Canadian Technical Report of
Fisheries and Aquatic Sciences 2546

August 2004

# Inter-laboratory Calibration of Redox Potential and Total Sulfide Measurements in Interfacial Marine Sediments and the Implications for Organic Enrichment Assessment

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This is the two hundred and fifty-fifth Technical Report of
The Biological Station, St. Andrews, New Brunswick

Cat. No. Fs 97-6/2546E ISSN 0706-6457

Correct citation for this publication:

Wildish, D.J., Akagi, H.M., Hargrave, B.T., and Strain, P.M. 2004. Inter-laboratory calibration of redox potential and total sulfide measurements in interfacial marine sediments and the implications for organic enrichment assessment. Can. Tech. Rep. Fish. Aquat. Sci. 2546: iii + 25 p.

## ABSTRACT

Wildish, D.J., Akagi, H.M., Hargrave, B.T., and Strain, P.M. 2004. Inter-laboratory calibration of redox potential and total sulfide measurements in interfacial marine sediments and the implications for organic enrichment assessment. Can. Tech. Rep. Fish. Aquat. Sci. 2546: iii + 25 p.

Inter-laboratory calibration experiments with interfacial sediments from salmon farm and reference locations in the Bay of Fundy were highly variable for redox potential (Eh), but less so for total sulfide measurements. The coefficient of variability for total sulfide was ≤ 38% using a standard method (1:1 volumetric standard/sample:SAOB, sulfide anti-oxidant buffer). In agreement with published results the high variability of Eh was due to two factors: probe "poisoning" (formation of coatings of sulfide or oxide on the platinum probe surface which altered the electrical response) and an unstable electrode response in poorly poised, oxic sediments. The probe "poisoning" could not be removed by polishing or chemical treatment with aqua regia. We conclude that measurements of total sulfide, made by the standard method, can be used to indicate the organic enrichment stage of each sample. The high variability for Eh precludes its use in defining sedimentary organic enrichment stages. Eh might be used semi-quantitatively as an internal validation of total sulfide concentrations where the sediments are anoxic or hypoxic.

## RÉSUMÉ

Wildish, D.J., Akagi, H.M., Hargrave, B.T., and Strain, P.M. 2004. Inter-laboratory calibration of redox potential and total sulfide measurements in interfacial marine sediments and the implications for organic enrichment assessment. Can. Tech. Rep. Fish. Aquat. Sci. 2546: iii + 25 p.

Les expériences d'étalonnage interlaboratoires sur des sédiments interfaciaux provenant de stations salmonicoles et de sites témoins dans la baie de Fundy ont donné des résultats très variables en ce qui concerne le potentiel rédox, mais moins variables dans le cas des sulfures. Le coefficient de variabilité pour les sulfures totaux était de ≤ 38 % quand on employait une méthode standard (1 : 1 étalon volumétrique/échantillon : solution tampon SAOB). Conformément aux résultats publiés, la forte variabilité du potentiel rédox était due à deux facteurs : « intoxication » de la sonde (formation de couches de sulfure ou d'oxyde sur la surface de la sonde de platine, ce qui altère la réponse électrique) et réponse instable de la sonde dans des sédiments oxiques peu équilibrés. Il a été impossible d'éliminer l'« intoxication » de la sonde par polissage ou traitement chimique à l'eau régale. Nous en concluons que les mesures des sulfures totaux, faites par la méthode standard, peuvent servir à indiquer le degré d'enrichissement organique de chaque échantillon. Par contre, la forte variabilité du potentiel rédox interdit d'utiliser cette mesure pour définir le degré d'enrichissement organique des sédiments. Le potentiel rédox pourrait toutefois servir de façon semi-quantitative pour une validation interne des concentrations de sulfures totaux quand les sédiments sont anoxiques ou hypoxiques.

## INTRODUCTION

Previously we presented a rationale for environmental monitoring of sediments to detect organic enrichment based on geochemical methods which was applicable to the salmon mariculture industry in New Brunswick (Wildish et al. 1999). The measurement of redox potentials (Eh) and total sulfides ($S^{=}$) in sediment pore water has been adopted by the N.B. provincial regulating authority for the salmon culture industry (Anon. 2001) and an operating protocol has been devised (Anon. 2002).

Following the publication of Wildish et al. (1999) we received feedback from users, or potential users, of geochemical methods for monitoring salmon culture organic enrichment effects. These included Brooks, K. (pers. comm. 2001) and Brooks and Mahnken (2003), who found Eh measurements to be highly variable. Part of the reason was thought to be that the large circular cross section (typically 6.5 mm diameter) of most commercial platinum electrodes was not suitable for sampling surface layers, especially if inserted sideways into the core tube, where Eh often rapidly changed over a few millimeters depth. Soo, A. (pers. comm. 2001) questioned the chemical conditions in which total sulfide was determined by ion analysis, in particular, whether sediment matrix effects influenced the sulfide results. Because of these and other more informal queries, we have undertaken the additional work reported here to investigate the suitability of geochemical methods for compliance monitoring purposes in the Bay of Fundy mariculture industry. The central part of this work was an inter-laboratory calibration experiment involving many of the contractors and regulatory personnel involved with the Bay of Fundy mariculture industry. Subsequent specific investigations for Eh involved varying probe filling solution concentration, the causes of variability among probes and the effect of sediment water content on the determination. In addition to the inter-calibration experiment for sulfide we compared different types of sulfide probes, different ratios of SAOB (sulfide anti-oxidant buffer) to standard $Na_2S$ solutions and the effect of seawater concentration on the determination.

As a result of the inter-laboratory calibration and further experiments reported here it became clear that redox potential could not be used as a variable to define the four stages of the organic enrichment gradient used in compliance monitoring. The reader will find the newly recommended field sampling and subsampling methods in the METHODS section. The rationale for the changes made is presented in the DISCUSSION section.

## METHODS

In the earlier publication (Wildish et al. 1999) we focused on practical sampling, but here stress two goals:

- the comparison of enriched and reference locations (see Wildish et al. 2001). The comparison method can be associated with a scientific hypothesis: the null being that the two locations are similar in geochemical characteristics and the alternative hypothesis being that they have different characteristics, with respect to organic enrichment; and
- application of geochemical methods to compliance monitoring by comparing farm sites with an empirically derived organic enrichment gradient, which is based on benthic macrofaunal characteristics.

## FIELD SAMPLING METHODS AT DEPTHS <30M

The diver should be positioned at the mid-cage point for sampling. This can be achieved by observing air bubbles from the submerged divers at the surface and guiding their movements by telephone contact. An approximate position at the time of sampling can be obtained by use of a handheld GPS or from the attending vessel. A 1- or 0.5-$m^2$ metal quadrat is laid out at the site and all replicate samples made from within it. The reference location is chosen so that it is not within the immediate zone of influence of organic enrichment, shares the same sediment and other environmental characteristics, as much as possible and is close (50-200 m) to the farm site. It too should have a 1-$m^2$ quadrat laid out, from which an equal number of replicate samples are taken.

The Hargrave corer for interfacial sediment sampling (Wildish et al. 2003) is deployed by divers. It is an open ended plexiglass box, 17.5 cm x 15 cm x 35 cm with a hinged lid (Fig. 1) and lower end which is cut diagonally with a sliding base to collect undisturbed sediment samples with the overlying water. The corer has stainless steel fittings and a large aluminum handle so that the diver can carry and position it in sediments (not shown in Fig. 1). After the diver pushes the corer into the sediment to about half to three quarters of its depth, the backplate is pushed closed and the hinged lid shut for transfer to the surface. It is stored in the upright position with the sediment surface uppermost, on a wooden stand. The volume capacity of the completely full corer shown in Fig. 1 is 6.2 L and the area sampled is 0.0263 $m^2$. The Hargrave corer can be fabricated by Plastic World and Design, Burnside, Dartmouth, Nova Scotia (Tel: 902-468-3233).

## FIELD SAMPLING METHODS AT DEPTHS >30 M

We have no further suggestions to add to the earlier report. The areas and volumes (assuming a 2 cm deep surface layer) sampled for the core tubes and Hunter-Simpson grab are:

- 50 cm long x 5 cm wide tube: Area $\pi r^2 = 3.14 \times 2.5^2$ cm $= 19.63$ cc
  Surface volume = Area x 2 cm = 39.25 cc
- Grab, 31 cm x 31 cm: Area = 961 $cm^2$
  Surface volume = Area x 2 cm = 1922 cc

## SUBSAMPLING

Subsamples are taken from the sediment surface (that is the top 0–2 cm from an intact sediment core). This is done by pushing a cut-off syringe into the sediment at an approximately $45^0$ angle, then gently withdrawing 5 cc of sediment slurry so as to exclude air spaces. Filled syringes are "capped" with a plastic lid, which fits tightly over the barrel of the syringe (to exclude air), washed, and then placed on ice in a cooler chest. These samples can be stored in a refrigerator for a maximum of 72 h before analysis (Wildish et al. 1999). We suggest taking a minimum of five replicate samples from each core/grab sample.

To prepare cut-off syringes: obtain plastic medical syringes, e.g. Becton-Dickson 5 cc (Fisher # 14-823-35). With a sharp, sturdy knife, cut off the Luer lock tip just to the zero mark on the

barrel (thus leaving the syringe barrel open ended). After washing, syringes and caps can be re-used.

## LABORATORY

The new subsampling method is designed to allow Eh and $S^{=}$ to be determined on the same replicate sample. After allowing the filled syringes to reach ambient temperature, each is extruded into a 50-cc plastic beaker (Fisher catalogue # 02-591-10A) and the temperature and Eh recorded. Push the Eh probe into the sediment for a few mm and wait until the mV reading on the meter has stabilized (Eh drift < 10 mV/min) before recording it. Once this is completed, add a 5 cc volume of SAOB to begin the sulfide determination (see below). We recommend a minimum of five replicate subsamples be analyzed for both Eh and total sulfide.

Eh is measured as in Wildish et al. (1999), except that the filling solution to be used is 4 M KCl (and not 0.2 M as stated in error in Wildish et al. 1999). Regular cleaning of the platinum probe surface is required by polishing (Anon. 1983). We have also undertaken the cleaning of a black precipitate which may form on inner surfaces of the probe. To do this, a small brush is used against the surface of the ceramic reference until clean, and then flushed with de-ionized water. The probe is re-assembled and fresh filling solution added, allowing 24 h for the probe to equilibrate. We have also used a more drastic treatment to recondition the probe (Nordstrom and Wilde 1998), which involves immersing the tip of the probe in aqua regia for <1 min, followed by flushing with deionized water. Aqua regia is carefully prepared by mixing 1 vol. concentrated nitric acid with 3 vol. concentrated hydrochloric acid in a fume hood. For short-term storage (<3 wk) redox potential probes are stored in beakers containing de-ionized water. For longer periods when not in use the filling solution is drained, the probes flushed clean with deionized water and stored dry. The results are expressed normal to the hydrogen electrode by the equation in Wildish et al. (1999). If solution temperatures are recorded, use equation (1) or (2) shown below. Instructions were given earlier for the Accumet ion meter which will not apply to other makes. Readers should follow the maker's instructions for their ion meter in setting up and making determinations.

Sulfide determinations are made as described in Wildish et al. (1999) using at least three points to establish the calibration curve from which unknown samples can be determined. We recommend the use of 0.1 M sodium sulfide as a standard for this purpose (to make a concentration range of 100, 1000, 10,000 µM). Readers with ion meters other than Accumet should follow the maker's instructions for their use. The practical lower detection limits of the Orion sulfide electrodes that we have used are ~50 µM total sulfide. If field samples <100 µM need to be accurately determined, then the results should be calibrated with $Na_2S$ standards in the 50–100 µM range.

## STATISTICAL ANALYSIS

The null hypothesis tested is generally that two independent, randomly drawn groups of samples are from the same population. Because of the known contagious distribution of geochemical variables in sediments at decimeter scales (Wildish, unpublished), non-parametric tests are recommended. We have used the non-parametric equivalent of the t-test, that is the Mann–

Whitney U-test, to determine between the null and alternative hypotheses. It is a test of the rank order between median values of farm and reference samples. The computations involved are shown in Elliot (1979) and if $n_1$ or $n_2$ is >20 the normal deviate can be used to calculate the U statistic. Where $n_1$ or $n_2$ is <20 the normal deviate cannot be used and individual $U_1$ and $U_2$ values must be calculated. Table 14 in Elliot (1979) gives tests of significance at the 5% level for sample sizes <20. The computations can be completed in MS Excel, although this software does not specifically include this test. Student t-tests were used for probe comparisons.

## RESULTS

### REDOX POTENTIAL

#### Probe filling solution (KCl) concentration

The internal filling solution for the redox potential ion probe is supplied as KCl of stated molarity, saturated with AgCl (e.g. Orion # 900011 is 4.0 M KCl). We diluted the latter solution with deionized water in the following experiments. This resulted in a precipitate which was removed by centrifuging. We tested three ion probes (DW #1, 2 and 3) all of which were Orion model 96-78 platinum redox electrodes, against freshly prepared Zobell's A and B solutions. The results are shown in Table 1. The Orion platinum redox electrode instruction manual (Anon. 1998) states that solution B should be ~66 mV greater than solution A. The mean and standard deviation, to the nearest millivolt at all molarities tested in Table 1 is: DW 1 = 66 ±4, DW 2 = 65 ±4, DW 3 = 66 ±4 mV. These values are within the specifications suggested by Orion (Anon. 1998).

Table 1. Redox potential as Eh, mV (unconverted) for the same batch of Zobell's solutions A and B. The probes are filled with different molarities of KCl. "Diff" is the difference between B and A readings. The probe time indicates the period for equilibration after changing the filling solution.

| KCl | Temp. | Probe | DW 1 | | | DW 2 | | | DW 3 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| molarity | $^0$C | time, h | A | B | Diff | A | B | Diff | A | B | Diff |
| 0.2 | 24.7 | 24 | 189 | 260 | 71 | 182 | 251 | 69 | 151 | 222 | 71 |
| 0.2 | 22.0 | 18 | 199 | 267 | 68 | 195 | 259 | 64 | 153 | 221 | 68 |
| 0.2 | 23.0 | 6 | 194 | 265 | 71 | 189 | 260 | 71 | 146 | 218 | 72 |
| 0.5 | 20.1 | 17 | 224 | 290 | 66 | 220 | 284 | 64 | 181 | 248 | 67 |
| 1.0 | 21.0 | 6 | 228 | 294 | 66 | 219 | 287 | 68 | 193 | 258 | 65 |
| 2.0 | 21.5 | 2 | 234 | 298 | 64 | 233 | 296 | 63 | 209 | 274 | 65 |
| 4.0 | 19.8 | 17 | 244 | 305 | 61 | 242 | 304 | 62 | 239 | 300 | 61 |
| 4.0 | 20.6 | 2 | 239 | 302 | 63 | 242 | 303 | 61 | 237 | 300 | 63 |
| 4.0 | 20.6 | 1 | 238 | 301 | 63 | 241 | 303 | 62 | 238 | 300 | 62 |

The values for 4.0 M KCl in Table 1 can be converted to $Eh_{NHE}$ by reference to Table 1 in Wildish et al. (1999), or for convenience we have expressed the data there as an equation:

$$Eh_{NHE}\ mV = 224 - (\text{temperature, } ^{o}C) \quad (1)$$

For other molarities based on data in Table 1 (Wildish et al. 1999) the following multiple regression equation can be used:

$$Eh_{NHE}\ mV = 285.4 - 16.23\ (\text{molarity}) - 0.825\ (\text{temperature, } ^{0}C) \quad (2)$$

**Inter-laboratory calibration experiment**

The aim was to determine the variability introduced by different ion probes and meters (operated by six groups of analysts) when measuring Eh and total sulfide in the same sediment samples. Hargrave cores, obtained by hard hat diving from the mid-point of net pens in Lime Kiln Bay on the 7th August, 2003, were taken from five different pens. Cores were also taken at a reference location ~50m away from the pens, with sampling in an area of a few square meters, to yield a total of five reference cores. Precise sampling locations were recorded with the aid of GAPS technology (McKeown et al., in prep.) and are given in Appendix 1. All 10 cores were transported to the lab for subsampling as described in the METHODS section. Subsampling was completed by others, so that each analyst was provided with five replicated, refrigerated, syringe subsamples from the farm (F) and five reference (R) subsamples. Zobell's A and B solutions were all pre-prepared as a single batch and provided to each analyst with a mercury bulb thermometer to record reaction temperatures. Six groups of analysts took part; they are not identified, but a complete list of persons involved is given alphabetically in the Acknowledgements section. Each analyst supplied his/her own ion meter and probes. These included the following meters: Orion (=ThermoOrion) SA 720, 290A+, Fisher - Accumet AP25, AP63, and 1003, and Hanna Instruments HI 9025. Redox potentials in Zobell solutions at the beginning, during and at the end of the inter-laboratory experiment for each analyst are given in Appendix 2. All of the Zobell results are within the expected range for acceptable probe responses by this test. Probes used (the figure in brackets indicates the probe in Table 2) included: Cole Parmer ORP Redox/pH electrode (1), Orion 96-78 (2), Orion 96-78 (3), Hanna HI 3230 (4), Orion 96-78 (5) and Hanna HI 3230 (6). All used 4 M KCl filling solution.

Results are shown in Table 2. The median for six probes, each with five replicates is for Farm -161 mV and for Reference 69 mV. The Mann-Whitney U-test shows that $H_0$ can be rejected ($U_1=0$, $U_2=36$, $p<0.05$) and the $H_1$ accepted, that the farm and reference locations differ in $Eh_{NHE}$. Two other pertinent results from Table 2 are that:

- probes 3 and 4 (Table 2), which were recently purchased and used for the first time during the inter-laboratory calibration experiment ("new" probes), gave significantly higher values in reference sediments than "old probes". This included all of the other probes, which had been used prior to the inter-laboratory experiment, often exhaustively, in both anoxic and oxic sediments. The differences were >200 mV ("new" probes 3 and 4 = 189±24.7 and "old" probes 1, 2, 5 and 6 = -24±49.0 mV; mean ± standard deviation); and

- the farm replicates showed less variability (S.D. ranging from 3–32 mV) than the reference replicates (S.D. from 14–71 mV).

Table 2. Inter-laboratory calibration experiment. $Eh_{NHE}$ mV from Lime Kiln Bay on 7th August, 2003, as determined by six independent analysts/probes. Five replicated determinations were made on the two, mixed farm and reference sediments taken from the surface 0–2cm depth. Sediment water content (mean ± S.D., N = 3) for farm = 70.7 ±1.97 and reference = 63.5 ± 0.75. See Appendix 2 for Zobell values recorded during this experiment.

| Farm | Probe | | | | | |
|---|---|---|---|---|---|---|
| Replicate | #1 | #2 | #3 | #4 | #5 | #6 |
| 1 | -156 | -150 | -153 | -91 | -274 | -161 |
| 2 | -153 | -176 | -161 | -99 | -236 | -196 |
| 3 | -147 | -181 | -164 | -98 | -206 | -206 |
| 4 | -154 | -170 | -165 | -97 | -203 | -196 |
| 5 | -163 | -171 | -170 | -95 | -198 | -211 |
| Mean | -155 | -170 | -163 | -96 | -223 | -194 |
| Std.Dev | 6 | 12 | 6 | 3 | 32 | 20 |
| Std.Err | 3 | 5 | 3 | 1 | 14 | 9 |
| | | | | | | |
| Reference | | | | | | |
| Replicate | | | | | | |
| 1 | 42 | 32 | 177 | 245 | -70 | 52 |
| 2 | -6 | 14 | 181 | 197 | -41 | -46 |
| 3 | -14 | 61 | 185 | 203 | -61 | -110 |
| 4 | 17 | 18 | 159 | 200 | -81 | -112 |
| 5 | -4 | 17 | 154 | 186 | -80 | -107 |
| Mean | 7 | 28 | 171 | 206 | -67 | -65 |
| Std.Dev | 23 | 20 | 14 | 23 | 16 | 71 |
| Std.Err | 10 | 9 | 6 | 10 | 7 | 32 |

To determine if the approximately 3 h that cores were in transport to the lab affected the results, Eh and temperature were measured as soon as the cores were brought on deck. This was done by inserting the probe vertically into the top few millimeters of the sediment interface. Results with two probes (both Orion 96-78), DW 2 and DW 3 (probe # 3 in the inter-calibration experiment) gave the following results as $Eh_{NHE}$ for the mean ± standard deviation: DW 2 ("old" probe which was not used in Table 3), Farm = -117± 28 ,Reference = -15 ± 11 mV; DW 3 ("new" probe as #3 in Table 3), Farm = -97 ± 38, Reference = 142 ± 22 mV (see Appendix 3). After storing for 24 h in the refrigerator subsamples (N = 5) from the intercalibration experiment were re-tested with BH 5 ("old" probe # 5 in the inter-calibration experiment) with the result: BH 5, Farm = -206 ±6, Reference = - 48 ± 5 mV (Appendix 3). Despite differences in preparation of the sediment the results are not significantly different from the mean for the same probes in Table 2, for both immediate sampling and storage experiments.

Table 3 $Eh_{NHE}$ mV at $23^0$C for Lime Kiln Bay intertidal sediments with untreated and physically cleaned probes. The mean water content of the sediment was 46.10%. N = 5. See Appendix 4 for the original data

| Treatment | Statistic | DW 2 - old | DW 3 - new | BH 5 - old |
|---|---|---|---|---|
| Untreated | Mean | 174 | 274 | 127 |
| | S.D. | 33 | 24 | 34 |
| | S.E. | 15 | 11 | 15 |
| Probe polishing | Mean | 200 | 301 | 135 |
| | S.D. | 38 | 28 | 10 |
| | S.E. | 17 | 13 | 5 |

## Causality of differences among probes in the inter-laboratory calibration experiment

We tested the working hypothesis that "old" probes had been poisoned to a variable degree by the deposition of oxides or sulfides on the platinum surfaces (see DISCUSSION for references). The poisoning effect could cause a slowing of the electromotive response and thus reduce the mV reading during the limited measurement times that were used. The tests involved using the cleaning procedures outlined in the METHODS section as well as using a different filling solution. We had two probes available from the inter-laboratory experiment: #3 (= DW 3 "new") and #5 (=BH 5 "old"). In addition, we used two "old" probes: DW 1 and DW 2 and purchased a new one (DW 4).

We obtained oxic, interface, silt/clay/sand sediments (top 2 cm scraped into a bucket) from the intertidal zone of Lime Kiln Bay on 25th August, 2003. The oxic layer above the RPD at the sampling site was deeper than 5 cm. On return to the laboratory the sediment was gently mixed to a smooth consistency and subsampled into replicated 5-cc cut-off syringes which were sealed by capping. These subsamples were refrigerated and analyzed after the syringe samples were equilibrated to lab temperature 48 h later. A test was made to determine if conventional polishing of the platinum tip removed the poisoning effect. After recording the redox potential of oxic sediments with each of the untreated probes (Table 3), each was disassembled, drained, cleaned with deionized water, and the platinum electrode surface polished before re-filling with 4 M KCl. Redox potential measurements with the cleaned probes are shown in Table 3. The results do not support the view that cleaning had any significant effect on the response of any of the 3 probes. All of the probes were checked against Zobell's A and B solution during these tests and gave potential differences, B-A, of 62-69 mV.

The "new" probe in Table 3 consistently gave significantly higher readings than the "old" probes, both before and after probe polishing and changing the filling solution.

Following communication with Orion, we tested a new filling solution. It was prepared by adding $KNO_3$ (10% weight/volume) to deionized water and then adding enough KCl to make a $10^{-3}$ M solution. Prior to adding the new filling solution in the second part of the experiment, we

also disassembled the old probes and used a fine brush to clean a black deposit from the inner probe surfaces. The results are shown in Table 4. As found earlier, the Zobell B–A differences were satisfactory with 4 M KCl as the filling solution. For oxic sediments the new probes responded with Eh values which were >100 mV higher than for "old" probes. In sulfide-amended sediments this difference disappeared as expected (due to better poising in sulfide rich sediments, see DISCUSSION). Results with the $KNO_3$/KCl filling solution show that the Zobell B–A differences are also satisfactory (63 mV), although the numbers are an average of 14 (range 2–29) mV higher. For the oxic sediment test with the new filling solution the difference between old and new probes had risen to >200 mV.

Table 4. Comparison of four Orion 96-78 probes with different filling solutions. Top half of table filling solution (KCl), bottom half with ($KNO_3$/KCl). "Old" probes reconditioned for the bottom half of the table. Units are uncorrected Eh mV at a temperature of 25$^0$C (where C = +199 mV). The standard solutions results are single readings and the sediment readings a mean of three determinations (see Appendix 5).

| | | "Old" Probes | | "New" Probes | |
|---|---|---|---|---|---|
| Solution | Treatment | DW # 1 | DW # 2 | DW # 3 | DW # 4 |
| | | FILLING SOLUTION (4M KCl) | | | |
| Zobell's A | | 204 | 222 | 214 | 231 |
| Zobell's B | | 279 | 289 | 287 | 296 |
| Orion Standard | | 211 | 208 | 210 | 214 |
| Sediment | Mixed | -322 | -348 | -208 | -239 |
| Sediment | Add $Na_2S$* | -500 | -445 | -512 | -514 |
| Zobell A | | 228 | 224 | 220 | 232 |
| Zobell B | | 292 | 286 | 290 | 296 |
| Orion Standard | | 210 | 210 | 210 | 215 |
| | | FILLING SOLUTION ($KNO_3$/KCl) | | | |
| Zobell A | | 233 | 233 | 229 | 233 |
| Zobell B | | 296 | 296 | 293 | 296 |
| Orion Standard | | 217 | 217 | 217 | 219 |
| Sediment | Mixed | -63 | -65 | 248 | 215 |
| Sediment | Add $Na_2S$* | -372 | -299 | -182 | -195 |
| Zobell A | | 227 | 230 | 226 | 229 |
| Zobell B | | 293 | 294 | 291 | 292 |
| Orion Standard | | 217 | 218 | 217 | 219 |

*10 mL 0.3 M $Na_2S.9H_20$

We also tested chemical cleaning with aqua regia (Nordstrom and Wilde 1998). The sediments used were oxic, sandy/silt particles scraped from the surface of intertidal deposits at Pottery Creek, collected just before use and well mixed with a glass spatula. The Eh readings shown in Appendix 6 were made by placing the probes in Zobell's solution A, then B, then sediment, followed by repeats in the same sequence until 10 readings of each solution and sediment had

been obtained. After each reading, the probes were rinsed with deionized water and dried with Kimwipes. All four of the probes used gave results with Zobell's solutions A and B which are within the Orion specifications (A = 234±9 mV and B = 300±9 mV), both before and after the aqua regia treatment. The old probes again gave lower readings in oxic sediment than the newer ones (Table 5) both in the "before" and "after" parts of this test. In the "before" part of the test for sediments each probe gave different readings and this persisted into the "after" part of the test. In oxic sediments probe # 1 became more negative, # 2 less negative, #3 was unchanged and # 4 became more negative after acid treatment.

Table 5. Comparison of four Orion 96–78 probes with 4 M KCl filling solution and before/after treatment for 1 min with aqua regia (1 vol conc. $HNO_3$ : 3 vol conc.HCl). Units are uncorrected Eh mV (to correct to $_{NHE}$ at $25^0$C use c = 199 mV). All readings are means corrected to the nearest mV (± standard error) of the 10 observations shown in Appendix 6.

| | "Old" Probes | | "New" Probes | |
|---|---|---|---|---|
| Solution/Treatment | DW # 1 | DW # 2 | DW # 3 | DW # 4 |
| | | BEFORE | | |
| Zobell A | 241(1.67) | 231(0.52) | 233(1.07) | 238(1.86) |
| Zobell B | 305(1.37) | 298(0.46) | 300(0.87) | 306(1.88) |
| Sediment, mixed | -35(31.05) | -257(14.13) | 235(5.27) | 171(18.15) |
| | | AFTER | | |
| Zobell A | 232(0.82) | 237(0.42) | 235(0.31) | 233(0.52) |
| Zobell B | 297(0.54) | 299(0.390 | 299(0.19) | 300(0.30) |
| Sediment, mixed | -341(9.79) | -129(12.5) | 227(7.06) | 9(21.0) |

**Effect of water content in oxic sediments**

The aim was to test the hypothesis that the lower water content of reference sediments, such as those from the Lime Kiln Bay intertidal (see also Table 2) affected Eh measurement and its variability. The probe filling solutions were 4 M KCl and these experiments were run prior to the tests shown in Tables 4 and 5. We changed the water content of the mixed sediment by adding locally available seawater (PSU = 30 ppt) in batches of 50 mL, followed by slow stirring. We tested three probes as is shown in Table 6. The raw data on which the means are calculated is shown in Appendix 4.

Again mV values measured with "new" probes were higher than for the "old" probes. T tests for difference between the means show that within each probe increasing water content had no effect on the Eh. This is shown graphically in Fig. 2 and confirms that there is little effect of water content for DW 2, BH 5 and for DW 3.

Table 6. $Eh_{NHE}$, mV at $23^0$ C for Lime Kiln Bay intertidal sediments (interfacial sample). Means are based on N = 5. Percentage water content is varied by addition of seawater to the sediment. Original data is in Appendix 4.

| | Probe | | | Percent water |
|---|---|---|---|---|
| Statistic | DW 2 old | DW 3 new | BH 5 old | content |
| Mean | 174 | 274 | 127 | 46.10 |
| SD | 33 | 24 | 34 | 0.27 |
| SE | 15 | 11 | 15 | 0.12 |
| Mean | 217 | 361 | 103 | 49.78 |
| SD | 35 | 3 | 44 | 0.28 |
| SE | 16 | 1 | 20 | 0.12 |
| Mean | 202 | 366 | 148 | 55.69 |
| SD | 36 | 5 | 61 | 0.35 |
| SE | 16 | 2 | 27 | 0.16 |
| Mean | 139 | 380 | 183 | 60.86 |
| SD | 34 | 28 | 62 | 0.06 |
| SE | 15 | 13 | 28 | 0.03 |

**Recognition of "poisoned" probes**

One way to determine the functional status of a probe platinum surface is to record potentials over time when continuously immersed in oxic seawater. Typical results of this treatment are shown in Table 7. The results suggest a probe-specific time to reach an equilibrium, with a clear influence of Zobell's solution on the observed potential. From Table 7 the two BH probes are "old" and the two DW probes are "new".

Table 7. Eh as unconverted mV in aerated seawater (PSU = 30 ‰).

| Elapsed time, h | Temp. $^0$C | BH 3 | BH 5 | DW 3 | DW 4 |
|---|---|---|---|---|---|
| 0* | 11.7 | 59 | 31 | 296 | 250 |
| 1 | 15.5 | 46 | -128 | 297 | 236 |
| 2 | 19.0 | 43 | -128 | 284 | 239 |
| 11 | 22.4 | -27 | -131 | 185 | 209 |
| 24 | 19.2 | -55 | -121 | 156 | 208 |
| 44 | 20.3 | -115 | -124 | 151 | 201 |
| 66 | 21.8 | -111 | -122 | 107 | 212 |
| 67* | 21.9 | 74 | 29 | 256 | 264 |
| 69 | 21.7 | 36 | -117 | 72 | 216 |
| 70* | 22.9 | 74 | 32 | 226 | 248 |

*All probes immersed in Zobell's solution prior to this measurement.

## TOTAL SULFIDE CONTENT OF SEDIMENT PORE WATER

### Comparison of different ion probes

Accumet ion meters (Fisher model AP25, see Anon. 1997) were used as previously (Wildish et al. 1999). The following ion probes were tested:

1. Orion #9616 combination silver/sulfide electrode with Orion # 900061 or 900062 filling solutions.
2. Orion #9416 silver/sulfide half cell and Orion # 90-02 double junction reference electrodes. The latter is filled with Orion # 900002 (AgCl) in the inner, and Orion # 900003 ($KNO_3$) in the outer chamber. This was the probe used in Table 10 probe comparison tests.
3. Orion # 9416 silver/sulfide half cell with Orion # 90-01 single junction reference electrode filled with Orion #900001 solution (contains Na, K, $NO_3$, Cl ions saturated with Ag). This probe was not used in the Table 10 calibration tests.
4. Thomas combination electrode (gel, does not require filling solution)
5. DetectIon combination electrode (gel, does not require filling solution)

Probes were compared by measuring the electrode responses in the same freshly prepared solution of $Na_2S.9H_2O$ (1000 µM). The sulfide solution was prepared with de-ionized water through which nitrogen gas in a fine stream was bubbled for 10 min (degassing). The determinations were made by mixing SAOB and standard in a 1:1 ratio. The results are shown in Fig. 3 and suggest that all four probes tested have a similar slope response to sulfide, although intercept values are different. Thus at 1000 µM total sulfide, there is a spread of ~70 mV with DetectIon showing the greatest and Orion # 9616 the least. Reproducibility of the measure seems to be higher for the liquid filled Orion probes (Table 8) and, consequently, we have not pursued the gel-filled probes further.

Table 8. mV results for three concentrations of sulfide standard. The second set of measurements was approximately 15 min after the first.

| Concentration µM | Probe | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Orion # 9616 | | Thomas | | Orion # 9416 | | DetectIon | |
| | First | Second | First | Second | First | Second | First | Second |
| 100 | 741 | 740 | 749 | 772 | 774 | 775 | 808 | 827 |
| 1000 | 774 | 773 | 789 | 807 | 808 | 807 | 843 | 858 |
| 10000 | 805 | 804 | 821 | 838 | 840 | 839 | 876 | 888 |

### Effect of Varying Ratio of SAOB: standard

All determinations of sulfide for testing the effect of varying the ratio of SAOB to standard were made with an Orion # 9616 probe. A new stock of sodium sulfide standard (1000 µM) was made in degassed de-ionized water. A determination of total sulfide was then made in the standard way

for a 1:1 volume ratio of SAOB: standard. More SAOB was then added to the beaker so that the ratios tested were 3:1, 7:1, 11:1, 15:1 and 19:1. This took approximately one-half hour, after which another 1:1 SAOB:fresh standard was measured. We have accounted for the dilution by the serial addition of SAOB. The results are shown in Fig. 4 and Appendix 7 and suggest that as more SAOB is used with a fixed volume (5 mL) of sample, more sulfide is extracted, with the process becoming asymptotic at >7:1.

**Effect of varying salinity**

We determined the effect of possible carryover of seawater in the sediment sample by replacing dilution water (deionized water) by seawater (locally available, filtered, ~30 PSU) as shown in Table 9. The results show that there is no effect of salinity on this determination

Table 9. Effect of seawater concentration at a 1:1 ratio of SAOB: standard (10,000 µM $Na_2S.9H_20$) on measurement by ion analysis of total sulfide.

| SAOB, mL | Seawater, mL | $Na_2S$ $9H_20$ (10,000 µM), mL | Deionized water, mL | Total sulphide, µM |
|---|---|---|---|---|
| 10 | 9.0 | 1 | 0.0 | 1030 |
| 10 | 7.5 | 1 | 1.5 | 1140 |
| 10 | 5.0 | 1 | 4.0 | 1220 |
| 10 | 2.5 | 1 | 6.5 | 1200 |
| 10 | 1.0 | 1 | 8.0 | 1200 |
| 10 | 0.0 | 1 | 9.0 | 1000 |

**Inter-laboratory calibration experiment**

All of the probes used in this experiment were either Orion # 9616 combination electrodes (with either filling solution A or B), or Orion # 9414 with a double junction reference electrode (Orion # 90-02 with inner filling solution of AgCl, Orion # 900002, and outer filling solution of $KNO_3$, Orion # 900003).Probe # 6 results were omitted from Table 10 below because it transpired that they were made without adding SAOB to the sediment samples.

For the remainder of the samples, N = 25, the median is 3266 for the farm and 705 µM for the reference station. A Mann-Whitney U test showed that total sulfides in farm sediments are significantly greater ($U_1 = 0$, $U_2 = 625$ $p < 0.001$) and the $H_0$ that the two sets of samples are from the same population, rejected. The overall coefficient of variation for this data is 38% for farm and 32% for reference samples.

**Checking the sulfide standard concentrations**

A potential problem with sulfide standards is that they may oxidize in air. This instability of sulfide standards is more pronounced the more dilute the concentration becomes. We

Table 10. Inter-laboratory calibration experiment. Total sulfide in sediment pore water as μM, from Lime Kiln Bay as determined by six independent analysts/probes (#6 was omitted because of a mistake during analysis). Five replicated determinations were made on farm and reference sediments.

| Farm | Probe | | | | |
|---|---|---|---|---|---|
| Replicate | #1 | #2 | #3 | #4 | #5 |
| 1 | 3000 | 3266 | 6150 | 6320 | 2950 |
| 2 | 2220 | 2504 | 6170 | 3420 | 3020 |
| 3 | 2020 | 3266 | 6180 | 3510 | 2530 |
| 4 | 3000 | 3490 | 4650 | 4110 | 2500 |
| 5 | 2180 | 2860 | 5240 | 3500 | 2440 |
| Mean | 2484 | 3077 | 5678 | 4172 | 2688 |
| SD | 477 | 393 | 701 | 1232 | 274 |
| SE | 213 | 176 | 313 | 551 | 123 |
| Reference | | | | | |
| Replicate | | | | | |
| 1 | 318 | 925 | 734 | 705 | 488 |
| 2 | 373 | 709 | 560 | 516 | 1030 |
| 3 | 568 | 621 | 754 | 713 | 773 |
| 4 | 445 | 758 | 1130 | 563 | 720 |
| 5 | 469 | 709 | 1240 | 629 | 630 |
| Mean | 435 | 744 | 884 | 625 | 728 |
| SD | 95 | 112 | 288 | 86 | 200 |
| SE | 43 | 50 | 129 | 39 | 90 |

independently check the concentration of sulfide standards by titration with Pb $(ClO_4)_2.6H_20$ (Orion 948206; Fisher # C13 641 773). The reactants are:

25 mL 0.03M $Na_2S.9H_20$
25 mL SAOB
Lead standard in the burette (Metrohm 645 Multi-Dosimat – Auto-buret)

Near the sharp end-point of the titration the lead standard is added in 0.1-mL increments. Calculations are as follows:

Volume Pb standard added = $V_{std}$ mL
Volume sulfide standard added = $V_S$ mL
Concentration of Pb standard = $C_{std}$ M

Then concentration of standard sulfide = $(V_{std}/ V_S)*C_{std}$

## DISCUSSION

### REDOX POTENTIAL MEASUREMENTS

According to Schulz (2000), unexplainable fluctuations of Eh ± 50 mV can be expected when measuring reduction potentials in sediment with platinum electrodes. Schulz's results were

obtained with well maintained and polished electrodes and use of a glove box. Thus, sediments were examined in an inert atmosphere (nitrogen or argon) free of oxygen. All of the results presented in our study were made in air without a glove box. During the inter-laboratory calibration experiment (Table 2) two probes (# 3 and # 4) consistently gave higher Eh potentials (>100 mV) in reference sediments. Both probes were recently purchased (Orion 96-78 and Hanna Instruments HI 3230). Further testing showed that "new" and "old" probes behaved differently. Cleaning "old" probes by polishing resulted in small (<100 mV) temporary increase in potential, although as soon as they were placed in sediments with a high sulfide content the potentials rapidly became negative again. Doyle (1968) considered the performance of platinum redox surfaces in iron-containing freshwaters and described the effect of oxide coatings which influenced potentials. Whitfield (1974) and Enriquez et al. (2001) mention the formation of oxides on probes in marine, oxic sediments and sulfides in anoxic conditions, which influences the performance of these probes. Thus "old" probes which have repeatedly been exposed to oxic and anoxic sediments will become "poisoned" in this way and respond more negatively than "new" ones, particularly in poorly poised sediments. During this study we sought ways to remove the poisoning effect and found that physical (polishing) and chemical (with aqua regia) treatment did not alter this effect in old probes. Internal cleaning and use of a different filling solution ($KNO_3$/KCl) also failed to rejuvenate poisoned probes. The use of standard solutions, e.g. Zobell's, failed to detect the poisoning effect because in well poised solutions (such as Zobell's and anoxic sediments where the $HS^-/SO_4^=$ couple is dominant) the response is rapid, because of the single redox couple present. In poorly poised oxic surface sediments, where macrofaunal bioturbation has carried dissolved oxygen to a few centimeters depth, the probe poisoning effect becomes noticeable. Thus standard solutions are only useful in detecting physical or electrical damage to the probe and not to probe poisoning. Probe poisoning effects could be recognized by observing potentials after immersion in aerated seawater. According to Whitfield (1974), oxide coated platinum surfaces are acting as oxide electrodes in oxic sediments and are actually measuring pH.

Another finding from the inter-laboratory calibration experiment (Table 2) was that variation within each probe replicate was greater from sediment reference samples (SD = 14-71 mV) than for farm samples (SD = 3-32 mV). This is consistent with the interpretation that oxic sediments, above the redox potential continuity, are poorly poised (Sigg 2000). Possible multiple redox potential couples are present including $O_2/H_20$, $NH_4^+NO_3^-$, $NH_4^+/N_{2\,(aq)}$, $HS^-/SO_4^=$, $Fe^{3+}/Fe^{2+}$, $CH_{4(aq)}/HCO_3$ (Sigg 2000). The oxic sediment is not at equilibrium because of the many redox couples present and electrochemical changes do not react fast enough to yield a stable redox potential endpoint. By contrast, highly anoxic sediments, that is, those below the redox potential discontinuity (RPD), where dissolved oxygen is absent (or nearly so) are better poised because of the dominance of the $HS^-/SO_4^{2-}$ couple.

Although it is true that commercially available redox potential probes cannot resolve the sometimes mm scale changes of Eh profile in surface sediments, this will usually not be a problem of such magnitude as probe poisoning. One obvious solution to this smearing effect is to physically homogenize the surface 0–2 cm sediment, as was done in the inter-calibration experiment.

## TOTAL SULFIDE MEASUREMENTS

The evidence from the inter-laboratory calibration experiment suggests that reproducibility of the method is sufficiently good for it to be used to indicate the stages of the organic enrichment gradient shown in Tables 11 and 12. The coefficient of variability for total sulfide measurements in the inter-laboratory calibration was <38%. We have also shown that some of the sediment matrix effects, e.g., by seawater carryover, do not affect the total sulfide measurement. Whether ions from a wider range of sediment types would affect this measurement remains to be determined. We have also shown previously that sediment samples in capped, cut-off syringes, as described in the METHODS section can be stored in a refrigerator for 72 h, without change of total sulfide concentration (Wildish et al. 1999, see Table 4). We have also shown that by varying the volumetric proportions of SAOB: sample, more total sulfide can be extracted from the sample. We recommend; however, that the volumetric ratio used remains at 1:1, to maintain comparability with older data.

## GEOCHEMICAL METHODS APPLIED TO COMPLIANCE MONITORING

Despite the problems identified with redox potential, both Eh and total sulfide measurements show that there are significant differences between the farm and reference sediments examined during the inter-laboratory calibration experiment. A negative Eh and high sulfide value in the farm sediment confirms that organic enrichment is present.

Compliance monitoring for the Bay of Fundy salmon culture industry (Anon. 2001; Anon. 2002) is not based on comparison of treated versus reference locations, but by comparing measurements of Eh and $S^{=}$ in sediments with ranges of values for each variable, empirically derived from observations in the Western Isles region of the Bay of Fundy (Tables 11 and 12). Individual farm sites are rated independently using Eh and total sulfide (E. Parker, pers. commun.) and the criteria listed in Table 11. In the case of a disagreement in designating the organic enrichment grouping between Eh and total sulfide, the less enriched group is the one selected.

Table 11. Correspondence between $Eh_{NHE}$ and total sulfide values for the four classically defined organic enrichment stages (Wildish et al. 2001).

| Organic enrichment stage | $Eh_{NHE}$, mV | Total Sulfide, µM |
|---|---|---|
| A Normal | >+100 | <300 |
| B+ Oxic | 0–100 | 300–1300 |
| B- Hypoxic | -100–0 | 1300–6000 |
| C Anoxic | <-100 | >6000 |

The inter-laboratory calibration results obtained are expressed as organic enrichment index groupings in Table 12. Consistency among the measured geochemical variables is greatest for total sulfide (all the same) and least for redox potential (Farm: 4 C's, 1 B-; Reference: 2 A's, 2 B's and 2 B-). It is clear from Table 12 that redox potential cannot be used as a reliable and

reproducible indicator of the organic enrichment stage of a given sediment sample, particularly in reference sediments where oxic conditions prevail. $S^{=}$ on the other hand provided a consistent measure of enrichment status. Hargrave et al. (1997) also found that $S^{=}$ was the single most sensitive variable for detecting differences between sediments at salmon farms and reference locations. Although the causes of Eh variability are reported in the literature (probe poisoning and poor probe performance in poorly poised sediments) and confirmed here, we did not find a way to overcome either problem. Consequently, we recommend that redox potential not be used as an indicator of organic enrichment stage. Redox potential measurements may still prove to be useful in well poised sediments, e.g. those where interface potentials are in the B- and C range. Eh can be used as a check on total sulfide concentration (there should be an inverse relationship between Eh on log. total sulfide). Total sulfide should be the only variable used to define the organic enrichment stage.

Table 12. Inter-laboratory calibration mean values of geochemical variables expressed as the equivalent organic enrichment group (see Tables 2, 10, and 11).

| Geochemical variable | Analyst/Probe | | | | | |
|---|---|---|---|---|---|---|
| | #1 | #2 | #3 | #4 | #5 | #6 |
| Farm | | | | | | |
| $Eh_{NHE}$ | C | C | C | B- | C | C |
| $S^{=}$,µM | B- | B- | B- | B- | B- | |
| Reference | | | | | | |
| $Eh_{NHE}$ | B+ | B+ | A | A | B- | B- |
| $S^{=}$,µM | B+ | B+ | B+ | B+ | B+ | |

## FURTHER RESEARCH

Our results for Bay of Fundy sediments show that total sulfide measurements can be used quantitatively to define organic enrichment endpoints. Additional research is needed to see whether the enrichment groups defined by $S^{=}$ measurements in the local area apply over a wider range of sediment types. In sediments from other locations, there is the possibility that other ions present might interfere with $S^{=}$ determinations made by electrochemical analysis.

Although "poisoned" redox potential probes can be recognized comparatively by immersion and monitoring their performance in aerated seawater, it is not known how to re-condition them. If the process of poisoning proves to be irreversible, a method of recognizing when the probe reaches the irreversible point would be of value in deciding when to discard it. It is clear from the literature and our own results that poorly poised, oxic sediment presents an inherent problem in compliance monitoring with redox potential measurements using ion analytical methods.

Research should also be undertaken to replace the redox potential method in defining the organic enrichment gradient, so that an independent approach is available to support total sulfide measurements in interfacial sediments. Candidate methods include; pH in interfacial sediments, sediment profile imaging (Wildish et al. 2003) and benthic macrofaunal community structure (Wildish et al. 2001).

Finally, the value of inter-laboratory experiments in detecting problems which could affect a particular methods ability to define the stages of the organic enrichment gradient are demonstrated in the work reported here. In future we recommend that all new candidate methods undergo an inter-laboratory calibration test similar to the one reported here.

## ACKNOWLEDGEMENTS

The following is a list of the analysts who took part in the intercalibration experiment of 7th August, 2003: Hugh Akagi, Marie Archambault, Mark Chenhall, Lisa Doucette, Eric Garnier, Chantale Imbeault, Denise Joy, Angela Martin, Ed Parker, Georgina Phillips and Susan Richardson. Others who helped organize the intercalibration experiment included Derek Knox and Ken McKeigan. The Huntsman Marine Science Centre provided a large laboratory where the experiment was conducted. Eric Garnier and Mark Chenall undertook the diving in Lime Kiln Bay to collect the sediment samples. Ian Stewart of Ocean Legacy provided access to a salmon farm, and Captain Wayne Miner and Danny Loveless of Pandalaus III carried divers, GAPS equipment and support party to the sampling locations. We thank all of the above for their collegial help in completing this project successfully, and Ed Parker and Joe Crocker for reviewing the manuscript.

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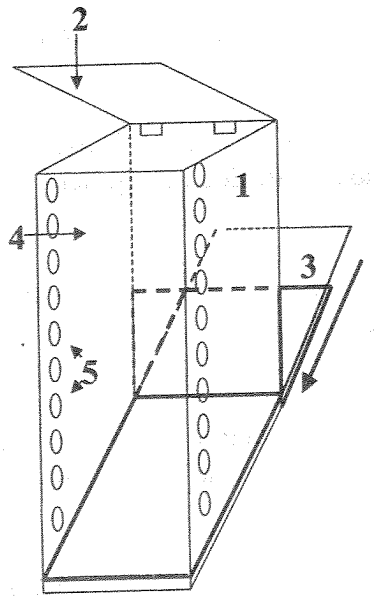


1 - open-ended plexiglass box (17.5 cm x 15 cm x 35 cm)

2 - hinged top lid

3 - bottom cut diagonally (45 °) with sliding base

4 - clear front face for photography (SPI)

5 - pre-drilled holes for Eh and sample removal

Fig. 1. Hargrave corer diagram.

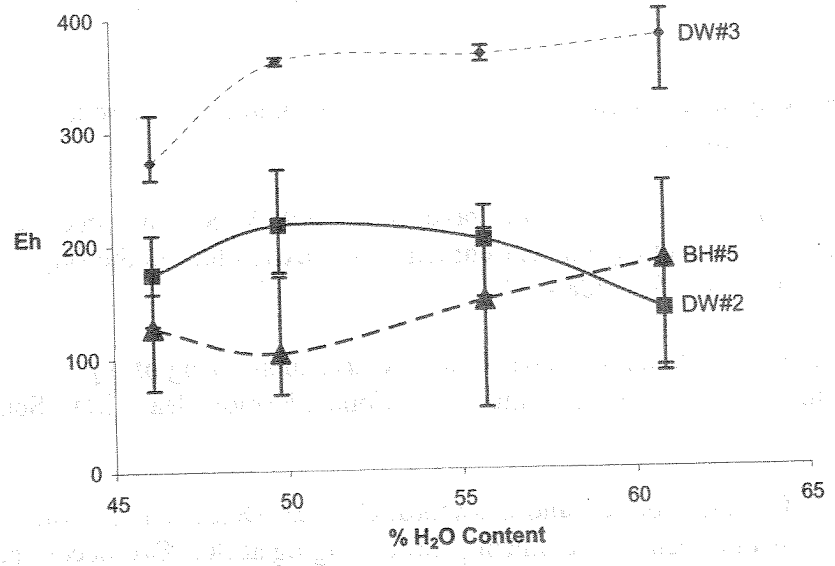


Fig. 2. Effect of sediment water content on observed Eh by three different probes.

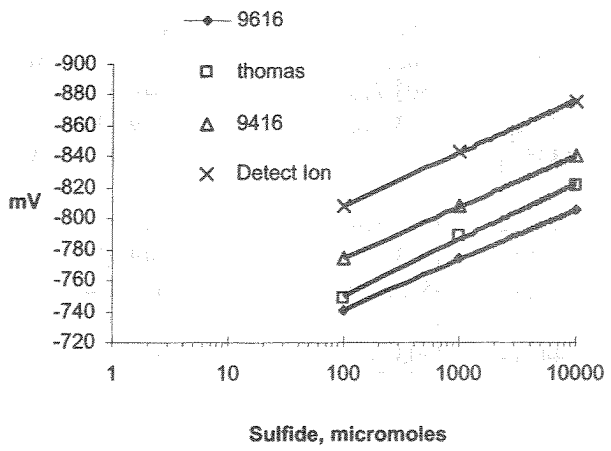


Fig. 3. Measurement of total sulfide in standard solutions by four types of sulfide probe. The volumetric ratios present were 1:1 SAOB:standard.

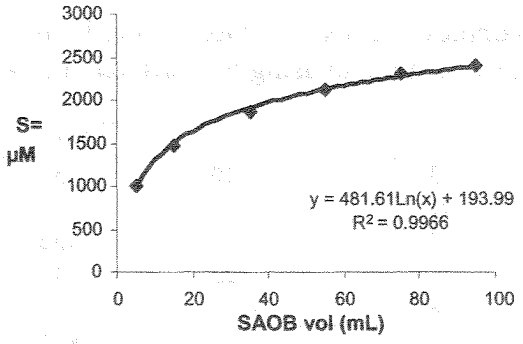


Fig. 4. The effect of the ratio of SAOB:standard.

Appendix 1. GAPS precisely determined locations sampled on 7th August, 2003.

| Location | Year | Day | Time | Lat. | | Long. | |
|---|---|---|---|---|---|---|---|
| Farm1 | 2003 | 219 | 132143 | 4503.719 | N | 6649.937 | W |
| Farm2 | 2003 | 219 | 132318 | 4503.722 | N | 6649.938 | W |
| Farm3 | 2003 | 219 | 132920 | 4503.719 | N | 6649.935 | W |
| Farm4 | 2003 | 219 | 133127 | 4503.718 | N | 6649.935 | W |
| Farm5 | 2003 | 219 | 133525 | 4503.719 | N | 6649.935 | W |
| Ref1 | 2003 | 219 | 144212 | 4503.884 | N | 6649.938 | W |
| Ref2 | 2003 | 219 | 143555 | 4503.886 | N | 6649.938 | W |
| Ref3 | 2003 | 219 | 144243 | 4503.719 | N | 6649.934 | W |
| Ref4 | 2003 | 219 | 144313 | 4503.885 | N | 6649.940 | W |
| Ref5 | 2003 | 219 | 144344 | 4503.887 | N | 6649.942 | W |

Appendix 2. Redox potential as Eh, mv, (uncorrected) for the same batch of Zobell A and B solutions. From inter-laboratory comparison of 7th August, 2003.

| Probe | | Initial | | | Second | | | Final | | |
|---|---|---|---|---|---|---|---|---|---|---|
| group | T (°c) | A | B | Diff | A | B | Diff | A | B | Diff |
| 1 | 20-25 | 247 | 309 | 62 | 238 | 302 | 64 | 231 | 296 | 65 |
| 2 | 25-26 | 230 | 296 | 66 | - | - | - | 232 | 296 | 64 |
| 3 | 16-25 | 245 | 310 | 65 | - | - | - | 230 | 295 | 65 |
| 4 | - | - | - | - | - | - | - | - | - | - |
| 5 | 20-24 | 242 | 304 | 62 | 230 | 294 | 64 | 230 | 296 | 66 |
| 6 | 24-25 | 231 | 299 | 68 | 230 | 298 | 68 | 230 | 297 | 67 |

Appendix 3. $E_{nhe}$ from Lime Kiln Bay samples (August 7th, 2003). Stored refrigerated for 24 h in a syringe (BH 5), or analyzed immediately in the field using two different probes (DW2 and DW3).

| Sample # | BH5 | DW2 | DW3 |
|---|---|---|---|
| F1 | -213 | -10 | 51 |
| F2 | -191 | -127 | -127 |
| F3 | -223 | -157 | -156 |
| F4 | -206 | -123 | -117 |
| F5 | -198 | -169 | -135 |
| Mean= | -206.2 | -117.20 | -96.80 |
| Std.Dev= | 12.52 | 63.03 | 83.86 |
| Std.Err.= | 5.60 | 28.19 | 37.50 |
| R1 | -64 | -42 | 136 |
| R2 | -36 | -34 | 104 |
| R3 | -38 | -19 | 155 |
| R4 | -56 | 21 | 218 |
| R5 | -45 | 0 | 97 |
| Mean= | -47.8 | -14.80 | 142.00 |
| Std.Dev= | 11.97 | 25.63 | 48.61 |
| Std.Err.= | 5.35 | 11.46 | 21.74 |

Appendix 4. Laboratory recorded $Eh_{NHE}$ (+199 @ 23°C) for Lime Kiln Bay intertidal sediments collected on 25th August, 2003. Measurements made on samples stored in capped syringes in a refrigerator for 48 h. The last series was completed after each of the platinum probe surfaces was polished.

| TTL seawater added (mL) | | Probe # DW#2 | DW#3 | BH#5 | Percent water content |
|---|---|---|---|---|---|
| 0 | | 129 | 315 | 125 | 46.47 |
| | | 153 | 261 | 151 | 45.90 |
| | | 183 | 260 | 157 | 46.28 |
| | | 197 | 258 | 128 | 45.81 |
| | | 209 | 274 | 72 | 46.05 |
| | Avg | 174.20 | 273.60 | 126.60 | 46.10 |
| | SD | 32.79 | 23.99 | 33.56 | 0.27 |
| | SE | 14.66 | 10.73 | 15.01 | 0.12 |
| 50 | | 199 | 363 | 67 | 50.06 |
| | | 237 | 358 | 76 | 49.98 |
| | | 175 | 359 | 77 | 49.54 |
| | | 207 | 362 | 171 | 49.43 |
| | | 266 | 365 | 126 | 49.86 |
| | Avg | 216.8 | 361.4 | 103.4 | 49.78 |
| | SD | 35.32 | 2.88 | 44.31 | 0.28 |
| | SE | 15.79 | 1.29 | 19.82 | 0.12 |
| 100 | | 224 | 373 | 164 | 56.09 |
| | | 225 | 359 | 54 | 55.66 |
| | | 232 | 366 | 125 | 55.40 |
| | | 176 | 363 | 187 | 55.98 |
| | | 151 | 368 | 211 | 55.29 |
| | Avg | 201.6 | 365.8 | 148.2 | 55.69 |
| | SD | 36.02 | 5.26 | 61.46 | 0.35 |
| | SE | 16.11 | 2.35 | 27.49 | 0.16 |
| 150 | | 135 | 331 | 174 | 60.95 |
| | | 165 | 387 | 84 | 60.81 |
| | | 130 | 390 | 209 | 60.85 |
| | | 176 | 403 | 252 | 60.81 |
| | | 89 | 391 | 195 | 60.90 |
| | Avg | 139 | 380.4 | 182.8 | 60.86 |
| | SD | 34.07 | 28.28 | 62.17 | 0.06 |
| | SE | 15.23 | 12.65 | 27.81 | 0.03 |
| 0 | | 211 | 325 | 131 | Not recorded |
| | | 151 | 331 | 136 | Not recorded |
| | | 215 | 277 | 145 | Not recorded |
| | | 174 | 306 | 120 | Not recorded |
| | | 248 | 268 | 143 | Not recorded |
| | Avg | 199.8 | 301.4 | 135 | |
| | SD | 37.84 | 28.13 | 10.07 | |
| | SE | 16.92 | 12.58 | 4.51 | |

Appendix 5. Uncorrected Eh mV for sediments comparing "old" and "new" probes in the experiments of Table 5. Reaction temperature = 25°C. Three replicated readings were made for each of the four treatments. Sediments came from the intertidal of Lime Kiln Bay.

| Treatment | "Old" probes | | "New" probes | |
|---|---|---|---|---|
| | DW # 1 | DW # 2 | DW #3 | DW #4 |
| **4 Molar KCl filling solution** | | | | |
| 40 mL sediment - well mixed | -330 | -375 | -181 | -216 |
| | -316 | -339 | -223 | -272 |
| | -321 | -330 | -221 | -229 |
| 40 mL sediment with 10 mL 0.3M $Na_2S$ added | -500 | -432 | -513 | -528 |
| | -500 | -450 | -514 | -507 |
| | -500 | -453 | -510 | -507 |
| **$KNO_3$/KCl filling solution** | | | | |
| 40 mL sediment - well mixed | -370 | -292 | -347 | -331 |
| | -367 | -294 | -340 | -314 |
| | -378 | -311 | -354 | -319 |
| 40 mL sediment with 10 mL 0.3M $Na_2S$ added | -519 | -483 | -475 | -452 |
| | -530 | -485 | -475 | -454 |
| | -521 | -486 | -467 | -448 |

Appendix 6. Eh mV unconverted for the aqua regia experiment as summarized in Table 6. A is Zobell's A, B is Zobell's B and Sed is a fresh, well-mixed sediment interfacial sample from Pottery Creek, St. Andrews. Four different probes were used as shown.

| | "Old" Probes | | | | | | "New" Probes | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | DW #1 | | | DW # 2 | | | DW #3 | | | DW #4 | | |
| Replicate | A | B | Sed | A | B | Sed | A | B | Sed | A | B | Sed |
| | BEFORE PROBE TREATMENT | | | | | | | | | | | |
| 1 | 248 | 309 | 48 | 228 | 295 | -179 | 236 | 302 | 205 | 246 | 313 | 105 |
| 2 | 244 | 309 | 58 | 232 | 298 | -240 | 235 | 302 | 242 | 242 | 310 | 117 |
| 3 | 244 | 308 | 50 | 232 | 299 | -256 | 235 | 303 | 226 | 241 | 309 | 152 |
| 4 | 243 | 307 | 8 | 232 | 298 | -266 | 234 | 302 | 241 | 240 | 308 | 141 |
| 5 | 242 | 306 | -91 | 231 | 298 | -271 | 235 | 302 | 242 | 238 | 306 | 167 |
| 6 | 240 | 304 | -59 | 231 | 298 | -285 | 230 | 298 | 241 | 235 | 302 | 198 |
| 7 | 239 | 303 | -75 | 231 | 298 | -294 | 230 | 298 | 234 | 235 | 302 | 213 |
| 8 | 237 | 302 | -61 | 231 | 298 | -258 | 231 | 299 | 235 | 236 | 303 | 213 |
| 9 | 237 | 302 | -123 | 231 | 298 | -268 | 231 | 299 | 241 | 234 | 302 | 207 |
| 10 | 237 | 301 | -106 | 231 | 298 | -249 | 231 | 299 | 243 | 233 | 301 | 198 |
| Mean | 241.1 | 305.1 | -35.1 | 231 | 297.8 | -256.6 | 232.8 | 300.4 | 235 | 238 | 305.6 | 171.1 |
| SD | 3.73 | 3.07 | 69.43 | 1.15 | 1.03 | 31.60 | 2.39 | 1.96 | 11.79 | 4.16 | 4.20 | 40.59 |
| SE | 1.67 | 1.37 | 31.05 | 0.52 | 0.46 | 14.13 | 1.07 | 0.87 | 5.27 | 1.86 | 1.88 | 18.15 |
| | AFTER AQUA REGIA PROBE TREATMENT | | | | | | | | | | | |
| 1 | 232 | 296 | -330 | 236 | 298 | -206 | 234 | 299 | 203 | 233 | 300 | 102 |
| 2 | 235 | 297 | -356 | 235 | 298 | -115 | 235 | 299 | 226 | 233 | 300 | 68 |
| 3 | 230 | 296 | -351 | 236 | 298 | -111 | 235 | 299 | 212 | 233 | 300 | 40 |
| 4 | 234 | 297 | -326 | 236 | 299 | -119 | 235 | 299 | 203 | 232 | 300 | 19 |
| 5 | 235 | 299 | -350 | 237 | 299 | -116 | 236 | 300 | 238 | 232 | 299 | -13 |
| 6 | 230 | 296 | -331 | 237 | 299 | -126 | 235 | 300 | 228 | 232 | 299 | -15 |
| 7 | 232 | 298 | -371 | 237 | 300 | -135 | 234 | 299 | 241 | 234 | 300 | -15 |
| 8 | 233 | 298 | -320 | 238 | 300 | -117 | 234 | 299 | 237 | 234 | 300 | -24 |
| 9 | 231 | 297 | -303 | 237 | 300 | -120 | 234 | 299 | 238 | 235 | 301 | -30 |
| 10 | 232 | 295 | -367 | 238 | 300 | -122 | 234 | 299 | 245 | 235 | 301 | -40 |
| Mean | 232.4 | 296.9 | -340.5 | 236.7 | 299.1 | -128.7 | 234.6 | 299.2 | 227.1 | 233.3 | 300 | 9.2 |
| SD | 1.84 | 1.20 | 21.88 | 0.95 | 0.88 | 27.96 | 0.70 | 0.42 | 15.78 | 1.16 | 0.67 | 46.95 |
| SE | 0.82 | 0.54 | 9.79 | 0.42 | 0.39 | 12.50 | 0.31 | 0.19 | 7.06 | 0.52 | 0.30 | 21.00 |

Appendix 7. Typical data for the SAOB:standard experiment as shown in Fig.4. The volume (5 mL) of standard is kept constant, SAOB volume varied. Before/after standards are with 5 mL each of SAOB:standard taken at the beginning and end of this test. The concentrations are in µM of sulfide, the "adjusted" column is adjusted for dilution involved in adding SAOB.

| | Sulfide before | | Sulfide after | |
|---|---|---|---|---|
| Standard µM | µM | mv | µM | mv |
| 10000 | 10000 | -802 | 10900 | -803 |
| 1000 | 1000 | -769 | 1010 | -772 |
| 100 | 100 | -737 | 111 | -739 |

| | Standard (5 mL), 1000 µM | | | |
|---|---|---|---|---|
| SAOB(mL) | mv | $S^{=}$ | Dilution | Adjusted |
| 5 | -769 | 1000 | 1 | 1000 |
| 15 | -766 | 735 | 2 | 1470 |
| 35 | -760 | 465 | 4 | 1860 |
| 55 | -756 | 354 | 6 | 2124 |
| 75 | -753 | 288 | 8 | 2304 |
| 95 | -750 | 240 | 10 | 2400 |