Fisheries and Oceans Canada | Pêches et Océans Canada
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Canadian Science Advisory Secretariat (CSAS)

Research Document 2025/016

National Capital Region

Storage Stability: Effect of Storage Time, Temperature and Preservation Method on Total Free Sulfide Measurements in Marine Benthic Sediment

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December 2025

Foreword

This series documents the scientific basis for the evaluation of aquatic resources and ecosystems in Canada. As such, it addresses the issues of the day in the time frames required and the documents it contains are not intended as definitive statements on the subjects addressed but rather as progress reports on ongoing investigations.

Published by:

Fisheries and Oceans Canada
Canadian Science Advisory Secretariat
200 Kent Street
Ottawa ON K1A 0E6

http://www.dfo-mpo.gc.ca/csas-sccs/
DFO.CSAS-SCAS.MPO@dfo-mpo.gc.ca

ISSN 1919-5044
ISBN 978-0-660-75876-3 Cat. No. Fs70-5/2025-016E-PDF

Correct citation for this publication:

Wong, D., and Page, F. 2025. Storage Stability: Effect of Storage Time, Temperature and Preservation Method on Total Free Sulfide Measurements in Marine Benthic Sediment. DFO Can. Sci. Advis. Sec. Res. Doc. 2025/016. iv + 43 p.

Aussi disponible en français :

Wong, D. et Page, F. 2025. Stabilité à l'entreposage : Effet du temps d'entreposage, de la température et de la méthode de conservation sur la mesure des sulfures libres totaux dans les sédiments benthiques marins. Secr. can. des avis sci. du MPO. Doc. de rech. 2025/016. iv + 46 p.

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ABSTRACT

Within Canada, the main regulatory indicator of impact to soft bottom benthos, used as a marker to gauge the oxic state of the seabed stemming from finfish aquaculture activities, is total free sulfide in sediment. Each region follows their own standard operating procedure (SOP) for monitoring this parameter, whether federally as part of the Aquaculture Activities Regulations (DFO 2018a) which British Columbia follows (DFO 2018b) or provincially, New Brunswick (NB DELG 2018) or Nova Scotia (NS DFA 2021). Determination of sulfide requires collection of sediment followed by quantification using ion selective methodology (Wong and Page, 2025). At present, depending on the jurisdiction, samples must be analysed within 5 minutes of collection in British Columbia (DFO 2018b), within 36 h as stipulated in the federal AAR (DFO 2018a), or within 72 h in New Brunswick (NB DELG 2018) and Nova Scotia (NS DFA 2021). Differences in these time frames have raised questions regarding the effect of storage on the accuracy of the generated sulfide data. The only known research conducted to try to answer this knowledge gap has been performed by Wildish et al. (1999) – sediment details not explicit in their report, and Wong (not published) whose experiments focused on muddy sediment types obtained from intertidal and subtidal sediments collected using hand scoops and surface deployed grabs respectively. Their work investigated whether sulfide could be stabilised in sediment by adding sulfide antioxidant buffer (SAOB), a solution comprising alkaline EDTA and L-ascorbic acid, to sediment samples at the start of storage. The function of SAOB is detailed in Wong and Page (2025). Wong (not published) also explored the possibility of maintaining sulfide levels by vacuum sealing sediment to remove oxygen and thus potentially reducing the oxidation of sulfide. Both of these procedures failed to maintain sulfide levels at, or near the initial determined concentration (hereafter referred to as ‘baseline’ in this report) during the investigated storage periods. Storage of sediment without any preservation methods also did not maintain sulfide levels. Data derived from experiments conducted by Wildish et al. (1999) and Wong (not published) indicated that sulfide’s degradation response is highly variable and unpredictable, and is most likely dependent on substrate type, and environmental/aquaculture setting (e.g., degree of organic loading, oxic/anoxic). Therefore to derive the most accurate sulfide concentration for regulatory purposes, it is recommended that sediment samples should be analysed for total free sulfide immediately after collection and not stored.

INTRODUCTION

Aquaculture in Canada is regulated under a number of acts, legislations, regulations and programs that are administered by various federal, provincial and territorial bodies. Fisheries and Oceans Canada (DFO) is responsible for the federal regulation and management of aquaculture throughout Canada under the Federal Aquaculture Activities Regulations (AAR; DFO 2018a). These regulations define the conditions under which an aquaculture operator may deposit organic material and are regulations under section 36 and section 35 of the Federal Fisheries Act.

DFO's regulatory mandate recognises that there are interactions between aquaculture operations and the natural environment. The risks associated with these interactions are considered and addressed through a suite of regulatory tools. An example of which is the requirement for aquaculture operation owners, or operators to monitor the oxic state of the seafloor if the aquaculture facility is located over soft bottom substrates.

Under the AAR (DFO 2018a), the oxic state is classified by measuring the concentration of total free sulfide in the upper 2 cm layer from collected benthic sediment samples. The protocols for conducting the sampling and for measurement of sulfide from sediment samples are outlined in the regulations and associated monitoring standards documents of British Columbia (DFO 2018b), New Brunswick (NB DELG 2018), and Nova Scotia (NS DFA 2021). The observed states are then compared to a set of oxic thresholds associated with predefined management actions.

The method required by DFO for determining the total free sulfide concentration in sediments is often referred to as the ion selective electrode (ISE) method for sulfide determination. The method was initially recommended by Wildish et al. (1999) as a method to detect organic enrichment resulting from particulate waste from salmon aquaculture activities, and subsequently suggested as a more cost effective method for this type of monitoring (Wildish et al. 2001). It was thereafter refined, described more fully, and tailored for management use by Wildish et al. (2004).

The Wildish et al. (1999) method is referred to in Annex 8 of the AAR (DFO 2018b) which British Columbia follows, and in the standard operating procedures (SOP) of New Brunswick (NB DELG 2018), and Nova Scotia (NS DFA 2021). A detailed discussion of the ISE methodology is presented in Wong and Page (2025).

Based on the regulations and monitoring documents, the time frame from collection of sediment samples to their analysis by the ISE method differs between regions. The AAR allows up to 36 hours, except in British Columbia, where samples must be analysed within 5 min of collection (DFO 2018a). However, in New Brunswick (NB DELG 2018) and Nova Scotia (NS DFA 2021) sample analysis can be conducted up to 72 h post collection.

The Aquaculture Management Directorate (AMD) within DFO has requested science advice specific to several questions pertaining to the ISE method used to quantify total free sulfide in benthic sediment, and also the stability of total free sulfide in said samples. This parameter is used in Canada as a regulatory indicator of soft bottom oxic state arising from organic enrichment due to finfish aquaculture activities and provides a statistical relationship for infauna/epifauna bio-diversity. These questions are also a result of management's recognition of inconsistencies with the method, and some practical implementation concerns raised by the private sector. Answering these questions should pave the way for the development of a nationally harmonised and robust approach for the testing of total free sulfide in benthic sediment.

This paper focuses on the questions of whether storage of collected sediment samples prior to analysis by the ISE method impacts the accuracy of the generated data compared to analysis immediately after collection, and whether preservation and/or alternate storage methods can help stabilize total free sulfide prior to analysis.

Questions from this CSAS meeting's Terms of Reference:

1. What are the effects of sediment sample storage time and conditions (i.e. temperature, vacuum-sealed, etc.) on the measurement of total free sulfide as compared to total free sulfide measured immediately upon sample collection?
2. Are these relationships consistent across sediment types and/or total free sulfide concentrations?
3. Is there a combination of storage conditions and storage time post collection that would result in expected total free sulfide measurements within +/- 5%, 10% and 15% of the value obtained from measuring total free sulfide immediately following sediment sample collection?
4. Are there steps in the ISE total free sulfide measurement protocol that are open to interpretation by the analyst and to which differences will result in different measured concentrations of total free sulfides?
5. Review ISE total free sulfide measurement methodologies and develop standard procedures for sample storage time, storage conditions, and analyses.
6. Characterize the natural spatial variability of total free sulfide concentrations in sediment.

This paper addresses questions 1, 2 and 3 of the above questions.

STORAGE STABILITY

REGULATORS

The main question asked as part of this CSAS process is whether total free sulfide is stable in sediment when stored prior to analysis. This is most relevant to New Brunswick and Nova Scotia where SOPs, NB DELG (2018) and NS DFA (2021) respectively, allow analysis of sediment samples within 72 hours of their collection. The AAR stipulates that samples must be analysed within 36 hours after collection for provinces other than British Columbia (BC) which follows Annex 8 of the AAR (DFO 2018b), which stipulates that samples in that province must be analysed within 5 minutes of collection. Therefore, the question of sulfide stability is less of an issue in BC.

There has been inconsistency in the literature pertaining to statements of when sediment samples should be analyzed relative to the time of collection. Wildish et al. (1999, p. 5) stated that the preferred approach is to make the sulfide measurements as soon as possible after the sediment sample has been recovered from the seabed and appears on the deck of the sampling vessel. They also indicate that their research gave suggestions that the sulfide concentrations changed considerably within the first 3 h after recovery and continued to change for some time thereafter. This information seemed to contribute to a consultant's suggestion, in a report providing a suggested environmental monitoring program for the New Brunswick finfish aquaculture industry, that sulfide measurements be taken within 3 h of sample retrieval (CoastalSmith Inc. 2002). Neither of these perspectives were adopted in the first New Brunswick SOP for environmental monitoring of finfish net pen operations since the first SOPs indicated measurements were to be made within 72 h (NB DELG 2004); this perspective has persisted through to the most recent version available at the time of this writing (NB DELG 2018). It should also be noted that the Wildish et al. (1999) report indicated that for storage >3 h, sulfide measurements appeared to stabilise, although at a value considerably different from the 0 h measurement. We are not aware of any documentation that discusses the actual reasoning for the differences between the Wildish et al. (1999) findings, the initial consultant's suggestions, and what was actually incorporated into regulations.

PREVIOUS RESEARCH

There is limited published research investigating the stability of total free sulfide in the porewater fraction of sediment during storage, therefore it is recognised that there is a significant knowledge gap in this area. However, Jørgensen et al. (2019) did indicate that significant fractions of sulfide in marine sediments are reoxidised via numerous biological and geochemical pathways. The magnitude of this reoxidation is dependent on the type and quantity of oxidant and the complement and abundance of microorganisms present.

We are only aware of three reports that allow an estimate of bias to be determined for the ISE sulfide method. The first report examined how sediment stored with and without sulfide antioxidant buffer (SAOB) affected the stability of total free sulfide (Wildish et al. 1999). The second one investigated how pure solutions of sulfide (no sediment present) and storage of the electrodes used to measure total free sulfide concentration biases measurements (Chang et al. 2014). A third source of information is the previously unpublished information provided below.

Storage of Sediment Samples With, and Without SAOB

The data presented in Wildish et al. (1999) for sediments stored with, and without SAOB indicated that concentrations of total free sulfide determined over the storage periods did not remain at, or near, the initial concentration (hereafter referred to as 'baseline' in this report) of the respective investigations over the investigated time periods. This suggested that total free sulfide was not stable under the storage conditions and temperatures used in their experiments (on ice in a chest freezer - temperature not reported, or in a refrigerator at 5°C). Sediment samples stored without SAOB exhibited degradation of sulfide over the storage period (Figures 1a and 1b within this paper). Sediment stored in the presence of SAOB on the other hand showed positive bias from the initial measured concentrations indicating that sulfide increased during storage (Figures 1c and 1d within this paper). The hourly rates of change ($\mu M\ S^{2-} \cdot h^{-1}$) of total free sulfide during these two storage experiments were highest within the first few hours as indicated by change between the first two storage time points i.e., 0 h and the first subsequent analysis timepoint after that. This suggests that in the sediments stored with SAOB, dissolution of bound sulfides may be occurring resulting in elevated levels of total free sulfide (Figure 1e). This interpretation is consistent with the findings of Brown et al. (2011) where their laboratory experiments, using model sediment, determined elevated levels of sulfide in sediments buffered to pH 10 and 14 compared to the controls.

For the sediment samples stored in the absence of SAOB, the hourly rate of change was very consistent during the storage period (Figure 1f). Based on these results, Wildish et al. (1999) recommended that sediments should be stored without the addition of SAOB. Interestingly, in a paper published a decade later, Lutz et al. (2008) also recommended that SAOB not be added prior to sediment storage, but they also stated that for porewater, SAOB should be added and samples analysed within 24 h.

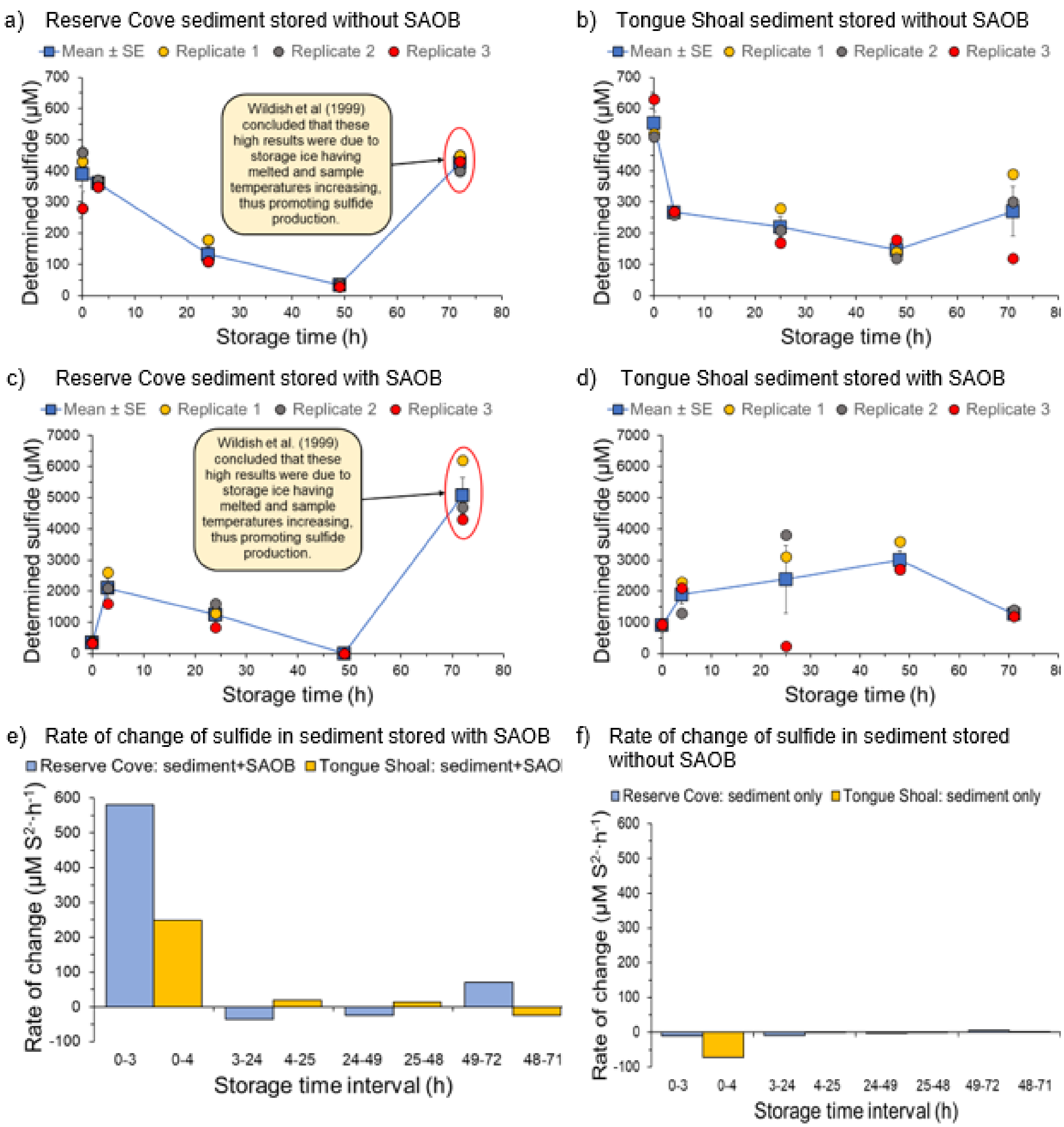


Figure 1. Stability of total free sulfide over 72 h in two sediments collected from southwest New Brunswick. Sediments were stored either (a) on ice in a freezer chest, or (b) in a fridge at 5°C. The same sediments were stored in the presence of SAOB (c) on ice in a freezer chest, or (d) in a fridge at 5°C. The rate of change of total free sulfide per hour (µM $S^{2-}\cdot h^{-1}$) in each sample is presented in graphs (e) and (f). Graphs reproduced from data reported in Wildish et al. (1999).

Storage of Standard Solutions

Chang et al. (2014) performed an interlaboratory experiment to determine the stability of sulfide over a range of concentrations within and above the calibration range of the sulfide ISE method, 100 to 10 000 µM and 20 000 to 30 000 µM respectively. Solutions were prepared using deoxygenated deionised water which reflects the matrix used for preparation of calibration standards and eliminated any possible matrix effects associated with sediment. This data indicate a consistent negative bias from the nominal concentrations (i.e., measurements underestimated the true value), the magnitude of the bias increased with storage time but decreased with sulfide solution concentration, and finally the magnitude of the bias varied between analytical laboratories (Figure 2). The relative bias for storage times of between 36 and 72 hours ranged from a few percent to over 60%.

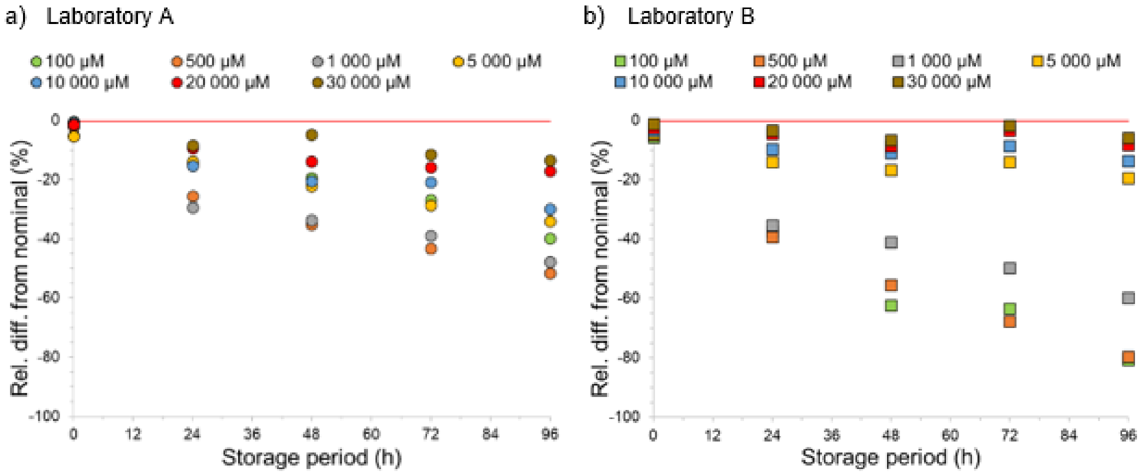


Figure 2. Interlaboratory comparison of sulfide stability in standard solutions prepared using deoxygenated deionised water and stored at ca. 4°C for 96 h.

No further research could be found which tested the stability of sulfide over prolonged storage followed by quantification of the sulfide anion (S^{2-}) using ISE. Numerous studies have however investigated the oxidation of sulfide in the form of hydrogen sulfide in fresh water and seawater under various conditions, although these studies focused on very low concentrations with spectroscopic determination by the methylene blue method (Cline 1969). Cline and Richards (1969) conducted experiments at constant salinity, pH, and temperature (30.27 ‰, pH 7.5 to 7.8, and 9.8 ± 0.5°C) using seawater collected from Lake Nitinat, BC. They determined that sulfide followed a logarithmic decay from ca. 62 µM to ca. 2 µM within 70 h and gave the following predominant reaction products, sulfate being the most abundant followed by thiosulfate and sulfite.

1. $\mathrm{HS^- + 2O_2 \rightarrow 2SO_4^{2-}\ (sulfate) + H^+}$
2. $\mathrm{2HS^- + 2O_2 \rightarrow S_2O_3^{2-}\ (thiosulfate) + H_2O}$
3. $\mathrm{2HS^- + 3O_2 \rightarrow 2SO_3^{2-}\ (sulfite) + 2H^+}$

Millero et al. (1987) conducted similar experiments to Cline and Richards (1969) but instead determined the rate of sulfide oxidation as a function of temperature, pH, and ionic strength. Their experiments used seawater with a constant salinity (35 ‰) buffered to pH 8 at 25°C which gave a half-life of 26 h ± 9 h for sulfide. Other studies reported the half-life of H_2S in seawater from 3.3 min to as long as 60 to 70 h (Siang et al. 2017). This suggests that the oxidation of sulfide (in seawater at least) is not a constant, but varies due to many factors including dissolved oxygen concentration, pH, and temperature. Sulfide degradation in sediment would be further complicated by other factors such as benthic microorganisms, organic matter, trace metals, etc.

Research regarding the effect of temperature on sulfide production in sediment was again very limited. However, one study by Nedwell and Floodgate (1972) incubated marine sediment samples in anaerobic conditions at 5, 10, 20, and 30°C. They determined sulfide production increased rapidly from 5 to 30°C with the optimal temperature for its production at 20°C. At 5°C, the rate of production was negligible with only around 10% of the total sulfate-reducing bacteria capable of reducing sulfate at that temperature.

NEW RESEARCH

Effect of Adding SAOB Prior to Sediment Storage

In an effort to further explore the effect of adding SAOB to sediments prior to storage, a soak time experiment was conducted by Wong (not published) using sediment sourced from an inter-tidal zone upriver from the mouth of the St Croix river in Oak Bay, NB and Shelburne, NS (Figure 3). See Appendix 1 for collection data, trace metals, and grain size analyses for the sediments used.

These experiments added SAOB to the sediment and used the ISE method to measure total free sulfide concentrations at two minute intervals for a total of 31 minutes post addition of SAOB (Appendix 5). The sample was left unstirred with the ISE probe in contact with the supernatant layer (above the sediment fraction) for the full 31 minutes. The results showed that levels of total free sulfide increased in the Shelburne sediment but decreased in the Oak Bay sediment (Figure 4). It is unclear whether the rise in concentration in the Shelburne sediment was due to solubilisation of bound sulfides in the high pH environment as determined by Brown et al. (2011), or whether the ISE was taking a long time to stabilise due to potential sediment matrix effects since this phenomenon was not evident in the Oak Bay sample. This occurrence of increasing levels of total free sulfide past the usual 2 minute recording window for ISE measurements has been commonly observed by one of us (Wong) in other studies involving low sulfide concentration samples. The New Brunswick sediment demonstrated stable total free sulfide concentrations after about five minutes post SAOB addition but then started to decrease steadily. It is unclear why this decrease was occurring since the Shelburne sediment maintained constant levels without any apparent degradation once concentrations had plateaued. These contrasting results suggest factors other than time post SAOB addition may be driving changes in sediment sulfide. Although this was a limited test using only two sediment types, the results support the previously reported recommendations that SAOB should not be added prior to sediment storage and it also highlights the importance of taking ISE measurements immediately after SAOB addition. Further research will be required to better understand the variability of results associated with this type of experiment and to identify the factors associated with the variability. However, this effect could best be avoided by not adding SAOB to sediment samples until measurements are about to be taken.

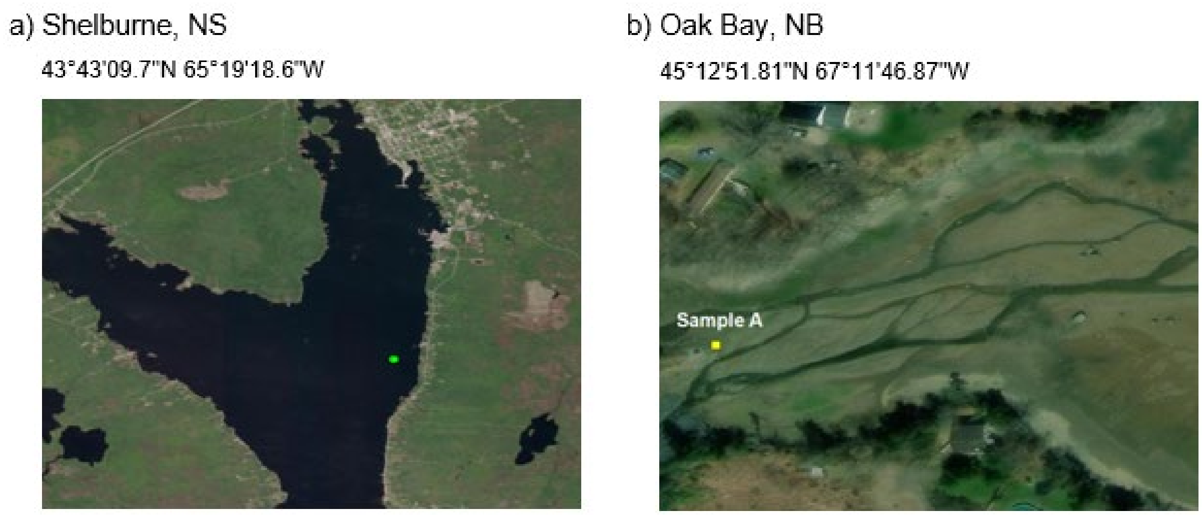


Figure 3. Locations of sediment sampling for SAOB soak time experiments.

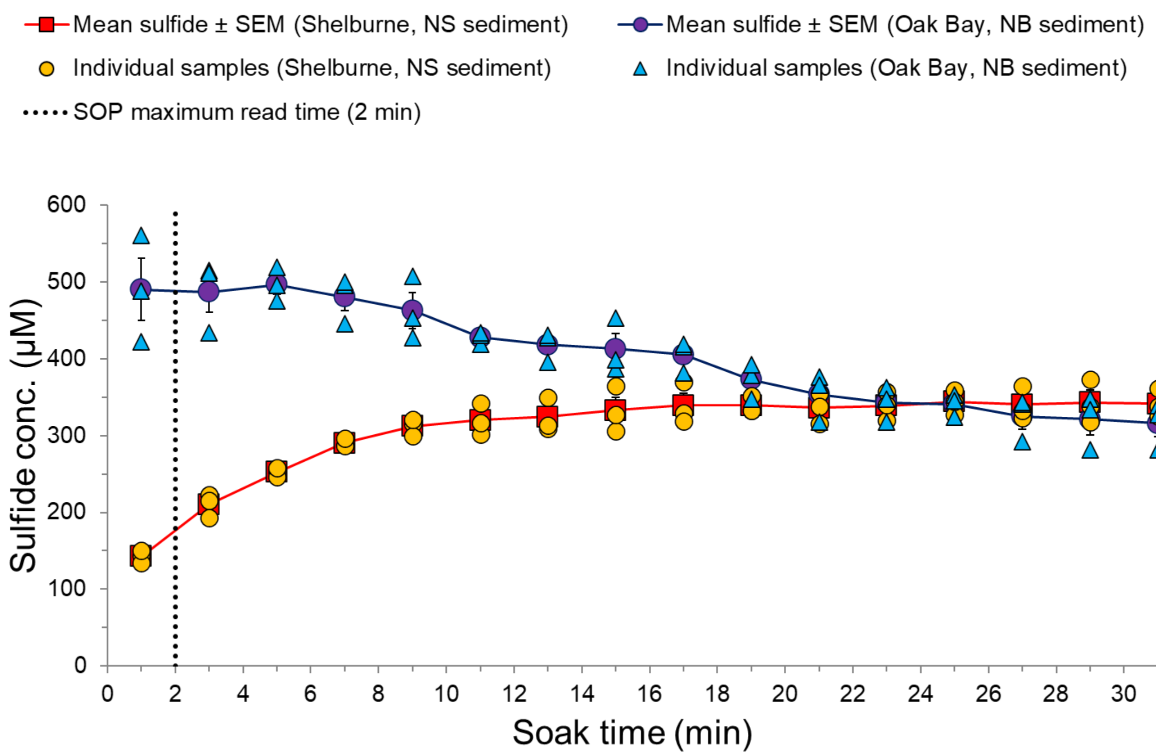


Figure 4. Soak time experiment to determine the effect of prolonged exposure of SAOB to total free sulfide in sediment

Sediment Storage Stability Experiments Without Adding SAOB prior to Storage

Experimental Protocols

To assess sulfide stability up to and beyond the 72 h limit which encompasses the storage limits as defined by NB DELG (2018) and NS DFA (2021), and which exceeds the 36 h limit set out by the AAR (DFO 2018a), Wong conducted a series of controlled experiments in which bulk samples of sediment were collected from two southwestern New Brunswick locations. One location was in an intertidal zone in Oak Bay which is located upriver from the mouth of the St Croix river (Figure 5a). It is recognised that the 35 day interval from collection to analysis of the Oak Bay sediments is quite long, unfortunately this was the first opportunity available to conduct experiments using this sediment. The other locations were in Lime Kiln Bay at an aquaculture site actively growing Atlantic salmon (*Salmo salar*) at the time of sampling. Sampling locations at the cage site were typically at cage edge with one exception which was just outside the lease boundary (Figure 5b). The sediment from these locations was fine grained and showed visual signs of low oxygen and olfactory smells of sulfide. See Appendix 1 for collection data, trace metals, and grain size analyses for the sediments used.

a) Oak Bay, NB

<u>Experiment 1</u>
Sample 1; 45°12'51.81"N 67°11' 46.87"W (35 day)
Sample 2; 45°12'52.37"N 67°11'41.50"W (35 day)

b) Lime Kiln Bay, NB

<u>Experiment 2</u>
Cage 5; 45°03'41.2"N 66°49'58.7"W (24 h)

<u>Experiment 3</u>
Cage 5; 45°03'40.8"N 66°49'58.9"W (3 h)
Cage 9; 45°03'36.5"N 66°49'58.2"W (3 h)

<u>Experiment 4</u>
Cage 5; 45°03'41.2"N 66°49'58.3"W (5 min)
Cage 9; 45°03'36.3"N 66°49'59.7"W (5 min)

Figure 5. Sediment sampling locations for storage stability experiments 1, 2, 3, and 4.

Experiments 1, 2, and 3

The sediment samples from Oak Bay were fine grained, muddy sediments and are referred to as experiment 1. The sediments from Lime Kiln Bay were also fine grained, muddy sediments and are referred to as experiments 2 and 3 for sediments processed 24 h, and 3 h respectively after collection. All samples were collected into plastic buckets with lids and transported to the St Andrews Biological Station (SABS). These were stored at ca. +4°C until processed for storage stability determination; the temperatures during transport and subsampling (described below) were not monitored during these periods. For the storage stability experiments, the bulk samples were thoroughly mixed using a paint stirrer attached to an electric drill to minimise spatial variation when collecting the sub-samples. It is recognised that mixing the sediment in such a way can introduce additional oxygen into the sample which could result in oxidation of in situ sulfide above that of undisturbed sediment. However, to minimise variation in obtained analytical results due to the patchiness of sulfide in the environment, it was decided that mixing was the most appropriate method to employ. The bulk sediments had an overlying layer of water when received, therefore it was not possible to ascertain whether the sediments had dewatered or separated from the interstitial porewater when it came to using them in these experiments.

After homogenisation, replicate sub-samples were taken from the bulk sediment using plastic 5 mL syringes cut-off at the ‘zero’ mark, so when full of sediment no headspace (air) existed. The filled syringes were then capped to prevent oxygen ingression. Baseline total free sulfide concentrations were determined in triplicate samples using the method detailed in Wong and Page (2025). The remaining syringes were placed in Ziploc bags and stored at ca. +4°C in a refrigerator until analysed in triplicate at 3 h, 6 h, 1, 2, 3, 4, 5 and 6 days post start of storage. The storage temperatures were monitored using RBR*solo* temperature loggers. In all cases SAOB was added immediately prior to sample analyses by ISE, i.e., sediments were not stored with SAOB present.

Experiment 4

For experiment 4, bulk samples were collected from Lime Kiln Bay; one sample was collected at the edge of cage 5, and the second one was collected away from cages at a location just outside the farm lease boundary in the vicinity of cage 9 (referred to as the cage 9 sample). The cage 5 sample was expected to have a high sulfide concentration and the cage 9 sample a low sulfide concentration. All bulk sediment samples were collected using a surface deployed Ponar grab then transferred to separate plastic buckets for processing using the same procedures as described for experiment 1 to 3. Sub-aliquoted sediment in syringes were placed in a cooler filled with ice for storage with the temperature monitored using an RBR*solo* temperature logger.

To obtain the most accurate in-situ concentration of total free sulfide, processing of collected sediments and early timepoint ISE analyses were conducted onboard the CCGS Viola Davidson. Analysis procedures onboard ship encompassed preparation of the sulfide stock standard, titration of the prepared stock, preparation of calibration standards, calibration of the ISE, and analysis of processed sediment samples at baseline and 1 h timepoints (n=3 per timepoint) using methodology by Wong and Page (2025). The 1 h timepoint, as opposed to 3 h which was used in experiments 1 to 3 was selected due to time constraints on the boat. The ISE was not calibrated for analysis of the 1 h timepoint samples since this time period was within the recommended 2 h operational period recommended by the manufacturer.

Once onshore, the syringed samples were transferred to refrigerated storage at ca. +4°C with temperature monitoring conducted using the same RBR*solo* temperature logger as used in the cooler. Triplicate samples were analysed at 5 h, 1, 2, 3, 4, 5 and 6 day post start of storage for total free sulfide concentrations using methodology detailed in Wong and Page (2025). As with experiments 1 to 3, SAOB was added to the selected samples immediately prior to ISE analysis.

Results

The sediments examined in these experiments ranged in oxic classification from Oxic A to Anoxic (Chang et al. 2011).

Storage temperatures experienced during each experiment were fairly constant but exhibited regular fluctuations (range -4.2 to 5.6°C) which was attributed to the refrigerator thermostat cycling on and off to maintain the set temperature (Figure 6). The Lime Kiln samples (cage 5 and 9, 5 min) which were stored in a cooler containing ice during the ship board phase experienced high temperatures (6.6 to 19.1°C) prior to refrigerator storage onshore (Figures 6a and 6b).

Initial observations from these experiments showed that the sediments from cage 5 and 9 produced very different degradation profiles to each other over the 6 days of storage, even though they were sampled spatially quite close to each other (Figures 6a, 6c, 6e – cage 5; 6b, 6d – cage 9). All sediments, with the exception of the Lime Kiln cage 5 (3 h) sample (Figure 6c) exhibited a consistent negative bias from the baseline concentrations typically from the first analytical timepoint which generally, but not in all cases, increased over the storage period. A continuous loss over the storage period was not observed, but rather total free sulfide concentrations reached a minimum level then plateaued. The exception to these results was the Lime Kiln cage 5 (3 h) sample which presented a positive bias from baseline which increased significantly until the end of storage (Figure 6c). A composite graph normalised to the respective baseline concentrations showing the trends for each experiment is presented in Appendix 2. Determined total free sulfide concentrations from the investigated samples are presented in Appendix 6.

When the data were plotted as relative difference from baseline (%) with boundaries of ±5%, ±10%, and ±15% (as per CSAS Terms of Reference question 3) then overlaid according to the sampling location, i.e. Lime Kiln cage 5, cage 9 and Oak Bay, a better picture of the similarities within and differences between sampling locations was obtained (Figure 7). Relative differences were calculated as:

$$\text{Rel. diff. (\%)} = \left[\frac{\left([S^{2-}]_x - [S^{2-}]_0\right)}{[S^{2-}]_0}\right] \times 100$$

Where $[S^{2-}]_x$ is the concentration of total free sulfide at timepoint x, and $[S^{2-}]_0$ is the baseline concentration of total free sulfide.

Sediments acquired from Lime Kiln Bay, cage 5 contained similar baseline concentrations of total free sulfide. They showed a stable period up to day 2 post start of storage with total free sulfide concentrations falling consistently within -15% of baseline. Some values did fall within ±5% and ±10% but not consistently due to fluctuations within the data. From day 3, the 5 min sample was still within the ±15% limit, but the 3 h and 24 h samples had exceeded it, +40.0% and -23.1% respectively. From day 4 onwards, total free sulfide concentrations in all the samples had exceeded the ±15% boundary with final values of -30.7% (5 min), +51.2% (3 h) and -26.6% (24 h). The 5 min and 24 h samples followed the expected trend of total free sulfide loss during storage whereas the 3 h sample contradicted this. This was assumed to be due to the anoxic nature of the sediment (baseline total free sulfide concentration = 6 443 µM) which may have resulted in sulfide generation exceeding its degradation (Figure 7a).

Sediments from Lime Kiln Bay, cage 9 differed in baseline total free sulfide concentrations from each other, they also responded differently to those collected from around cage 5. Total free sulfide concentrations declined within the early timepoints without any indication of a stable period. By 6 h, this had declined below -15% of baseline in the 3 h sample and by day 1 both were outside this limit where they stayed until day 3 when total free sulfide increased back up to within -15%. Up to this point the two profiles mirrored each other, however after this point they diverged with total free sulfide in the 3 h sample remaining similar to the day 3 level up to day 6. In contrast, total free sulfide in the 5 min sample decreased again to below -15% of baseline until day 6 (Figure 7b).

The Oak Bay samples (Figures 6f and 6g) also contained different concentrations of sulfide to each other although the difference was less than the Lime Kiln cage 9 samples. The degradation profiles for these two samples, unlike the Lime Kiln samples did not show any similarities. Total free sulfide in the lower concentration sample (sample 1) decreased below -15% of baseline within the first analytical timepoint then increased and remained within ±10% of baseline from day 1 to 6. The higher concentration sample (sample 2) exhibited a gradual decline from the first timepoint but was still within -15% of baseline by day 2. From this point on, sulfide was outside this limit but did stabilise up to day 6 (Figure 7c).

Two of the analysed sediment samples, Lime Kiln, cage 9 (5 min), and Oak Bay – sample 1 (35 d) contained sulfide near the limit of quantification (LOQ) of the ISE method, 100 µM. Sulfide levels over the course of the storage period did not fall below the LOQ, therefore quantification of the analyte in these samples was not compromised in any way.

The rate of change of total free sulfide per hour was calculated using the following equation:

$$\text{Rate of change of } S^{2-} \text{per hour } (\mu M\ S^{2-}\cdot h^{-1}) = \frac{([S^{2-}]_b - [S^{2-}]_a)}{(T_b - T_a)}$$

Where $[S^{2-}]_a$ is the concentration of total free sulfide at timepoint 1 (e.g. 0 h), $[S^{2-}]_b$ is the concentration of total free sulfide at timepoint 2 (e.g. 3 h), and $(T_b - T_a)$ is the time difference, in hours, between the 2 timepoints (e.g. 3 h).

When the rate of change of total free sulfide per hour was calculated for each experiment, results showed that total free sulfide changed the most during the first 2 analytical timepoints (Figure 8). The highest changes were seen in the Lime Kiln samples (both cage 5 and 9). The Oak Bay samples demonstrated very little hourly change in both samples. It is unclear why the highest rates of change occurred within 5 to 6 hours post start of storage. One theory is that the sediment samples experienced elevated temperatures during processing which may have increased total free sulfide degradation. Rates would then decrease once samples were cooled when placed into ca*.* +4°C storage.

Based on the data presented from these experiments, it can be assumed that the rate and pattern of total free sulfide degradation in sediments is unpredictable and predominantly dependent on sampling locations and/or sediment type. Predicting if total free sulfide will fall within ±5%, ±10% or ±15% of baseline for a particular sediment may not be possible. Other factors which would affect the accuracy of results falling within set limits is the variability associated with the analytical method used to determine total free sulfide, this is discussed further in Wong and Page (2025) as part of this CSAS process.

These stability experiments were conducted in soft muddy sediments containing high organic content. Experiments were not conducted using sandy sediment since this matrix is considered not to typically contain appreciable concentrations of total free sulfide due to its nature. Fedorov et al. (2019) stated that low sulfide concentrations are usually found in sandy sediments since these substrates are normally located in hydrodynamic areas which facilitates the removal of organic matter thus resulting in reduced anaerobic bacterial production. Martinez-Garcia et al. (2015) conducted organic enrichment experiments and determined that sulfide production was always higher in muddy than sandy sediments when low and high organic enrichment took place. Program specific analysis performed by Wong also demonstrated that sandy sediments exhibited total free sulfide concentrations <750 µM, equivalent to an Oxic A environmental rating (Chang et al. 2013), compared to muddy sediments. Lin et al. (2022) determined through their experiments using the sulfate reducing bacteria *Desulfovibrio bizertensis* that sulfide generation increased as the ratio of kaolinite to calcite increased, and that bacteria favoured clay dominated substrates due to the higher surface area and buffering capacity. Sandy sediments typically contain large grain sizes, have low surface area and low porosity. Muddy sediments on the other hand comprise small grain sizes, high surface area and increased porosity, they are also generally cohesive and impermeable thus allowing porewater to be retained during sampling, Anschutz and Charbonnier (2020).

Statistical analyses of the data generated from each of the experiments was tested with a significance level of $\alpha = .05$. Two-way ANOVA analysis determined there was a significant effect (p <.05) on total free sulfide concentrations from the interaction of storage time and sediment type. The Kruskal-Wallis post-hoc Dunn's Test determined there was not a statistical significance in sulfide concentrations up to 48 hours post start of storage (p >.05). See Appendix 3 for the statistical results.

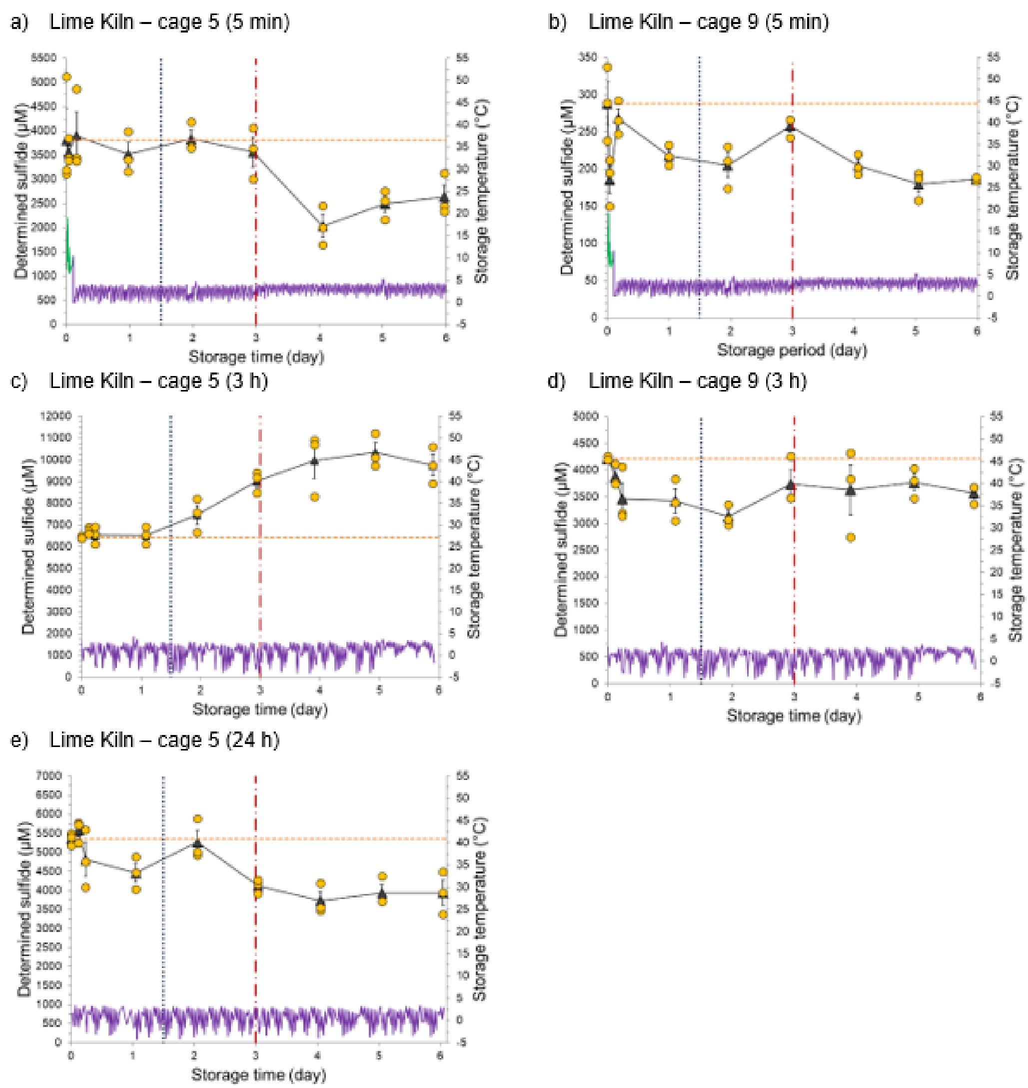


Figure 6. Stability profiles of total free sulfide in sediments stored for 6 days at ca. +4°C collected from Lime Kiln (figures a to e), and Oak Bay (figures f and g), NB. The legend for the charts are as follows: = Replicate (n=3); = Mean ± SEM; = Baseline conc.; = AAR storage limit (36 h); = NB and NS storage limit (72 h); = Temperature of cooler containing ice ; = Storage temperature.

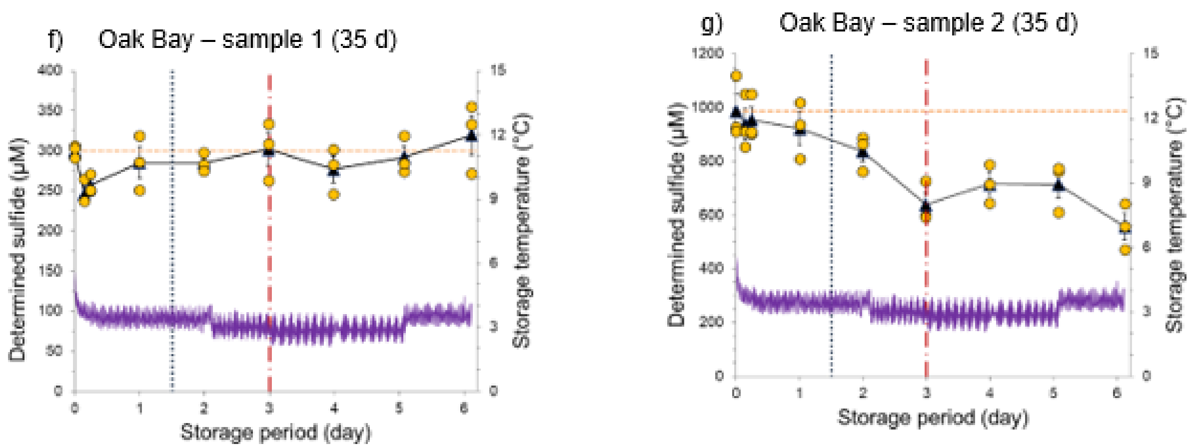


Figure 6 (continued). Stability profiles of total free sulfide in sediments stored for 6 days at ca. +4°C collected from Lime Kiln (figures a to e), and Oak Bay (figures f and g), NB. The legend for the charts are as follows: = Replicate (n=3); = Mean ± SEM; = Baseline conc.; = AAR storage limit (36 h); = NB and NS storage limit (72 h); = Temperature of cooler containing ice ; = Storage temperature.

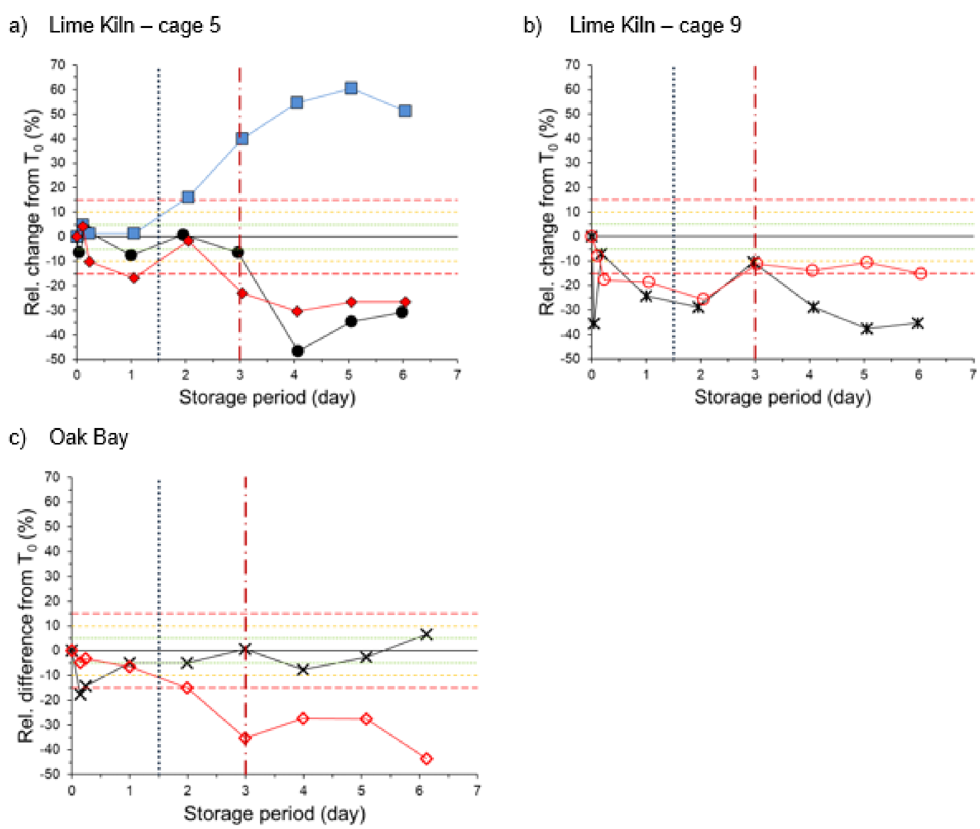


Figure 7. Relative differences (%) from baseline concentrations of total free sulfide in sediments collected from Lime Kiln (figures a and b), and Oak Bay (figure c), NB over a 6 day storage period at ca. +4°C with assigned boundaries of ±5%, ±10%, and ±15%. The legend for the charts are as follows: = Lime Kiln, cage 5 (5 min); = Lime Kiln, cage 5 (3 h); = Lime Kiln, cage 5 (24 h); = Lime Kiln, cage 9 (5 min); = Lime Kiln, cage 9 (3 h); = Oak Bay, sample 1 (35 d); = Oak Bay, sample 2 (35 d); = AAR storage limit (36 h); = NB and NS storage limit (72 h).

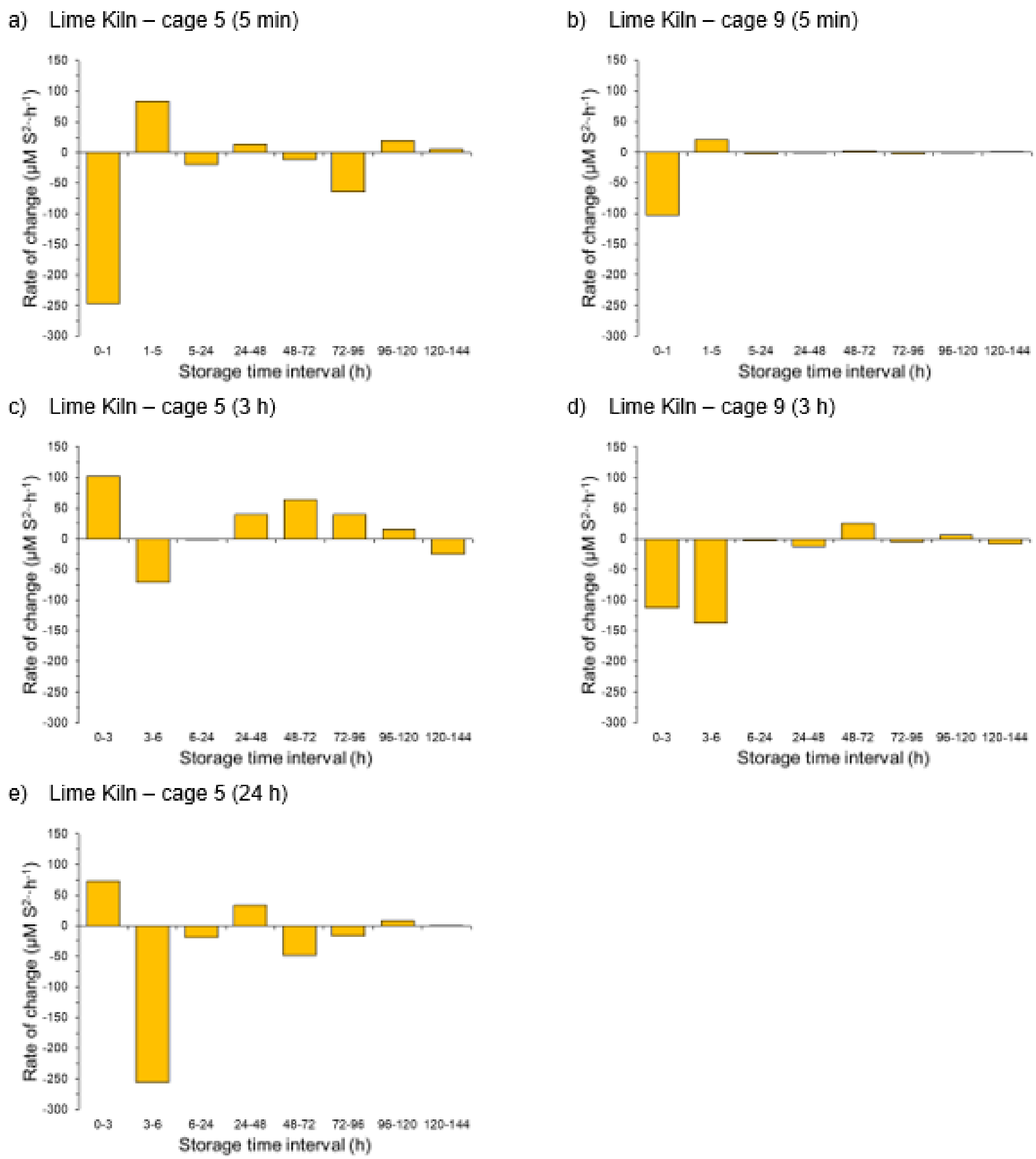


Figure 8. Rates of change (µM S^{2-}·h^{-1}) of total free sulfide in sediment samples collected from Lime Kiln, NB (figures a to e), and Oak Bay, NB (figures f and g) which were stored for 6 days at ca. +4°C.

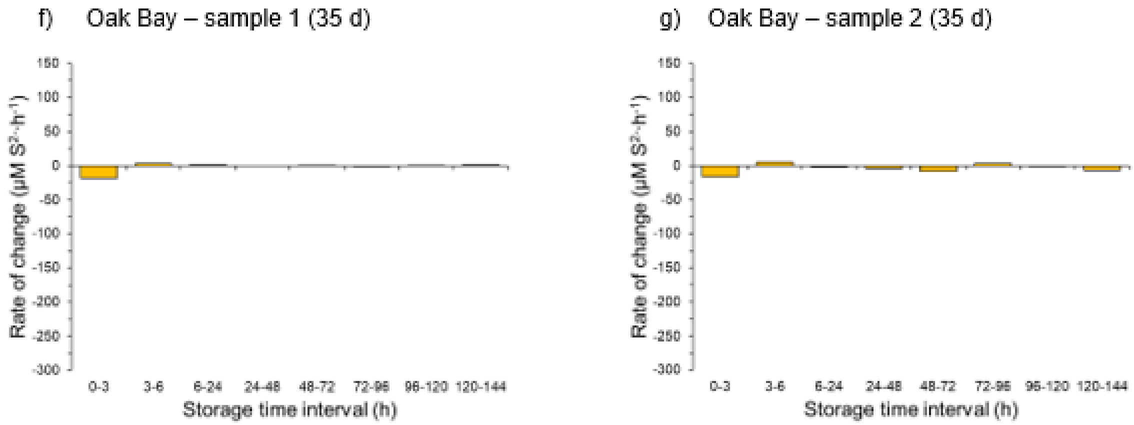


Figure 8 (continued). Rates of change (μM S2-·h-1) of total free sulfide in sediment samples collected from Lime Kiln, NB (figures a to e), and Oak Bay, NB (figures f and g) which were stored for 6 days at ca. +4°C.

Potential mitigation of sediment storage effects using alternative preservation methods

Since storage of sediment samples appears to cause changes in the total free sulfide concentrations relative to initial values, the following options for mitigation of the changes have been considered.

Storage of Sediment With SAOB Added

Due to the solubilisation effect of bound sulfides in sediment in a high pH environment as determined by Brown et al. (2011), and the effects observed in the previous section, "Effect of Adding SAOB Prior to Sediment Storage", storage of sediment samples with SAOB present to preserve total free sulfide levels is considered to be unsuitable.

Chemical Precipitation

Due to the volatile and unstable nature of sulfide, it is readily degraded which raises the question of its stability in the porewater fraction of collected sediment samples over time as discussed previously. To mitigate this, it is possible to precipitate the porewater sulfide as an insoluble salt which would preserve it until its analysis. Typically, aqueous solutions of transition metal cations, e.g., iron (Fe^{2+}), and zinc (Zn^{2+}), will precipitate soluble sulfide as low solubility (in water) metal-sulfide salts, e.g. iron sulfide (FeS) and zinc sulfide (ZnS). This method has been used to precipitate dissolved sulfide in sediment samples by Lasorsa and Casas (1996), or to trap liberated H_2S gas from acidified sediment for determination of acid volatile sulfides (Tisserand et al. 2021). A similar method was employed by Wong and Page (in press) to precipitate sulfide in sediment porewater collected using Rhizon samplers (Rhizosphere Research Products, The Netherlands) for analysis by the methylene blue method (Cline 1969). The reagent used, zinc acetate:disodium EDTA dihydrate:sodium hydroxide (1:1:0.8% w/v), precipitated the soluble sulfide as the insoluble zinc sulfide (ZnS) salt and was shown to stabilise the free sulfide for up to 6 months at ca. +4°C storage.

The use of a precipitating reagent in conjunction with the ISE method is deemed not suitable. The precipitation method relies on acidification of the sample to release the bound sulfide from the salt for quantification. In contrast, the ISE method is dependent on high alkaline conditions to enable conversion of the dominant bisulfide ion (HS^-) and to a lesser extent hydrogen sulfide

(H_2S), to the dianionic form, S^{2-} for detection. Given the previous logic, use of a precipitating reagent is considered unsuitable if analysis is to be performed by ISE.

Use of Vacuum Sealing

Due to the volatility and ease of oxidation of sulfide, the viability of vacuum sealing sediment in capped syringes to limit its degradation was also considered. To assess this, a series of experiments were performed using sediments collected from Shelburne, NS (Figure 9a) and from a intertidal zone at Oak Bay, NB (Figure 9b). See Appendix 1 for collection data, trace metals, and grain size analyses for the sediments used.

a) Shelburne, NS
43°43'09.7"N 65°19'18.6"W

b) Oak Bay, NB
Sample A; 45°12'51.81"N 67°11'46.87"W
Sample B; 45°12'52.37"N 67°11'41.50"W

Figure 9. Locations of sediment sampling for vacuum sealed storage stability experiments.

Bulk sediments from each of the locations were collected into plastic buckets with lids then transported to SABS for storage at ca. +4°C (temperatures not monitored) until used. As for the stability experiments, the sediments were mixed to obtain a homogenous sample prior to sub-sampling into 5 mL plastic syringes cut-off at the ‘zero’ mark. The sub-samples were capped and triplicate syringes were vacuum sealed together. Baseline total free sulfide concentrations were determined in triplicate samples by ISE using the method detailed in Wong and Page (2025). The batches of sealed packages were either stored in the dark at ambient temperature on the bench or in covered water baths to occlude light set to maintain constant temperatures of 10°C and 0°C. For each of the Oak Bay sediments, an additional set of samples was prepared that were capped, but not vacuum sealed, and stored at ambient temperature in the dark. Storage temperatures were monitored using RBR*solo* temperature loggers. Determination of total free sulfide concentrations in the samples were performed at 3, 6 h, 1, 2, 3, 4, 5, and 6 days post start of storage.

Shelburne sediment

During quantification of total free sulfide in the Shelburne samples, SAOB was added to the triplicate samples simultaneously which resulted in them soaking in SAOB for up to 2, 4 and 6 minutes while analysis was taking place. Because of this, increases in total free sulfide concentrations was observed from replicates 1 to 3. The same samples were re-read at 8, 10 and 12 minutes post SAOB addition. These later readings were less variable indicating that total free sulfide concentrations had stabilised within this time frame (Figure 10). The 2 sets of readings, i.e., the 2, 4 and 6 min readings (the square red symbols in Figure 10) and the 8, 10 and 12 minute readings (the circular orange symbols in Figure 10) resulted in respective baseline total free sulfide concentrations of 195 μM and 338 μM. Determined total free sulfide concentrations from the investigated samples are presented in Appendix 7.

Data from the 1st readings (2, 4, and 6 min post SAOB addition) showed that samples stored vacuum sealed at ambient temperature exhibited a consistent positive bias relative to baseline for the full 6 days of storage. The percent relative difference between readings and baseline values exceeded ±15% up to day 3 post start of storage. After this, total free sulfide concentrations fell to between 10% and 15% of baseline (Figure 11a). Data from the 2nd readings (8, 10, and 12 min post SAOB addition) showed more of a negative bias compared to their baseline concentrations. For the 2nd readings, the degradation profiles for each of the storage temperature mirrored each other with little variation between them. All of the 2nd readings were within ±15% of baseline (Figure 11b). The rate of change was highest within the first two storage time intervals (0-3 h and 3-6h) during the 1st readings. By the 2nd readings, this initial increase in rate was less pronounced with the rate of change very similar at all storage intervals (Figure 12).

Overall, the temporal variation in total free sulfide concentration associated with samples stored at 10°C and 0°C was very similar for the 1st and 2nd readings over the course of the 6 days. The 2nd readings mirrored those of the 1st except at higher concentrations. Total free sulfide concentrations at each analytical timepoint was similar to the respective baseline concentrations indicating no significant loss, or production of sulfide. Data for the 1st readings demonstrated fluctuations below, and above ±15% limit. In contrast, the 2nd readings fell within ±10% of baseline with the exception of the final storage day (6 d) when data started falling below -15% (Figure 11). As with the samples stored at ambient temperature, the highest rates of change in total free sulfide were seen during the first 2 time intervals during the 1st readings with no marked difference during the 2nd readings (Figure 12).

Statistical analyses of the data generated from each of the experiments was tested with a significance level of $\alpha = .05$. Two-way ANOVA analysis determined there was not a significant effect ($p > .05$) on total free sulfide from the interaction between storage time and storage temperature. The Kruskal-Wallis Test failed to reject the null hypothesis. See Appendix 4 for the statistical results.

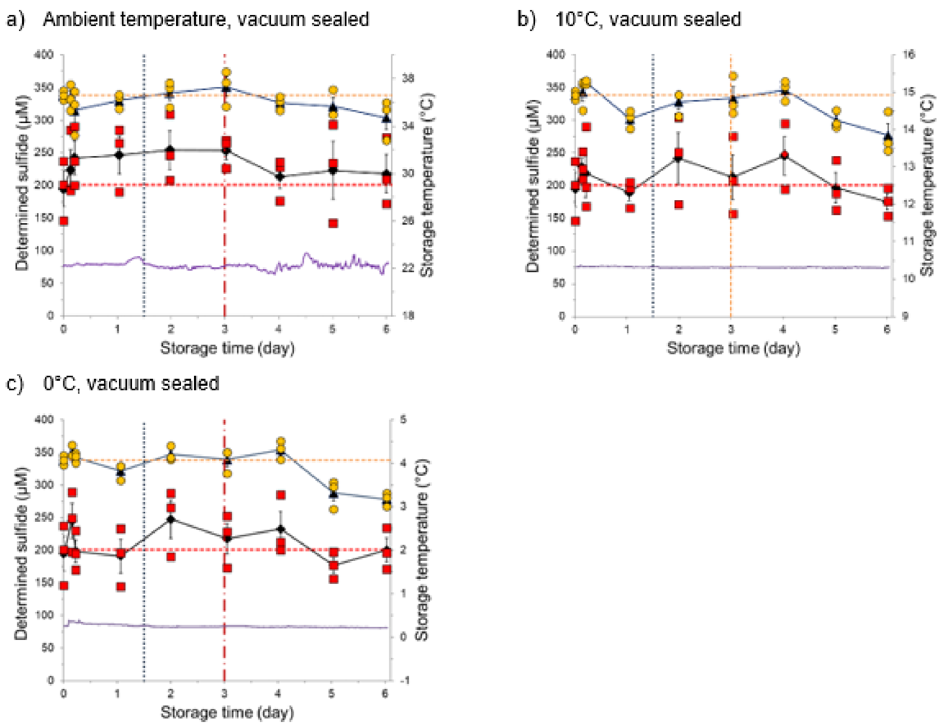


Figure 10. Stability profiles of total free sulfide in vacuum sealed sediment samples stored for 6 days at ambient, 10°C and 0°C collected from Shelburne, NS. First readings were taken ca. 2, 4, and 6 min after SAOB addition. Second readings were taken after the initial determinations equivalent to ca. 8, 10, and 12 min after SAOB addition. The legend for the charts are as follows: ■ = Replicate (n=3, 1st reading); ◆ = Mean ± SEM (1st reading); - - - = Baseline conc. (1st reading); ● = Replicate (n=3, 2nd reading); ▲ = Mean ± SEM (2nd reading); - - - = Baseline conc. (2nd reading); • • • • • = AAR storage limit (36 h); — · = NB and NS storage limit (72 h); —— = Storage temperature.

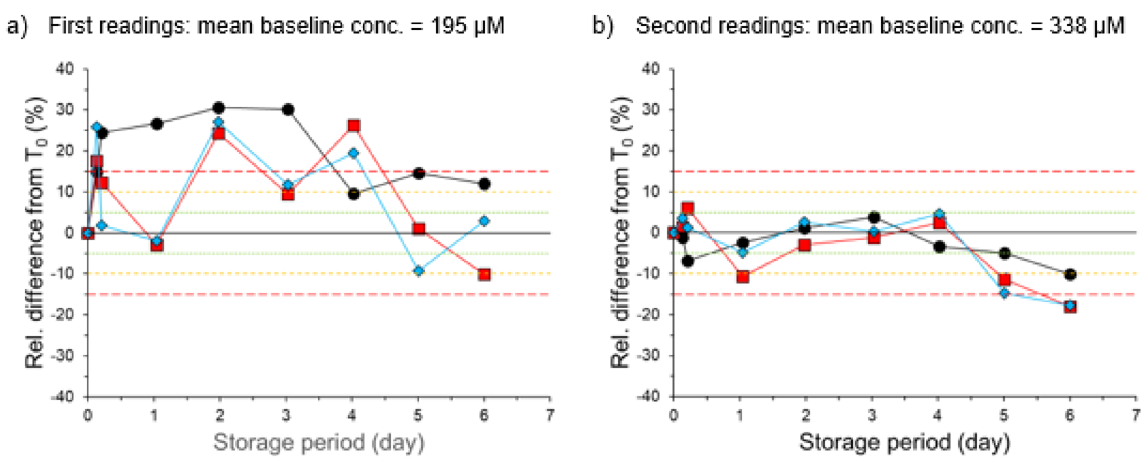


Figure 11. Relative differences (%) from baseline concentrations, with limits of ±5%, ±10%, and ±15% of total free sulfide in vacuum sealed sediment samples collected from Shelburne, NS over a 6 day storage period at ambient, 10°C, and 0°C. Samples were read at (a) 2, 4, 6 min, and (b) 8, 10, and 12 min post SAOB addition. The legend for the charts are as follows: ● = Samples stored at ambient, vacuum sealed; ■ = Samples stored at 10°C, vacuum sealed; ◆ = Samples stored at 0°C, vacuum sealed.

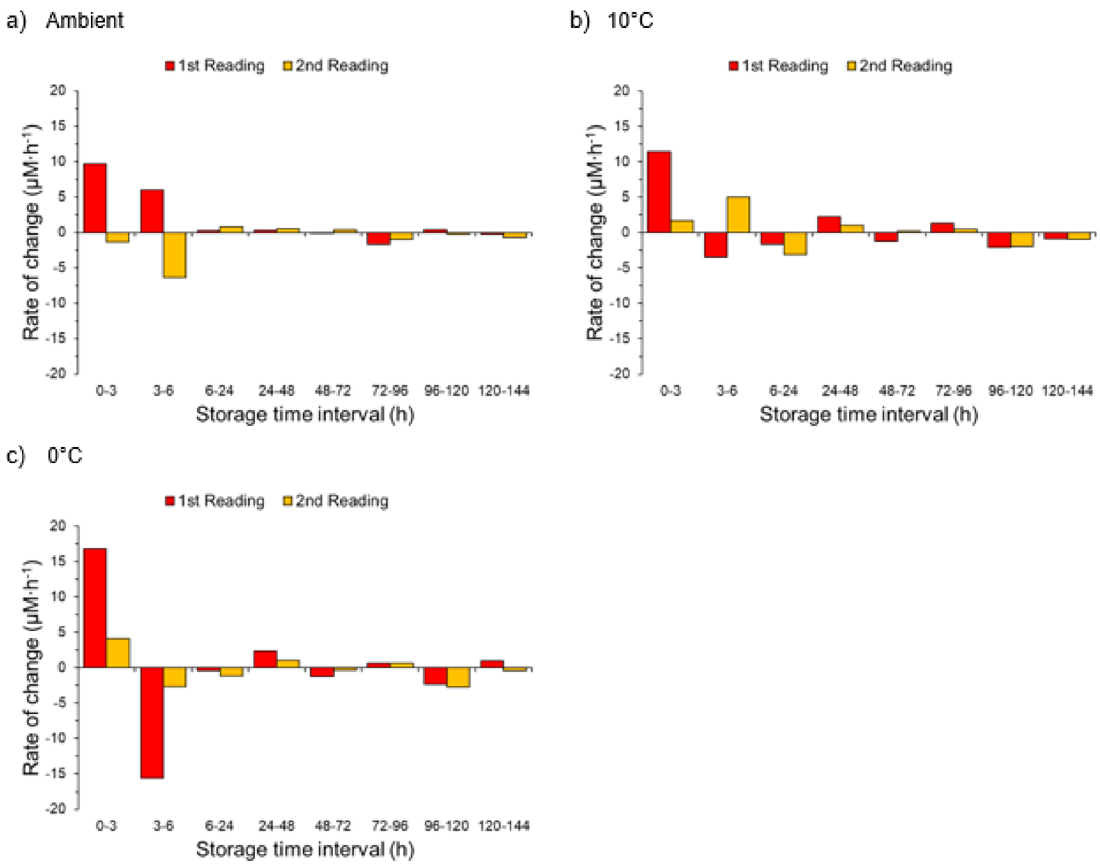


Figure 12. Rates of change of total free sulfide per hour (µM $S^{2-}\cdot h^{-1}$) in vacuum sealed sediment samples stored for 6 days at ambient, 10°C, and 0°C collected from Shelburne, NS. First readings were taken at 2, 4, and 6 min post SAOB addition and the second readings taken from the same samples at 8, 10, and 12 min post SAOB addition.

Oak Bay sediment

Unlike the analysis of the Shelburne samples, SAOB was added to each Oak Bay sample immediately before each sample was analysed by ISE, with total free sulfide concentration readings taken within the first 1 to 2 min after SAOB addition. The baseline total free sulfide concentrations in the Oak Bay sediment samples were 478 µM (sample A) and 1 700 µM (sample B) for the two sediments investigated. The temporal variation in the ISE total free sulfide concentrations associated with these sediments responded very differently from that of Shelburne; the Shelburne sediments reacted similarly to each of the storage temperatures, whereas the Oak Bay sediments behaved very differently. Determined total free sulfide concentrations from the investigated samples are presented in Appendix 8.

Sample A sediment

When sample A sediments were stored vacuum sealed at ambient temperature, the ISE derived total free sulfide concentrations exhibited positive bias from the baseline concentration beginning within the first analysis timepoint of 3 h (Figure 13a). Total free sulfide concentration increased steadily up to day 3 post start of storage to a maximum of 1 037 µM and then declined on day 4 to 742 µM when levels plateaued. Only the 3 h timepoint data fell within either

±5%, ±10%, or ±15% limits, with a value of +9.0%. The remainder of the results up to day 6 were all greater than +15%, ranging from +18.9% to +117.0% (Figure 14a).

The samples stored at ambient temperature but not vacuum sealed responded differently from the sealed samples at the same temperature (Figure 13b). Total free sulfide declined below the baseline concentration to a relative difference of -0.6 to -14.8%, up to 1 day post start of storage, then increased above baseline by day 2 and remained quite stable to the end of storage with the majority of readings greater than +15% (relative differences varied between +13.2% and +23.0% (Figure 14a).

The stability profile for the samples stored vacuum sealed at 10°C (Figure 13c) was similar to the ambient, unsealed samples (Figure 13c). An initial decline below baseline occurred within the first 2 analytical timepoints (relative difference -11.0% and -5.3%) followed by an increase above the baseline concentration that remained quite consistent to day 6, although again the majority of readings were greater than the ±15% boundary (relative difference +2.7% to +34.9% (Figure 14a).

Vacuum sealed samples stored at 0°C produced a stable profile that was negatively biased from baseline within the first analytical timepoint (Figure 13d). All readings were either at, or below the -15% limit with the exception of one result (relative differences ranged from -0.4% to -31.7% (Figure 14a).

When the relative differences from these experiments were overlaid and compared, it can be seen that there was considerable fluctuations with no single storage condition producing results that consistently fell within ±5%, ±10%, or ±15% limits (Figure 14a).

In contrast to the storage stability experiments (described in the Storage Stability section of this report), the hourly rates of change of total free sulfide were very low with no discernable period where significant total free sulfide change was taking place. However, the profiles for the samples stored at 10°C and 0°C did mirror each other (Figure 15).

Sample B sediment

The sample B sediment elicited a different response compared to sample A; it produced similar stability profiles for all storage conditions with consistent positive bias, but with varying magnitudes of response. The vacuum sealed samples stored at ambient temperature (Figure 13e) gave rise to the highest response with a steep increase in total free sulfide concentrations which peaked by 6 h post start of storage (+173.3% of baseline). Concentrations then declined gradually but showed an increase on the last day, day 6 (+80.0% of baseline), but overall total free sulfide concentrations remained above the baseline concentration (Figure 14b).

The unsealed samples stored at ambient temperature (Figure 13f) produced the next highest response with the maximum total free sulfide concentrations obtained again by 6 h post start of storage (+91.0% of baseline). Total free sulfide then decreased up +21.2% of baseline, on day 6, (Figure 14b).

The profiles for the vacuum sealed samples at 10°C and 0°C (Figures 13g and 13h) mirrored each other quite consistently with the same steep increase by 6 h post start of storage seen in the ambient samples. Their highest total free sulfide concentrations however, were observed at day 3 (+149.8%, 10°C; +73.3%, 0°C) followed by a decrease on day 4 then a period of increasing total free sulfide up to the last day of storage (Figure 14b).

When the relative differences from these experiments were overlaid and compared, it can be seen that all data points for all experiments were positively biased and exceeded +15%, up to ca. +180% in one case (Figure 14b).

Where sample A sediment did not display significant rates of change of total free sulfide, the sample B sediment did. The highest rates were seen during the first 2 time point intervals (similar to the storage stability experiments) at 0-3 h and 3-6 h in all the samples. The order for the rates of change were, ambient (sealed) > ambient (unsealed) >10°C (sealed) >0°C (sealed) (Figure 15).

Statistical analyses of the data generated from each of the experiments was tested with a significance level of α = .05. Two-way ANOVA analysis determined there was significant interactions between the storage time and storage temperatures for both sediments 1 and 2. The Kruskal-Wallis Test was rejected for the null hypothesis of no temporal differences for all storage conditions (*p* <.05) with the exception of one experiment which was not rejected by a small probability margin. See Appendix 4 for the statistical results.

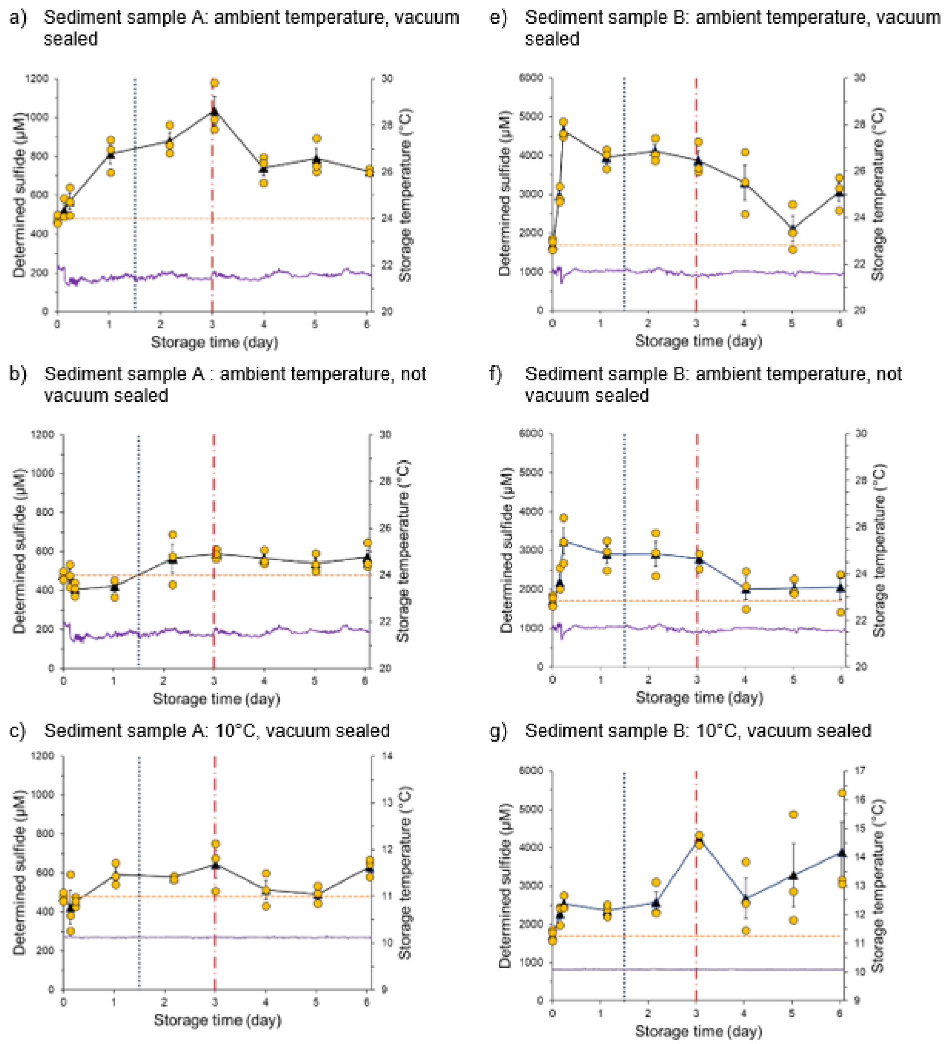


Figure 13. Stability profiles of total free sulfide in two sediment samples collected from Oak Bay, NB and stored vacuum sealed at ambient, 10°C and 0°C and unsealed at ambient temperature for 6 days. Plots a) to d) belong to sediment sample A (baseline concentration = 478 µM), and plots e) to h) to sediment sample B (baseline concentration = 1 700 µM). The legend for the charts are as follows: ○ = Replicate (n=3); ▲ = Mean ± SEM; ---- = Baseline sulfide concentration (µM); • • • • • = AAR storage limit (36 h); — · = NB and NS storage limit (72 h); — = Storage temperature.

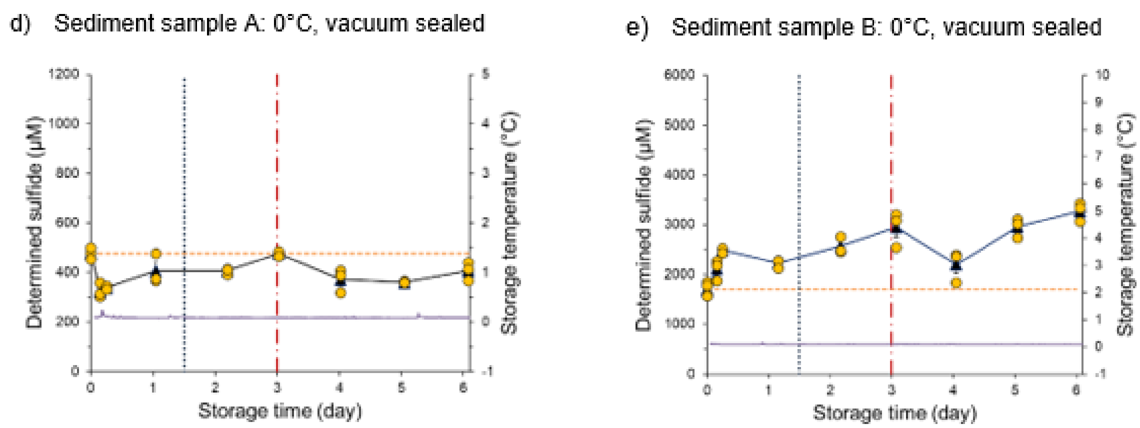


Figure 13 (continued). Stability profiles of total free sulfide in two sediment samples collected from Oak Bay, NB and stored vacuum sealed at ambient, 10°C and 0°C and unsealed at ambient temperature for 6 days. Plots a) to d) belong to sediment sample A (baseline concentration = 478 µM), and plots e) to h) to sediment sample B (baseline concentration = 1 700 µM). The legend for the charts are as follows: ● = Replicate (n=3); ▲ = Mean ± SEM; - - - = Baseline sulfide concentration (µM); • • • • • = AAR storage limit (36 h); — · = NB and NS storage limit (72 h); —— = Storage temperature.

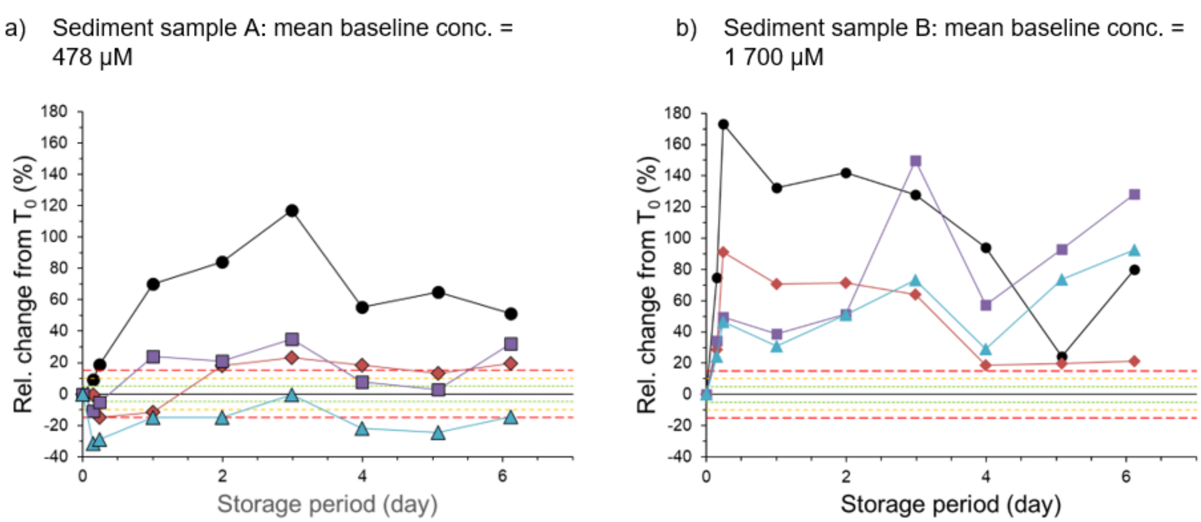


Figure 14. Relative difference (%) from baseline concentrations, with limits of ±5%, ±10%, and ±15%, of total free sulfide in sediment samples A and B collected from Oak Bay, NB. Samples were stored vacuum sealed over a 6 day storage period at ambient, 10°C and 0°C, and unsealed at ambient temperature. The legend for the charts are as follows: ● = samples stored at ambient, vacuum sealed; ◆ = samples stored at ambient, not vacuum sealed; ■ = samples stored at 10°C, vacuum sealed; ▲ = samples stored at 0°C, vacuum sealed.

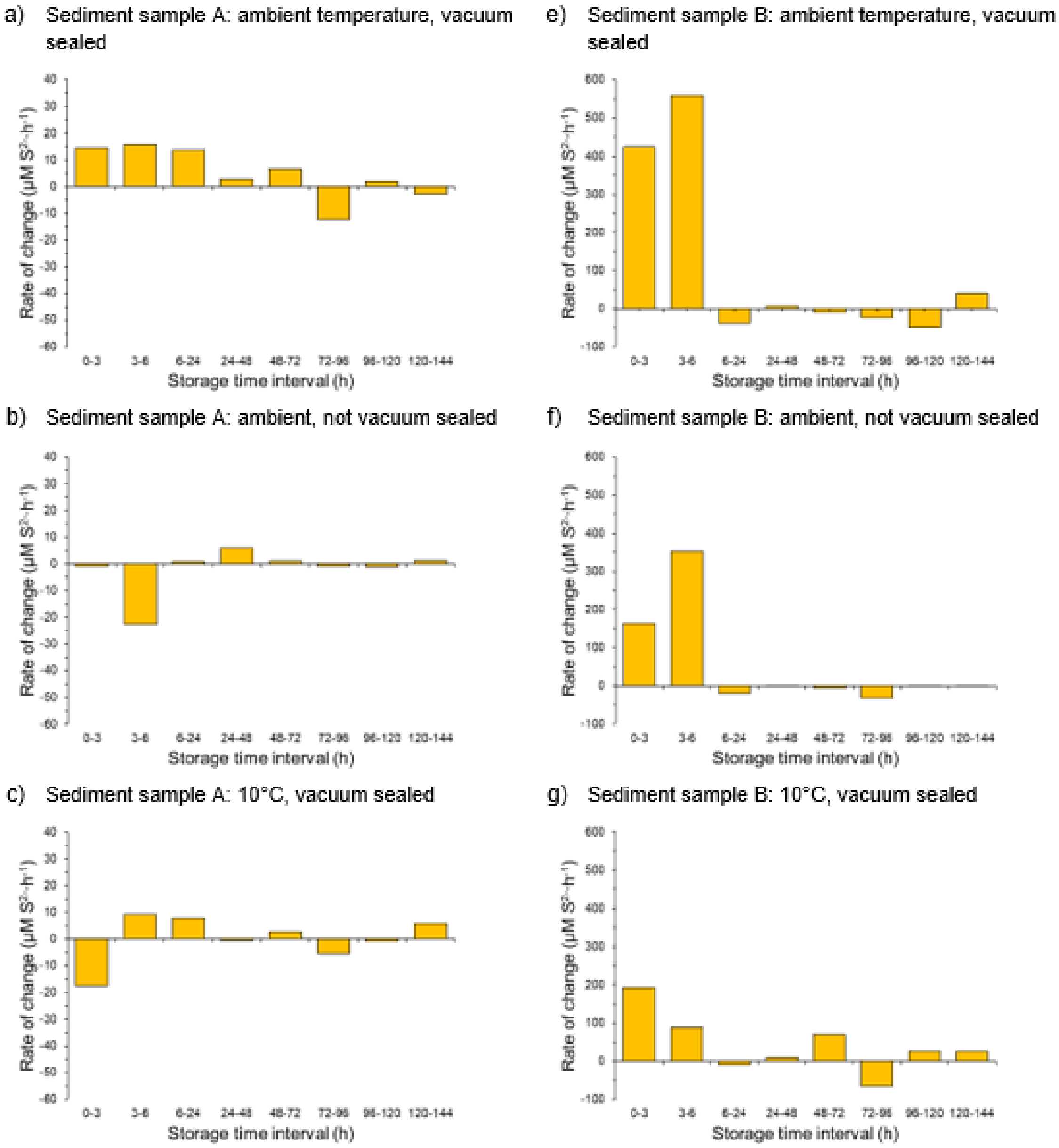
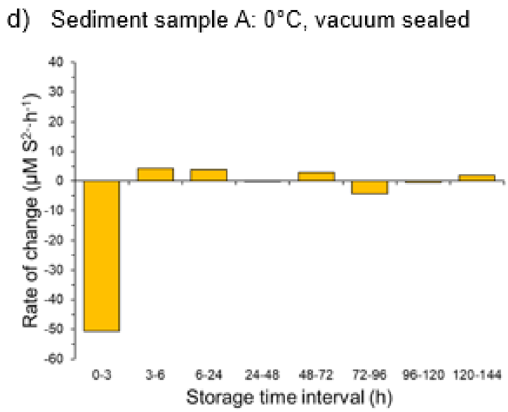


Figure 15. Rates of change (µM S^{2-}·h^{-1}) of total free sulfide in sediment samples A and B collected from Oak Bay, NB and stored vacuum sealed at ambient, 10°C and 0°C and unsealed at ambient temperature for 6 days. Plots a) to d) belong to sediment sample A (baseline concentration = 478 µM), and plots e) to h) to sediment sample B (baseline concentration = 1 700 µM).



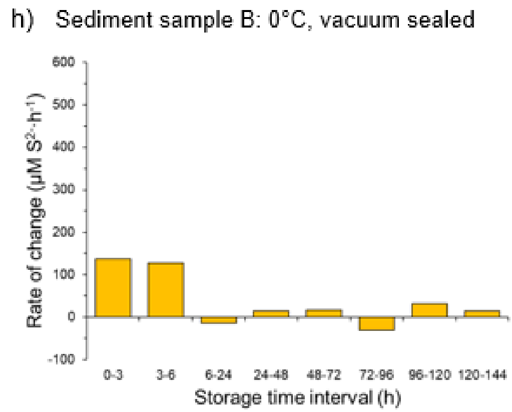


Figure 15 (continued). Rates of change (μM $S^{2-}\cdot h^{-1}$) of total free sulfide in sediment samples A and B collected from Oak Bay, NB and stored vacuum sealed at ambient, 10°C and 0°C and unsealed at ambient temperature for 6 days. Plots a) to d) belong to sediment sample A (baseline concentration = 478 μM), and plots e) to h) to sediment sample B (baseline concentration = 1 700 μM).

SUMMARY AND CONCLUSIONS

- Storage of samples does not give a magnitude of change of zero, as shown in these experiments. The degree of change is inconsistent, unpredictable, and can exceed ±15% of the initial (baseline) total free sulfide measurement. This magnitude of change is based on the results of a single analyst. Given the variability of the methodology (whether analyst or instrumental) used to quantify the concentration of total free sulfide as detailed in Wong and Page (2025) and Page and Wong (In press), the variability will likely be increased if results included experiments and analyses conducted by a range of analysts and instruments.
- The question regarding acceptability of data derived from stored samples depends on how much variation and accuracy Aquaculture Management is willing to accept based on the previous bullet point.
- Based on the data derived from the storage stability experiments, the response of total free sulfide to short term storage appears to be dependent on the sampling location, and sulfide concentration present. Therefore predicting how sediments collected from spatially different locations and with different matrix characteristics will respond to storage is not possible.
- Total free sulfide concentration in anoxic sediments has the potential to be overestimated if stored due to possible sulfide production resulting from anaerobic conditions. Whether this production is consistent for all anoxic sediments is unclear at this time, and needs to be investigated further.
- Ascertaining what ‘perfect’ storage conditions give total free sulfide concentrations within ±5%, ±10%, or ±15% immediately after collection is not possible since each sediment responds differently to the same storage conditions.
- Storage stability determination was not conducted in large grain size (sandy) sediments since appreciable levels of total free sulfide are not expected in this matrix type.
- Storing sediment in the presence of SAOB is not a viable method of preserving total free sulfide while awaiting analysis.

- Vacuum sealing sediment samples to occlude oxygen is not a viable method to stabilise total free sulfide either.

RECOMMENDATIONS

- Do not store sediment samples. Conduct ISE measurements on sediment samples as soon as they are collected. In this way, all the uncertainties associated with sediment storage are avoided. If a 'no storage' situation is not feasible, interpretations of the monitoring results need to take into consideration a variable bias of 10% to 50%, or greater.
- Do not mix or homogenise sediment samples after collection, doing so can introduce oxygen and thus has the potential to elevate the rate of sulfide degradation.
- Use of a standardised matrix (i.e., porewater) should reduce any matrix effects associated with sediment type (grain size, organic matter, etc.) or sampling location. This will also overcome sediment porosity affecting porewater content and thus accurate quantification of total free sulfide as determined in Wildish et al. (2004), and Hargrave et al. (2008). Many methods are available for the collection of porewater, therefore a suitable robust method will have to be investigated and evaluated. Also, a matrix change would require re-evaluation of the relationship between biodiversity and total free sulfide concentration.
- If porewater is chosen as a standardised matrix, other analytical techniques other than ISE could be used to quantify total free sulfide, e.g., UV spectroscopy (Cranford et al. 2020), and methylene blue assay (Wong and Page in press).

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APPENDIX 1. DETAILS OF SEDIMENTS USED IN THE EXPERIMENTS

Table A1.1. Collection details.

Parameter	Lime Kiln, NB					Oak Bay, NB*	Shelburne, NS
	Cage 5 (5 min)	Cage 9 (5 min)	Cage 5 (3 h)	Cage 9 (3 h)	Cage 5 (24 h)		
Sampling Co-ordinates	N45 03.686’ W66 49.971’	N45 03.605’ W66 49.995’	N45 03.680’ W 66 49.981’	N45 03.608’ W66 49.970’	N45 03.686’ W66 49.978’	45°12'51.81"N 67°11' 46.87"W 45°12’52.37"N 67°11’41.50"W	43°43'09.7"N 65°19'18.6"W
Collection depth	Not recorded - subtidal	Not recorded - subtidal	17.2 m - subtidal	15.4 m - subtidal	~ 16 m - subtidal	0 m - intertidal, with air exposure	Not recorded - subtidal
Water type	Seawater, salinity not determined					Brackish, salinity not determined	Seawater, salinity not determined
Sampler type	Ponar grab	Grab, type not specified				Hand scoop	Ponar grab
Sediment characteristics	Not recorded	Not recorded	Dark brown/black mud, with mussel shells, containing feed and faeces	Black mud with a lot of feed and faeces, and off-gassing	Not recorded	Not recorded	Not recorded
Sample container type, volume, and seal	10 L White plastic pails (HDPE), with sealable lids. Sediment samples were collected into sealed pails with varying amounts of headspace present.						
Pre-storage conditions	No storage, analysed immediately after collection		Kept at ambient after collection, and during transportation to SABS. Processed within 3 h of collection		Kept at ambient after collection, and during transportation to SABS. Stored at ~4°C until processed 24 h after collection	Kept at ambient after collection, and during transport to SABS. Stored at ~4°C until processed	Kept at ambient after collection, and stored at ~4°C. Transported back to SABS at ambient. Stored at ~4°C until processed
Grain size characterisation	N	N	Y	Y	Y	Y#	N
Trace metals	N	N	Y	Y	Y	Y	Y
Porewater	1) Collection: Potential loss of sediment integrity and porewater during sampling using the aforementioned collection methods. 2) Storage: Potential loss of sediment integrity, and separation of porewater during storage – not an issue since sediments were homogenised prior to analysis.						

* = Sediments used in the Oak Bay experiments were collected on a couple of occasions. Only one set of GPS co-ordinates was recorded by the person collecting these samples. However it was informed that the sampling locations were spatially very similar, therefore these co-ordinates are representative for all the samples used.

= Grain size only performed for sediments used in Experiment 1 (samples 1 and 2) of the non-vacuum sealed storage experiments.

SABS = St. Andrews Biological Station, St Andrews, NB.

Table A1.2. Trace metals. Analysis performed by RPC - Fredericton, NB using their in-house SOPs with method references to EPA 3050B, and EPA 200.8/EPA 200.7.

Analytes	Units	Lime Kiln, NB					Oak Bay, NB				Shelburne, NS
		Cage 5 (5 min)	Cage 9 (5 min)	Cage 5 (3 h)	Cage 9 (3 h)	Cage 5 (24 h)	Sample A*	Sample B$	Sample 1#	Sample 2#	
Aluminum	mg/kg	NP	NP	19600	13000	18600	11500	11850	12800	12500	10600
Antimony	mg/kg	NP	NP	< 0.1	< 0.1	< 0.1	< 0.1	0.1	0.2	0.1	< 0.1
Arsenic	mg/kg	NP	NP	9	5	8	9	14.5	17	9	10
Barium	mg/kg	NP	NP	63	42	61	27	38	24	34	50
Beryllium	mg/kg	NP	NP	1.0	0.7	1.0	0.5	0.5	0.6	0.4	0.5
Bismuth	mg/kg	NP	NP	< 1	< 1	< 1	< 1	<1	< 1	< 1	< 1
Boron	mg/kg	NP	NP	43	37	43	14	21.5	17	11	80
Cadmium	mg/kg	NP	NP	0.41	0.34	0.41	0.34	0.87	0.17	0.51	0.62
Calcium	mg/kg	NP	NP	13200	26600	19900	13400	42750	2370	29000	5090
Chromium	mg/kg	NP	NP	37	26	35	25	25.5	25	27	27
Cobalt	mg/kg	NP	NP	11.8	7.5	11.0	9.6	10.6	9.4	9.6	5.7
Copper	mg/kg	NP	NP	95	53	74	26	34.5	23	32	18
Iron	mg/kg	NP	NP	30800	19900	28800	24600	24850	24900	25900	19200
Lead	mg/kg	NP	NP	22.6	14.0	21.1	11.1	12.75	20.6	10.7	21.6
Lithium	mg/kg	NP	NP	38.7	25.0	37.1	27.2	27.55	29.1	29.2	26.4
Magnesium	mg/kg	NP	NP	11800	10300	12200	7610	7980	6940	8560	8800
Manganese	mg/kg	NP	NP	368	255	349	259	457	232	400	224
Molybdenum	mg/kg	NP	NP	3.2	5.1	4.4	1.6	1.9	1.6	1.3	3.7
Nickel	mg/kg	NP	NP	31	20	29	28	26	27	29	21
Potassium	mg/kg	NP	NP	5640	4510	5710	1860	2090	2180	1570	4180
Rubidium	mg/kg	NP	NP	29.0	20.0	27.8	13.5	14.7	16.6	11.5	21.2
Selenium	mg/kg	NP	NP	1	< 1	1	< 1	1.5	< 1	< 1	2
Silver	mg/kg	NP	NP	< 0.1	< 0.1	< 0.1	< 0.1	0.1	0.1	< 0.1	< 0.1
Sodium	mg/kg	NP	NP	25700	32600	29700	4430	8095	6060	5570	24600
Strontium	mg/kg	NP	NP	88	151	114	92	233	25	144	57
Tellurium	mg/kg	NP	NP	< 0.1	< 0.1	< 0.1	< 0.1	<0.1	< 0.1	< 0.1	< 0.1
Thallium	mg/kg	NP	NP	0.2	0.2	0.2	0.1	0.1	0.1	< 0.1	0.2
Tin	mg/kg	NP	NP	< 1	< 1	< 1	< 1	<1	< 1	< 1	< 1
Uranium	mg/kg	NP	NP	2.0	3.0	2.4	1.7	2.15	1.8	1.9	1.8
Vanadium	mg/kg	NP	NP	51	33	47	28	27.5	33	29	32
Zinc	mg/kg	NP	NP	181	327	229	165	313	80	245	54

NP = Not performed.

* = Oak Bay sample A used in the SAOB soak time and vacuum sealed storage stability experiments.

$ = Oak Bay sample B used in the vacuum sealed storage stability experiments.

= Oak Bay samples 1 and 2 were used in the non-vacuum sealed storage stability experiments.

Table A1.3. Grain size. Analysis performed by RPC - Fredericton, NB using their in-house method based on: Walton, A (1978). Methods for Sampling and Analysis of Marine Sediments and Dredged Materials – Ocean Dumping Report 1. Department of Fisheries and the Environment.

Size classification	Units	RL	Lime Kiln, NB					Oak Bay, NB				Shelburne, NS
			Cage 5 (5 min)	Cage 9 (5 min)	Cage 5 (3 h)	Cage 9 (3 h)	Cage 5 (24 h)	Sample A*	Sample B$	Sample 1#	Sample 2#	
PHI -2 (4mm)	% Finer	0.1	NP	NP	100.	100.	100.	NP	NP	96.6	98.9	NP
PHI -1 (2 mm)	% Finer	0.1	NP	NP	99.9	100.	99.9	NP	NP	92.0	87.6	NP
PHI 0 (1 mm)	% Finer	0.1	NP	NP	99.8	99.8	99.8	NP	NP	88.0	72.5	NP
PHI 1 (0.5 mm)	% Finer	0.1	NP	NP	99.6	99.2	99.4	NP	NP	85.2	52.7	NP
PHI 2 (0.25 mm)	% Finer	0.1	NP	NP	98.8	96.9	97.7	NP	NP	79.0	31.5	NP
PHI 3 (0.125 mm)	% Finer	0.1	NP	NP	98.1	94.4	95.8	NP	NP	57.9	24.0	NP
PHI 4 (62.5 µm)	% Finer	0.1	NP	NP	96.2	92.5	93.9	NP	NP	33.7	19.4	NP
PHI 5 (31.25 µm)	% Finer	0.1	NP	NP	90.5	91.9	91.2	NP	NP	24.4	16.9	NP
PHI 6 (15.6 µm)	% Finer	0.1	NP	NP	73.1	69.6	71.7	NP	NP	18.6	13.7	NP
PHI 7 (7.8 µm)	% Finer	0.1	NP	NP	47.7	37.0	35.9	NP	NP	13.6	6.6	NP
PHI 8 (3.9 µm)	% Finer	0.1	NP	NP	38.9	28.1	25.2	NP	NP	11.4	3.7	NP
PHI 9 (1.9 µm)	% Finer	0.1	NP	NP	16.3	17.9	8.2	NP	NP	9.5	0.7	NP
Gravel	%	0.1	NP	NP	< 0.1	< 0.1	< 0.1	NP	NP	8.0	12.4	NP
Sand	%	0.1	NP	NP	3.7	7.5	6.0	NP	NP	58.3	68.2	NP
Silt	%	0.1	NP	NP	57.3	64.4	68.6	NP	NP	22.3	15.7	NP
Clay	%	0.1	NP	NP	38.9	28.1	25.2	NP	NP	11.4	3.7	NP

RL = Reporting limit.
NP = Not performed.
* = Oak Bay sediment sample A was used in the SAOB soak time and vacuum sealed storage stability experiments.
$ = Oak Bay sediment sample B was used in the vacuum sealed storage stability experiments.
# = Oak Bay sediment samples 1 and 2 were used in the non-vacuum sealed storage stability experiments.

APPENDIX 2. COMPOSITE GRAPH OF NORMALISED TOTAL FREE SULFIDE STABILITY IN THE EXAMINED SEDIMENTS

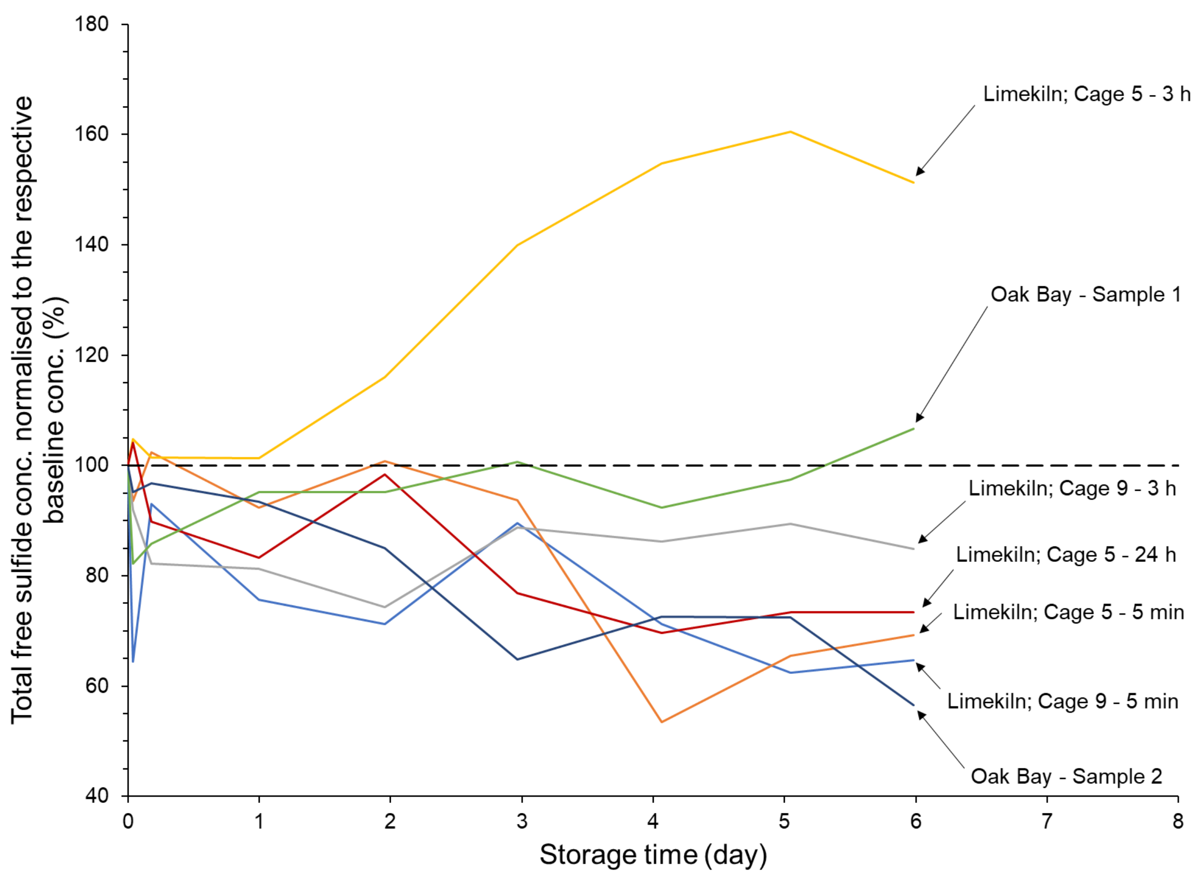

Figure A2.1. Composite graph of normalized total free sulfide concentrations as a function of time post baseline analysis. Total free sulfide concentrations were normalized by dividing each derived timepoint concentration by the respective baseline concentration and multiplying by 100%. Baseline concentrations therefore have a value of 100%.

APPENDIX 3. STATISTICS ASSOCIATED WITH STORAGE STABILITY ANALYSES

To test for statistical significance resulting from the storage of sediment samples the following statistical tests were performed using SPSS version 24 (IBM Corp., USA):

1. Two-way ANOVA, the null hypotheses are:
   a. the storage time will have no significant effect on total free sulfide concentration.
   b. the sample type will have no significant effect on total free sulfide concentration.
   c. storage time and sample type interaction will have no significant effect on total free sulfide concentration.
2. Kruskal-Wallis one-way ANOVA on ranks (Kruskal and Wallis, 1952) with a post hoc Dunn's Multiple Comparisons Test (Dunn, 1964). The null hypothesis for this test is:
   a. that the mean ranks of the groups are the same.

Two-way ANOVA

Two separate tests (α = .050) were conducted since two slightly different storage regimes were used during these experiments (Table 1 of this appendix).

The first test compared samples from Lime Kiln cage 5 and 9 (3 h) and Lime Kiln cage 5 (24 h) and Oak Bay samples 1 and 2 (35 day). These results showed that there was a significant interaction between the sample type and storage time on total free sulfide concentrations, $F(_{df\ between}\ 32,\ _{df\ within}\ 90) = 8.304$, $p = .000$. However, the simple main effects analysis indicated that there was not a significant storage time effect on total free sulfide concentrations ($p = .061$). This was in contrast to the sample type which showed that there was significant effect ($p = .000$) on total free sulfide concentrations.

The second two-way ANOVA test was performed on Lime Kiln samples from cage 5 and cage 9 (5 min). Results showed that again, there was a significant interaction between sample type and storage time on total free sulfide concentrations, $F(8, 36) = 3.1016$, $p = .009$. Main effect analysis showed a significant effect on total free sulfide concentrations as a result of sample type ($p = .000$) and storage time ($p = .000$).

Kruskal-Wallis one-way ANOVA

Results for each of the samples investigated showed that Oak Bay sample 1 (35 d) and Lime Kiln cage 9 (3 h) samples failed to reject the null hypothesis, $H(8) = 12.964$, $p = .113$ and $H(8) = 11.981$, $p = .152$ respectively.

However the rest of the samples rejected the null hypothesis; Oak Bay sample 2 (35 d), $H(8) = 21.597$, $p = .006$; Lime Kiln cage 5 (5 min), $H(8) = 17.818$, $p = .023$; Lime Kiln cage 9 (5 min), $H(8) = 20.775$, $p = .008$; Lime Kiln cage 5 (3 h), $H(8) = 21.970$, $p = .005$; and Lime Kiln cage 5 (24 h), $H(8) = 19.075$, $p = .014$.

For the post hoc multiple comparisons test, only results from the pairwise comparisons against the baseline groups are presented (Table 6). SPSS did not conduct the Dunn's Test on Oak Bay sample 1 and Lime Kiln cage 9 (3 h) since they failed to reject the Kruskal-Wallis null hypothesis. Dunn's Test results showed that there were significant differences ($p <.05$) observed from 72 h to 144 h of storage on total free sulfide concentrations in Oak Bay sample 2, Lime Kiln cage 5 (3 h) and Lime Kiln cage 5 (24 h). Lime Kiln cage 5 (5 min) only showed significance ($p <.05$) at 96 h whereas Lime Kiln cage 9 did so at 1 h, 48 h and 96 to 144 h.

Table A3.1. Results of Kruskal-Wallis post-hoc Dunn's Test showing the differences between groups when compared to baseline.

Storage time comparison	Oak Bay sample 2 (35 d)	Limekiln cage 5 (3 h)	Limekiln cage 5 (24 h)	Storage time comparison	Limekiln cage 5 (5 min)	Limekiln cage 9 (5 min)
0 – 3 h	.700	.396	.719	0 – 1 h	.817	.018*
0 – 6 h	.738	.625	.410	0 – 5 h	.700	.918
0 – 24 h	.681	.681	.181	0 – 24 h	.959	.181
0 – 48 h	.198	.181	.959	0 – 48 h	.410	.051**
0 – 72 h	.006*	.024*	.057**	0 – 72 h	.918	.837
0 – 96 h	.035*	.005*	.012*	0 – 96 h	.035*	.064
0 – 120 h	.031*	.002*	.027*	0 – 120 h	.100	.005*
0 – 144 h	.002*	.007*	.031*	0 – 144 h	.123	.005*

* = Statistical significance ($p < .05$).
** = This value on the border of statistical significance ($p < .05$) and is therefore included as significant.

APPENDIX 4. STATISTICS ASSOCIATED WITH VACUUM SEALING ANALYSES

To test for statistical significance resulting from the storage of sediment samples with vacuum sealing, the following statistical tests were performed using SPSS version 24 (IBM Corp., USA):

1. Two-way ANOVA, the null hypotheses are:
   a. the storage time will have no significant effect on total free sulfide concentration.
   b. the sample type will have no significant effect on total free sulfide concentration.
   c. storage time and sample type interaction will have no significant effect on total free sulfide concentration.
2. Kruskal-Wallis one-way ANOVA on ranks (Kruskal and Wallis, 1952) with a post hoc Dunn's Multiple Comparisons Test (Dunn, 1964). The null hypothesis for this test is:
   a. the mean ranks of the groups are the same.

Two-way ANOVA

Two-way ANOVA were conducted using $\log_{10}$ transformed total free sulfide concentrations. The analyses were used to test for interaction effects between storage time and storage temperature as well as main effects within storage time and storage temperature.

Shelburne sediment analysis did not detect a significant interaction (α = .050) when analyses were restricted to vacuum sealed samples stored at ambient, 10°C and 0°C ($F(_{df\ between}\ 16,\ _{df\ within}\ 54) = 0.4185$, p = .971). Main effects analysis also showed that storage time and storage temperature did not have a significant (α = .050) effect on total free sulfide concentration (p = .174 and p = .260 respectively).

For the Oak Bay samples A and B, both samples showed significant interaction between the storage period and storage temperature on total free sulfide concentrations, F(24, 72) = 3.643, p = .000 for sample A, and F(24, 72) = 3.922, p = .000 for sample B. Main Effects analysis showed that for both samples, storage time and temperature had significant effects on total free sulfide concentrations, p = .000.

Kruskal-Wallis one-way ANOVA

Kruskal-Wallis one-way ANOVA were used to test for differences in total free sulfide concentrations taken at different times.

Analysis conducted in the vacuum sealed sediment from Shelburne failed to reject the null hypothesis (α=.05), that mean total free sulfide concentrations differed as a function of storage time, and therefore the variation in the temporal distribution of total free sulfide concentrations stored at a particular temperature is considered to be the result of samples drawn from a single population with a common mean and variance(for ambient, 10°C and 0°C; H(8) = 3.967, p = .860, H(8) = 7.185, p = .517 and H(8) = 8.010, p = .432 respectively).

For Oak Bay sample A (478 µM) the null hypothesis of no temporal differences was rejected for the ambient sealed (F(8) = 22.913, p = .003), ambient unsealed (F(8) = 17.421, p = .026), and 0°C sealed (F(8) = 21.147, p = .007) samples. The null hypothesis was however not rejected for the 10°C sealed samples (F(8) = 14.745, p = .064) by a small probability margin (i.e., p=064 only marginally greater than p=0,05). Analysis of Oak Bay sample B (1 700 µM) data rejected the null hypothesis of no temporal variation for all storage conditions. Ambient sealed (F(8) = 22.205, p = .005; ambient unsealed F(8) = 18.752, p = .016; 10°C sealed F(8) = 16.764, p = .033 and 0°C sealed F(*) = 23.859, p = .002.

For the post hoc multiple comparisons test, only results from the pairwise comparisons against the baseline groups are presented in Table 1 of this appendix. SPSS did not conduct the Dunn's Test on the Shelburne sediment samples, nor Oak Bay sample A, 10°C sample since they failed to reject the Kruskal-Wallis null hypothesis. Dunn's Test results showed that there were significant differences although there was no clear pattern to the obtained results with the exception of ambient storage (sealed, and unsealed) for sediment sample B.

Table A4.1. Results of Kruskal-Wallis post-hoc Dunn's Test showing the differences between groups when compared to the control group (baseline) in Oak Bay sediment samples containing 478 and 1 700 µM of total free sulfide.

Storage time comparison	Sample A (478 µM)			Sample B (1 700 µM)			
	Ambient	Ambient (unsealed)	0°C	Ambient	Ambient (unsealed)	10°C	0°C
	.003	.026	.007	.005	.016	.033	.002
0 – 3 h	.758	.877	.002*	.237	.237	.227	.471
0 – 6 h	.537	.269	.003*	.000*	.003*	.060	.040*
0 – 24 h	.018	.396	.198	.011*	.007*	.181	.328
0 – 48 h	.004*	.292	.258	.006*	.014*	.111	.027*
0 – 72 h	.000*	.076	.918	.016*	.018*	.001*	.002*
0 – 96 h	.064	.157	.040*	.100	.440	.064	.280
0 – 120 h	.024*	.341	.015*	.719	.537	.024*	.003*
0 – 144 h	.136	.190	.237	.217	.410	.002*	.000*

* = Statistical significance ($p < .05$).

APPENDIX 5. EFFECT ON TOTAL FREE SULFIDE CONCENTRATIONS IN SEDIMENTS COLLECTED FROM SHELBURNE, NS, AND OAK BAY, NB WHEN LEFT TO SOAK IN SULFIDE ANTI-OXIDANT BUFFER (SAOB)

Table A5.1. Oak Bay, NB.

Time post SAOB addition (min)	Determined free sulfide conc. (µM)				SD	SEM
	Replicate			Mean		
	1	2	3			
1 (Baseline)	561	489	422	491	69.515	40.134
3	515	512	434	487	45.924	26.514
5	476	519	496	497	21.517	12.423
7	446	496	500	481	30.089	17.372
9	428	453	508	463	40.927	23.629
11	419	431	434	428	7.937	4.583
13	396	428	431	418	19.399	11.200
15	387	399	453	413	35.157	20.298
17	382	416	419	406	20.551	11.865
19	348	379	393	373	23.029	13.296
21	318	377	366	354	31.374	18.114
22	318	363	348	343	22.913	13.229
25	324	353	346	341	15.133	8.737
27	292	341	343	325	28.885	16.677
29	282	348	334	321	34.775	20.078
31	282	339	327	316	30.050	17.349

Table A5.2. Shelburne, NS.

Time post SAOB addition (min)	Determined free sulfide conc. (µM)				SD	SEM
	Replicate			Mean		
	1	2	3			
1 (Baseline)	144	134	150	143	8.083	4.667
3	223	193	216	211	15.695	9.062
5	253	247	258	253	5.508	3.180
7	288	287	297	291	5.508	3.180
9	316	300	321	312	10.970	6.333
11	342	302	317	320	20.207	11.667
13	350	309	314	324	22.368	12.914
15	365	306	328	333	29.816	17.214
17	370	330	319	340	26.839	15.496
19	352	333	333	339	10.970	6.333
21	355	316	338	336	19.553	11.289
22	357	320	340	339	18.520	10.693
25	360	328	345	344	16.010	9.244
27	365	323	333	340	21.939	12.667
29	373	318	338	343	27.839	16.073
31	362	325	338	342	18.771	10.837

APPENDIX 6. STABILITY DATA FOR TOTAL FREE SULFIDE CONCENTRATIONS IN NON-VACUUM SEALED SEDIMENT SAMPLES COLLECTED FROM LIME KILN, NB, AND OAK BAY, NB STORED AT CA. +4°C OVER 6 DAYS

Table A6.1. Lime Kiln, NB – cage 5, processed 5 min post collection.

Nominal timepoint	Determined free sulfide conc. (µM)				SD	SEM
	Replicate			Mean		
	1	2	3			
Baseline	5120	3110	3190	3807	1138.083	657.1
1 h	3850	3440	3390	3560	252.389	145.7
5 h	4870	3440	3380	3897	843.465	487.0
1 day	3990	3160	3400	3517	427.122	246.6
2 day	4180	3680	3650	3837	297.714	171.9
3 day	3000	4060	3640	3567	533.791	308.2
4 day	2450	2000	1650	2033	401.040	231.5
5 day	2750	2170	2560	2493	295.691	170.7
6 day	3130	2440	2340	2637	430.155	248.4

Table A6.2. Lime Kiln, NB – cage 9, processed 5 min post collection.

Nominal timepoint	Determined free sulfide conc. (µM)				SD	SEM
	Replicate			Mean		
	1	2	3			
Baseline	337	289	238	288	49.508	28.6
1 h	150	195	212	186	32.036	18.5
5 h	265	247	292	268	22.650	13.1
1 day	217	232	205	218	13.528	7.8
2 day	230	211	174	205	28.478	16.4
3 day	266	266	242	258	13.856	8.0
4 day	202	220	193	205	13.748	7.9
5 day	194	187	158	180	19.088	11.0
6 day	185	185	189	186	2.309	1.3

Table A6.3. Lime Kiln, NB – cage 5, processed 3 h post collection.

Nominal timepoint	Determined free sulfide conc. (µM)				SD	SEM
	Replicate			Mean		
	1	2	3			
Baseline	6440	6500	6390	6443	55.076	31.8
3 h	6890	6770	6590	6750	150.997	87.2
6 h	6590	6910	6110	6537	402.658	232.5
1 day	6110	6910	6560	6527	401.040	231.5
2 day	8200	7560	6670	7477	768.397	443.6
3 day	8480	9400	9180	9020	480.416	277.4
4 day	10900	10700	8310	9970	1441.076	832.0
5 day	11200	9720	10100	10340	768.635	443.8
6 day	10600	8900	9750	9750	850.000	490.7

Table A6.4. Lime Kiln, NB – cage 9, processed 3 h post collection.

Nominal timepoint	Determined free sulfide conc. (µM)				SD	SEM
	Replicate			Mean		
	1	2	3			
Baseline	4260	4190	4190	4213	40.415	23.3
3 h	4120	3770	3740	3877	211.266	122.0
6 h	4060	3190	3140	3463	517.333	298.7
1 day	3390	3050	3830	3423	391.067	225.8
2 day	2970	3350	3070	3130	196.977	113.7
3 day	4260	3480	3480	3740	450.333	260.0
4 day	3830	2740	4320	3630	808.764	466.9
5 day	4030	3800	3470	3767	281.484	162.5
6 day	3680	3360	3680	3573	184.752	106.7

Table A6.5. Lime Kiln, NB – cage 5, processed 24 h post collection.

Nominal timepoint	Determined free sulfide conc. (µM)				SD	SEM
	Replicate			Mean		
	1	2	3			
Baseline	5490	5400	5180	5357	159.478	92.1
3 h	5770	5720	5240	5577	292.632	169.0
6 h	4760	5590	4080	4810	756.241	436.6
1 day	4880	4470	4030	4460	425.088	245.4
2 day	4920	5000	5880	5267	532.666	307.5
3 day	4170	4270	3920	4120	180.278	104.1
4 day	4190	3460	3540	3730	400.375	231.2
5 day	4370	3710	3710	3930	381.051	220.0
6 day	3940	4490	3370	3933	560.030	323.3

Table A6.6. Oak Bay, NB - sample 1, processed 35 day post collection.

Nominal timepoint	Determined free sulfide conc. (µM)				SD	SEM
	Replicate			Mean		
	1	2	3			
Baseline	306	303	291	300	7.937	4.583
1 h	265	238	237	247	15.885	9.171
5 h	271	250	251	257	11.846	6.839
1 day	251	286	319	285	34.005	19.633
2 day	283	275	298	285	11.676	6.741
3 day	263	309	334	302	36.014	20.793
4 day	283	302	246	277	28.478	16.442
5 day	319	274	284	292	23.629	13.642
6 day	272	333	355	320	43.000	24.826

Table A6.7. Oak Bay, NB – sample 2, processed 35 day post collection.

Nominal timepoint	Determined free sulfide conc. (µM)				SD	SEM
	Replicate			Mean		
	1	2	3			
Baseline	928	1120	912	987	115.747	66.826
1 h	855	1050	913	939	100.132	57.811
5 h	1050	903	911	955	82.658	47.723
1 day	1020	809	937	922	106.297	61.370
2 day	887	763	866	839	66.365	38.316
3 day	727	598	593	639	75.963	43.857
4 day	789	644	716	716	72.501	41.858
5 day	610	762	774	715	91.418	52.780
6 day	558	643	472	558	85.500	49.364

APPENDIX 7. STABILITY DATA FOR TOTAL FREE SULFIDE CONCENTRATIONS IN VACUUM SEALED SEDIMENT SAMPLES COLLECTED FROM SHELBURNE, NS STORED AT VARIOUS TEMPERATURES OVER 6 DAYS

Table A7.1. Ambient, vacuum sealed.

Nominal timepoint	Determined free sulfide conc. (μM)				SD	SEM
	Replicate			Mean		
	1	2	3			
Baseline	201, 331	237, 345	146, 338	195, 338	45.829, 7.000	26.460, 4.041
3 h	194, 332	192, 355	285, 315	224, 334	53.126, 20.075	30.672, 11.590
6 h	200, 324	237, 277	290, 344	242, 315	45.236, 34.395	26.117, 19.858
1 day	190, 317	265, 334	285, 339	247, 330	50.083, 11.533	28.916, 6.658
2 day	208, 320	246, 349	309, 357	254, 342	51.013, 19.468	29.452, 11.240
3 day	226, 358	269, 321	265, 374	253, 351	23.756, 27.185	13.715, 15.695
4 day	176, 329	228, 315	236, 336	213, 327	32.578, 10.693	18.809, 6.173
5 day	142, 309	234, 308	293, 347	223, 321	76.099, 22.234	43.936, 12.837
6 day	172, 316	209, 269	273, 327	218, 304	51.098, 30.806	29.501, 17.786

Table A7.2. 10°C, vacuum sealed.

Nominal timepoint	Determined free sulfide conc. (μM)				SD	SEM
	Replicate			Mean		
	1	2	3			
Baseline	201, 331	237, 345	146, 338	195, 338	45.829, 7.000	26.460, 4.041
3 h	210, 355	252, 358	225, 315	229, 343	21.284, 24.007	12.288, 13.860
6 h	198, 360	168, 355	290, 360	219, 358	63.571, 2.887	36.703, 1.667
1 day	166, 305	196, 287	205, 314	189, 302	20.421, 13.748	11.790, 7.937
2 day	171, 306	251, 339	304, 339	242, 328	66.955, 19.053	38.657, 11.000
3 day	157, 323	207, 311	275, 368	213, 334	59.228, 30.050	34.196, 17.349
4 day	194, 351	248, 329	295, 359	246, 346	50.540, 15.535	29.180, 8.969
5 day	188, 289	163, 295	239, 315	197, 300	38.734, 13.614	22.363, 7.860
6 day	153, 265	196, 253	176, 313	175, 277	21.517, 31.749	12.423, 18.330

Table A7.3. 0°C, vacuum sealed.

Nominal timepoint	Determined free sulfide conc. (μM)				SD	SEM
	Replicate			Mean		
	1	2	3			
Baseline	201, 331	237, 345	146, 338	195, 338	45.829, 7.000	26.460, 4.041
3 h	197, 361	249, 345	289, 345	245, 350	46.130, 9.238	26.633, 5.333
6 h	170, 349	195, 334	230, 344	198, 342	30.139, 7.638	17.401, 4.410
1 day	144, 307	196, 329	233, 329	191, 322	44.710, 12.702	25.813, 7.333
2 day	190, 339	265, 360	287, 342	247, 347	50.856, 11.358	29.362, 6.557
3 day	173, 350	228, 350	252, 318	218, 339	40.501, 18.475	23.383, 10.667
4 day	213, 367	200, 339	285, 356	233, 354	45.786, 14.107	26.434, 8.145
5 day	156, 304	197, 263	177, 297	177, 288	20.502, 21.932	11.837, 12.662
6 day	171, 287	196, 267	234, 281	200, 278	31.723, 10.263	18.315, 5.925

Note: the *x,y* format of data in the above tables relates to the 1st and 2nd readings taken. Where *x* = 1st readings taken within 2, 4, and 6 min of SAOB addition, and *y* = 2nd readings taken from the same samples, but within 8, 10, and 12 min of the initial SAOB addition.

APPENDIX 8. STABILITY DATA FOR TOTAL FREE SULFIDE CONCENTRATIONS IN VACUUM SEALED SEDIMENT SAMPLES COLLECTED FROM OAK BAY, NB STORED AT VARIOUS TEMPERATURES OVER 6 DAYS

Table A8.1. Sediment sample A; ambient, vacuum sealed.

Nominal timepoint	Determined free sulfide conc. (µM)				SD	SEM
	Replicate			Mean		
	1	2	3			
Baseline	479	500	455	478	22.517	13.000
3 h	490	490	583	521	53.694	31.000
6 h	642	496	567	568	73.009	42.152
1 day	718	837	886	814	86.396	49.881
2 day	860	962	817	880	74.474	42.997
3 day	992	940	1180	1037	126.259	72.896
4 day	796	765	664	742	69.024	39.851
5 day	894	721	721	789	92.446	53.374
6 day	715	737	715	722	12.702	7.333

Table A8.2. Sediment sample A; ambient, non-vacuumed sealed.

Nominal timepoint	Determined free sulfide conc. (µM)				SD	SEM
	Replicate			Mean		
	1	2	3			
Baseline	479	500	455	478	22.517	13.000
3 h	417	476	533	475	58.003	33.488
6 h	441	370	411	407	35.642	20.578
1 day	450	365	452	422	49.662	28.672
2 day	688	431	575	565	128.811	74.369
3 day	611	565	588	588	23.000	13.279
4 day	537	609	550	565	38.371	22.154
5 day	591	533	500	541	46.069	26.598
6 day	524	540	647	570	66.876	38.611

Table A8.3. Sediment sample A; 10°C, vacuum sealed.

Nominal timepoint	Determined free sulfide conc. (µM)				SD	SEM
	Replicate			Mean		
	1	2	3			
Baseline	479	500	455	478	22.517	13.000
3 h	303	381	592	425	149.514	86.322
6 h	426	476	456	453	25.166	14.530
1 day	542	583	652	592	55.591	32.095
2 day	584	584	567	578	9.815	5.667
3 day	507	675	752	645	125.285	72.333
4 day	512	599	430	514	84.512	48.793
5 day	496	533	444	491	44.710	25.813
6 day	580	647	667	631	45.567	26.308

Table A8.4. Sediment sample A; 0°C, vacuumed sealed.

Nominal timepoint	Determined free sulfide conc. (µM)				SD	SEM
	Replicate			Mean		
	1	2	3			
Baseline	479	500	455	478	22.517	13.000
3 h	303	316	360	326	29.872	17.247
6 h	338	347	333	339	7.095	4.096
1 day	368	478	376	407	61.330	35.409
2 day	394	415	412	407	11.358	6.557
3 day	476	486	466	476	10.000	5.774
4 day	320	409	389	373	46.694	26.959
5 day	366	358	360	361	4.163	2.404
6 day	369	442	414	408	36.828	21.263

Table A8.5. Sediment sample B; ambient, vacuum sealed.

Nominal timepoint	Determined free sulfide conc. (µM)					SD	SEM
	Replicate				Mean		
	1	2	3	4			
Baseline	1850	1780	1600	1570	1700	136.382	68.191
3 h	3210	2890	2810	NS	2970	211.660	122.202
6 h	4490	4880	4570	NS	4647	205.994	118.930
1 day	4160	3660	4030	NS	3950	259.422	149.778
2 day	4450	4030	3870	NS	4117	299.555	172.948
3 day	4360	3590	3680	NS	3877	420.991	243.059
4 day	4090	3320	2490	NS	3300	800.187	461.988
5 day	2740	2010	1580	NS	2110	586.430	338.575
6 day	3430	2590	3160	NS	3060	428.836	247.588

Table A8.6. Sediment sample B; ambient, non-vacuumed sealed.

Nominal timepoint	Determined free sulfide conc. (µM)					SD	SEM
	Replicate				Mean		
	1	2	3	4			
Baseline	1850	1780	1600	1570	1700	136.382	68.191
3 h	2010	2550	2010	NS	2190	311.769	180.000
6 h	3840	3220	2680	NS	3247	580.460	335.128
1 day	2490	2970	3250	NS	2903	384.361	221.911
2 day	3450	2950	2340	NS	2913	555.908	320.953
3 day	2530	2920	2920	NS	2790	225.167	130.000
4 day	2470	2090	1490	NS	2017	494.099	285.268
5 day	1940	2270	1890	NS	2033	206.478	119.210
6 day	1420	2370	2390	NS	2060	554.346	320.052

Table A8.7. Sediment sample B; 10°C, vacuum sealed.

Nominal timepoint	Determined free sulfide conc. (µM)					SD	SEM
	Replicate				Mean		
	1	2	3	4			
Baseline	1850	1780	1600	1570	1700	136.382	68.191
3 h	2450	1980	2410	NS	2280	260.576	150.444
6 h	2450	2750	2430	NS	2543	179.258	103.494
1 day	2380	2190	2510	NS	2360	160.935	92.916
2 day	2320	2300	3100	NS	2573	456.216	263.397
3 day	4330	4080	4330	NS	4247	144.338	83.333
4 day	2550	3630	1840	NS	2673	901.351	520.395
5 day	4870	2110	2860	NS	3280	1427.130	823.954
6 day	3160	5430	3050	NS	3880	1343.466	775.650

Table A8.8. Sediment sample B; 0°C, vacuumed sealed.

Nominal timepoint	Determined free sulfide conc. (µM)					SD	SEM
	Replicate				Mean		
	1	2	3	4			
Baseline	1850	1780	1600	1570	1700	136.382	68.191
3 h	2270	2180	1880	NS	2110	204.206	117.898
6 h	2510	2530	2430	NS	2490	52.915	30.551
1 day	2280	2280	2120	NS	2227	92.376	53.333
2 day	2460	2760	2480	NS	2567	167.730	96.839
3 day	3210	3080	2550	NS	2947	349.619	201.853
4 day	2390	2360	1840	NS	2197	309.246	178.543
5 day	2740	3110	3010	NS	2953	191.398	110.504
6 day	3430	3340	3060	NS	3277	192.959	111.405

NS = No sample