8	1485	4
-152.3	3	3730	2	1	1	100	119	1
-81	2	1377	1	4	4	95.1	214	1
-130	3	3885	2	4	4	64.4	49	1
-101	3	2310	2	31	25	71.9	2987	2
-121.7	3	4413	2	3	3	97.1	683	1
-135	3	1490	2	5	4	85.6	251	1
-14	2	1663	2	27	20	34.4	4856	3
-17.7	2	2420	2	10	9	8.3	169	3
269	1	180	1	43	31	3.9	1959	4
-33.7	2	2667	2	27	20	77.2	5839	5
-22.5	2	3035	2	24	20	16.1	1848	5
-61	2	1807	2	27	23	44.2	667	2
-15.7	2	1826	2	9	7	0	222	1
-133.7	3	3930	2	6	5	49.6	70	4
136	1	1121	1	44	36	0	5695	2
-67	2	1420	2	43	35	3.3	1255	3
-17	2	674	1	39	33	6.3	798	3
24	1	908	1	61	40	28.7	1407	5
72	1	1250	1	41	33	13.3	506	5
47	1	394	1	45	32	0.3	1493	4
-99	2	2213	2	3	2	64.2	119	1
-141	1	2413	1	2	2	98.7	218	1
-118	3	2713	2	2	2	63.6	103	1
-139	3	1689	2	19	16	80.7	1046	1
-108.7	3	1483	2	4	4	78.6	144	1
-161	3	1650	2	11	10	1.2	165	1
-12	2	775	1	29	28	27.8	403	3
21.3	1	1831	2	9	8	0	177	1
342	2	56	1	48	39	9.7	1152	4
-48	2	2920	2	34	27	54.3	1811	5
-62	2	2660	2	19	17	26.4	407	5