Stony Brook University

Comparisons of intermediate oxidation state sulfur compounds in the episodically anoxic Forge River and the Cariaco Basin

A Thesis Presented

by

Lan Thi Tong

to

The Graduate School

in Partial Fulfillment of the

Requirements

for the Degree of

Master of Science

in

Marine and Atmospheric Science

with a concentration in

Marine Science

Stony Brook University

May 2013

Stony Brook University

The Graduate School

Lan Thi Tong

We, the thesis committee for the above candidate for the

Master of Science degree, hereby recommend

acceptance of this thesis.

_____

Mary I. Scranton,
Professor
School of Marine and Atmospheric Sciences

_____

Gordon Taylor
Professor
School of Marine and Atmospheric Sciences

_____

Qingzhi Zhu
Associate Professor
School of Marine and Atmospheric Sciences

This thesis is accepted by the Graduate School

_____

Dean of the Graduate School

Abstract of the Thesis

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2013

Abstract

The anoxic Cariaco Basin located on the Venezuela continental shelf and the seasonally anoxic Forge River located on the south shore of Long Island are two quite different aquatic systems. However, they are both characterized by the pulsed intrusion of oxygen into sulfidic waters, where sulfur intermediates including sulfite (SO_3^{2-}), thiosulfate ($S_2O_3^{2-}$), and zero-valent sulfur (S^o) are usually found. In this study, I investigated the distribution of sulfur intermediates and the relative importance of chemical and biological hydrogen sulfide oxidation to the sulfur intermediates distribution in these two systems. The sulfide oxidation rate, determined using Forge River water with an incubation in the dark at three temperatures (2, 25 and 40^{o}C) and two initial ratios of H_2S/O_2 (0.7 and 2.8), was about 40- 300 times faster than the initial rate of chemical oxidation predicted from kinetic calculations, likely due to catalysis by trace metals. This observation was consistent with the lower activation energy of sulfide oxidation for two initial ratios of H_2S/O_2 which were 42±0.7 and 36±1.5 kJ mol^{-1} and were lower than the values of 66 ± 5 kJ mol^{-1} predicted for pure chemical oxidation of sulfide in air saturated seawater. In comparison with sulfide oxidation which could have been associated with chemoautotrophy, the chemical sulfide oxidation accounted for more than 90% of the total sulfide oxidation.

In the deep and permanently anoxic Cariaco, the sulfur species are likely result of chemical H_2S oxidation after oxygen intrusion. In a spiking experiment, chemoautotrophy was found to be either suppressed or undetectable in the presence of air and chloroform. However, under anoxic conditions, chemoautotrophy and the reaction of H_2S with trace metals (either biotic or abiotic) both could be important for particulate elemental sulfur formation. In the Forge River, particulate elemental sulfur was most likely to be the result of chemical sulfide oxidation by a metal catalyst rather than that of biological processes. In both systems, zero-valent sulfur was the dominant sulfur intermediate product with higher concentrations found at a higher ratio of H_2S to O_2.

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Acknowledgments

I would like to deeply thank my advisor, Mary I. Scranton, for her guidance, support and encouragement throughout my study. Without her, my project would not have been completed. I would also like to thank Gordon Taylor for his insightful suggestions and comments on this work, especially on microbial data. My thanks also go to Qingzhi Zhu for giving me a lot of useful comments on this paper. I would like to thank the staff and scientists of EDIMAR and the crew of the Hermano Hines for helping me with collecting data in Cariaco. A special thanks to all of my friends who have been providing me with unlimited help and comfort.

Introduction

Hydrogen sulfide oxidation and the resulting intermediate oxidation state sulfur compounds have been studied at oxic-anoxic interfaces in several anoxic marine systems including the Cariaco Basin (Zhang and Millero 1993; Percy et al. 2007; Hayes et al. 2006; Li et al. 2008), Mariager Fjord (Zopfi et al. 2001), the Black Sea (Jorgensen et al. 1990), and northern Rehoboth Bay (Ma et al. 2006). Oxidation of hydrogen sulfide can result in a variety of products of different oxidation states. Studies of the chemical oxidation of H_2S in the laboratory (Chen and Morris 1972; Zhang and Millero 1993) and in the environment (Sorokin 1970; Millero 1991) showed that sulfate (SO_4^{2-}), thiosulfate ($S_2O_3^{2-}$) and sulfite (SO_3^{2-}) are the major oxidation products, with polysulfides (S_n^{2-}) and elemental sulfur (S^0) also being produced under high sulfide to oxygen ratios (Chen and Morris, 1972). Overall, the distribution of sulfur intermediates is controlled by the ratio of H_2S to O_2, the pH of the solution (O'Brien and Birkner, 1973) and trace metal concentrations (Zhang and Millero, 1993).

Zopfi et al. (2001) investigated the mode (biological/chemical) of H_2S oxidation and the formation and distribution of sulfur species (thiosulfate, sulfite, S^0) in the stratified water column of Mariager Fjord. Concentration maxima of S^0, thiosulfate and sulfite were found in or below the chemocline following an intrusion of oxygen-containing water into sulfidic water (Zopfi et al., 2001). The results from this investigation suggested that, while biological sulfide oxidation was responsible for more than 88% of the total sulfide oxidation when reactant concentrations were low, such as in the suboxic zone, the proportion of chemical sulfide oxidation increased under conditions when there was mixing of oxic and sulfidic water masses. Although Zopfi et al. (2001) directly observed an oxygen intrusion event, this study was conducted over only 3 days surrounding a single event (one day before the intrusion event, one day during the event and two days after the event), giving limited information on the response of sulfur intermediates to a pulse of oxygen.

In another study of an anoxic coastal system that received pulses of oxygen, Ma et al. (2006) reported the removal of sulfide through an iron catalytic cycle and iron sulfide precipitation in two seasonally anoxic tributaries of northern Rehoboth Bay, a component of the Delaware Inland Bay. Their results indicated that iron played an important role in S redox cycling and precipitation. H_2S was oxidized at the oxic-anoxic interface by iron (III) hydroxides which were

dominant in the surface oxic waters. Elemental sulfur was the main product of hydrogen sulfide oxidation, with concentrations reaching as high as 30 µM. Ma et al (2006) suggested this was due to high concentrations of H_2S and a shallow water depth (5.5 m) which allowed extensive H_2S transport to the oxic-anoxic interface. No data on other sulfur intermediates were reported in this study.

In the Cariaco Basin (Fig.1), the intrusion of oxygenated water into the sulfidic zone also has been associated with the formation of sulfur intermediates (Zhang and Millero 1993; Hayes et al. 2006; Percy et al. 2007; Li et al. 2008). However, because of potentially long and unknown time lags between oxygen intrusions and sampling, and because measured concentrations of the sulfur intermediates are the net result of complex reaction processes that may selectively remove some species and/or may be subjected to multiple cycles (turnover), detailed mechanisms have been difficult to define. Oxygen intrusion events in the Cariaco are unpredictable, and the sampling time relative to oxygen intrusion events varies from cruise to cruise. This means that the sampling might have been conducted a week after an event for one cruise but one or several months (or even years) after the intrusion for other cruises. Li et al (2008) suggested that the fact that there are different "types" of profiles of sulfite and thiosulfate in the Cariaco Basin might be related to variable delays between oxygenated intrusions and observations of sulfur species. Sulfite and thiosulfate maxima have been found in oxic water (Li et al. 2008), at the O_2/H_2S interface, and below it. On some occasions, there are low values in oxic water and at the interface, and the compounds are present only in sulfidic water. However, the most common pattern is that both sulfite and thiosulfate concentrations are low but measurable across the interface, that sulfite concentrations are usually (but not always) well correlated with thiosulfate concentrations, and that S^0 has a sharp maximum at/or below the depth of first appearance of sulfide (Hayes et al. 2006; Li et al. 2008). Nevertheless, the controls on the distribution of thiosulfate, sulfite and S^0 in the Cariaco Basin remain unclear.

In order to determine whether there is a clear pattern of formation of sulfide oxidation products following an oxygen intrusion, I undertook a study of the Forge River (Long Island, New York) water column. Unlike in the Cariaco Basin, in the Forge River pulses of oxygen are added to sulfidic water on a regular basis since the water is shallow and both primary production and sulfide flux from the sediment are high. During the night, oxygen concentrations are very low. During daylight hours, however, oxygen is produced at very high rates by photosynthetic

activity. At a station at the mouth of Wills Creek of the Forge River (Fig. 2), Wilson et al. (2009) observed that oxygen concentrations were undetectable at dawn but were supersaturated by the end of the day. The sediment at the same station was very anoxic and hydrogen sulfide was very high in bottom sediments (1 mM) based on samples collected by Aller et al. (2006). (NOTE: sediment and water column samples were collected on different dates). A white precipitate (which was hypothesized to be elemental sulfur by Swanson, personal communication) has also been seen in the Forge River in late summer. These observations led us to believe that sampling in the Forge would allow a detailed study of temporal variation of sulfur intermediates in response to mixing of oxygenated and sulfidic waters. Overall, the goal of this study was to investigate the pattern of sulfide intermediates under different ratios of oxygen to hydrogen sulfide, as well as the relative importance of chemical and biological processes to the formation of intermediate products of sulfide oxidation.

Method and Materials

Study sites

The Cariaco Basin is a large permanently anoxic depression, located on the continental shelf of Venezuela (10°30’ N, 64°40’ W). The basin is a tectonically-formed with a depth of about 1,400 m. The basin is connected to the Caribbean Sea by two shallow (~140 m) sills, one to the north and one to the north-west (Fig.1). The oxic-anoxic interface generally lies between 220 and 350 m but the factors which control this depth are complex and remain poorly understood (Scranton et al. 2001).

The Forge River (Fig. 2) is a small estuary on the south shore of Long Island, New York, and is highly eutrophic due to the sluggish nature of the river and excessive nutrient loading from anthropogenic inputs including septic systems and duck farms. Because of the extremely high levels of photosynthesis by phytoplankton in the river, combined with intense oxygen removal by bacterial degradation of organic matter, oxygen concentrations in the river vary dramatically over a diel cycle.

Sampling

Cariaco Basin

Samples from the Cariaco Basin were collected during four cruises: CAR 175 (6-7 Dec 2010), CAR 180 (6 -7 May 2011), CAR 186 (10-11 Nov 2011) and CAR 191 (11-12 May 2012) on the B/O Hermano Gińes as a part of the international CARIACO Biogeochemical Time-Series program. Data links can be found at the project website: http://www.imars.usf.edu/CAR. Details of sampling strategies are provided in other CARIACO publications such as Scranton et al. (2001), Percy et al. (2007) and Li et al. (2008).

Forge River

Water samples were taken at the mouth of Wills Creek from the Brookhaven Town Dock (approximately Station 2 in Fig. 2) for most of a light cycle beginning before dawn on July 8, and August 1, 2011. In both samplings, we started around 7:00 and ended by 17:00. Sunrise was 5: 27 and 5:48 and sunset at 8: 24 and 8:46 on these dates, respectively. The measurements on July 8 used surface water from 20 cm below the surface and near bottom water collected 20 cm above the sediment. The measurements on August 1 used near bottom water which was collected 10 cm above the sediment and surface water collected about 60 cm above the deeper sample. Total water depth ranged from 1.1 to 1.8 m over a tidal cycle.

On both dates, a Masterflex peristaltic pump equipped with Masterflex tubing was used to pump water continuously onto the dock. The speed of the pump was adjusted to avoid bubbles in the tube. The residence time of water samples in the tubing was about 30 seconds. The tubing was labeled with depth markers and attached to a pole secured to the dock. Sampling occurred approximately every 2 hours at the two depths (near surface and near bottom). The temperature was measured with a mercury thermometer in water flowing from the tubing. Salinity was measured by refractometer.

Samples were collected at each sampling time for sulfide, $S_2O_3^{2-}$, SO_3^{2-}, elemental sulfur and total zero-valent sulfur. A 60 ml syringe held on a ring stand was connected to the pump tubing. Triplicate samples for sulfide and for $S_2O_3^{2-}$, SO_3^{2-} were taken from continuously flowing water using a 10 ml gas tight syringe. The total zero-valent sulfur samples were collected in duplicate 40 ml sub-samples by using a gas tight BD syringe in the same way as sulfide samples and fixed with two ml 2% (w/v) zinc acetate (Zopfi et al., 2001; Li et al. 2008). With this pretreatment, this method determines the sum of particulate sulfur, colloidal sulfur and the sulfane fraction of polysulfides (Li et al.2008).

To collect elemental sulfur samples, which required filtering larger volume of water, a Niskin bottle was filled using the pump tubing which extended to the bottom of the bottle. The Niskin was filled to overflowing; then samples (in duplicate) were drawn by gravity filtration through a GF/F prefilter and a 0.2 μM polycarbonate filter using a 47 mm inline filter holder. The volume of water filtered was measured by graduated cylinder (Scranton et al. 2001; Li et al. 2008).

Sulfide samples were analyzed with the methylene blue method (Cline, 1969). This method measures total sulfide species ($H_2S + HS^- + S^{2-}$ + reactive polysulfides) and thus throughout this paper, H_2S is equivalent to total hydrogen sulfide. Sulfite and thiosulfate were analyzed using the DTNP method as used by Hayes et al. (2006) which was modified from Vairavamurthy and Mopper (1990). Particulate sulfur and total zero valent sulfur were analyzed with a modification of the methods of Heneken et al. (1997) and Li et al. (2008).

For dissolved metal (Mn and Fe) analysis, syringes and sample vials first were acid washed using trace metal clean acid and MilliQ water in a trace metal clean lab and dried in a laminar hood. Once dried, syringes and vials were stored in clean Ziploc bags which were stored in larger Ziploc bags. In the field, samples were collected in syringes by two analysts using polyvinyl gloves and taking precautions to avoid touching any surfaces to limit trace metal contamination. Syringes were pre-rinsed with sample, and samples for metal analysis were filtered using 0.45 μm PES syringe filters directly into in acid-washed polypropylene vials. Upon return to Stony Brook, 60 μl of trace metal clean, concentrated HCl was added to samples which were stored for about a month prior to analysis. Trace metals were then analyzed on Graphite Furnace/ Flame Atomic Adsorption Spectrometer. Details of the method were described in Percy et al. (2007).

Incubation experiments

Cariaco Basin

We also performed a few incubation experiments to determine the oxidized sulfur products in response to injection of possible oxidants (oxygen, FeOOH). Water was taken from three depths (260, 285 and 385m) during CAR 191 for oxidant-spiking and dark incubation. The first two depths were just below the suboxic zone, where sulfide concentration ranged from 7-10 μM and particulate elemental sulfur and dark CO_2 fixation are usually at a maximum. The bottom depth was deeper in the sulfidic zone, where sulfide concentration was 30 μM and both particulate elemental sulfur and dark carbon fixation are usually undetectable.

Water was collected from Niskin bottles into 200 ml glass vials for incubation in the dark at surface temperature (27^{o}C). At each depth, each of 15 vials was subjected to one of the following (in triplicate): non-treatment (control), injection of 1 ml air, injection of combination of 1 ml air and 1ml chloroform, injection of a suspension of 5 µM of FeOOH, and injection of FeOOH suspension and chloroform. The iron suspension was formed immediately prior to the cruise by addition of NaOH into $FeCl_3$ solution (pH=7). After 30 hours of incubation, all vials were sacrificed to collect H_2S, TZVS, SO_3^{-2}, and $S_2O_3^{-2}$.

Water from 260, 285 and 385 m with same treatments were incubated with ^{14}C-bicarbonate ($H^{14}CO_3^-$) as previously described by Taylor et al. (2001) to investigate the effects of possible oxidants on dark carbon fixation.

Forge River

A series of incubations were done with Forge River water to try to identify the relative importance of biological and chemical oxidation under sulfide and oxygen limiting conditions (different ratios of sulfide to oxygen). Incubations were carried out at three different temperatures (2^{o}C, 25ºC (ambient) and 40^{o}C) and at two H_2S/O_2 ratios. The effect of iron and manganese on the rate of sulfide oxidation and on the products of sulfide oxidation was not assessed experimentally but concentrations of the metals were measured so oxidation rates could be compared to calculated rates using literature values for oxidation rate constants.

Subsurface water was collected for incubations from the Forge River in the early morning of July 29, 2012, when water typically has been most sulfidic. Water for incubation was first filtered through 20 µm Nitex mesh. Thus the incubated water was quite heterogeneous, and retained the various types of material present in the natural sample. O_2 and temperature were measured using a YSI ProODO electrode. pH was measured using a Orion pH electrode. Samples for determination of initial H_2S, total zero-valent sulfur and dissolved and total trace metal were also collected.

Total suspended matter was assayed by filtering 100 ml of water immediately after collection through a preweighed 0.7µm GF/F filter. The loaded filter was rinsed with distilled water to remove salt and was stored in a petri dish in the freezer. Later, filters were dried in an oven at 105^{0}C for one hour and reweighed to determine the weight of total suspended matter. The weight percent of organic matter was determined by loss on ignition. The dry filter was combusted at

450^0C for 2 hours. Weight loss is typically assumed to be 50% organic carbon. The remaining mass consists of particulate inorganic matter; in this area it is mostly clays and metal oxides.

As mentioned above, water for incubation was filtered through 20 μm pore size Nitex mesh cloth to remove the largest particles. Filtration through 20 μm pore size filters makes incubation water more homogeneous and will remove a portion of the terrigenous sediment but will retain smaller phytoplankton cells and some of the terrigenous material whose surface could stimulate H_2S oxidation. A liter of this filtrate was then filtered through a GF/F glass fiber filter to obtain a specific measure of the total suspended matter of the water to be incubated. Before use, the pooled filtered water was kept at about 3^oC in a cooler filled with ice.

Prior to the start of incubation at each temperature, 20 μm filtered Forge River water in a glass bottle was purged with a mixture of air/nitrogen to get the desired initial oxygen concentration (from 20- 60 μM O_2). pH was measured before and after bubbling to identify any change of pH due to removal of CO_2 from the incubation water. The initial pH of water was 7.5 and after bubbling the pH increased to 7.6.

A sulfide solution was prepared by dissolving $Na_2S.9H_2O$ in oxygen–free water. An aliquot of this solution was added to the bottle of pre-filtered, bubbled water to obtain the experimental initial H_2S concentration. After bubbling and sulfide spiking, water was transferred under argon into forty-five 125 ml Erlenmeyer flasks for incubation at assigned temperatures. A green-butyl low-sulfur rubber stopper was inserted into each flask in such a way to eliminate a headspace.

Initial concentrations of H_2S, O_2, SO_3^{-2}, $S_2O_3^{-2}$, and total zero-valent sulfur in the incubation water were determined after the bubbling and before sulfide addition and transfer to the 125 ml flasks. A subsample of water of each temperature and each H_2S/O_2 ratio also was collected into triplicate glass vials to measure microbial rates of dark carbon assimilation (using $H^{14}CO_3^-$ uptake) and heterotrophic production (using ^{3}H-leucine incorporation).

For the first experiment, the initial H_2S/O_2 ratio was 0.7, with H_2S and O_2 concentrations of 43 μM and 60 μM, respectively. This manipulation should mean the samples will be sulfide limited by the end of the incubation. For the second experiment, the initial H_2S/O_2 ratio was 2.8, with sulfide and O_2 concentrations of 73 μM and 25 μM, respectively, resulting in oxygen limitation by the end of incubation.

Incubations were done in the dark at three temperatures: 2^oC in a cooler containing ice, 25^oC in thermo-statically controlled water bath, and 40^oC in an incubator. Water was brought to the

desired temperature prior to the sacrifice of the first (time = 0) sample. This took from 15 to 60 minutes, and explains why the "initial" concentrations plotted in Fig.10 are different from the time zero concentrations. For incubations done at 25ºC and 40ºC flasks were continuously shaken on oscillatory shakers. For incubation at 2ºC, flasks were periodically shaken in a swirling motion by hand. Fifteen flasks were incubated at each temperature. At each time interval, triplicate flasks were sacrificed to monitor the disappearance of H_2S and O_2, the formation of sulfite, thiosulfate and total zero-valent sulfur, and the variation in pH.

Results

Cariaco data

Profiles of sulfur intermediates

In the Cariaco Basin, the suboxic zone is operationally defined as lying between the first depth where oxygen values were ≤ 2-3 µM and the first depth where H_2S values were ≥2-3 µM (Li et al., 2008). The estimates of the top and bottom of the suboxic zone are represented by the dashed lines in Fig. 3. The depth and position of the suboxic zone varied among cruises and indicated the dynamic nature of geochemical fluxes within the Cariaco Basin (Scranton et al., 1998, 2006; Percy et al., 2007).

Sulfite and thiosulfate were present in low concentrations in the suboxic zone but covaried throughout both suboxic and sulfidic water. Their concentrations tended to be higher in the upper sulfidic water. Kinetic calculation for a mixing of sea water with 250 µM O_2 and 60 µM sulfide by Li et al (2008) suggested sulfite and thiosulfate formation in upper sulfidic water did not result from artifacts during sample processing.

Particulate elemental sulfur was usually observed in the zone where the concentration of H_2S was greater than 1 µM, extending to the depth where H_2S around 10 µM. The zone of maximum elemental sulfur concentrations coincided with that of chemoautotrophic production (Taylor personal communication). Over the four cruises, S^0 concentrations reached values of 1 to 1.5 µM.

Spiking experiments

In order to distinguish the effects of oxygen and FeOOH on sulfur intermediate formation and the relationship between chemoautotrophy and elemental sulfur formation, we carried out several incubation experiments using CARIACO water during CAR191 (Fig. 4).

Sulfur intermediate distributions from incubation at three water depths in the Cariaco Basin are presented in Fig. 4. The controls at 260m and 280 m contained mainly total zero-valent sulfur while the control at 385m contained mainly sulfite and thiosulfate. Incubations using water collected at 260 and 285 m yielded the same sulfur intermediate distribution pattern: zero-valent sulfur was present in all treatments, sulfite was at trace concentration(<0.1µM) and thiosulfate concentrations were detectable only in treatments where chloroform was present. Incubation of 385 m water yielded measurable sulfite and thiosulfate in all treatments, but zero-valent sulfur was undetectable in the control incubation and present in only in trace concentrations in other treatments (Fig. 4).

Near the oxic/anoxic interface (260 m) where dark carbon fixation was at a maximum, the dark carbon fixation rate was stimulated by 40% by FeOOH (Fig.5). However, water from 285 m (also in the dark carbon fixation maximum) showed no significant stimulation with FeOOH. The addition of air, the combination of air and FeOOH or air and chloroform reduced dark carbon fixation by 30%, 20-40% and up to 95% respectively in incubations of water from both 260 and 285 m. For the deep water incubation (385m), the in situ dark carbon fixation rate was very low, and none of treatments significantly stimulated the carbon fixation rate.

Forge River data

Distribution of sulfur intermediates over a diel cycle

Two attempts were made to monitor conditions in the Forge River over a diel cycle. On 8 July 2011, the weather was cloudy almost the entire day. We began sampling at 0730 when the tide was falling. According to NOAA (http://tidesandcurrents.noaa.gov/ using Sandy Hook, NJ Station Id: 8531680 with correction time from the report of Swanson et al. (2009)) the morning low tide was at 0923 and the afternoon high tide was at 1513. The samples were collected over a period of 12 hours for near surface water (20 cm below the surface) and near bottom water (10 cm above the sediment). Salinity varied from 15 to 22 and temperature from 25- 27^0C over the sampling period. Unfortunately, I don't have enough data of salinity and temperature to plot their variation over a diel cycle. The variation of H_2S, O_2 and sulfur species collected for the Forge River on this date is shown in Fig. 6. While the surface water oxygen increased from 70 µM at dawn to 200 µM by 17:00, the near bottom water oxygen remained low (< 3 µM) the entire sampling period. In near bottom water, sulfide concentration increased from 13 µM in the

morning to 110 µM at noon and but dramatically declined to 7 µM 2 hours later. This dramatic changes in near bottom water sulfide concentration coincided with rising of tide when water mixing or sediment disturbance was likely to occur. Hydrogen sulfide concentration in the surface water was measured only twice and was about 1 µM both in early morning and at 16:00. Sulfite and thiosulfate were undetectable in surface water, while in the near bottom water, sulfite was constant at about 3 µM, while thiosulfate increased from 0.6 µM to 2 µM in the afternoon.

Particulate elemental sulfur was undetectable in the surface water, but particulate elemental sulfur varied from 6 µM to a maximum value of 16 µM in the near bottom water. Total zero-valent sulfur in the surface was about 2 µM, which is similar to the surface H_2S concentration. In the near bottom water, TZVS showed the same trend as particulate sulfur. Since the TZVS represents elemental sulfur in all forms, one might expect that TZVS should be equal to or greater than particulate S. In the surface water particulate S is zero but TZVS is relatively high implying significant quantities of polysulfides. In near bottom water TZVS is roughly equal to particulate S implying fewer polysulfides.

Dissolved Fe and Mn in near bottom water were constant over the sampling period with dissolved Mn concentrations of 5-6 µM and iron concentrations of 2-3 µM (Fig.7).

On August 2012, I repeated this experiment, beginning sampling at 0700 when the tide was rising. High tide was at 1036 and the low tide was at 1709. Samples were collected roughly every 2 hours over a period of 12 hours. T and S data are presented in table 7. The variation of H_2S, O_2 and sulfur species on this date is presented in Fig. 8.

The co-existence of H_2S and O_2 in the water column was observed in the Forge River over the sampling period. Oxygen increased from 90 µM at dawn to 250 µM by late afternoon near the surface and from about 90 µM to 150 µM near the bottom. H_2S concentration increased gradually from 5 to 13 µM in near bottom water over the day, while in near surface water the H_2S concentration was as low as 2 µM in the morning but abruptly increased to 8 µM shortly after the time of high tide and remained at this level till the end of sampling.

Concentrations of sulfite and thiosulfate in near surface water were comparable to those in near bottom water, and the sulfite concentration was 4-6 times higher than thiosulfate in both surface and near bottom waters. Sulfite and thiosulfate showed the same trends in both water depths with higher concentrations in the morning than in the afternoon. Sulfite exhibited a sharp

peak (4 µM) at high tide in both surface and near bottom waters. By the end of sampling, sulfite declined to < 1µM and thiosulfate was reduced to trace concentrations (<0.1 µM).

In the morning, the near bottom water particulate elemental sulfur concentration was less than 1µM and surface water particulate elemental sulfur was undetectable. As the tide started falling around 1030, particulate sulfur concentration started increasing at both water depths. Later in the day, particulate sulfur in near bottom water increased to values as high as 6 µM and became the predominant sulfur species, but particulate sulfur in near surface water was lower (2 µM). Total zero-valent sulfur showed the same trend as H_2S and particulate elemental sulfur in both near surface water and near bottom water, although TZVS tended to be about 1 µM higher than particulate S.

Dissolved Fe and Mn in near bottom water and surface water were similar and showed the same pattern as seen in July with manganese concentrations being higher than iron, although the concentrations of both dissolved metals was slightly lower in August than in July (Fig. 9).

Dark incubation of Forge River water

Water from the Forge River used for incubation contained 25.5 mg l^{-1} total suspended matter (TSM). Based on loss on ignition, 90% of the TSM was organic matter (although since the filter was not pre-combusted, LOI was overestimated as there is organic matter on the silicate filter in the box). Thus 10% of the TSM was made up of particles which could be metal oxides or clays whose surface could be reaction sites for H_2S oxidation. pH values changed slightly over the course of incubation (data not shown) with the range in a series of incubation from 7.6 - 8. Dissolved Fe and Mn were 0.75 µM and 3.5µM respectively.

Unlike the other incubations, the incubation at 25^0C with starting condition of severe oxygen limitation (H_2S/O_2 ratio 2.8) showed an initial drop in H_2S and then a reappearance of H_2S (Fig. 10d). This phenomenon was not observed at other temperatures or for an H_2S/O_2 initial ratio of incubation of 0.7 where oxygen was not limiting (Fig.10 a, b, c). As the incubation proceeded, thiosulfate concentrations significantly increased in all series of incubations (Fig.10, Table 5). The sulfite concentration was mostly either constant or decreased with time. However, at 2^oC when a considerable amount of H_2S was still available in solution, sulfite concentration increased significantly with time (Fig.10 a and d, Table 5). By the end of the incubation, for the incubations which initially had an H_2S/O_2 ratio of 0.7, both sulfite and thiosulfate were very low

at all three temperatures. For incubations with initial H_2S/O_2 ratios of 2.8, the complete disappearance of sulfite was observed at 40^oC.

The first time point was collected after the water samples were brought to the desired temperature. Since the water did not initially contain sulfur intermediates, the products of a mixture of sulfur intermediates (S^0, $S_2O_3^{2-}$, SO_3^{2-}) seen at the first time point were produced within the time it took to bring the samples to the desired temperature (Fig.10). As the incubation proceeded, thiosulfate concentration increased (Fig.10, Table 5), while the sulfite concentration was mostly either constant or decreased with time except in the 2^0C incubation when a considerable amount of H_2S was still available in solution (Fig.10, Table 5). At the end of the incubation, for the initial ratio of H_2S to O_2 of 0.7, both sulfite and thiosulfate were lost at all three temperatures, while at an initial ratio of H_2S to O_2 of 2.8, the loss of sulfite was observed only at 40^oC incubation. TZVS was the dominant sulfur intermediate product in all incubations with higher concentration observed at the higher initial ratio of H_2S to O_2. TZVS also persisted until the end of incubation at all incubation temperatures (Fig. 10).Within the same starting concentrations of H_2S/O_2, the zero-valent sulfur distribution was not clearly different at the three different temperatures even though a sharp peak was observed at 40^0C.

Dark carbon fixation was very low at 2^0C and slightly higher at 40^0C and showed a maximum at 25^0C with both initial ratios of H_2S/O_2 (Fig. 11). The carbon fixation rate under more oxygen limiting conditions at 25^0C was lower than that with more oxygen in the solution. Bacterial net production had the same trend as did the dark carbon fixation rate for the two sulfide oxygen ratios. With relatively more oxygen present, bacterial net production was lower at 2^0C but was similar at 25^0C and 40^0C to values measured with less oxygen.

Discussion

My goal was to determine the proportion of abiotic and biotic oxidation of reduced sulfur to sulfur products in the Forge River. I initially hypothesized that the pattern of sulfur intermediates in the Forge River over the light period would be controlled by the relative importance of chemical and biological sulfide oxidation, and the different ratios of H_2S/O_2 and the incubation experiments were chosen to test this. I suggested that while the chemical oxidation of H_2S would yield SO_3^{-2} and $S_2O_3^{-2}$ as products, elemental sulfur would be the main product of biological oxidation. I also hypothesized that FeOOH and MnO_2 might play an important role by reacting

with sulfidic water to produce zero-valent sulfur (Chen and Morris 1972; Zhang and Millero 1993; Yao and Millero 1993, 1996; Zopfi et al. 2001; Jost et al. 2010).

In Cariaco, the nature of detailed controls on sulfur intermediates distribution, such as the possible oxidants of sulfide in anoxic water and the role of chemoautotrophic and possibly sulfur oxidizing bacteria on H_2S oxidation and sulfur intermediate distribution was unclear. Thus, a comparison between the pattern of sulfur intermediates in the Forge River and in the suboxic zone in the Cariaco Basin also could give a greater understanding about the pattern of sulfur intermediates in systems where a pulse of oxygen is added to sulfidic waters.

Dark incubation at different temperatures and initial ratios of H_2S/O_2 in the Forge River

Comparison of predicted chemical oxidation and observed oxidation rate of H_2S

In our incubation experiments at all three temperatures and at both high and low initial H_2S/O_2 ratios, hydrogen sulfide concentrations decreased rapidly with time (Fig. 10). The observed sulfide oxidation rate can be calculated from the slope of the first two time points from the plot of $[H_2S]$ vs time.

The chemical rate of H_2S oxidation can be predicted (Table 2) according to a simple rate law, assuming it is dependent only on initial sulfide and oxygen concentrations (Zhang and Millero 1993):

$$\frac{d[H2S]}{dt} = k[H2S][O2] \quad (1)$$

Here k ($M^{-1}\,h^{-1}$) is the rate constant, H_2S is the concentration of total hydrogen sulfide species, and O_2 is the oxygen concentration. The rate constant k can be calculated from the equation given by Millero (1987):

$$\log k = 10.50 + 0.16pH - (3.0 \times 10^3)/T + 0.44I^{1/2} \quad (2)$$

where T is the absolute temperature in ^oK, pH is the measured pH and I is ionic strength is a measure of the concentration of ions in that solution (mol L^{-1}). The effect of pH on the rate of H_2S oxidation is mainly through its effect on chemical speciation which is related to acid-base equilibrium: $H_2S = HS^- + H^+$. The fractions of H_2S and HS^- can be calculated using the dissociation constant of Millero et al. (1988). For a pH range from 7.6 to 8 (observed in the Forge) HS^- is the predominant sulfide species (85-90%) in the incubation solution.

The observed rate of oxidation was compared to the theoretical rate by calculating the ratio of observed rate to predicted chemical rate based on laboratory experiments (Zhang and Millero

et al., 1993; Table 2). The observed rate for our experiments was 40-100 times higher than the theoretical rate for an initial H_2S/O_2 ratio of 0.7 and from 100 to 200 times higher than the theoretical rate for an initial H_2S/O_2 ratio of 2.8.

Since the presence of trace metals at concentrations greater than 100 nmol l^{-1} has been shown to increase the rate of sulfide oxidation in seawater, and iron is the most effective catalyst (Vazquez et al. 1989; Zhang and Millero 1993), it is to be expected that the presence of trace metals in Forge River incubation water should catalyze H_2S oxidation. The reaction rate is predicted to be enhanced 40 fold by Fe^{2+} (at 0.75 µM), enhanced 5 fold by particulate iron oxide (0.5µM) and enhanced 1.5 fold by Mn^{2+} (3.5 µM), based on the equation :

$$\log (k/k_o) = a + b \log [M] \quad (3)$$

where k_o is the rate constant without metal, [M] is the concentration of the metal in solution, and "a" and "b" are constants dependent on the metal. Vazquez et al. (1989) reported that a = 1.68, 6.55 and 5.18 and b = 0.28, 0.82 and 0.72 for Fe (II), Fe (III) and Mn (II) respectively.

The increase in rate (Table 2) is in reasonable agreement with the effect of trace metal presence in solution for an initial ratio H_2S/O_2 ratio of 0.7 but higher for an initial ratio H_2S/O_2 ratio of 2.8. This is probably due to the fact that the trace metal concentrations that I used to fit to equation (3) were collected from water taken before bubbling (and unfortunately we did not have trace metal data after bubbling). These values may not actually reflect their concentrations for incubations under two different initial ratios of H_2S/O_2 especially for incubation under oxygen limitation where concentration of dissolved iron is likely to increase in the solution (Caroll et al. 2002). In July 2011 (Fig.7) under anoxic condition, dissolved Fe in near bottom water was 3 µM which would accelerate the H_2S oxidation rate up to 100 times. Thus, the comparison suggests the importance of trace metal as a catalyst to sulfide oxidation in this incubation.

Activation energy calculation E_a

The effect of temperature on oxidation of H_2S in the Forge River water (S = 20) at pH = 7.8 is shown in Table 2. The measured rate of H_2S disappearance was lowest at 2°C, intermediate at 25°C and maximum at 40°C (Table 2). The activation energy E_a for H_2S oxidation with molecular oxygen in Forge River incubation can be calculated using an Arrhenius plot and this can be compared to values from the literature for pure chemical reactions without trace metal and biological components. The logarithmic form of the Arrhenius equation is:

$$\ln \quad k = -\frac{E_a}{RT} + \ln \quad A \quad (4)$$

where k is the rate constant, A is the exponential pre-factor or frequency factor, E_a is the activation energy, R is the ideal gas constant (8.3145 J $K^{-1}mol^{-1}$) and T is the temperature (K). Plotting ln (k) versus 1/T allows the calculation of activation energy by multiplying the slope with R (Fig. 11). Plots for different experiments yielded slopes of -5,000±96.5 (K) and -4,350±182.3 (K) for low and high initial ratios of sulfide to oxygen and thus the relevant activation energies are 42 ± 0.7 and 36 ± 1.5 kJ mol^{-1}, respectively. These values are slightly lower than the values of 66 ± 5 kJ mol^{-1} for oxidation of H_2S in air saturated seawater obtained by Millero et al. (1986). The lower values of E_a are consistent with the importance of trace metals as they would catalyze sulfide oxidation and lower the activation energy. On the other hand, my values are higher than the values of 14 kJ mol^{-1} and 4.5 kJ mol^{-1} for the oxidation of H_2S by manganese dioxide (Yao and Millero, 1993) and hydrous Fe(III) oxides (Yao and Millero,1996) possibly due to the presence of more complex mineral phases. This would be consistent with the composition of Forge River suspended matter water which contains 90% organic matter and 10% inorganic particles.

Q_{10} calculation

While chemical reaction rates will simply increase in proportion to increasing temperature, an enzyme-catalyzed reaction is expected to decline above the optimum temperature for the enzyme. To compare the temperature responses of bacterial net production, chemoautotrophy and H_2S oxidation in dark incubations, we used the Q_{10} values (temperature coefficient) to assess temperature dependence. Basically, the Q_{10} can be used to calculate the rate change in the incubation as the result of changing temperature. Q_{10} is calculated as:

$$Q_{10} = \left(\frac{R_2}{R_1}\right)^{10/(T_2 - T_1)} \quad (5)$$

where T is the temperature in degrees Celsius, R_1 and R_2 are the reaction rates at T_1 and T_2, and Q_{10} is a unitless ratio. It is generally accepted that the reaction rate of reactions mediated by microorganisms will typically increase by a factor of 2-3 for each 10^oC increase in temperature (Morita 1974). However it should be noted that Q_{10} is not necessarily constant over the range of temperature or over different microbial and biochemical processes (Apple et al. 2006; Abed et al. 2006). Since the Forge River water contains different groups of microbial communities

(phototrophic sulfur bacteria, chemoautotrophic sulfur bacteria, heterotrophic bacteria), the response to temperature changes might be complex.

The calculated values of Q_{10} for dark carbon fixation, bacterial net production and for H_2S oxidation for our dark incubation experiments are shown in Table 3. In my experiment, increasing the reaction temperature resulted in increases in H_2S oxidation rate, with Q_{10} values from 1.5-1.8 for two initial ratios of H_2S/O_2. This is consistent with a chemical reaction pattern.

The Q_{10} calculated for chemoautotrophy was between 1.7 and 3.0 between 2 and 25^{o}C but was less than 1 between 25 and 40^{o}C. In the range of 2 to 25^{o}C, the Q_{10} for heterotrophic bacteria was between 2.8 and 3 for both initial ratios of H_2S/O_2. However, in the range of 25 to 40^{o}C, Q_{10} was 1 if sulfide was limiting, but was less than 1 under the condition of limiting oxygen. It appears that above 25^{o}C cells are stressed and shutting down, Q_{10} becomes irrelevant in the 25-40^{0}C range. 40^{0}C is probably lethal to many Forge River microbes. Overall, this may suggest that H_2S oxidation in our dark incubation was largely abiotic.

Potential contributions of chemoautotrophy to sulfide oxidation

Jørgensen et al. (1991) suggested that 7 to 9 moles of H_2S are required to assimilate 1 mole of CO_2 by chemoautotrophy. Using carbon fixation rates measured by Elizabeth Suter during my Forge River experiments (Fig. 11), assuming all chemoautotrophy is associated with sulfide oxidation, and assuming 9 mole of H_2S were oxidized during fixation of one mole of carbon, we would predict that sulfide oxidation rates from dark carbon fixation in the Forge River water incubations would be on the order of 33 μM d^{-1} at 25^{0}C. The observed rate of H_2S oxidation is much higher (50 – 300 times) than the predicted rates (Table 4**)**, and consistent with the Q_{10} calculations, imply that dark carbon fixation is likely not the primary mechanism for sulfide oxidation in this system. There are other species of microbes whose metabolism is not similar to that studied by Jørgensen but may be involved in sulfide oxidation (aerobic sulfide oxidizers, anoxygenic photoautotrophs). However, it is tempting to speculate that abiotic oxidation catalyzed by metal oxides was responsible for more than 90 % of the H_2S oxidation.

Intermediate oxidation state products of sulfide oxidation in the incubations

In the present study, the initial ratio of H_2S/O_2 was adjusted to yield low (0.7) and high (2.8) values. These ratios were intended to represent sulfide and oxygen limiting conditions. In a

sulfide – oxidizing reactor with a mixed culture of *Thiobacilli*, Janssen et al (1995) found that at a ratio of H_2S/O_2 less than 0.7, sulfate and elemental sulfur were formed as end products, while under severe oxygen limiting conditions (H_2S/O_2 greater than 2), thiosulfate was abundantly formed. The formation of thiosulfate was due to chemical sulfide oxidation which is more important than biological oxidation under highly oxygen-limited conditions (Janssen et al. 1995). However, in continuous culture of *Thiobacillus thioparus* T5, a considerable amount of thiosulfate (17%) was observed *in vivo,* and 75% of H_2S oxidized under oxygen-limiting conditions (H_2S/O_2 greater than 2) went to zero-valent sulfur (Van de Ende et al. 1993). When more oxygen was present (H_2S/O_2 less than 0.7) sulfide was mainly oxidized by *Thiobacillus thioparus* T5 into sulfate with a minor amount of elemental sulfur (Van de Ende et al. 1993). In a co-culture of *Thiobacillus thioparus* T5 and *Thiocapsa roseopersicina* M1, Van de Ende et al. (1996) reported the products from H_2S oxidation by *Thiobacillus thioparus* T5 were mainly sulfate (96%) and a minor amount of zero valent sulfur (4%) under the condition of H_2S/O_2 less than 0.7. As oxygen availability decreased (H_2S/O_2 greater than 1.5), sulfur oxidation products changed from primarily sulfate (96% of oxidized H_2S) to increasing concentrations of zero-valent sulfur (up to 26% of oxidized sulfide) but sulfate remained the main product of sulfide oxidation.

In other studies, elemental sulfur was an important intermediate of sulfide oxidation by *Desulfobulbus propionious* and by green phototrophic sulfur bacteria in the Black Sea, by chemolithotrophy in Mariager Fjord, and was postulated to be important in Cariaco Basin (Jorgensen et al. 1991; Fuseler and Cypionka ,1995; Zopfi et al. 2001; Taylor et al. 2001; Li et al. 2008).

The pattern of sulfur species products in my incubation of Forge River did not correspond to the patterns seen by others who varied initial H_2S/O_2 ratios in culture studies. A calculation of the sulfur balance in my experiments (Table 1) suggests that the majority of sulfide was oxidized to a form not measured here (likely sulfate), although a variety of intermediates (S^0, $S_2O_3^{2-}$, and SO_3^{2-}) made up to 15% of the products. In my incubations of Forge River water, I found an initial increase in sulfite followed by relatively constant or slightly decreasing concentrations. For thiosulfate, the initial increase was followed by a continued increase, resulting in a ratio of thiosulfate to sulfite of 1.5 -2. These patterns are in good agreement with chemical studies of H_2S oxidation by molecular oxygen with and without presence of metal (Chen and Morris 1972;

O'Brien and Birkner 1977; Zhang and Millero 1993). In the prior studies, which were done in abiotic systems, thiosulfate was a stable product and predominated even with the presence of trace metal (Zhang and Millero 1993). In my incubations, thiosulfate formation was observed for both low and high starting ratios of H_2S/O_2 indicating its independence on possible reaction pathways present as the result of changing H_2S/O_2 conditions. That would suggest thiosulfate formation was more likely of abiotic origin.

Zero-valent sulfur (ZVS) formation for the high initial ratio of H_2S/O_2 was about 1.5- 2 times higher than that for low initial ratio ofH_2S/O_2, reaching about 10% of sulfide oxidized under oxygen limiting conditions. This is lower than the 26% - 75% of ZVS formed during sulfide oxidation by sulfur bacteria as observed by Van de Ende et al (1993, 1996). In the present study under conditions of both relatively high and low oxygen, ZVS was the predominant product at all incubation temperatures including at 2^0C where biological activities as measured by chemoautotrophic production and bacterial net production were the lowest. This would suggest that microbial activity may be relatively unimportant in controlling elemental sulfur formation in the present incubations (Fig. 10).

Our results are consistent with other studies in which the formation of ZVS was found to be an important product of chemical oxidation of H_2S by oxygen in the presence of trace metal as catalyst (Millero et al. 1990; Luther et al. 1991; Jørgensen et al. 1991). This is also consistent with the observation of lower activation energy and increased rate of H_2S oxidation in our experiment which was probably due to presence of metal catalyst. The independence of ZVS on incubation temperature supports our conclusion that its formation in Forge River incubations was likely of an abiotic origin since if ZVS was biologically formed, we would expect ZVS concentration at 25^0C would be much higher than at 2 and 40^0C. The increase in TZVS under higher initial ratio of H_2S/O_2 (oxygen-limiting) in our experiment was thus probably due to the fact that there was twice as much H_2S in the flasks with the higher ratio of H_2S/O_2 rather than because of enhanced biological activity under oxygen limited conditions.

Spiking experiment using Cariaco Basin water

Two experiments were conducted using Cariaco Basin waters to investigate the formation of sulfur intermediates in response to oxidant stimulation and the role of chemoautotrophy in elemental sulfur formation for waters in and below the suboxic zones (Li et al. 2010). Data are

shown in Fig. 4. In the controls (water incubated in vials without headspace or oxidant addition) the primary intermediate oxidation state sulfur compound observed was zero-valent sulfur. Zero valent sulfur also was an important product at 260 and 285 m in all treatments (O_2, FeOOH and combination of each oxidant with chloroform). Chemoautotrophy was inhibited by the addition of oxygen and chloroform but not by addition of iron (Fig. 5). Since particulate S was produced in all treatments, but chemoautotrophy was suppressed in the presence of chloroform, chemoautotrophy did not seem to be important to particulate sulfur formation following oxidant addition, but that, instead, chemical oxidation was likely to be most important. This finding is consistent with the previous study of Li et al. (2010) which suggested a more important role of chemical sulfide oxidation than biological oxidation within the suboxic zone in Cariaco water column. A similar observation was made in Mariager Fjord where an increase in the proportion of chemical sulfide oxidation was seen under conditions where there was mixing of oxic and sulfidic water masses (Zopfi et al. 2001).

Results for the other sulfur intermediates were somewhat different from particulate S. In the controls, thiosulfate was about 0.6 μM at 260 m and very low (0.1 μM) at 285 m. Thiosulfate was only observed in treatments at 260 and 285 m that included the combination of an oxidant (O_2 or FeOOH) and chloroform, situations in which dark carbon fixation was strongly inhibited. The inhibition of thiosulfate oxidizing bacteria by the chloroform could explain the fact that thiosulfate produced by chemical oxidation of sulfide accumulated in these treatments. This is consistent with the finding that thiosulfate was an important substrate for chemoautotrophs (Thamdrup et al. 1994). Low thiosulfate concentrations are frequently seen in the suboxic/oxic transition zone in Cariaco and this may similarly reflect efficient consumption by chemoautotrophic bacteria (Taylor et al. 2001; Hayes et al. 2006; Li et al. 2008). I do not believe that thiosulfate was an artifact of chemical reaction between H_2S and chloroform under low oxygen concentrations, as an experiment in the lab where chloroform was added to water containing sulfide showed no difference in production of thiosulfate between the water with and without addition of chloroform (data not shown).

In the controls, sulfite concentrations were similar to those of thiosulfate at both 260 and 285m. In all treatments at these two depths, sulfite was observed at blank level (< 0.2 μM) in all

treatments. This could be due to the fast kinetics of chemical sulfite oxidation in which sulfite is rapidly oxidized to sulfate in the presence of oxygen and trace metals (Zhang and Millero 1993).

We also did incubations with Cariaco water from 385 m. At this depth, water is permanently anoxic and sulfide concentrations are typically about 30 μM. In the control, sulfite and thiosulfate were main products. In contrast to the importance of particulate sulfur at 260 and 280 m, the predominant sulfur species at 385 m were thiosulfate and sulfite in all treatments (Fig.3). In these same experiments, a trace concentration of zero-valent sulfur was seen in the bottles with oxidant additions indicating its production under a high ratio of H_2S/O_2 (Chen and Morris 1972). Chemoautotrophy was not evident at this depth in any treatment (Fig. 4). That suggests that, at high H_2S/O_2 ratios, oxygen and FeOOH additions will result in sulfite and thiosulfate production. Lack of change in these species in the presence of chloroform is consistent with the hypothesis that bacterial consumption of sulfite and thiosulfate is low at these depths. Previously Li et al. (2008) reported that thiosulfate significantly stimulated carbon fixation in suboxic but not in sulfidic waters.

Comparisons of sulfur species between Cariaco Basin and Forge River

In Cariaco, a strong correlation between sulfite and thiosulfate with a ratio of about 2 for most samples was noted by Hayes et al. (2006), Percy et al. (2007) and Li et al. (2008). Over four more recent cruises (CAR 175, CAR 180, CAR 186, CAR 191), this close relationship was also observed (Table 6). Sulfite was usually 2 fold greater than thiosulfate near the oxic/anoxic interface but the ratio of sulfite to thiosulfate decreased to 1:1 in upper sulfidic water (down to 400 m). A relationship between sulfite and thiosulfate was found not only in the Cariaco Basin suboxic zone but also in the shallow and turbid system of the Forge River. As in the Cariaco Basin, sulfite and thiosulfate in the Forge River were closely correlated (Table 6) although here sulfite concentrations were 4- 6 times greater than thiosulfate concentrations over the sampling period in both near surface and near bottom water. The strong relationship between those two compounds may indicate coupled production or inter-conversion of the two compounds (Thamdrup et al., 1994).

Based on a Pearson Correlation Matrix (Table 5), the sulfite and thiosulfate also were closely correlated with sulfide in both systems. However, in the Cariaco Basin, sulfite and thiosulfate are both positively related to sulfide, but in the Forge River these parameters are negatively

correlated with sulfide (Table 5). That means that in Cariaco, sulfite and thiosulfate increased with depth as sulfide increased (down to 400m) even though oxidants were probably limited (Fig. 3). In contrast in the Forge, higher sulfide was associated with lower sulfite and thiosulfate. The higher values of sulfite and thiosulfate in sulfidic water might be due to the fact that their consumption by bacteria was less efficient at depth compared to that in the oxic/suboxic layer (as observed in the spiking incubation).

I do not believe the association is related to analytical issues as Li et al. (2008) calculated that sample processing is typically very fast relative to chemical conversion of sulfide to the oxidation products. The distribution of sulfite and thiosulfate on our recent cruises (CAR175, 180, 186, and 191) was similar to that seen by Li et al. (2008) during CAR128, CAR132, CAR 139, 145, and 153 (unpublished data) with low concentrations in the suboxic zone and a tendency of increasing values with depth.

In a few Cariaco cruises, there was evidence for oxygen intrusions into the suboxic zone. For these cruises (CAR118, CAR122) Li et al. (2008) found maxima of sulfite and thiosulfate at the oxic/anoxic interface which was not the typical pattern. Based on nutrients and other parameters, we speculate that part of the difference might have been related to the occurrence of an oxygen containing intrusion prior to sampling. Since density increases accompany the introduction of oxygenated water from Caribbean, the calculation of month to month differences in potential density is one way to examine the intrusion effect (Scranton et al. 2001; Percy et al. 2007). Comparison of density profiles between CAR 121, CAR 122 and CAR 128 showed that while there was no evidence of oxygen intrusion immediately before CAR128, deep intrusion (down to 400m) had occurred before CAR 122 (Fig. 13). In CAR 122, maxima of sulfite and thiosulfate were found in both the oxic and suboxic zones. Sulfite and thiosulfate concentrations were much lower at the interface in CAR 128 than in CAR 122 (likely the result of continued oxidation between the two expeditions).

However, it appears that the effect of oxygen intrusion may not always give the same signal. We also saw evidence for an oxygen intrusion in CAR 180 (Fig. 14). A secondary maximum in oxygen was seen at 275 m, and the first appearance of H_2S was 40 m deeper than that seen in CAR 175 (collected 6 months earlier). For CAR 180, the sulfite and thiosulfate concentrations in the suboxic zone were low, unlike in CAR 122 when there was a maximum within suboxic zone. Comparing the intrusion of CAR 122 and CAR 180 suggests that the depth of intrusion and the

relationship between sampling time and the occurrence of an oxygen intrusion need to be considered in accounting for the difference in the sulfur species profiles between any cruises. Unfortunately I could not calculate $\Delta \sigma_\theta$ for CAR 180, since temperature and salinity sensors were new and were not calibrated correctly (Y. Astor, personal communication). However, based on phosphate, oxygen and dissolved Mn^{2+} data it appears that the intrusion depth was at least 310 m (Fig. 13). If that observation is correct, the intrusion before CAR 122 was deeper than that before CAR 180. The deeper intrusion in CAR 122 could have oxidized sulfide down to 400 m where sulfide concentrations usually reach 30 µM, and where one would expect more abundant sulfur intermediate formation than in the suboxic zone. The gradient of sulfur species could cause the upward diffusion of those sulfur species to the suboxic zone from sulfidic water. The H_2S inventory of CAR 122 calculated from the onset of H_2S to 500 m was about 23% smaller than for CAR 128 (representing a loss of hydrogen sulfide greater the standard error of analytical measurement (10-15%)).

In the Forge River, when sulfide increased, sulfite and thiosulfate decreased. Unlike in the Cariaco, the increase in sulfide in the Forge was accompanied by an increase in oxygen which potentially would cause the further oxidation of sulfite and thiosulfate, resulting in their loss in the water column (Fig.8). This was again consistent with the incubation experiments with oxygen in excess and H_2S limiting where the disappearance of both sulfite and thiosulfate was observed.

Particulate sulfur in Cariaco was observed consistently in both suboxic and sulfidic water, and particulate sulfur in Forge River was observed when both oxygen and H_2S were present in water. In both systems, it appears to depend on the ratio of H_2S to O_2 with higher concentrations observed at higher ratios or where oxygen is more limiting (Fig.16).

The persistence of ZVS as well as particulate S in the water columns and in our incubations using Cariaco and Forge River water may imply low turnover and low bioavailability**.** In CAR180, a sharp peak of particulate sulfur coincided with the depth of a phosphate and Mn^{2+} minimum, likely caused by precipitation of a manganese oxide phase during an oxygen intrusion mentioned above (Fig. 14). Thus we may have possible formation of particulate sulfur (elemental S) in the Cariaco water column via a metal oxide reaction pathway. In our incubations, despite a significant stimulation in carbon fixation by suspended FeOOH observed for Cariaco water from 260 m, the corresponding elemental sulfur formation in the FeOOH treatment was comparable to

those in the control (untreated water) and in the treatment combining FeOOH and chloroform This might suggest that oxidation of H_2S by FeOOH in the Cariaco water column was probably not mediated by chemoautotrophs.

In Cariaco, the inventory of elemental sulfur significantly correlated with chemoautotrophy (n=11, r^2=0.7) (Fig.15), suggesting that elemental sulfur might be produced by chemoautotrophy under anoxic condition and stored as sulfur globules. This is consistent with my incubations in which the addition of air and chloroform to sulfidic water inhibited carbon assimilation significantly, whereas the control incubation showed carbon fixation occurred at and below the suboxic zone, the depths where sulfur was seen in the depth profile. It would be useful to observe filters of water from depths where sulfur is detectable to confirm the existence of sulfur globules. Li et al. (2008) explained the close relationship of chemoautotrophy and particulate elemental sulfur by proposing that elemental sulfur was likely to be an important substrate for chemo-autotrophs in Cariaco water column via sulfur disproportionation ($S^0 + H_2O \rightarrow SO_4 + HS^- + 5H^+$). However, they tested the hypothesis of sulfur intermediate disproportionation near the interface in Cariaco water column by examining the stable S isotope composition of the HS^- and suggested that disproportionation was not important. The importance of chemoautotrophy to particulate sulfur formation has also been reported in other anoxic systems such as Mariager Fjord (Denmark), Gotland Basin (Baltic Sea) (Zopfi et al. 2001; Jost et al. 2010).

Recently, Milucka et al. (2012) reported evidence for the formation of zero-valent sulfur as the intermediate product of coupling of anaerobic methane oxidation and sulfate reduction by a consortium of methanotrophic archaea. This could be an alternative explanation for formation of ZVS in anoxic and sulfidic water.

Unlike in Cariaco chemoautotrophy seems unlikely to be important for ZVS formation in the Forge River. Our dark incubation experiment did demonstrate that sulfur formation in the Forge River was likely from chemical oxidation of H_2S with metal oxide catalysts.

Conclusions

The goal of this study was to investigate sulfur products including sulfite, thiosulfate, and zero- valent sulfur in the Forge River and in Cariaco Basin to provide an understanding of the pattern of sulfur intermediates and the relative roles of chemical and biological oxidation of H_2S

on this distribution in systems characterized by intrusion of pulses of oxygen into sulfidic water. The dark incubations at three temperatures (2, 25 and 40^{o}C) and two initial ratios of H_2S/O_2 (0.7 and 2.8) using Forge River water showed that in the Forge, a shallow turbid system containing high levels of trace metal oxidants, the abiotic rates exceeded the biotic sulfide oxidation . The oxidant spiking experiments using possible oxidants (O_2, FeOOH) in Cariaco Basin demonstrated that, sulfide oxidation was more likely chemically driven with presence of oxygen and under anoxic condition, chemoautotrophy was important for particulate elemental S formation. In both systems, sulfur intermediates were likely products of abiotic oxidation of H_2S with the catalysts of metal oxides.

Future research

Hydrographic profiles over a diel cycle are needed to examine the vertical stratification in water column of the sampling station 2 in the Forge River. The profiles also help to provide more understandings on tidal effect on hydrogen sulfide and oxygen fluxes in the Forge River. Further determination of dark incubation at three temperatures using Forge River water filtered through 0.2 µm filters which should remove the biological component is needed to investigate responses of chemical oxidation of H_2S to temperature changes. The concentration of dissolved trace metal under different concentrations of oxygen in initial water, after bubbling and over the course of dark incubation should be determined. This further study is necessary to confirm the effect of dissolved trace metal in acceleration of H_2S oxidation and to sulfur intermediate distribution.

Meanwhile, in the Cariaco, further investigations on the different pathways of H_2S oxidation by possible sulfur oxidizing bacteria are needed to elucidate the relative contributions of biotic and abiotic sulfide oxidation.

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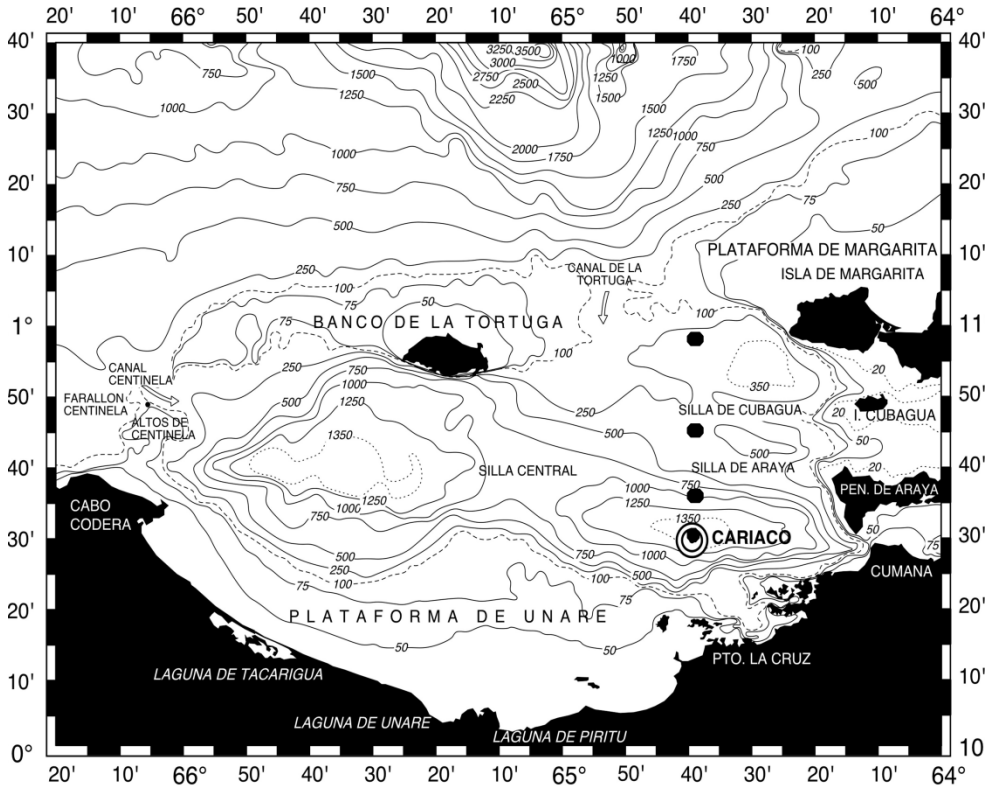


Fig.1: Map of Cariaco Basin showing the sampling location (open circle)

Fig. 2: Map of Forge River from report of Wilson et al. 2009. Sampling for this experiment was close to station 2

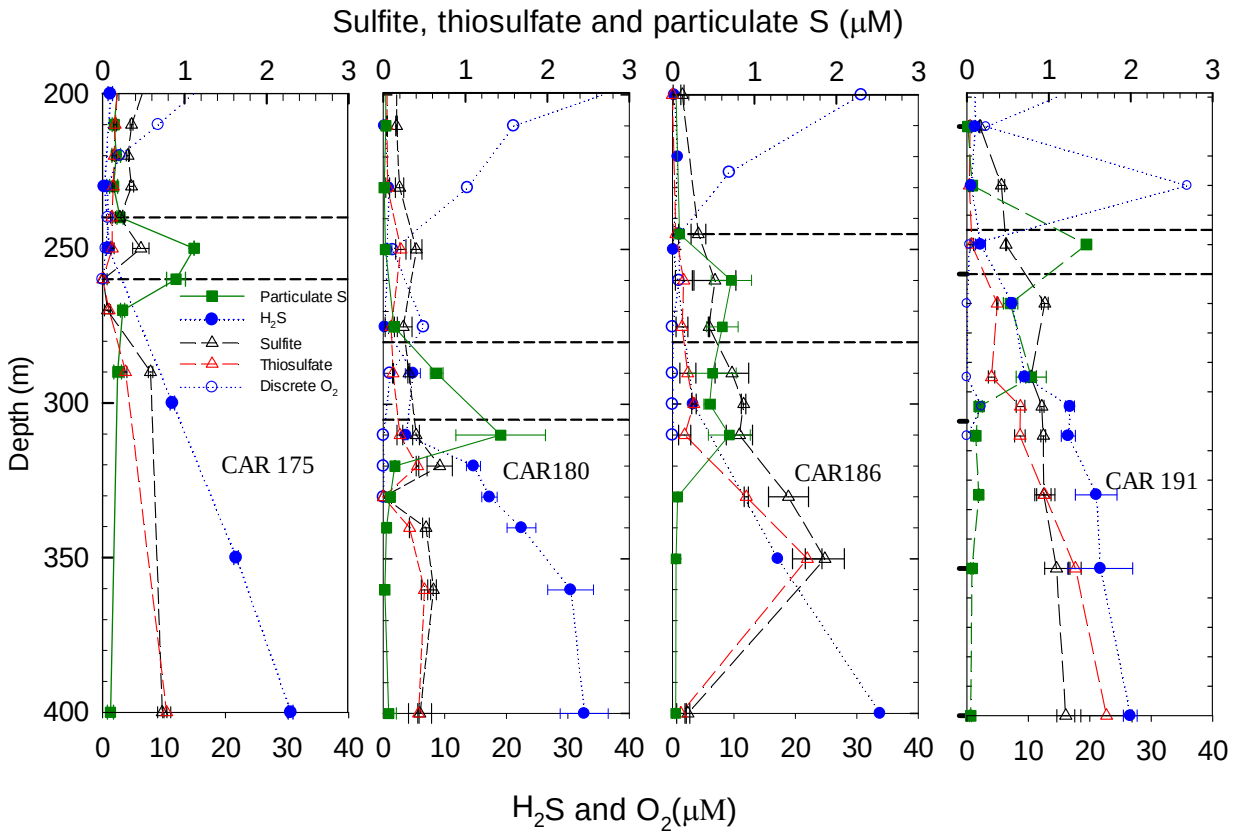


Fig. 3: Depth profiles of sulfur intermediates distribution in the Cariaco basin. Dashed lines represent the upper and lower boundaries of suboxic zone in which both hydrogen sulfide and O_2 are about 2- 3 μM.

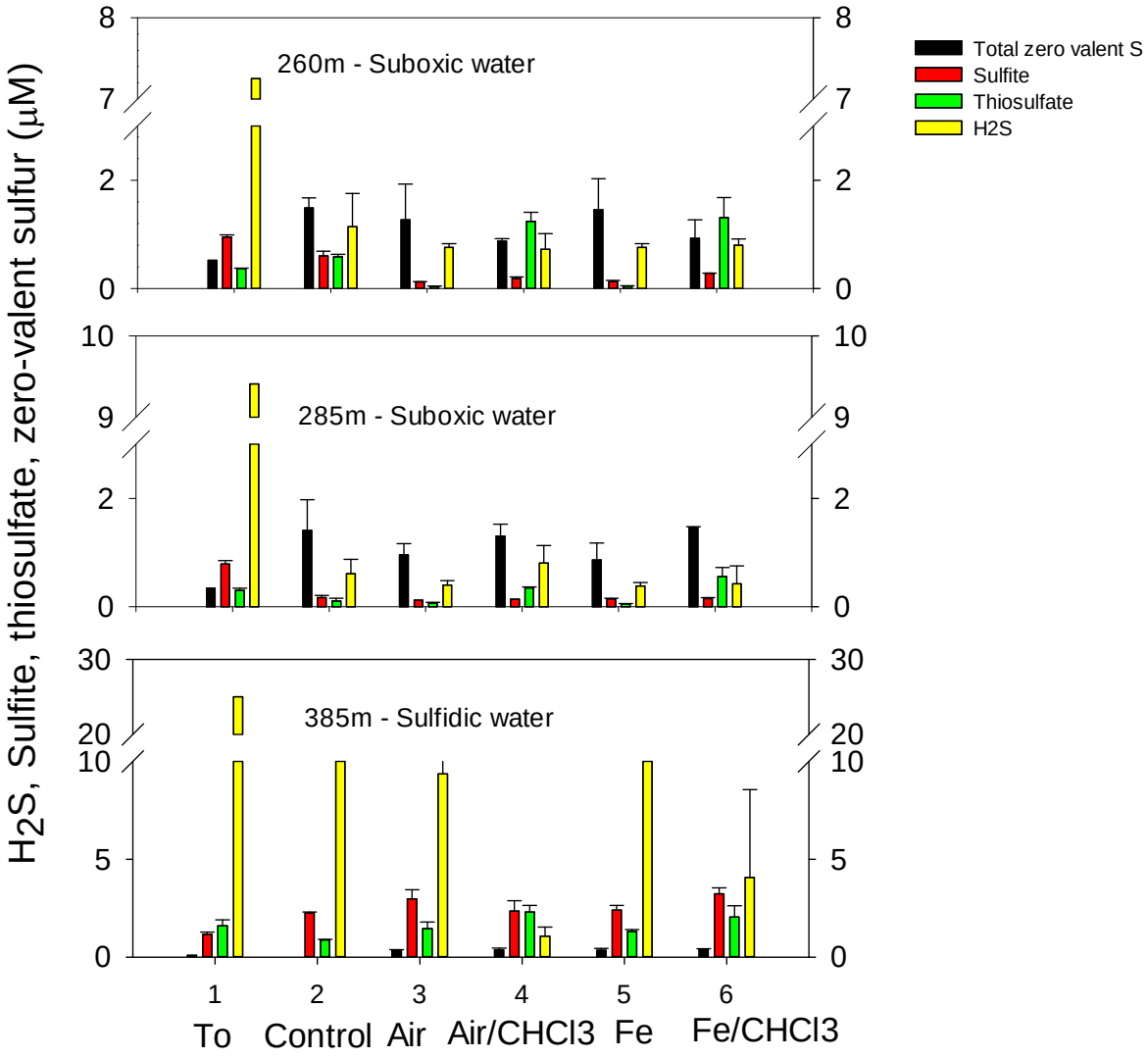


Fig.4: Sulfur species from incubation using three water depths of Cariaco Basin during CAR 191

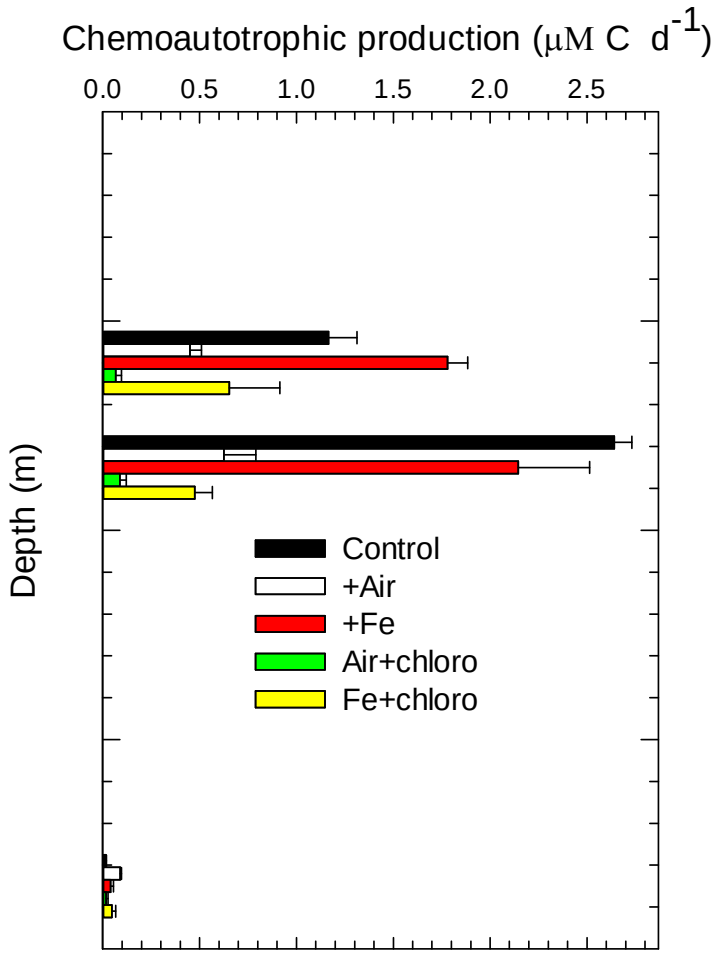


Fig.5: Chemoautotrophic production of CAR191 with the different treatments (data are provided by Gordon Taylor)

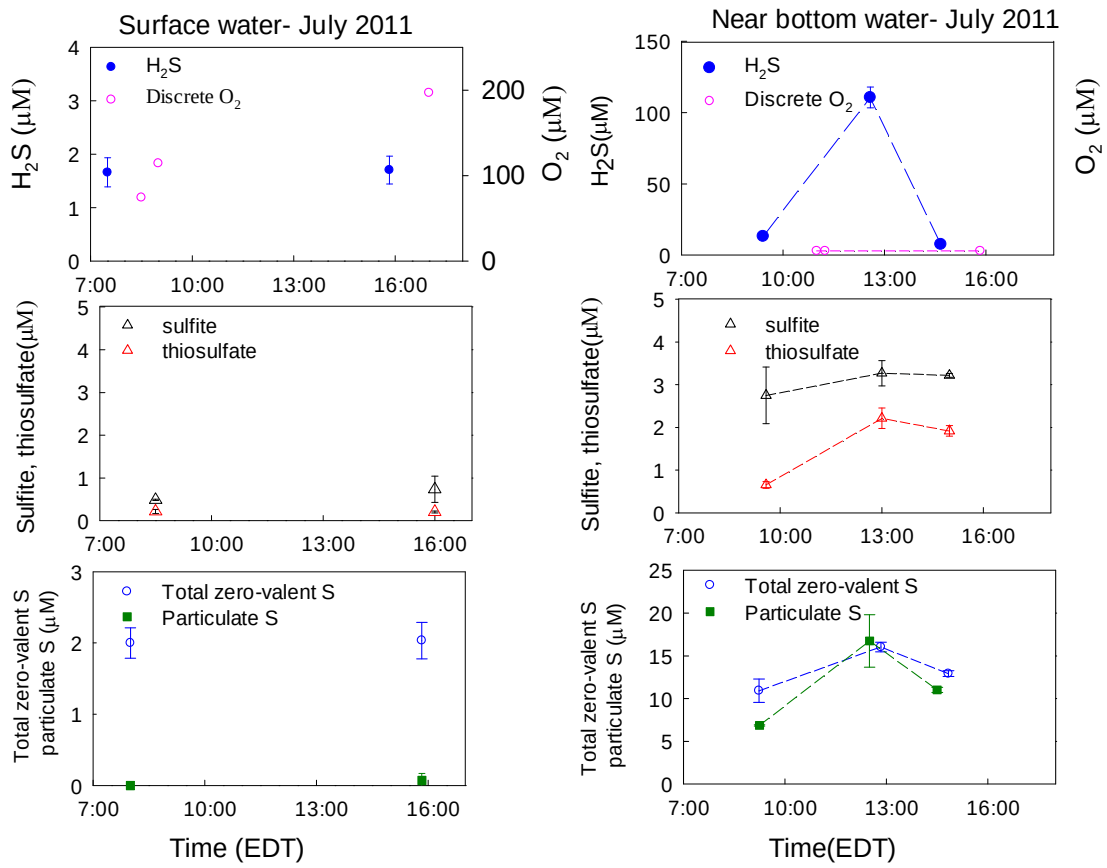


Fig. 6: Sulfur intermediates distribution over a diel cycle in sampling station 2 in the Forge River (July 8th 2011)

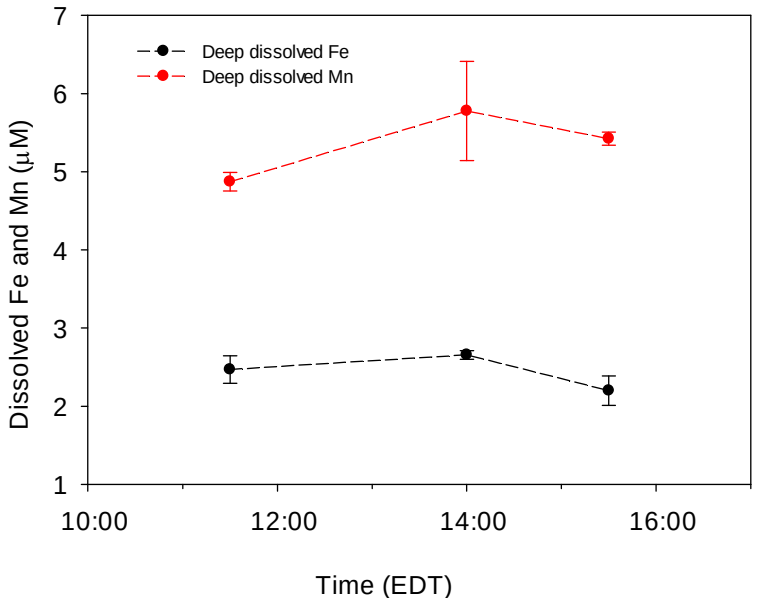


Fig.7: Trace metal in near bottom water of sampling station 2 in the Forge River (July 8th 2011)

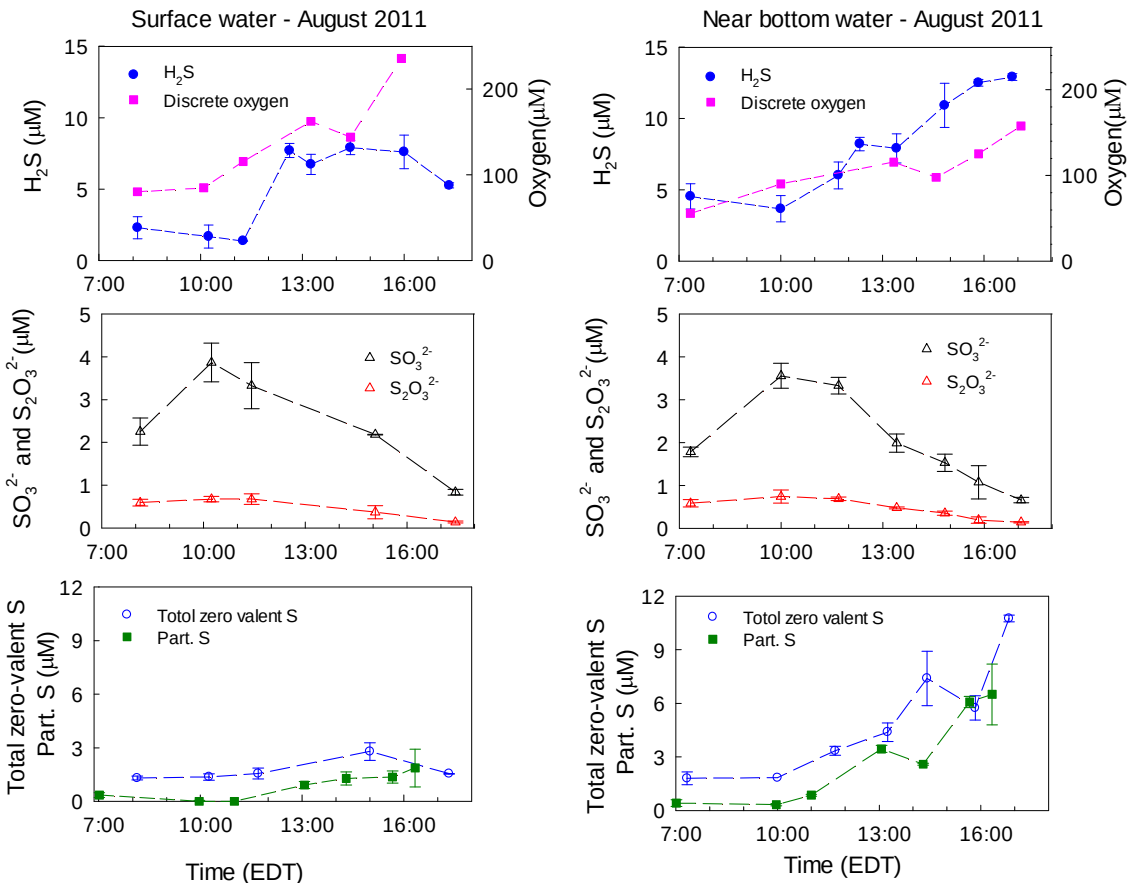


Fig. 8: Sulfur distribution over the diel cycle in sampling station 2 in the Forge River (August 1st 2011)

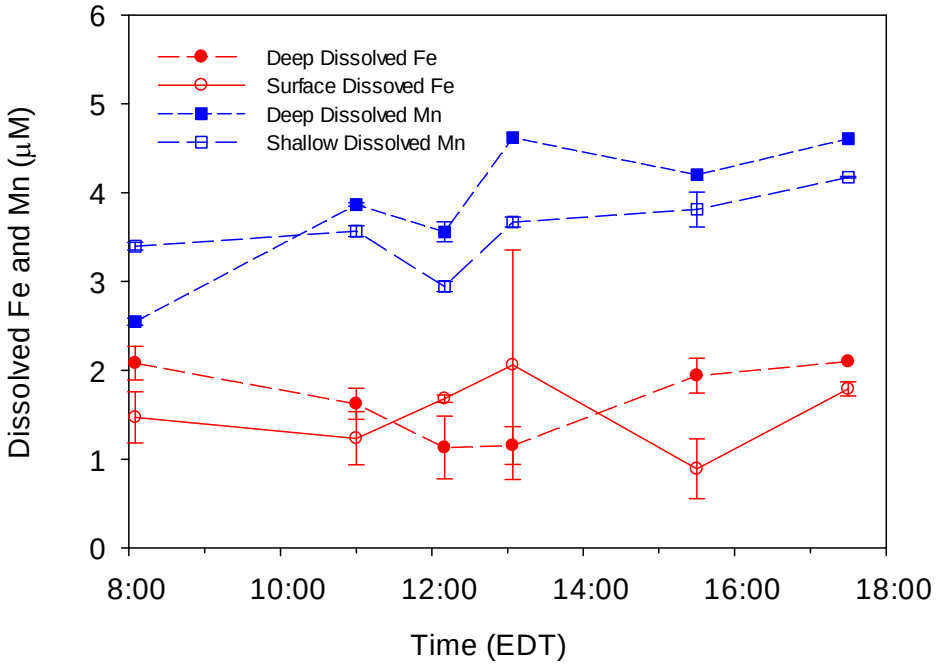
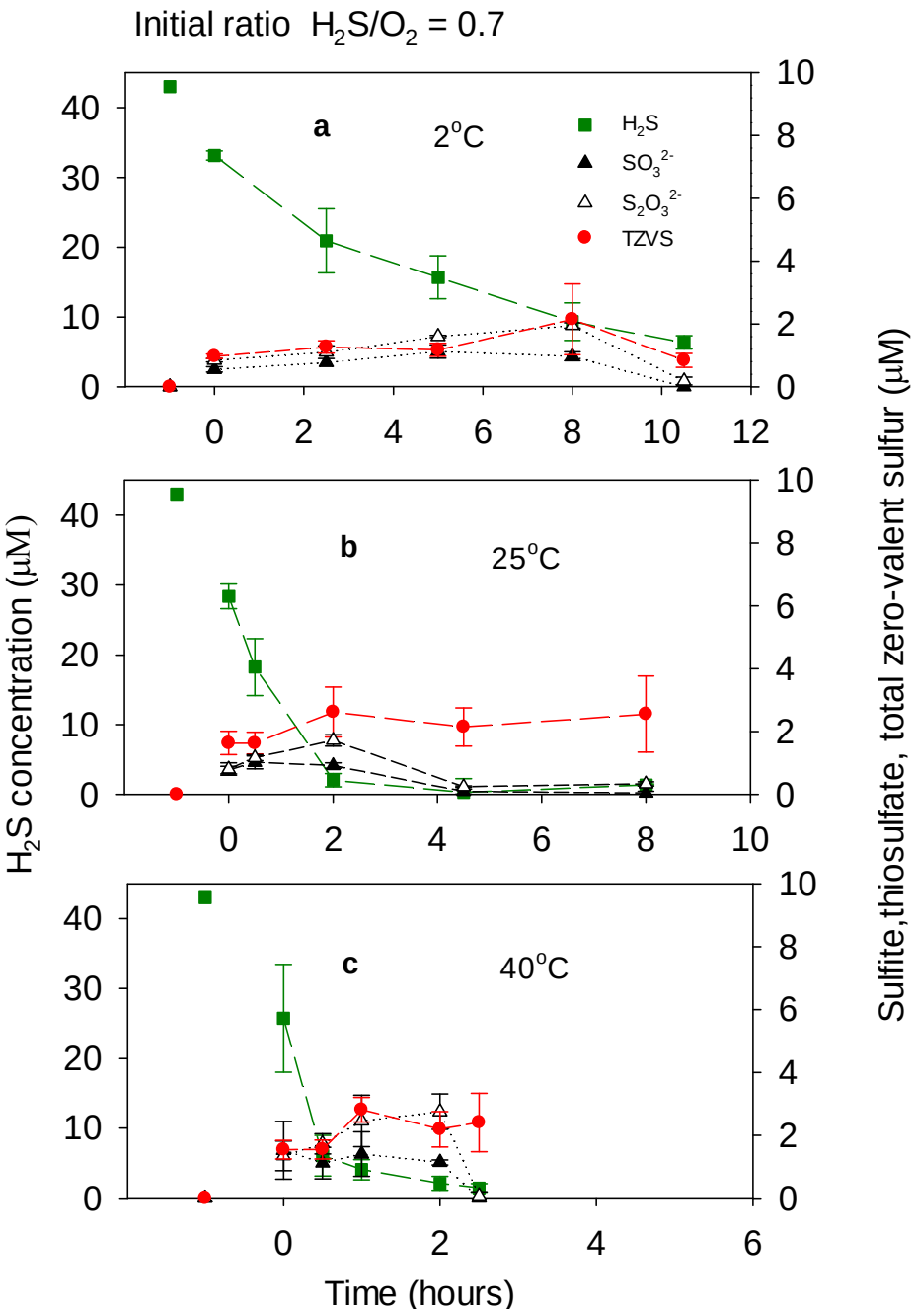


Fig. 9: Trace metal in sampling station 2 in the Forge River August 1st 2011


Initial ratio H2S/O2 = 0.7
a
2°C
b
25°C
c
40°C
H2S
SO3 2-
S2O3 2-
TZVS
H2S concentration (μM)
Sulfite,thiosulfate, total zero-valent sulfur (μM)
Time (hours)

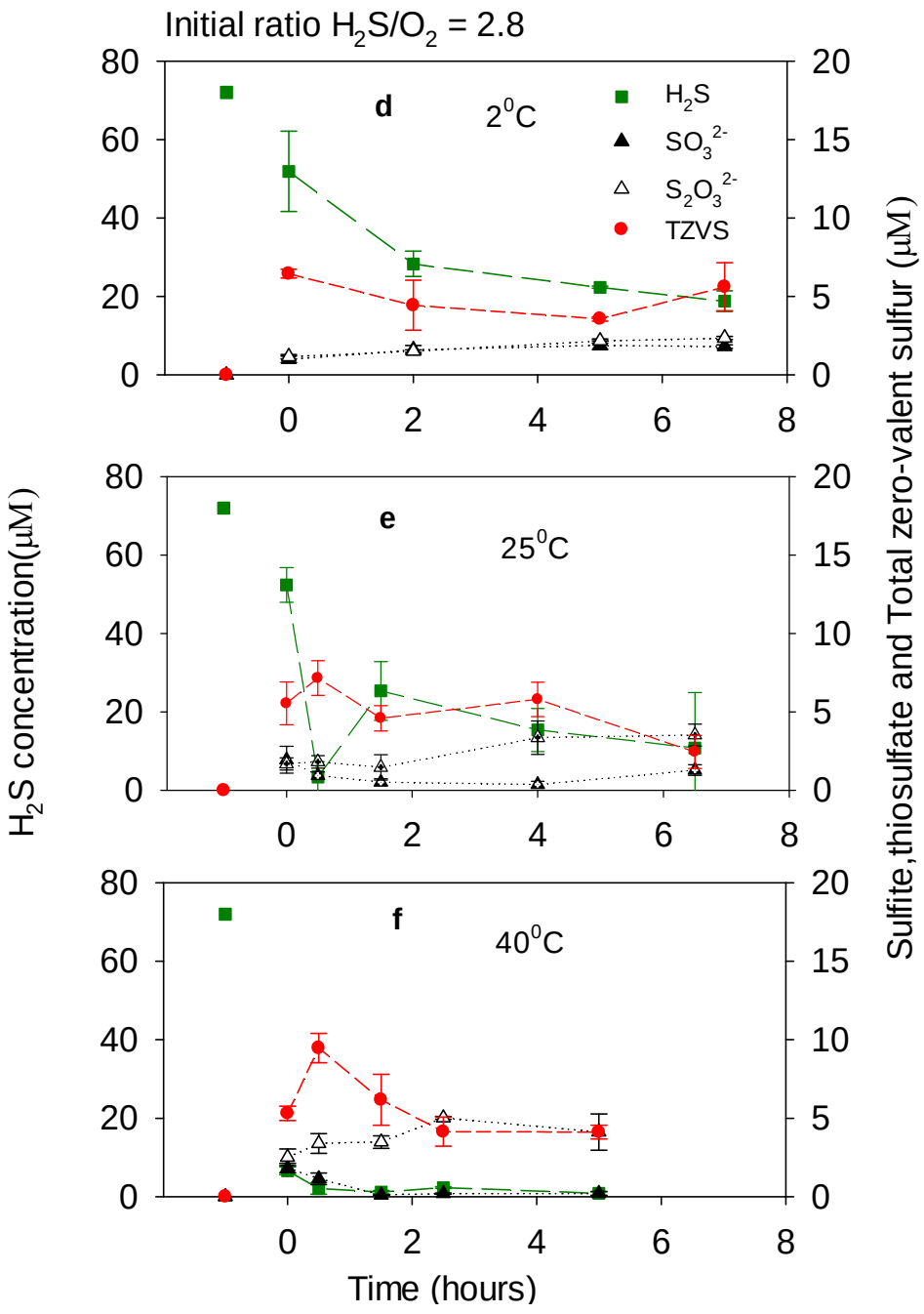


Fig.10: Sulfur intermediates distribution from the Forge river incubation for initial ratio of □H_2S/O_2 = 0.7 (a, b, c) and for initial ratio □H_2S/O_2 = 2.8 (d, e, f). Note: the unconnected points on left of graph represent the initial concentrations of H_2S and sulfur intermediates measured before the time point T = 0.

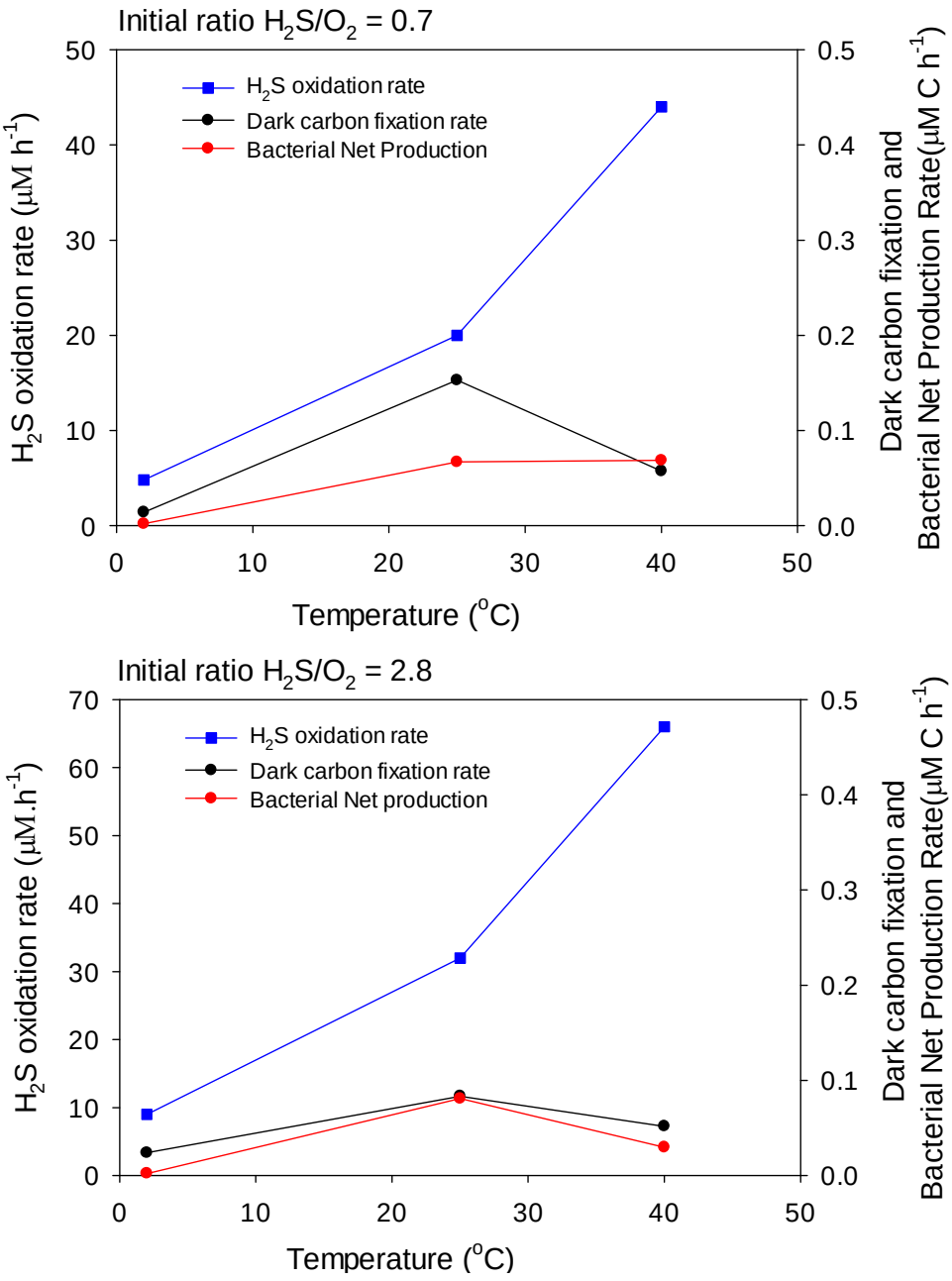


Fig.11: Measured H_2S oxidation rate, dark carbon fixation rate and bacterial net production (microbial data are from E. Suter)

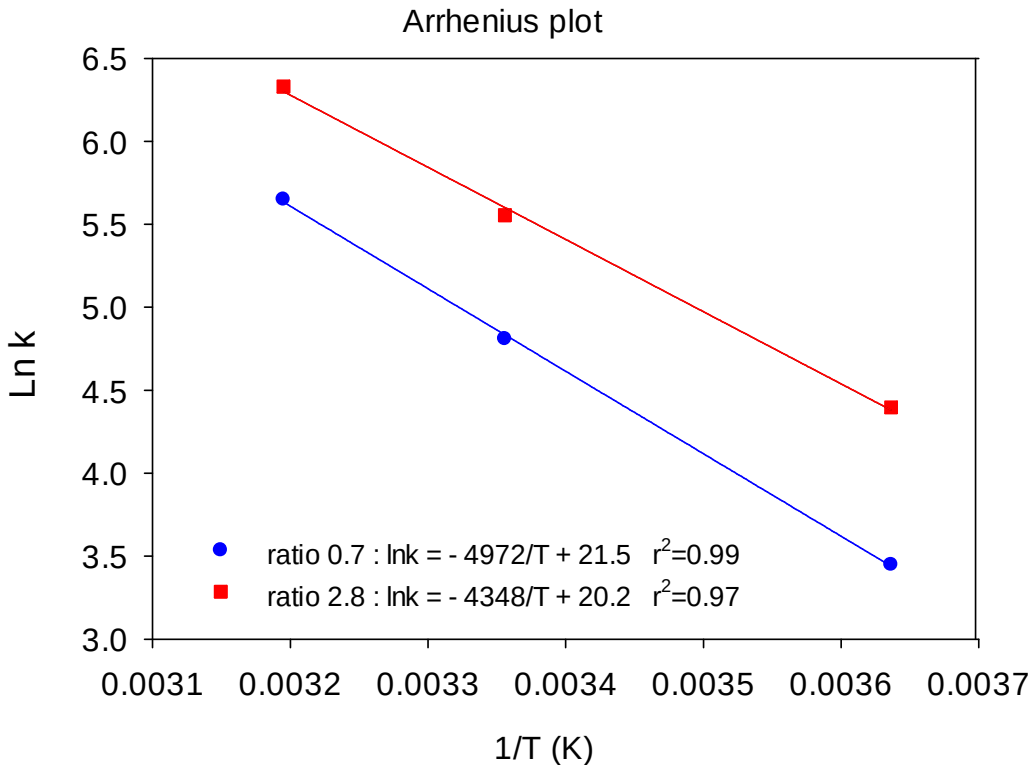


Fig. 12: Arrhenius plot for sulfide oxidation for data from Fig. 11

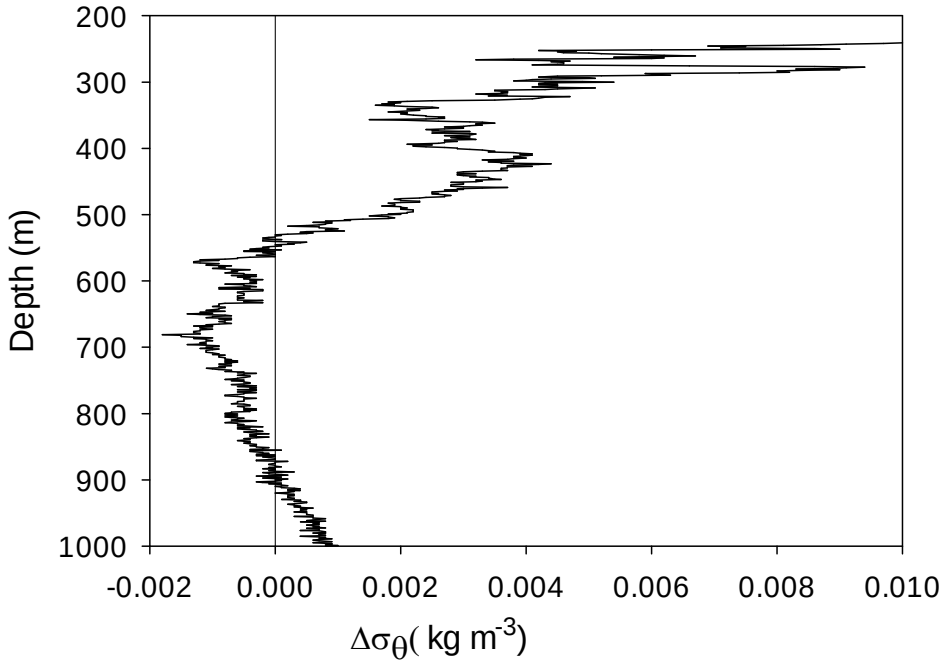


Fig.13: Change in □θ between CAR 122 (May 2006) and CAR 121(April 2006)

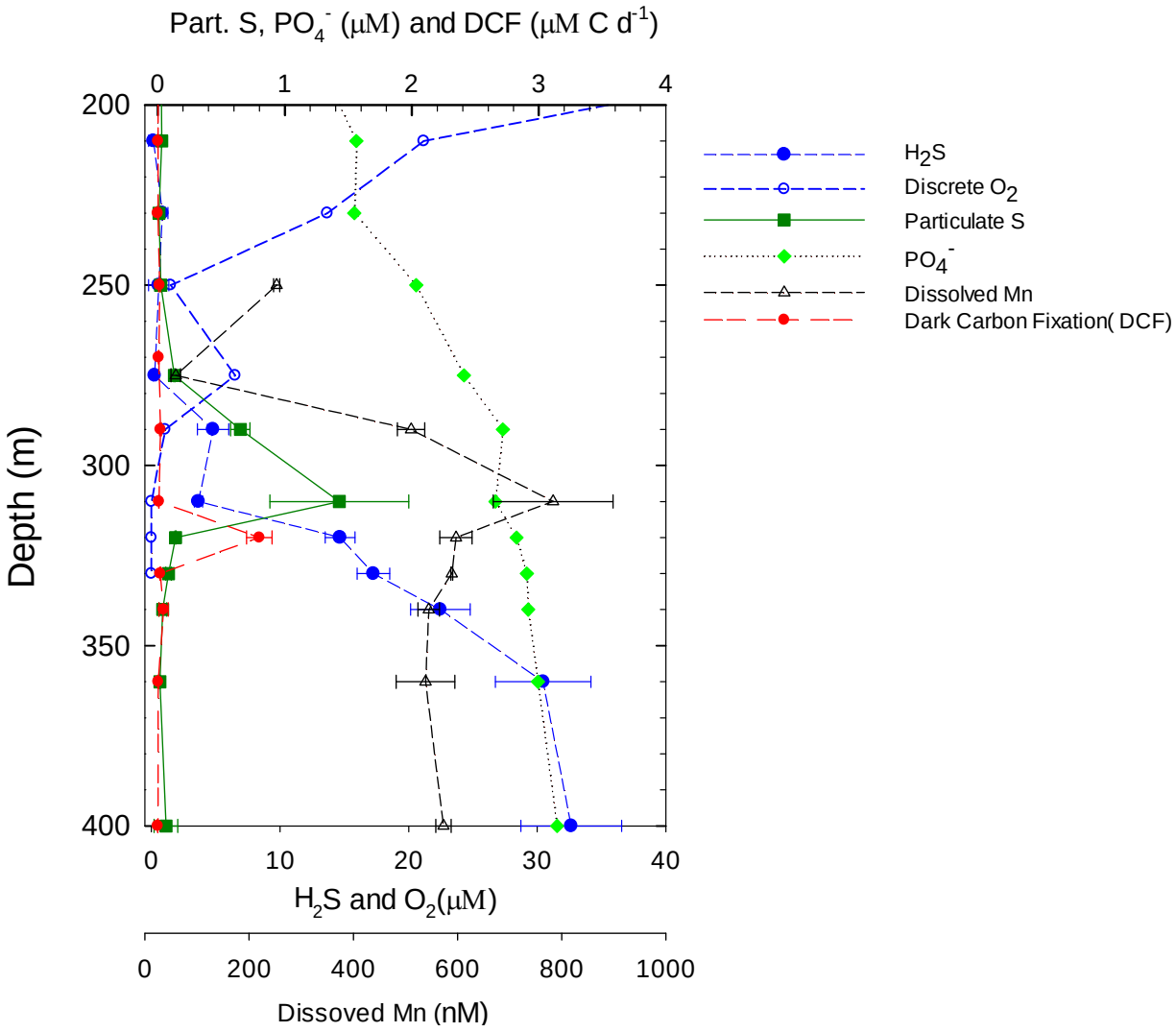


Fig.14: Oxygen intrusion observed in CAR180 (Dark carbon fixation data from GT Taylor)

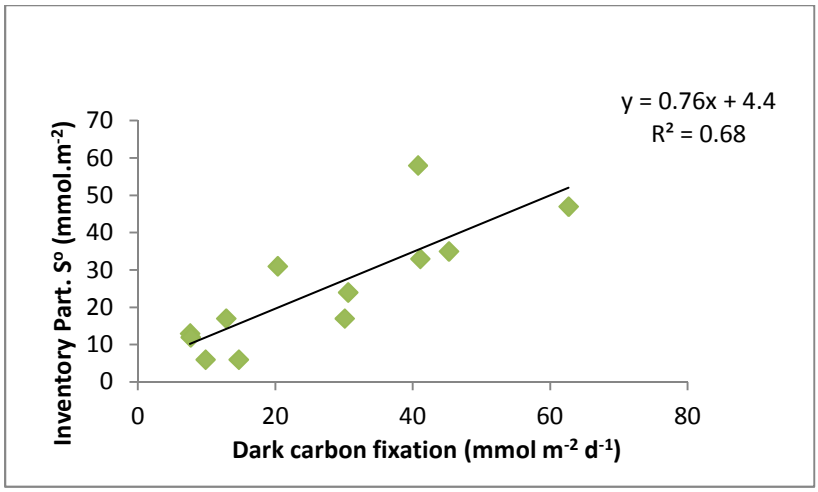


Fig.15: Relationship between chemoautotrophy and particulate elemental sulfur in Cariaco Basin cruises. Integrated carbon fixation rates were compared to inventories of particulate S between 200 and 400 m for 11 cruises (CAR 118, 122, 128,132, 145, 153, 163, 169, 175, 186 and 191). Data from CAR180 was excluded from this figure since dark carbon fixation was unusually low.

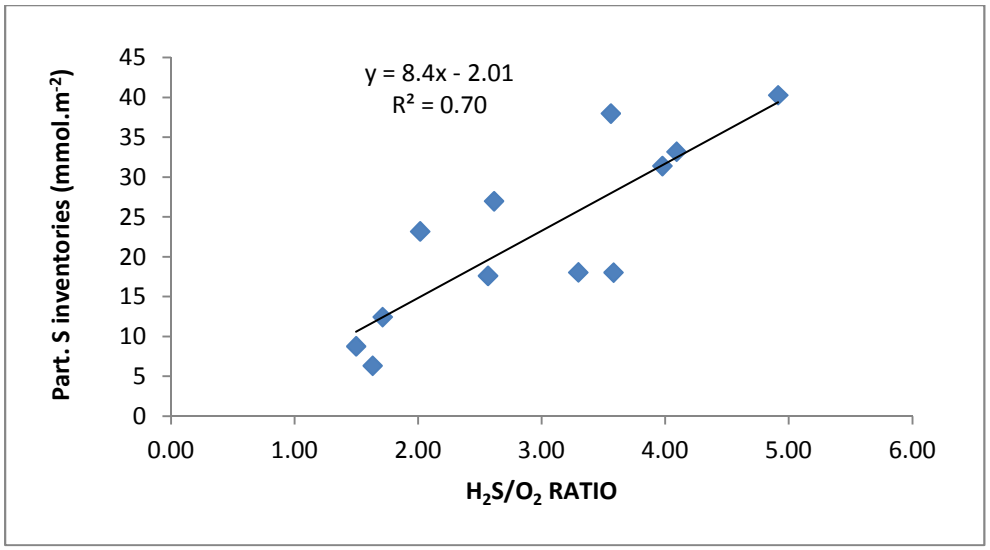


Fig.16: Relationship between H_2S/O_2 ratio and particulate elemental sulfur in Cariaco Basin (Integration was done for 12 cruises over the zone with both O_2 and H_2S concentration $\leq$10 µM)

Table 1: Sulfur products from dark incubation for the Forge River for two initial ratios of H_2S/O_2. TZVS = total zero valent sulfur. H_2S loss (or the amount of sulfide no longer present in fractions analyzed) was calculated by the difference between oxidized H_2S and the sum of sulfur intermediates.

A. Products from oxidation of H_2S for initial ratio $H_2S/O_2 = 0.7$

Temp (^{0}C)	Time (hours)	Measured H_2S (µM)	TZVS (µM)	SO_3^{2-} (µM)	$S_2O_3^{2-}$ (µM)	H_2S loss (µM)
2	0	33.16	0.97	0.56	0.85	7.46
	2.5	20.94	1.27	0.78	1.18	18.83
	5	15.70	1.18	1.13	1.61	23.38
	8	9.35	2.15	0.98	1.95	28.57
	10.5	6.39	0.85	0.00	0.19	35.57
25	0	25.74	1.64	0.78	0.81	14.03
	0.5	18.25	1.63	1.02	1.17	20.93
	2	2.03	2.63	0.92	1.73	35.69
	4.5	0.29	2.15	0.09	0.25	40.22
	8	1.35	2.56	0.04	0.33	38.72
40	0	22.40	1.54	1.52	1.34	16.20
	0.5	6.06	1.55	1.11	1.73	32.55
	1	4.07	2.81	1.40	2.45	32.27
	2	2.11	2.19	1.13	2.75	34.82
	2.5	1.44	2.40	0.00	0.07	39.09

B. Products from oxidation of H_2S for initial ratio $H_2S/O_2 = 2.8$

Temp (^oC)	Time (hours)	Measured H_2S (µM)	TZVS (µM)	SO_3^{2-} (µM)	$S_2O_3^{2-}$ (µM)	H_2S loss (µM)
2	0	51.91	6.45	1.00	1.20	11.44
	2	28.32	4.44	1.60	1.50	36.14
	5	22.35	3.57	1.90	2.20	41.98
	7	18.80	5.62	1.80	2.40	43.38
	9	18.80	4.58	1.50	3.10	44.02
25	0	52.41	5.55	1.95	1.69	10.40
	0.5	3.42	7.16	1.60	1.51	58.31
	1.5	25.32	4.60	0.52	1.47	40.09
	4	15.38	5.80	0.78	3.78	46.26
	6.5	10.80	2.44	0.97	2.72	55.07
40	0	6.58	5.32	1.82	2.50	55.78
	0.5	2.06	9.47	1.14	3.39	55.94
	1.5	1.13	6.17	0.42	3.69	60.59
	2.5	2.29	4.14	0.19	5.02	60.36
	5	0.81	4.11	0.20	4.12	62.76

Table 2: The observed rate of H_2S oxidation in the incubation and predicted rate from chemical kinetic data in literature

Initial ratio H_2S/O_2	Temperature (^oC)	Predicted H_2S oxidation rate ($\mu M\ h^{-1}$)	Observed H_2S oxidation rate ($\mu M\ h^{-1}$)	Observed/Predicted rate
0.7	2 25 40	0.04 0.31 0.89	4.8 ± 1.85 20 ± 8.8 44 ± 5.8	120± 46 65± 28 49± 6.5
2.8	2 25 40	0.03 0.24 0.68	9±5.4 32±11.8 66±1	297± 178 136± 38 97± 3

Table 3: Calculation of Q_{10} for observed H_2S oxidation rate, dark carbon fixation and bacterial net production.

T (^{o}C)	Q_{10}					
	Initial ratio H_2S/O_2 = 0.7			**Initial ratio H_2S/O_2 = 2.8**		
	Dark carbon fixation	BNP	H_2S oxidation	Dark Carbon fixation	BNP	H_2S oxidation
2- 25	2.95*	2.81	1.85	1.73	3.04	1.73
25- 40	0.52	1.02	1.51	0.73	0.51	1.62

Table 4: Comparison between the observed rate of sulfide disappearance and the potential biotic rate associated with chemoautotrophy

Initial ratio H_2S/O_2	Temperature (^oC)	Observed rate H_2S oxidation ($\mu M.d^{-1}$)	Predicted biotic rate related with Chemoautotrophy ($\mu M.d^{-1}$)
0.70	2 25 40	115 480 1056	3 33 12
2.80	2 25 40	288 2352 3168	5 18 11

Table 5: Regression analysis of SO_3^{-2} and $S_2O_3^{-2}$ concentration versus time ($\mu M\ h^{-1}$)

	Initial ratio 0.7			Initial ratio 2.8		
SO_3	2^0C	25^0C	40^0C	2^0C	25^0C	40^0C
Slope	0.11	0.89	-0.14	0.12	-0.89	-0.7
r^2	0.79	0.02	0.02	0.6	0.54	0.7
P value	<.001	0.67	0.67	<.001	<.001	<.0001
n	11	10	12	18	14	13

	Initial ratio 0.7			Initial ratio 2.8		
S_2O_3	2^0C	25^0C	40^0C	2^0C	25^0C	40^0C
Slope	0.15	0.43	0.76	0.18	0.41	0.86
r^2	0.97	0.86	0.53	0.95	0.43	0.75
P value	<.001	<.001	<.005	<.0001	<.05	<.0001
n	12	10	12	18	14	13

Table 6: Pearson product–moment correlation between sulfur species at each depth (200-400 m) in Cariaco over 4 cruises (CAR 175, 180, 186, 191) (n= 41) and over the daylight cycle in the Forge River (n =12)

The Cariaco Basin

	O_2	Part.S	$S_2O_3^{-2}$	SO_3^{-2}
Part.S	-0.28			
$S_2O_3^{-2}$	-0.28	-0.27		
SO_3^{-2}	-0.27	-0.08	0.85**	
H_2S	-0.36*	-0.34*	0.60**	0.38*

Forge River

	O_2	Part.S	$S_2O_3^{-2}$	SO_3^{-2}
Part.S	0.38			
$S_2O_3^{-2}$	-0.71**	-0.80**		
SO_3^{-2}	-0.52	-0.75**	0.92**	
H_2S	0.44	0.91**	-0.85**	-0.79**

Part.S: Particulate elemental sulfur

* Correlation is significant at the 0.05 level

**Pearson product–moment correlation is significant at the 0.01 level

Table 7: Salinity and temperature in sampling station 2 on August 1st 2011 (Temperature was not recorded after 7:00)

Time	Surface water		Near bottom water	
	Temperature (^oC)	Salinity	Temperature (^oC)	Salinity
5:35	21	20	23	20
7:00	28.5	20	28	20
12:20		15		13
14:25				17
15:50				17.5
17:20		17		20