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Sulfur biogeochemistry in the Cariaco Basin

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Abstract of the Dissertation

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Spatial and temporal variations of sulfur cycling, as well as factors influencing these processes, were studied in the Cariaco Basin. Four major questions were addressed: 1) what is the distribution pattern of sulfur intermediates in the Cariaco Basin? 2) are there variations in stable isotope signals in various sulfur pools and how are they related to *in situ* processes? 3) is formation of iron sulfide minerals important in the water column? 4) what is the temporal variability of oxidant and reductant supply to the redoxcline and how does this influence chemoautotrophy rates?

Measurement of sulfite and thiosulfate concentrations on four cruises at four stations showed that their distributions were always closely related. However, no consistent distribution pattern or relationship with chemoautotrophic production in the redoxcline was observed. In contrast, particulate elemental sulfur maxima were consistently found near the O_2/H_2S interface. Higher concentrations of particulate

elemental sulfur at stations closer to the edges of the basin seemed to be associated with higher inventories of sulfide at these stations. Comparisons of profiles of total zero-valent sulfur to particulate elemental sulfur suggest that a large fraction of total S^0 is either in the form of colloidal S or polysulfides. Although thiosulfate stimulated bacterial activity in amendment experiments, the role of thiosulfate and sulfite in chemoautotrophic production is not yet clear. A strong relationship was found between chemoautotrophic production and particulate elemental sulfur, suggesting that sulfur disproportionation may be an important fueling reaction for carbon fixation within the redoxcline.

In close agreement with results from the Black Sea, $\delta^{34}S_{H2S}$ and $\delta^{34}S_{SO4}$ were relatively constant in deep anoxic waters, with $\delta^{34}S_{H2S}$ depleted in ^{34}S relative to $\delta^{34}S_{SO4}$ by roughly -53.6‰. However, near the oxic-anoxic interface, $\delta^{34}S_{H2S}$ was 3-4 ‰ heavier than that in deeper anoxic waters, which was probably due to sulfide oxidation and/or *in situ* sulfide production. Repeated detection of a minimum in $\delta^{34}S_{SO4}$ at the O_2/H_2S interface on four cruises may reflect sulfide oxidation. We also measured four sulfur isotopes (^{32}S, ^{33}S, ^{34}S, and ^{36}S) in sulfide and sulfate on one cruise to explore the sulfur isotope fractionation effects associated with sulfate reduction, sulfur disproportionation, and sulfur oxidation. Our results strongly suggest that sulfide oxidation and sulfate reduction are the two dominant processes controlling sulfur isotope composition in the Cariaco Basin water column.

While particulate acid volatile sulfur (AVS) was low throughout the water column, greigite and pyrite showed distinct maxima near the interface, with concentrations 1-2 orders of magnitude higher than AVS. Pyrite comprised nearly 0.2-0.4 % of the total particulate flux in the anoxic water column, as measured in time-series sediment trap

materials. The sulfur isotope composition of the sinking particulate sulfide fluxes confirmed the major formation zone of pyrite to be near the oxic-anoxic interface. Pyrite and elemental sulfur distribution patterns, together with other environmental factors, suggest pyrite forms via the reaction of FeS with polysulfides or particulate elemental sulfur near the interface. This is consistent with other data from this study of sulfur species and chemoautotrophic production, which suggest the reaction of sulfide with FeOOH may be an important pathway for sulfide oxidation and sulfur intermediate disproportionation near the interface.

Vertical fluxes of oxidants and reductants over the past ten years (24 cruises, 4 stations) using a simple one dimensional model were compared to the chemoautotrophic production rates. In general, downward fluxes of the major oxidants balanced well with upward electron fluxes from reductants when calculated as electron equivalents. However, vertical fluxes of electron donors/acceptors never explain more than 10% of the measured chemoautotrophy if a theoretical stoichiometry of $1H_2S$: 1 CO_2: 1 O_2 is assumed and if 'normal' vertical diffusivities are used. In exploring several possible explanations, we estimated the energy yield for chemoautotrophs near the interface under environmental conditions. Our results suggest that aerobic oxidation of methane, sulfide and hydrogen is thermodynamically favorable at the Cariaco interface. A hypothetical high energy yield f of chemoautotrophic microbes together with a local, efficient cycling of carbon produced near the interface may provide an explanation for the calculated imbalance between biological demand and chemical supply.

In summary, the sulfur cycle in the Cariaco Basin is very dynamic. At redox gradients, sulfur intermediates may be important substrates for chemoautotrophic

production, and they could also be important for sulfur iron mineral formation in the water column. Sulfide isotope signal at the interface appears to be mainly controlled by sulfide oxidation and sulfate reduction.

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CHAPTER ONE: Introduction and background

1. INTRODUCTION

Chemoclines between oxic and anoxic waters are especially interesting environments, where a wide range of microbial processes take place within sharp gradients, and cycles of a variety of elements, including C, N, S and metals, are linked. Previous studies focusing on elemental cycling within redox gradients have investigated how redox-sensitive species are transformed and how microbes are involved in those processes. Most prior studies have been of chemoclines in sediment. However, the redox transition zone (RTZ) or redoxcline in sediment can be very thin and that makes sampling and analysis difficult. In anoxic water column settings, such as the Cariaco Basin and the Black Sea, the RTZ is greatly expanded. For example, the RTZ in the Cariaco Basin spans several tens of meters. In addition, the Cariaco Basin has fundamental scientific value for oceanographers, paleoceanographers, and paleoclimatologists as a natural sediment trap, and is relatively stable, allowing repeatable measurements and experimentation.

In the Cariaco RTZ, an active secondary microbial food web is supported by microbial chemoautotrophy, which probably depends largely on cycling of sulfur species (sulfide, thiosulfate, sulfite, elemental sulfur) coupled with inorganic electron acceptors (e.g. O_2, NO_3, metal oxides) (Taylor et al., 2001, 2006; Madrid et al., 2001; Ho et al., 2004; Hayes et al., 2006; Lin et al., 2006) . However, previous studies have not examined

in much detail the manner in which sulfur intermediates are produced, consumed and cycled in the Cariaco Basin, how this sulfur cycling is related to chemoautotrophic production near the redoxcline, how the sulfur isotopic composition of different sulfur pools is affected by this sulfur cycling, and what processes control the sulfur budget in the anoxic water column. This study aims to provide answers to some of these questions.

2. BACKGROUND

2.1 The Cariaco Basin study site

The Cariaco Basin is located on the continental shelf north of Venezuela and is the second largest permanently anoxic basin on Earth. Because of restricted horizontal circulation below the sill depth (about 90-140m), a density gradient that inhibits vertical mixing, and oxidative degradation of organic matter, the Cariaco Basin contains no oxygen below depths of about 275m (Richards, 1975), and the depth of oxygen depletion oscillates up and down with time.

Anoxic bottom conditions and lack of bioturbation in the Cariaco Basin favor the accumulation of varved sediments (Peterson et al., 1991). It has been argued that the varves reflect seasonal to annual variations in surface water conditions and that they can be used to study paleoclimatology and paleoceanography (Hughen et al., 1996). However, most inferences from the sediment record are based on the assumption that the composition and rate of delivery of biogenic debris to the seabed are exclusively driven by surface processes. In order to properly interpret the varves of the Cariaco Basin sediment, we need to identify whether there are other sources of POC flux. It has been suggested, for example that chemoautotrophy is an important contributor to water column POC fluxes below the oxic/anoxic interface (Taylor et al., 2001) based on the observation

that, at times, carbon flux at 475 m is greater than at 275m. This phenomenon seems to be seasonal and it implies a source of material between these two depths (Thunell et al., 2000; Taylor et al., 2001). As in the Black Sea (Sorokin et al., 1995), elevated concentrations of autotrophic and heterotrophic bacteria, and high rates of dark DIC assimilation have been found in the Cariaco RTZ (Taylor et al., 2001). Dark carbon fixation in the RTZ is high (9-159 mmol C m^{-2} d^{-1}), which is equivalent to 10-333% (median of 50%) of contemporaneous primary production, depending on the station and the season (Taylor et al., 2001). There is a dynamic microbial redoxcline community that must be supported by chemoautotrophic bacteria since the interface is located well below the euphotic zone, mostly utilizing oxidants and reductants to obtain energy for the fixation of CO_2 (Taylor et al., 2001, 2003, 2006; Ho et al., 2004).

Craig (1969) pointed out that horizontal advection can be negligible over linear regions on the T-S diagram. Fig. 1.1 illustrates that, thoughout the redoxcline of the Cariaco Basin (200-400 m, which is the focus of this thesis), the T-S plot is linear. Therefore, vertical diffusive transport of the dissolved species is more dominant controlling supply than horizontal advection. If that is the case, fluxes of reductants and oxidants calculated from vertical concentration profiles and estimates of vertical eddy diffusion should balance biological demand estimated from the measured chemoautotrophic production. However, previously calculated vertical diffusion rates of measured electron donors (H_2S, Fe^{2+}, Mn^{2+}) and electron acceptors (O_2, MnO_2, Fe_2O_3, NO_3^-) could support only a few percent of the measured chemoautotrophic production at the CARIACO station (Taylor et al., 2001). In fact, based on the organic matter flux and stoichiometry of sulfate reduction, Hayes et al. (2006) calculated that the particulate

carbon sinking flux at the CARIACO site was too low to support the required H_2S supply. Imbalance between chemoautotrophic production and diffusive fluxes of substrates has also been observed in other anoxic water bodies, such as the Black Sea (Jørgensen et al., 1991; Murray et al., 1995) and the Mariager Fjord (Zopfi et al., 2001). In the remainder of this thesis, I will use the short term 'energy imbalance' when discussing the apparent discrepancy between the calculated vertical fluxes of chemical species and biological demand.

In addition to symbiotic associations (Taylor et al., 2001), another possible explanation for the imbalance is that horizontal supply of these species is most important. Transient lateral intrusions of oxic water or high wind events that circulate and introduce oxygenated water into the suboxic or anoxic zone may supply electron acceptors (Scranton et al., 2001; Astor et al., 2003, Scranton et al., 2008). Oxygen in these waters could oxidize H_2S, either directly, or in reactions catalyzed by MnO_2/ Fe_2O_3, to produce sulfur intermediates, such as S_n^{2-}, S^0, $S_2O_3^{2-}$ and SO_3^{2-} (Chen and Morris, 1972; Millero et al., 1991). Calculations have suggested that enough O_2 may enter the basin through intrusions to produce sufficient thiosulfate to support the chemoautotrophic growth of bacteria (Hayes et al., 2006). However, the source of sulfide is still unclear.

Since the maximum dark carbon fixation is always slightly deeper than the depth of detectable O_2 and nitrate (Taylor et al., 2001; 2006), the primary oxidants are likely to be those metal oxidants and sulfur intermediates which have been proposed to play important roles in the sediment (Jørgensen, 1990a, b). To date, enrichment cultures from the Cariaco Basin have yielded microaerophilic thiosulfate-oxidizers, sulfur and thiosulfate disproportionators, thiosulfate-oxidizing manganese reducers and denitrifying

thiosulfate-oxidizers (Madrid, 2000; Lin, unpubl. data). Results from stimulation/inhibition experiments, 16S rDNA libraries and enrichment cultures all support the viewpoint that the redoxcline is inhabited by highly stratified guilds of diverse chemoautotrophs that organize in response to resource availability (Taylor et al., 2006). They also appear to have phylogenetic affinities to organisms with known sulfur and hydrogen metabolisms which rely on energy from S in reduced (sulfide) and intermediate oxidation states (S_n^{2-}, S^0, $S_2O_3^{2-}$, SO_3^{2-}) (Madrid et al., 2001, Lin et al., 2006).

2.2 Production, cycling and distribution of sulfur intermediates within the RTZ

In euxinic water bodies, sulfur intermediates, typically including S_n^{2-}, S^0, $S_2O_3^{2-}$, SO_3^{2-} and $S_nO_6^{2-}$, can originate from oxic or anoxic sulfide oxidation near the chemocline. It is common to observe maximum concentrations of these compounds near the oxic/anoxic interface (Zopfi et al., 2001; Hayes et al., 2006). Several lines of evidence (rate measurements, molecular fingerprinting) suggest the oxidation process may be either mediated by microbes or may occur through pure chemical reactions (Jørgensen et al., 1979; Madrid et al., 2001). At steady state, biological sulfide oxidation is likely to dominate since chemical oxidation is a second-order reaction (Zhang and Millero, 1993) and the reactant concentrations are low (Sørensen and Jørgensen, 1987). This is consistent with Nelson et al. (1986), who found that biological oxidation of HS^- in stratified microcosms of *Beggiotoa* is 3 orders of magnitude faster than purely chemical oxidation. The conclusion that biological oxidation is more important is also consistent with the observation of high rates of dark $^{14}CO_2$ fixation in the lower part of the chemocline (Sorokin et al., 1995; Ramsing et al., 1996; Taylor et al., 2001). However,

when oxygen intrusions occur, oxic and sulfidic water masses are mixed and the importance of chemical sulfide oxidation is likely to increase.

There is considerable evidence showing that the Cariaco Basin is ventilated from time to time. Holmen and Rooth (1990) convincingly demonstrated from tritium data that relatively young water must reach the basin deep on a decadal time scale. They proposed two distinct sources: a high-salinity but low-volume source, originating along the Venezuela coast sinking to the bottom and a larger source from water injected at the sills, providing water that sinks to mid-depths. Since 1995, repeated observations of small maxima in dissolved oxygen in the vicinity of the chemocline have suggested the occurrence of intrusions of oxygenated water into the region of the oxic-anoxic interface (Scranton et al., 2001), although no direct observations of sinking high salinity water have been made. Ventilation events occurring in 1997 and 1998 were associated with eddies near the shelf in the southeastern Caribbean Sea. It was hypothesized that this would result in colder and denser Caribbean Sea water being drawn up over the sill, subsequently sinking within the Cariaco Basin and spreading along isopycnals (Astor et al., 2003). Other intrusions, not apparently associated with eddies, have also been observed (Astor, unpubl. data).

After sulfur intermediates are produced, they have one of three fates awaiting them (Zopfi et al., 2004). First, in the presence of extra electron donors, such as organic matter, they could be reduced to sulfide. Second, sulfur intermediates could be further oxidized to sulfate by oxygen, nitrate or manganese/iron oxides (Lovley and Phillips, 1994). The third possibility is that sulfur intermediates can undergo disproportionation to sulfide and sulfate (Thamdrup et al., 1993). All three processes are likely to be catalyzed by

microorganisms to yield metabolizing energy. Radiotracer studies have shown that these three reactions can co-occur throughout sediment but with changing proportions from oxidizing to reducing zones (Jørgensen, 1990b). Canfield et al. (2005) recently described the sulfur cycle schematically as in Figure 1.2.

Although all these three processes may be occurring, the relative importance of production and consumption will influence the distribution of sulfur intermediates. Sulfur intermediates are mostly produced within fluctuating environmental conditions (Van den Ende and Van Gemerden, 1993). In an early study of Mariager Fjord, a maximum of 0.6 μM S^0 was measured (Ramsing et al., 1996). In contrast, with the same method, 17.6 μM S^0 was measured immediately following an apparent oxygen intrusion, decreasing to 11 μM on the following next day (Zopfi et al., 2001). Thus elevated concentrations of sulfur intermediates may only be transient, and high rates of consumption and particle aggregation appear to be the main factors governing their distributions after formation.

2.3 Roles of metals in sulfur cycling

Metal oxidants can be important in sulfur cycling. In the Cariaco Basin, manganese and iron are quantitatively the most important metals (Jacobs and Emerson, 1982). Iron and manganese oxides may be supplied by aeolian transport, or advected in by local river discharge, or can form *in situ*.

The concept of a "Mn/Fe redox shuttle" has been studied broadly for anoxic environments (Nealson and Myers, 1992; Aller, 1994; Neretin et al., 2003). In the model, particulate Mn/Fe oxidants can sink through the chemocline, providing oxidants for chemoautotrophs. The sinking rate can be as fast as 1.0-10 m/d in the water column (Yakushev, 1998). Once reduced, the soluble iron and manganese species can then

diffuse back up to the oxic/suboxic zone and get oxidized by oxygen or nitrate under the catalysis of specific groups of prokaryotes (Emerson, 2000). For the Black Sea, model results (Oguz et al., 2001) suggested that, without particulate manganese, it was impossible to form the interface structure with oxygen alone. In sulfide-containing marine systems, maxima in the concentration of particulate manganese and iron are usually observed above the oxic-anoxic interface (Spencer and Brewer, 1971; Jacobs and Emerson, 1982; Lewis and Landing, 1991; Ramsing et al., 1996), presumably reflecting this shuttle.

Through the use of such electron shuttles, organisms can utilize reductants that are spatially separated from dissolved oxidants. As is the case with sulfur transformations, both the reduction and oxidation rate of iron and manganese can be stimulated to a large extent by specific microbes, but abiotic transformations, such as reduction by sulfide, are also important and can sometimes compete with biological processes (Yao and Millero, 1995).

The cycling between reduced and oxidized metal species probably affects the distribution of sulfur species. Elemental sulfur is the main product of sulfide oxidation with Mn (IV) (Burdige and Nealson, 1986) (Equation 1), but with an increasing ratio of Mn (IV) to H_2S, thiosulfate and even sulfate become the main products (Yao and Millero, 1995). Compared to Mn (IV), Fe (III) is a weaker oxidant and can barely oxidize sulfide completely to sulfate (Aller and Rude, 1988). During the reaction of sulfide with Fe (III), elemental sulfur is also produced first (Equation 2) (Luther et al., 1991).

$$MnO_2 + HS^- + 3H^+ \rightarrow Mn^{2+} + \tfrac{1}{8} S_8 + 2H_2O \qquad (1)$$

$$2FeOOH + HS^- + 5H^+ \rightarrow 2Fe^{2+} + \tfrac{1}{8} S_8 + 4H_2O \qquad (2)$$

Examination of particulate Mn and elemental sulfur data in the western Black Sea indicated that particulate manganese was the direct oxidant of sulfide but that oxygen acted as the ultimate oxidant through the catalysis of Mn oxide formation (Konovalov et al., 2003). In addition to reacting directly with sulfide, metal oxidants can also provide surface area, which acts as a catalyst for sulfide oxidation (Bratina et al., 1998). In the Black Sea, sinking particulate Mn can oxidize about 25% of the total vertical upward flux of sulfide (Konovalov et al., 2006). The flux of Mn is also important for redox transformations of Fe, as reduced Fe can be oxidized by MnO_2 (Lovely and Phillips, 1988). Iron concentrations can be affected by other processes in the suboxic/anoxic water column by forming insoluble sulfide minerals (discussed below in 2.5).

2.4. Sulfur isotopes

Sulfur isotope composition has proven to be a robust tool for studying biogeochemical cycling of sulfur (Fry et al., 1991; Sørensen and Canfield, 2004). Sulfate-reducing bacteria produce sulfide depleted in ^{34}S compared to the initial sulfate (Kaplan and Rittenberg, 1964). A wide range of fractionation factors (between 2‰ and 49‰) have been observed for pure cultures and natural populations of sulfate-reducing bacteria (Kaplan and Rittenberg, 1964; Canfield, 2001). However, sulfide in sediments and anoxic waters is commonly depleted in ^{34}S by 45-70‰ relative to sulfate. Brunner and Bernasconi (2005) argued that isotope fractionation during sulfate reduction well in excess of the -46‰ predicted by Rees (1973) was possible. However, this large fractionation is mostly likely to occur in hyper-sulfidic conditions. It is widely accepted

at present that the large isotope difference between sulfide and sulfate in nature is due to reactions associated with the oxidative part of sulfur cycle- i.e., disproportionation reactions of intermediate sulfur species (S^0, $S_2O_3^{2-}$, SO_3^{2-}) (Canfield and Thamdrup, 1994; Habicht and Canfield, 2001; Bottcher et al., 2005). Typical fractionations obtained for bacterial cultures of sulfur intermediate disproportionators are shown in equation 3-5 (Habicht and Canfield, 2001):

$$4S^0 + 4H_2O \rightarrow 3HS^- + SO_4^{2-} + 5H^+$$

$$(\delta^{34}S^0 - \delta^{34}HS^- = 7‰;\ \delta^{34}S^0 - \delta^{34}SO_4^{2-} = -21‰) \qquad (3)$$

$$S_2O_3^{2-} + H_2O \rightarrow HS^- + SO_4^{2-} + H^+$$

$$(\delta^{34}S_2O_3^{2-} - \delta^{34}HS^- = 3‰–15‰;\ \delta^{34}S_2O_3^{2-} - \delta^{34}SO_4^{2-} = -3‰–-15‰) \qquad (4)$$

$$4SO_3^{2-} + H^+ \rightarrow H_2S + 3SO_4^{2-}$$

$$(\delta^{34}SO_3^{2-} - \delta^{34}HS^- = 28‰;\ \delta^{34}SO_3^{2-} - \delta^{34}SO_4^{2-} = -10‰) \qquad (5)$$

Thus the isotopic composition of both sulfide and sulfate records the initial fractionation during sulfate reduction, followed by additional fractionations generated through repeated cycles of sulfide oxidation to sulfur intermediates and then disproportionation of these compounds to sulfide and sulfate.

Analysis of the $\delta^{34}S_{\sum H2S}$ and $\delta^{34}S_{SO4}$ values in aquatic systems can help identify the microbial or abiotic processes involved in sulfur cycling as well as temporal and spatial

changes in these processes. Through study of the sulfur isotope composition of dissolved sulfur species in the Black Sea water column, Neretin et al. (2003) found that dissolved sulfide averaged -39.6 ± 1.3‰ over all depths and the isotopic difference between sulfide and sulfate was about -60‰. Their data corroborated earlier results of Fry et al. (1991) and they argued that the large isotopic difference between sulfide and sulfate was due to disproportionation of sulfur intermediates. A sulfate reduction rate study in the Mariager Fjord indicated that 80% of sulfate reduction occurred in the sediment, and that sulfide in the water column came mainly from the sediment (Sørensen and Canfield, 2004). Therefore, the sulfide in the water column should have had a similar isotope composition to that in the sediment. However, the sulfide isotope composition in the water column varied between -13‰ and -21‰, while the sulfide diffusing from the sediment had a mean isotope value of -11.3‰. Together with most probable number data showing that disproportionators were present in all depths of the fjord, Sørensen and Canfield (2004) suggested that the excess fractionation could be accounted for by the disproportionation of sulfur intermediates in the water column, and was not due to slow sulfate reduction rate alone in the water column.

Few previous reports of sulfur isotope composition in the Cariaco Basin exist, which is largely due to the methodological constraints imposed by the small pool size of sulfide and rapid turnover of sulfur intermediates like sulfite, thiosulfate and elemental sulfur in this system. Fry et al. (1991) analyzed the sulfide isotopes for the Cariaco Basin water column and found that the isotope difference between sulfide and sulfate was about -52‰. However, only samples below 400 m were collected and analyzed. Therefore, the sulfur isotope signal near the interface, where disproportionation of sulfur intermediates

might be important based on concentration profiles and enrichment experiments (Li et al., 2008), was not examined. Werne et al. (2003) analyzed sulfur isotopes in Cariaco sediments to investigate the timing and possible pathways of organic matter sulfurization but did not measure isotopes in the water column. Therefore, the result of our study will assist in the interpretation of isotopic evidence preserved in Cariaco Basin sediment.

2.5 Pyrite formation in the anoxic water column

The relationship between pyrite and organic carbon has been used broadly to reconstruct the paleo-environments under which sediments have accumulated (Berner, 1982; Raiswell and Berner, 1985; Lyons et al., 2003). In marine anoxic basins such as the Black Sea and the Cariaco Basin, pyrite in the sediment has been suggested to have two sources, syngenetic (pyrite formation in the anoxic water column) and diagenetic (formed in the sediment after deposition) (Muramoto et al., 1991; Perry and Pedersen, 1993; Henneke et al., 1997; Lyons, 1997; Werne et al., 2003). If pyrite that is formed in the anoxic water column sinks to the overlying sediment, it may complicate the interpretation of past environmental conditions using pyrite.

Formation of pyrite at the oxic-anoxic interface has been reported for several environments where metabolisable organic matter, elemental sulfur and reactive iron are abundant. Based on isotope data, Muramoto et al. (1991) argued that iron sulfide precipitation in the water column of the Black Sea occured near the oxic anoxic interface. A similar conclusion was reached by Lyons and Berner (1992) who studied pyrite isotopes in Black Sea sediment. Calvert et al. (1998) suggested that most of the syngenetic pyrite in the Black Sea was formed in the upper part of the anoxic zone (upper 200 m), while others argued the formation zone was wider and could happen down to

1000-1500 m in the water column (Tambiev and Zhabina, 1998). In the Orca Basin, a high density of particulate FeS was found at the brine-seawater interface (Trefry et al., 1984; Hurtgen et al., 1999). In the Framvaren fjord, based on SEM, Skei (1988) concluded that pyrite is most likely to be formed near the redoxcline. In addition, several researchers have used the concentrations of dissolved Fe and sulfide to calculate the saturation state of Fe-sulfide minerals (Jacobs et al., 1985; Landing and Lewis, 1991). In the Cariaco Basin, temporal fluctuations in Fe and sulfide concentrations seem to be an indication of water column mineral formation (Jacobs et al., 1987; Scranton et al., 2001; Percy et al., 2008). However, due to the requirement for large sized samples for traditional methods (hundreds of milligrams of iron sulfides), only a few attempts at direct quantification of particulate sulfur minerals in the water column have been made so far (Perry and Pedersen, 1993; Cutter and Kluckhohn, 1999).

Sulfur isotope composition of particulate sulfide fluxes could be used as a tracer for the origin of the sulfide. Werne et al. (2003) calculated the contribution of syngenetic pyrite in the sediment based on the assumption that the isotopic composition of syngenetic pyrite should be similar to the isotope signal of deep water sulfide. This predicted that pyrite in the surface sediment was all from the water column. However, prior to the present study, no direct measurement of pyrite concentrations has been made in the water column of the Cariaco Basin, or of the magnitude of particulate sulfur flux to the sediment, or the isotope composition these pyrite fluxes might carry when it sinks from the water to the sediment.

3. **RESEARCH QUESTIONS**

Using the Cariaco Basin as a model system, this thesis aims to add more information of sulfur speciation, metal cycling and chemolithtrophic production to the library of the anoxic water bodies (just to name a few, the Black Sea, the Mariager Fjord, the Baltic Sea, Framvaren Fjord, Jellyfish Lake and Saanich Inlet) and major oxygen minimum zones (such as the Arabian Sea, the gulf of Mexico, and the equatorial pacific ….).

Several specific questions are addressed in this dissertation:

1) What is the distribution pattern of different sulfur intermediates in the Cariaco Basin water column? How do the distributions vary with space and time? What factors control the concentration of different sulfur intermediates? How do different sulfur intermediates relate to chemoautotrophic production at the redoxcline?

2) What is the extent of ^{34}S depletion in sulfide compared to sulfate in the water column of the Cariaco Basin? Are there variations in sulfur isotope compositions among different sulfur pools that may be related to different processes, such as sulfide oxidation and sulfur intermediate disproportionation?

3) Is pyrite forming in the anoxic water column? Where is the major formation zone: near the oxic-anoxic interface or in the deeper anoxic water column? How important is a syngenetic pyrite flux to the Cariaco sediment? Are the sources and losses of sulfur balanced well with each other in the anoxic Cariaco water column?

4) Are there significant temporal and spatial variations of chemical flux to the interface? How does the 'energy imbalance' issue vary with time and space?

4. THESIS ORGANIZATION

This thesis investigates sulfur cycling in the water column of the Cariaco Basin, with high depth resolution across the oxic-anoxic interface over four years, to better understand the interplay between geochemical cycling, biological production and microbial ecology. Chapter 1 provides background information on sulfur cycling and on the research questions addressed in this dissertation. Chapter 2 deals with concentration profiles of sulfur intermediates, and their relationship with chemoautotrophic production and other hydrographic conditions. The goal was to explore which sulfur intermediates are important in supporting the growth of chemoautotrophic microbes. Chapter 3 extends the sulfur cycling study to stable sulfur isotope measurement (dissolved sulfide and sulfate), especially by using a multiple sulfur isotope techniques, to give insight as to how different processes are controlling the isotope signal. Chapter 4 investigates pyrite formation in the anoxic water column. This chapter provides information on where pyrite is mainly formed and its possible formation mechanism, and how important syngenetic pyrite flux is to the Cariaco sediment. At the end of this chapter, a preliminary sulfur budget is presented. Chapter 5 compares the vertical chemical flux to the interface with the chemoautotrophic production for 24 cruises and 4 stations to explore whether the downward electron fluxes of oxidants balance well with upward reductants fluxes. We also revisited the 'energy imbalance' question based on what we learned from the previous three chapters of the thesis. Chapter 6 summarizes the thesis, and suggests future research directions.

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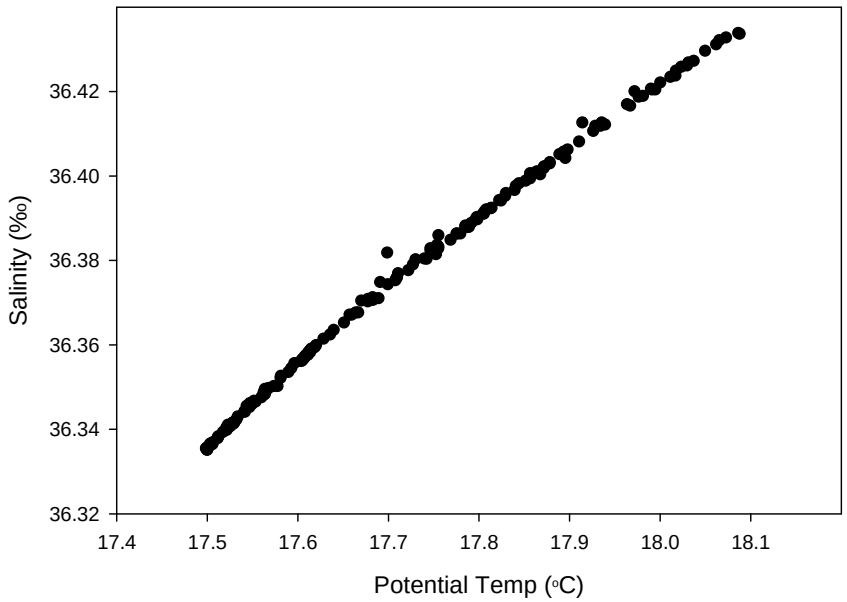

Figure 1.1. Potential tempeture-salinity correlation with data from April 2007 in the redoxcline (200-400 m) of the Cariaco Basin.

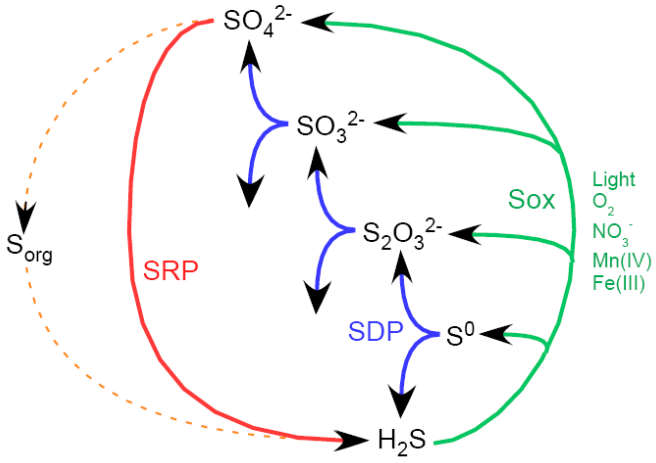

Figure 1.2. A cartoon depicting the primary forms of sulfur (the pools) and microbial processes (the transformations or pathways) involved in the sulfur cycle (modified from Canfield et al., 2005). Sulfide is formed from sulfate either through dissimilatory sulfate reduction (SRP, in red) or assimilatory sulfate reduction, which is a minor pathway (shown in dashed orange). Sulfide that is not sequestered as Fe-S minerals or organic sulfides in sediments is reoxidized through either biological or abiological processes (S ox, in green). A number of sulfur intermediate compounds can be formed as well, and these are either oxidized, reduced, or in many cases disproportionate to sulfide and sulfate (SDP, in blue).

CHAPTER TWO: Relationship of sulfur speciation to hydrographic conditions and chemoautotrophic production in the Cariaco Basin

(Li, X.N., Taylor, G.T., Astor, Y., Scranton, M.I., 2008. Marine Chemistry 112: 53-64)

Abstract

Sulfur species are likely to be important microbial substrates at redox transition zones. In this study, we measured thiosulfate ($S_2O_3^{2-}$), sulfite (SO_3^{2-}), particulate elemental sulfur (S^0), total zero-valent sulfur (particulate S^0 + polysulfides + colloidal S^0), and hydrogen sulfide (H_2S) while chemoautotrophic production and heterotrophic bacterial production were also measured at several locations in the Cariaco Basin as part of the on-going CARIACO (CArbon Retention in a Colored Ocean) time series project. Sulfite and thiosulfate concentrations always covaried within the redoxcline, but no reproducible distribution pattern was observed nor did it correlate with chemoautotrophic production. In contrast, particulate elemental sulfur maxima were consistently found at the interface and were highly correlated with chemoautotrophic production. Higher concentrations of particulate elemental sulfur at stations closer to the basin's margin were associated with higher inventories of hydrogen sulfide. Comparisons of profiles of total zero-valent sulfur and of particulate elemental sulfur suggest that a large fraction of zero-valent sulfur is either colloidal or in the form of polysulfides. Although thiosulfate stimulated bacterial activity in amendment experiments, the role of thiosulfate and sulfite in chemoautotrophic production is not yet clear.

1. Introduction

In anoxic basins, a pronounced peak in the rate of dark CO_2 fixation has been repeatedly observed in the redoxcline of the water column (Jørgensen et al., 1991; Fenchel et al., 1995; Taylor et al., 2001). However, the relatively high rates observed for chemoautotrophic production are problematic, since vertical fluxes of reductants (H_2S, NH_4^+, Fe^{2+}, Mn^{2+}) and oxidants (O_2, MnO_2, Fe_2O_3, NO_3^-) calculated from concentration profiles and estimates of vertical eddy diffusion can only support a few percent of the measured carbon fixation (Murray et al., 1995; Zopfi et al., 2001; Taylor et al., 2001). In addition, based on the organic matter flux and stoichiometry of sulfate reduction, Hayes et al. (2006) calculated that the particulate carbon sinking flux at the CARIACO site is too low to support rates of sulfate reduction required to satisfy the putative microbial demand for H_2S.

In addition to symbiotic associations (Taylor et al., 2001), another possible explanation for the apparent discrepancy between vertical fluxes of chemical species and biological demand is that horizontal supply of these species is important (Taylor et al., 2001, 2006). Transient lateral intrusions of oxic water or high wind events that circulate and introduce oxygenated water into the suboxic or anoxic zone may supply electron acceptors (Holmen and Rooth, 1990; Scranton et al., 2001; Astor et al., 2003). Calculations have suggested that enough O_2 may be able to enter the basin through intrusions to produce sufficient thiosulfate to support the chemoautotrophic growth of bacteria (Hayes et al., 2006). However, the origin of the required sulfide is still unclear. Taylor et al. (2001) calculated that vertical sulfide flux can account for only 0.7-2.8% of the estimated demand for reductants at Cariaco Station. Percy et al. (2007) pointed out

that higher inventories of sulfide (between 250 m to 340 m) were found in shallower, more productive water. However, the observed variations in sulfide inventory were not large. Even at the more productive, shallower station B (Fig. 2.1), the inventory of sulfide is only twice that of station A.

Since the maximum dark carbon fixation rate is always slightly deeper than the depth of detectable O_2 and nitrate (Taylor et al., 2001; 2006), our recent microbiological and geochemical studies have explored the cycling of S, Fe and Mn, which have been proposed to play important roles in sediments (Jørgensen 1990b; Nealson and Myers, 1992; Aller, 1994). Sulfide oxidation results in the formation of sulfur intermediates such as polysulfides (S_n^{2-}), elemental sulfur (S^0), thiosulfate ($S_2O_3^{2-}$) and sulfite (SO_3^{2-}). In recent investigations of thiosulfate and sulfite in the Cariaco (Hayes et al., 2006; Percy et al., 2007), no apparent relationship was found between either thiosulfate or sulfite and chemoautotrophic production within the redoxcline. Percy et al. (2007) suggested that elemental sulfur and polysulfides might be important, although no direct measurement of elemental sulfur had been carried out in the Cariaco Basin since Hastings and Emerson (1988).

In the present study, abundances and distributions of thiosulfate, sulfite and elemental sulfur were investigated during both upwelling and non-upwelling periods at four sites in the Cariaco Basin, and concentrations were compared to rates of chemoautotrophic and heterotrophic microbial production. Our objective is to identify, among the many potential factors, those hydrographic, chemical and microbial processes that can explain the observed distributions of sulfur intermediates in this unique environment. Based on the results to date, we suggest that CO_2 fixation is at least in part a

result of efficient cycling of sulfur species by chemoautotrophs in the Cariaco, and that elemental sulfur plays an important role either as a product or as a substrate.

2. Materials and Methods

2.1 Field site

The Cariaco Basin (Fig. 2.1) is a large, marine anoxic system on the continental shelf off the northern coast of Venezuela. A 900 m deep saddle separates the eastern and western basins. The deepest parts of the basin reach 1400 m and are isolated by a shallow (90 to 150 m) sill that restricts the entrance of Caribbean water. Channels to the northeast (La Tortuga channel: about 135 m) and the west (Centinela channel: about 146 m) provide pathways for denser water to penetrate into the deep basin (Richard, 1975). The stability of the basin is controlled by temperature but density is uniform to within 0.1 σ_θ units below about 250 m (Richards, 1975). The depth of first appearance of hydrogen sulfide has ranged between about 200 and 350 m (Ho et al., 2004), and a suboxic zone in which both oxygen and sulfide concentrations are below 1–2 μmol L^{-1} has varied in thickness from zero to over 50 m between 1995 and 2007 (Scranton et al., 2006; Percy et al., 2007).

2.2 Sample collection

Water samples were collected in the Cariaco Basin as part of the international CARIACO (CArbon Retention in a Colored Ocean) time series program. In CARIACO, a single station (station A) has been sampled monthly, and oxygen, temperature, salinity, nutrients, primary production and CO_2 data have been collected since 1995. A general description of the CARIACO program and data links can be found at the web site *(http://www.imars.usf.edu/CAR/).* In addition to the data available from monthly cruises,

we have undertaken two or three additional cruises annually during which more detailed chemical and microbiological measurements have been made. Some of the data from these cruises are described by Taylor et al. (2001, 2006), Hayes et al. (2006), Scranton et al. (2006), Lin et al. (2006, 2007), and Percy et al. (2007) as well as in other publications.

For the present study, samples were collected at four stations (Fig. 2.1, Table 2.1), including the CARIACO time-series station (station A, maximum depth ca. 1400 m, 10°30′ N 64°40′ W), a station southeast of La Tortuga Channel (station B, maximum depth ca. 600 m, 10°40′ N 64°45′ W), a station in the western basin (station C, maximum depth ca. 1400 m, 10°40′ N 65°35′ W) and a station to the northeast of the CARIACO station (station D, maximum depth ca. 500 m, 10°43′ N 64°32′ W). Dates of sample collection are summarized in Table 2.1. Water samples were collected in 12 8-L Teflon-lined Niskin bottles mounted on a Seabird rosette system equipped with a SBE 25 CTD, a SBE 43 oxygen probe, a WetLab profiling fluorometer for chlorophyll-a, and a WetLab c-beam transmissometer (660 nm). The Niskin bottles were slightly pressurized with N_2 during sampling to minimize O_2 contact. In the Cariaco Basin, peaks in beam attenuation (BAT) from the transmissometer have proven to be reliable proxies for bacterial maxima near the interface, and sampling depths were adjusted accordingly to resolve features of interest.

2.2.1 Oxygen

Seawater samples for dissolved oxygen were drawn into standard oxygen bottles in duplicate and Winkler reagents were added immediately. Details of methods are provided in Astor et al. (2003). Samples were stored in an air-conditioned room with seawater around the caps to minimize oxygen transfer across the stoppers and were run within 48 h

of returning to the Fundación La Salle laboratory on Margarita Island. The oxygen probe on the CTD also provided continuous O_2 profiles which were corrected for response time and calibrated with Winkler O_2 data.

2.2.2 Sulfide, thiosulfate and sulfite

Samples for sulfide, thiosulfate and sulfite analyses were collected as described by Hayes et al. (2006) and Percy et al. (2008) by placing the tip of a 10 ml all-glass Hamilton Gas-Tight syringe below the surface of water flowing upward through a 60 ml plastic syringe barrel which was attached to the Niskin bottle by Tygon tubing. Sampling from flowing seawater was used to minimize contact of samples with O_2. Sulfide samples were taken in triplicate and were analyzed using Cline (1969) methylene blue method. Thiosulfate and sulfite were collected in triplicate and were analyzed with the DTNP method of Vairavamurthy and Mopper (1990) as modified by Hayes et al. (2006).

Kinetic calculations for a mixing of sea water with 250 μM O_2 and 60 μM sulfide (full oxygenation of anoxic bottom water) suggest a maximum production rate of sulfite of 0.017 μmol L^{-1} min^{-1} (Zhang and Millero, 1993b), indicating that under the worst conditions during our derivatization about 0.1 μmol L^{-1} SO_3^{2-} could be produced by the reaction of sulfide with oxygen during a 5 min reaction time. Since oxygen was carefully excluded during sample collection and the sulfide concentration was always lower than 10 μM above 350 m where we observed sulfite concentration of as high as ca. 4 μmol L^{-1} (see Fig. 2.3 CAR 122), this calculation suggests that concentrations of sulfite in the upper anoxic water are not a consequence of sample collection artifacts.

2.2.3 Elemental sulfur

Duplicate particulate elemental sulfur samples were acquired by gravity filtering directly from the Niskin bottles as described by Trouwborst (2005) and were analyzed by a modification of the method of Henneke et al. (1997). Filter holders, loaded with 0.2 μm polycarbonate filters, were attached to the Niskin bottle by Tygon© tubing. Filtrate was collected for each filter in a graduated cylinder to determine the filtered volume. The filters were rinsed with de-ionized water, dried by passing argon gas through the filters and stored in 15 ml centrifuge tubes at -20 °C. After return to Stony Brook University, 6 ml methanol was added to each centrifuge tube to extract elemental sulfur from the filter. The centrifuge tubes were shaken for 2.5 hours on a mechanical shaker and the S^0 concentration of each sample was analyzed on a Shimadzu HPLC consisting of a SCL 10A-VP system controller, two LC-10AT pumps, an SPD-10AV/VP ultraviolet detector, and a SIL-10A auto-injector. We used a ODS hypersil C_{18} reverse phase, 250 mm × 4.6 mm, 5 μm column (Supelco Co.) at room temperature. Twenty μl samples were injected into the chromatograph and eluted with 100% methanol at a pump speed of 1 ml/min. Retention time of the elemental sulfur peak was typically about 2.2 min. Elemental sulfur was detected at 226 nm, with a detection limit of about 1 μmol L^{-1}, and a precision of 0.5% relative standard deviation among replicates. Standard solutions, made by dissolving sulfur powder in methanol and serially diluting, are linear in the range of 1–100 μmol L^{-1}.

We also measured total zero-valent sulfur at station A during cruise CAR-132. Duplicate 40 ml sub-samples were taken with a gas tight BD syringe in same manner as sulfide samples and fixed with 1 ml 2% (w/v) zinc acetate (Ramsing et al., 1996; Henneke et al., 1997; Zopfi et al., 2001). With this treatment, the sulfane-S components

of polysulfides are transformed to elemental sulfur. Therefore, this method determines the sum of particulate, colloidal and sulfane fractions of the polysulfides. Samples were stored at -20 °C in the dark. After return to Stony Brook University, total elemental sulfur was extracted twice using 1 ml chloroform, and the pooled chloroform extract was diluted 1:1 with methanol to optimize chromatography. The HPLC configuration was basically similar to that used for the measurement of particulate elemental sulfur, but the mobile phase was changed to 90% methanol: 10% DI water at a flow rate of 0.5 ml/min. The retention time of sulfur (0) was around 8.5 min.

2.2.4 Microbiological analysis

Heterotrophic bacterial net production (BNP) and chemoautotrophic production (CHEMO) were determined by assimilation of ^{3}H- leucine into protein and ^{14}C-bicarbonate into particles, respectively. Details of the methods have been described previously (Taylor et al., 2001).

Amendment experiments with thiosulfate and sulfite were performed during CAR-132 at station A to assess the role of sulfur intermediates on chemoautotrophic metabolism at 6-7 depths, spanning the redoxcline. In addition to ^{14}C-bicarbonate, duplicate samples were spiked with 100 µl of N_2 -purged thiosulfate or sulfite (final concentration: 50 µmol L^{-1}) using a gas tight syringe, and then were incubated in the dark at in situ temperature (17 °C) for 18 hours. Samples were processed in the same manner as chemoautotrophy samples to test whether addition of thiosulfate or sulfite stimulated dark carbon fixation. Previous incubation experiments with additions of S^0 did not stimulate chemoautotrophic carbon fixation (Taylor et al., 2001), although this result may have been affected by the low bioavailability of the crystalline S^0 used.

2.2.5 Inventory calculation

Concentrations of sulfur species (sulfide, particulate elemental sulfur) were integrated from 250 m to 340 m to calculate their "inventory" for each of the four cruises. We picked this depth range because it brackets the full suboxic and upper sulfidic zones, the depth interval most impacted by temporal variability and in which chemolithotroph production is typically most active. We also integrated BNP and chemo to estimate rates per meters squared per day.

3. Results

3. 1. Suboxic zone variability

For the Cariaco Basin, we have operationally defined the suboxic zone as lying between the first depth where O_2 was determined to be ≤ 2 μmol L^{-1} by Winkler titration and the first depth where H_2S was greater than 1 μmol L^{-1}. Therefore, the water column can be classified into three zones (Fig. 2.2): an oxic zone where O_2>2 μmol L^{-1}, a sulfidic zone where H_2S> 1 μmol L^{-1}, and a suboxic zone, where sulfide and O_2 are both very low (or are both absent) with little perceptible vertical gradient (Murray et al., 1995; Glazer et al., 2006). Because we sampled at ten meter intervals across the interface, in some cases we did not have measurements at these specific concentrations. Under those circumstances, when there was a clear change in the slope of the oxygen or sulfide profile, we used an oxygen value of 2-3 μmol L^{-1} or a sulfide value of 1-2 μmol L^{-1}. Estimates of the top and bottom of the suboxic zone are marked using dashed lines on Figure 2.2 for each cruise. As found by Scranton et al. (2006) and Percy et al. (2008), depth and position of the suboxic zone varied depending on location and time, exhibiting changes in both the depth of oxygen disappearance and of sulfide appearance. Changes from cruise

to cruise were not uniform at all stations. As pointed out by Percy et al. (2007), variations in the suboxic zone demonstrate the dynamic nature of geochemical fluxes within the Cariaco Basin, and indicate that intrusions, other advective processes and episodic events all likely contribute to spatial and temporal variability of chemical properties. Since changes in the suboxic zone thickness caused by intrusions likely result in oxidation of sulfide, sulfide inventories as well as those of sulfur intermediates are expected to vary spatially and temporally.

3.2. Sulfur intermediates

3.2.1 Thiosulfate and sulfite

As found previously by Hayes et al. (2006) and Percy et al. (2007), several distribution patterns were evident for thiosulfate and sulfite in the Cariaco (Fig. 2.3). During Jan 2006 (CAR-118), thiosulfate and sulfite profiles showed distinct maxima at the bottom of the station A suboxic zone. In May 2006 (CAR-122), thiosulfate and sulfite concentration maxima were observed again at station A, apparently within the suboxic zone. However, the maximum concentration of sulfite was twice that observed in CAR-118. The concentration of thiosulfate during CAR-122 at station A was comparable to the concentration of sulfite, while in all other situations, thiosulfate was typically half of the concentration of sulfite. During Nov 2006 (CAR-128), at station B, maxima in thiosulfate and sulfite concentrations were found at the bottom of the suboxic zone, but the amounts of thiosulfate/sulfite were much lower than seen during CAR-118 and CAR-122.

We also observed minima of thiosulfate and sulfite near the interface on a few occasions. For example, during CAR-118, there was a distinct decrease for thiosulfate and sulfite in the suboxic zone of station B, although this was the only occasion where we

found minimum of thiosulfate and sulfite in the suboxic zone compared to concentrations in the oxic and anoxic zone. At Station C, during the same cruise, a maximum of sulfite/thiosulfate was found at the top of the suboxic zone which was followed by a minimum at the base of the suboxic zone.

Finally, on several other occasions, no clear pattern was found around the interface. For example, the distribution of thiosulfate and sulfite at Station B and D (CAR-122), Station A (CAR-128), Station A, B and D (CAR-132) fell into this category. For these stations, the vertical distribution of sulfite/thiosulfate was irregular, with low concentration near the interface, and a tendency to increase with depth. The concentration of thiosulfate and sulfite for these occasions was always below 1 μmol L^{-1} for most of the sampling depths.

3.2.2 Particulate elemental sulfur

A particulate elemental sulfur peak was consistently found within the redoxcline at all stations during all four cruises (Fig. 2.3). Maximum concentrations of elemental sulfur at each station are listed in Table 2.2 and ranged from a low of 0.22 μmol L^{-1} at station C during CAR-118 to a high of 1.22 μmol L^{-1} at station B during CAR-128. Particulate elemental sulfur was measured at Station A and B during all four cruises, and the highest sulfur peak was detected during CAR-128 at both stations (Nov 2006).

During CAR-118, 122 and 128, higher particulate elemental sulfur was always found in the suboxic zone of station B than other stations, possibly related to a higher sulfide source at this station (Fig. 2.4). During April 2007 (CAR-132), the elemental sulfur maximum was highest at station D, then station A, followed by station B. Station B is closer to La Tortuga Channel and more susceptible to effects of oxygen intrusion than

station A. The suboxic zone of station B is always shallower and broader than station A (Percy et al., 2007; this work, Fig. 2.2), suggesting higher particulate organic flux and lateral intrusion of oxic water, respectively. However, in contrast to previous cruises, the upper boundary of sulfide at station B during CAR-132 was deep, even deeper than that of station A (Fig. 2.2), and the sulfide inventory was lower than the previous three cruises (Fig. 2.4). Chlorophyll *a* data derived from SeaWiFS *(http://imars.marine.usf.edu/*) revealed that in the two weeks before CAR-132, chlorophyll *a* concentrations at station D were 2-8 times of that at station B, while before CAR-122, the chlorophyll *a* concentrations at these two stations were similar. This result highlights the dynamic nature of the Cariaco Basin and is supplementary to the results of Percy et al. (2007) who found that higher inventories of sulfide were found in more productive areas.

3.2.3 Total zero-valent sulfur and particulate elemental sulfur

A more detailed analysis of the distribution of total zero-valent sulfur compared to particulate elemental sulfur was carried out at Station A during CAR 132 (Fig. 2.5). Particulate elemental sulfur showed a sharp peak of 0.5 μmol L^{-1} at 290 m, which was the depth of first appearance of sulfide, but was almost undetectable below 400 m. In comparison, the peak in total zero-valent sulfur was broader, with a maximum observed at a shallower depth (270 m). Total zero-valent sulfur (which includes particulate elemental sulfur, the sulfane fraction of polysulfides and colloidal sulfur) was still detectable to 400 m, the deepest depth we sampled for this parameter. Particulate elemental sulfur made up from 2 to 63% of the total zero-valent sulfur. In the water below 305 m, most zero-valent sulfur (70-98%) was present in the form of polysulfides

or colloidal sulfur since particulate elemental sulfur was undectable (Fig. 2.5). Unfortunately, we can't differentiate between polysulfides and colloidal sulfur.

3.3 Amendment experiment with thiosulfate and sulfite

Amendment experiments have been done on a number of CARIACO cruises to assess the role of thiosulfate and sulfite on chemoautotrophic metabolism (Taylor, unpub. data). The result from CAR-132 station A is shown as an example here to demonstrate the effect of $S_2O_3^{2-}$ and SO_3^{2-} on dark carbon fixation rates (Fig. 2.6). Thiosulfate stimulation was apparent in the deeper suboxic and upper anoxic zones. In contrast, in the upper suboxic and deeper anoxic zones, the stimulation effect by $S_2O_3^{2-}$ was marginal, suggesting that the organisms at these depths either are not using $S_2O_3^{2-}$ or are limited by other resources, e.g., oxidants. Stimulation of carbon fixation by sulfite at all sampling depths was minimal, suggesting that sulfite may not be an important substrate for chemoautotrophs in the Cariaco Basin.

4. Discussion

4.1 Distributions of sulfur species in the Cariaco Basin

Oxygen in intruding water can oxidize H_2S, either directly or in reactions catalyzed by MnO_2/ Fe_2O_3 to produce sulfur intermediates such as S_n^{2-}, S^0, $S_2O_3^{2-}$ and SO_3^{2-} (Chen and Morris, 1972; Millero, 1991). Under situations when mixing was enhanced due to temporarily varying oxygen intrusion, elemental sulfur was by far the most abundant sulfur intermediate in Mariager Fjord (Zopfi et al. 2001). However, in some environments, thiosulfate can be the major intermediate oxidation product and can represent as much as 50% of all sulfur intermediates (in lake sediments, Jørgensen 1990a, b). Biological activity can also cause conversion of one sulfur species to another (Steudel,

1989). During the four cruises reported here, relative abundances of thiosulfate, sulfite and particulate elemental sulfur varied among stations and temporally.

4.1.1 Thiosulfate and sulfite

Thiosulfate/sulfite findings corroborate earlier results of Hayes et al. (2006) and Percy et al. (2007). It remains unclear why profiles of sulfite and thiosulfate do not consistently show either maxima or minima within the redoxcline. Sulfide oxidation is likely to be dependent on vertical mixing and lateral intrusions within the redoxcline and this variability is high both spatially and temporarily (Fig. 2.2). Therefore, sulfur intermediates are expected to be dynamic, and high variability for thiosulfate and sulfite is likely.

The distribution of thiosulfate and sulfite in the Cariaco Basin probably reflect a balance between production (abiotic or biotic oxidation of sulfide) and consumption (chemical transformation or the reaction time for microbial population to access the resource). The extremely low thiosulfate near the suboxic zone at some stations is probably due to its value as substrate for bacteria (Jørgensen, 1990b). Our thiosulfate amendment experiments stimulated both cell growth rates (data not shown) and dark carbon dioxide fixation (Fig. 2.6). Thus, the depth distribution of thiosulfate may reflect the high efficiency of its consumption (Thamdrup et al., 1994).

While there was no apparent dependence of thiosulfate/sulfite on sulfide (Table 2.3), sulfite concentration was highly correlated with that of thiosulfate ($R=0.93$, $n=106$, Table 2.3). This has been seen previously (Hayes et al., 2006; Percy et al., 2007). Sulfite concentrations were typically 2-3 times higher than thiosulfate (Fig. 2.3) although this varied somewhat (see CAR-122 station A data in Fig. 2.3). This strong relationship may

suggest coupled production or inter-conversion of the two compounds (Thamdrup et al., 1994). As suggested by Jørgensen (1990b), the two compounds can be produced by sulfide oxidation at a certain ratio which depends on the oxidants and sulfide availability. Similar distribution patterns of thiosulfate and sulfite have been reported for the Black Sea (Vairavamurthy and Mopper, 1990), the sediments of a Danish salt marsh (Thamdrup et al., 1994), and previously in the Cariaco Basin (Zhang and Millero, 1993a; Hayes et al., 2006, Percy et al., 2007). Sulfite was not detected by the DTNP method in Framvaren Fjord (where sulfide concentrations are millimolar) while thiosulfate was found to increase with depth (Millero, 1991).

4.1.2 Elemental sulfur

So far, most studies of elemental sulfur have been carried out in the sediments and very few measurements in anoxic water are available (Hastings and Emerson, 1988; Zopfi et al., 2001). In the present study, concentrations of particulate elemental sulfur were similar to previously reported values for the Cariaco Basin, in spite of the different sampling and analytical method used (Hastings and Emerson, 1988). Particulate elemental sulfur maxima near the redoxcline of the Cariaco were similar to the highest values in the Black Sea in spite of lower sulfide concentration (Jørgensen et al., 1991; Konovalov et al., 2003).

The inventory of particulate elemental sulfur is significantly correlated to sulfide inventory below the interface (250-340 m) (Fig. 2.4). Higher elemental sulfur inventories are always found in the more productive areas where there was higher sulfide, such as in Station B and D. This is consistent with the result of Zopfi et al. (2004), who found that

the S^0 content was higher in an environment where the sulfate reduction rate and therefore the sulfide production was higher.

Seasonal variability of particulate elemental sulfur also may be due to temporal changes of water circulation patterns in the Cariaco. Over four cruises in different parts of the Basin, maximum particulate sulfur near the interface varied by a factor of 5 (Fig. 2.3, Table 2.2), with higher values of particulate elemental sulfur seen in November at both station A and B. Preliminary interpretation of Acoustic Doppler Current Profiler data suggests that currents are lowest during winter and reach their maximum value during late summer (Charles Flagg, pers. comm.). These currents seem to be associated with a gyre in the Basin which could result in Ekman transport of O_2-containing water from the sill into the suboxic zone. If this is the case, then variations in S^0 may be explained by greater pumping of oxidants into the suboxic zone during summer and fall (CAR 128) (Wang et al., 2008).

Elemental sulfur is an important product of both chemical and biological sulfide oxidation. From the similarity of sulfide oxidation rates in poisoned and unpoisoned water samples, Sorokin (1972) concluded that bacteria played no significant role in the initial step of sulfide oxidation to elemental sulfur. However, kinetics of sulfide oxidation in water samples from the redoxcline of Solar Lake, where sulfide fluxes were high enough to sustain a large population of sulfide-oxidizing bacteria, demonstrate the strong involvement of bacteria in elemental sulfur formation (Jørgensen et al., 1979). Under in situ conditions in the Black Sea, chemical oxidation of sulfide appeared to be more important, but when amended with bacterial isolates with a modest density (> ca. 10^4 ml^{-1}), biological sulfide oxidation could compete successfully with spontaneous chemical

oxidation (Jannasch et al., 1991). Intracellular pools of sulfur granules from chemoautotrophic sulfur bacteria can also be extracted using our protocol of particulate element sulfur. Steudel (1989) found that elemental sulfur can be produced by sulfide-oxidizing bacteria, and is present as intracellular storage products. However it is not possible with present data to tell whether the observed particulate S fraction represents intracellular granules or extracellular inorganic S (0). Based on the fact particulate elemental sulfur is only a fraction of total zero-valent sulfur, we speculate that the particulate sulfur pool we quantified is not all intracellular. Another piece of evidence supporting this speculation is that total bacterial abundances in the redoxcline of the four cruises do not correlate with particulate elemental S (data not shown). Further investigation using environmental SEM and elemental sulfur isotope probes would help to clarify this question.

Several oxidants, such as O_2, MnO_2 or Fe_2O_3, could be used to oxidize sulfide (chemically or biologically) near the oxic-anoxic interface to produce elemental sulfur. For example,

$$MnO_2 + HS^- + 3H^+ \rightarrow Mn^{2+} + S^0 + 2H_2O \qquad (1)$$

$$2FeOOH + HS^- + 5H^+ \rightarrow 2Fe^{2+} + S^0 + 4H_2O \qquad (2)$$

In the Black Sea, oxidized Mn species have a significant relationship with the sulfur concentration near the interface. Konovalov et al. (2003, 2006) argued that, instead of oxygen, the particulate Mn was the direct oxidant of sulfide in the western Black Sea. Also in the Black Sea, dissolved Mn (III) complexes were shown to account for most of the oxidizing power (Trouwborst et al., 2006; Yakushev et al., 2007). Unfortunately, we

do not have particulate Mn/Fe and Mn (III) data to test these hypotheses in the Cariaco Basin. However, estimates of upward fluxes of dissolved Mn and Fe are not strongly correlated with elemental sulfur concentrations (data not shown).

Elemental sulfur has been reported to be the main product of sulfide oxidation with Mn (IV) (Burdige and Nealson, 1986) (Equation 1), although at high abundances of Mn (IV) relative to H_2S, thiosulfate and even sulfate become the main products (Yao and Millero, 1995). Compared to Mn (IV), Fe (III) is a weaker oxidant and oxidation of sulfide completely to sulfate is marginally thermodynamically favored (Aller and Rude, 1988). During the reaction of sulfide with Fe (III), elemental sulfur is produced first (Equation 2) (Luther et al., 1991). In the Cariaco Basin and Black Sea, dissolved Fe concentrations are similar, but dissolved Mn in the Black Sea is an order of magnitude higher than that in the Cariaco (Lewis and Landing, 1991; Percy et al., 2007). Thus the quantity of Mn/Fe oxidant may only be sufficient to oxidize sulfide to elemental sulfur in the Cariaco, possibly resulting in a greater role for this species.

4.1.3 Total zero-valent sulfur and particulate elemental sulfur

As mentioned earlier, measured total zero-valent sulfur includes particulate elemental sulfur, colloidal sulfur and the sulfane part of polysulfides. Quantification of both total zero-valent sulfur and particulate elemental sulfur during CAR-132 allows us to assess the potential significance of colloidal sulfur and polysulfides in the Cariaco.

In sulfidic waters, the solubility of elemental sulfur can be greatly increased by its reaction with sulfide to form polysulfides (Chen and Gupta, 1973):

$$HS^- + (n\text{-}1)\, S^0 \leftrightarrow S_n^{2-} + H^+ \qquad (3)$$

Sulfur speciation data from Rogoznica Lake (Croatia) obtained using a voltammetric technique support the hypothesis that at depths above the oxic-anoxic interface, a part or even all elemental sulfur is in the form of soluble or colloidal sulfur while in deeper layers, when sulfide concentrations are several times higher than that of elemental sulfur, zero-valent sulfur is probably in the form of polysulfides (Ciglenečki et al., 1996). In Solar Lake (Jørgensen et al., 1979), particulate element sulfur peaked near the oxic-anoxic interface while in deeper waters, constant and high polysulfide S^0 concentrations (around 100 μmol L^{-1}) were observed. In the Black Sea, polysulfides were observed at the base of the suboxic zone (Glazer et al., 2006). Zopfi (pers. comm.) examined partitioning of elemental sulfur between particulate and dissolved fractions in the Mariager Fjord's redoxcline where concentrations of particulate elemental sulfur and total sulfur were highest, and found that particulate elemental sulfur (trapped on 0.2 μm polycarbonate filter) only accounted for 30-40% of the total sulfur, with the rest appearing in the filtrate as either colloids or polysulfides. In the Cariaco redoxcline, particulate sulfur:total sulfur ratios varied from 0.1 to 0.6. In other environments where the sulfide concentration is low, elemental sulfur is typically found in the particulate or colloidal phases (Troelsen and Jørgensen, 1982).

To estimate polysulfide speciation in Cariaco water, we used in situ pH, temperature and sulfide concentrations from CAR 132 to calculate thermodynamically the concentration of each polysulfide (n=2-8) (Table 2.4). The calculated concentrations are based on the assumption that our system is saturated with elemental sulfur and therefore the activity of elemental sulfur is assumed to be 1. The equilibrium constant for equation 3 thus is given by

$$K_n = \frac{\{S_n^{2-}\} \cdot \{H^+\}}{\{HS^-\}} \quad (4)$$

Then we can calculate the concentration of each polysulfide as:

$$[S_n^{2-}] = [HS^-]*K_n/[H^+] \quad (5)$$

We used the thermodynamic constants from Kamyshny et al. (2007). Our calculations indicate that in Cariaco, at all depths below the first appearance of sulfide, sulfide concentration is high enough to support polysulfide formation (Table 2.4). Consistent with observation, at 290 m where sulfide first appears, approximately 60% of the total elemental sulfur was calculated to be present as particulate elemental sulfur, but at depths of higher sulfide, the particulate S^0 fraction decreases and most elemental sulfur should be present in the form of polysulfides.

We can test the assumption that our system is saturated with elemental sulfur by comparing calculated and measured polysulfide. Here, we define potential maximum concentration of polysulfides as the difference between measured total zero-valent sulfur and particulate elemental sulfur. Column *14* in Table 2.4 summarizes the amount of zero-valent sulfur in calculated polysulfides (In S_2^{2-} there is one S^0 atom, in S_3^{2-} there are two S^0 atoms, and so on). When we compare this value to the potential maximum concentration of polysulfides, we find that, in the upper 290 m, our system is in equilibrium with elemental sulfur, while below 305 m the sum of zero-valent sulfur for calculated polysulfides is more than the measured potential maximum concentration of polysulfides. Therefore, our system is likely not to be saturated with S^0 below ca. 305 m.

We need to point out that only limited thermodynamic data are available for polysulfide speciation (Kamyshny et al., 2007). We can not be certain that constants picked here are representative of the Cariaco Basin. Therefore, the thermodynamic

calculations are meant to provide a general idea of the form of sulfur rather than a rigorous quantitative assessment. Direct measurement of specific polysulfides is problematic since the detection limit is relatively high (ca. 1 μmol L^{-1} when expressed as S^0 (Kamyshny et al., 2006)), compared to the potential maximum concentration of polysulfide in the Cariaco (ca. 0.5 μ mol L^{-1}).

If zero-valent sulfur is present in the deep anoxic waters, it could act as a potential oxidant in the deep water column, or even in the sediment. Based on the sulfur isotope value of organic sulfur compounds in Cariaco sediment, Werne et al. (2003) suggested that organic matter sulfurization might be taking place via sulfur intermediates, such as elemental sulfur. Our data support the possibility of the existence of a water column source of elemental sulfur to the sediment.

4.2 Sulfur species and chemoautotrophic production

We are aware of the fact that the redoxcline of anoxic basins harbors a tremendous diversity of marine microbes and different functional groups of bacteria, archaea, and protists arise from this diversity to dominate various habitats and drive important biogeochemical cycles. Exploration of the distribution of microbial taxa and possible microbial interactions should be encouraged when exploring the relatively high rates of chemoautotrophic production for future research. In this communication, based on the depth distribution and relationship of particulate elemental sulfur with chemoautotrophic production and heterotrophic bacterial production, we want to discuss elemental sulfur as a potential substrate for chemoautotrophic bacteria, a process which has not been considered in Cariaco previously.

Chemoautotrophic and heterotrophic bacterial production at a particular depth co-varied strongly with concentration of particulate elemental sulfur at that depth, but not with thiosulfate or sulfite concentrations (Table 2.3). The position and the shape of the peak of chemoautotrophic production, heterotrophic bacterial production and particulate sulfur agree reasonably well in CAR-118, CAR-122 and CAR-132 (Fig. 2.7). However, in CAR-128, the chemoautotrophic production was lower than other dates (note the scale of dark carbon fixation for CAR-128 is three times lower than for other cruises, Fig. 2.7).

Based on the depth distribution and relationship with chemoautotrophic production and heterotrophic bacterial production, we speculate that elemental sulfur is an important substrate for chemoautotrophic bacteria around the oxic-anoxic interface and upper anoxic zone. One possible reaction coupling chemoautotrophic production and elemental sulfur is sulfur disproportionation. Under standard conditions, the $\Delta G^{0'}$ value of elemental sulfur disproportionation is positive, while the $\Delta G^{0'}$ value of thiosulfate/sulfite disproportionation is negative (Rabus et al., 2006). However, environmental concentrations are substantially lower than the 1 M standard values. We used pH measured during monthly CARIACO cruises, [SO_4^{2-}] concentration measured gravimetrically (Li, unpublished data), and measured dissolved sulfur intermediate concentrations to recalculate the energy yield of sulfur intermediates disproportionation under in situ conditions based on equations 6-8. For simplicity, we assume that the concentrations of S^0, $S_2O_3^{2-}$, SO_3^{2-} and HS^- are all 1 μmol L^{-1}, which is reasonable considering typical conditions near the interface (Fig 2.2, 2.3). Under these circumstances, the energy yield of sulfur intermediate disproportionation turns out to be:

$$4\ SO_3^{2-} + H^+ \rightarrow 3SO_4^{2-} + HS^- \qquad (6)$$

$$\Delta G^{0\prime} = -29.6\ KJ/\ mol\ sulfite$$

$$S_2O_3^{2-} + H_2O \rightarrow SO_4^{2-} + HS^- + H^+ \qquad (7)$$

$$\Delta G^{0\prime} = -72.8\ KJ/\ mol\ thiosulfate$$

$$4S^0 + 4H_20 \rightarrow SO_4^{2-} + 3HS^- + 5\ H^+ \qquad (8)$$

$$\Delta G^{0\prime} = -70.2\ KJ/\ mol\ sulfur$$

These calculations show that at the in situ conditions of the Cariaco redoxcline, the energy yield of elemental sulfur disproportionation is similar to that of thiosulfate, but is 2-3 times higher than sulfite, although all three reactions are thermodynamically favorable. The lower yield for sulfite may explain the minimal stimulation observed in enrichment experiment with sulfite. These results suggest that elemental sulfur might be as important a substrate for chemoautotrophic production as thiosulfate. Other workers have suggested that sulfur disproportionation should occur preferentially where the sulfide concentration is maintained at low levels (less than 1 μmol L^{-1}) either by oxidation with O_2 or by reaction with MnO_2 and FeOOH (Thamdrup et al., 1993). However, further calculation with the in situ conditions in the Cariaco Basin indicated that even when sulfide concentration is 10 μmol L^{-1}, the energy yield of disproportionation of sulfur intermediates does not change much ($S_2O_3^{2-} \sim S^0 > SO_3^{2-}$). Therefore, from the redoxcline to the upper anoxic zone, where we always observe maxima of both elemental sulfur and chemoautotrophic production, and occasionally

maxima in SO_3^{2-} and $S_2O_3^{2-}$, disproportionation of sulfur intermediates is always thermodynamically favored.

We do not yet understand why the elemental sulfur concentrations were more closely related to chemoautotrophic production than were concentrations of thiosulfate. Based on the stoichiometry, the measured quantity of particulate MnO_2 and FeOOH (Percy et al., 2007) is only adequate to oxidize sulfide to zero-valent sulfur, but not further to thiosulfate or sulfite. It is likely that there is a metal/sulfur redox shuttle operating in our system where dissolved sulfide could diffuse up to oxic/suboxic water and rapidly get oxidized by either O_2 or metal oxidants (MnO_2 and FeOOH), forming particulate zero-valent sulfur, which would then sink across the interface and be utilized by chemoautotrophic bacteria. This mechanism permits deeper and faster penetration of potential oxidant into the chemocline than diffusion alone and could be associated with repetitive cycling of the redox pairs. Direct kinetic measurements at sea with radiotracer technique under controlled conditions might help resolve this possibility.

Although we don't have direct evidence for sulfur disproportionation in the Cariaco system, microorganisms whose energy metabolism is based on the disproportionation of inorganic sulfur compounds have been found to be numerically abundant in some similar environments (Jørgensen, 1990a). Disproportionation of elemental sulfur in pure cultures was first reported by Bak and Cypionka (1987). In the Mariager Fjord, Ramsing et al. (1996) pointed out that elemental sulfur could be an important sulfur intermediate with a high turnover rate. Based on stable isotope mass balances, Sørensen and Canfield (2004) indicated that half of the sulfide is oxidized to either elemental sulfur or thiosulfate and subsequently disproportionated. To date, enrichment cultures from the Cariaco Basin

have yielded microaerophilic thiosulfate-oxidizers, sulfur and thiosulfate disproportionationers, thiosulfate-oxidizing manganese reducers and denitrifying thiosulfate-oxidizers (Madrid et al., 2001; Lin, unpubl. data). However, there is a variety of pathways involving sulfur, metal oxides and nitrogen species which are possible, and should also be further investigated.

5. Conclusion

The concentrations of several sulfur intermediates have been determined in the Cariaco water column, including particulate elemental sulfur, zero-valent sulfur, thiosulfate and sulfite. Results showed that the concentrations of sulfur intermediates near the interface vary with space and time, and thiosulfate and sulfite profiles near the interface lack a reproducible distribution pattern, likely reflecting the dynamic and complicated nature of the Cariaco Basin. In contrast, a particulate element sulfur peak was consistently observed near the interface. Based on the distribution profile and relationship analysis between sulfur species and chemoautotrophic production, we postulate that elemental sulfur is important in supporting the chemoautotrophic bacterial near the interface and upper anoxic zone.

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Table 2.1. Sampling dates and stations

Cruise #	*Date*	*Station*	*Note*
CAR-118	Jan 14-16 2006	A, B, C	Relaxation
CAR-122	May 19-20 2006	A, B, D	Upwelling
CAR-128	Nov 10-12 2006	A, B	Relaxation
CAR-132	Apr 11-13 2007	A, B, D	Upwelling

Table 2.2. Particulate elemental sulfur maximum concentration (μmol/L) and the depth where sulfur maximum was located (meters, in parenthesis) during the four cruises at the Cariaco Basin

	CAR-118 (Jan 2006)	*CAR-122 (May 2006)*	*CAR-128 (Nov 2006)*	*CAR-132 (Apr 2007)*
Station A	0.38 (280)	0.55 (260)	0.56 (275)	0.51 (290)
Station B	0.45 (265)	0.99 (245)	1.22 (260)	0.25 (315)
Station C	0.22 (270)	ND	ND	ND
Station D	ND	0.5 (270)	ND	0.92 (285)

ND: not determined (station not occupied).

Table 2.3. Correlation Analysis at each depth (200m-400m)
(CAR-118, CAR-122, CAR-128 and CAR-132, N=106)

	BAT	*CHEMO*	*BNP*	*O_2*	*H_2S*	*S^0*	*SO_3^{2-}*
CHEMO	0.28**						
BNP	0.10	0.28**					
O_2	-0.01	-0.12	-0.07				
H_2S	-0.23	-0.19*	-0.12	-0.24*			
S^0	0.55**	0.51**	0.31**	-0.14	-0.11		
SO_3^{2-}	-0.01	0.02	-0.12	-0.16	0.01	0.02	
$S_2O_3^{2-}$	-0.07	-0.04	-0.08	-0.11	0.02	0.03	0.93**

CHEMO: Dark carbon fixation rate; BNP: Bacterial net production; BAT: Beam attenuation

** Pearson product-moment correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

Table 2.4. Calculated polysulfide species under "sulfur saturation" assumption (CAR-132)

Measured parameters						*Calculated polysulfides (µmol/L)*							*S(0) * (µmol/L)*	*S(0) Saturation*
Depth (m)	sulfide (µmol/L)	Part. S^0 (µmol/L)	Total S^0 (µmol/L)	pH	Temp (°C)	S_2^-	S_3^-	S_4^-	S_5^-	S_6^-	S_7^-	S_8^-		
260	0.0	0.01	0.68	7.65	17.7	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	Yes
270	0.0	0.22	1.24	7.65	17.7	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	Yes
280	0.0	0.05	0.77	7.65	17.7	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	Yes
290	1.1	0.51	0.80	7.65	17.7	0.03	0.01	0.02	0.03	0.02	0.00	0.00	0.34	Yes
305	5.9	0.16	0.58	7.65	17.7	0.14	0.03	0.11	0.16	0.09	0.02	0.00	1.76	No
320	9.7	0.11	0.75	7.65	17.7	0.23	0.05	0.18	0.26	0.14	0.04	0.01	2.87	No
340	11.0	0.06	0.60	7.65	17.7	0.26	0.06	0.20	0.30	0.16	0.04	0.01	3.28	No
400	19.6	0.01	0.50	7.65	17.7	0.46	0.10	0.35	0.53	0.29	0.07	0.02	5.82	No

*: Maximum theoretically possible S (0) in all polysulfide species

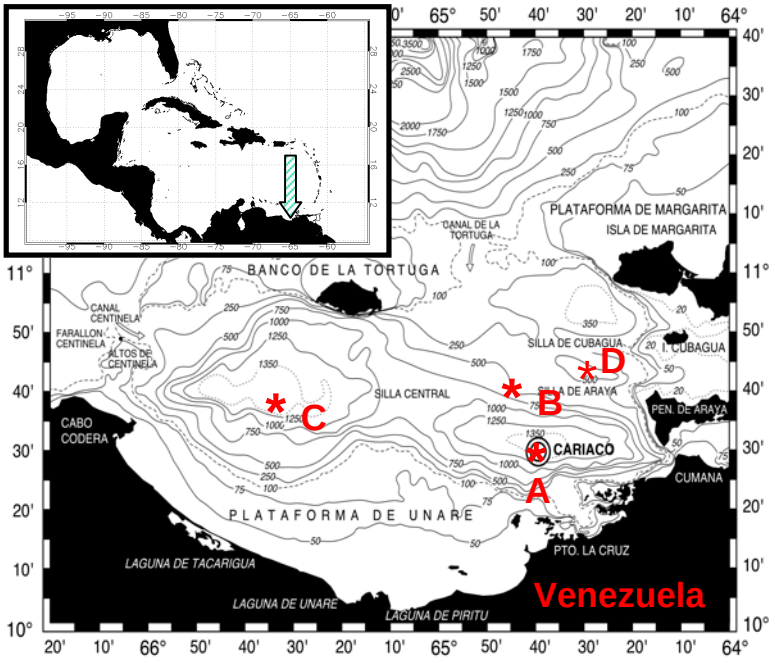

Figure 2.1. Station locations in the Cariaco Basin. Sampling was conducted during both upwelling and relaxation periods**.**

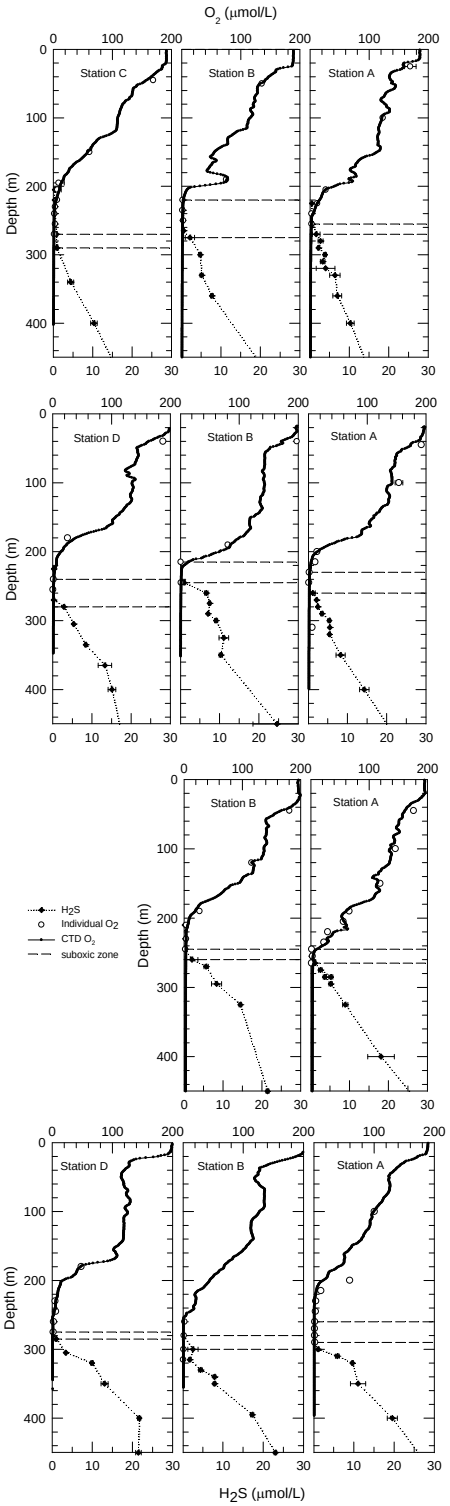

Figure 2.2. Vertical profiles of sulfide and oxygen concentrations during the four reported cruises. The dashed lines indicate the suboxic zone. From top to bottom: CAR-118, CAR-122, CAR-128, and CAR-132. Symbols and error bars represent means and 1 standard deviation of triplicate samples.

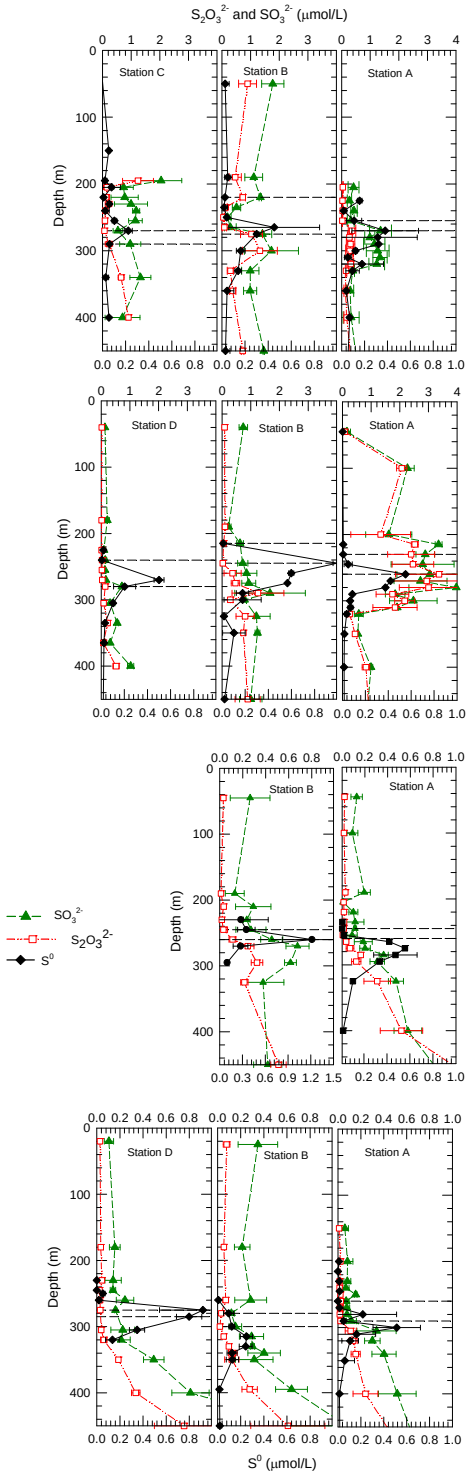

Figure 2.3. Depth profiles of thiosulfate, sulfite and particulate elemental sulfur concentrations. From top to bottom: CAR-118, CAR-122, CAR-128, and CAR-132. Symbols and error bars represent means and 1 standard deviation of replicate samples.

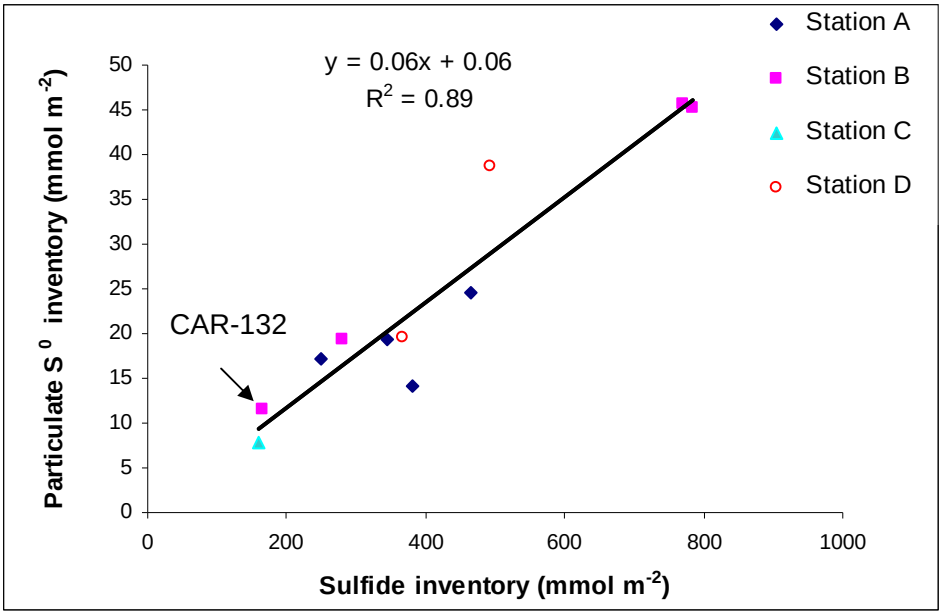

Figure 2.4. Comparison of sulfide and particulate elemental sulfur integrated through the redoxcline at all stations during the four cruises. As discussed in the text, sulfide inventory during CAR 132 is low.

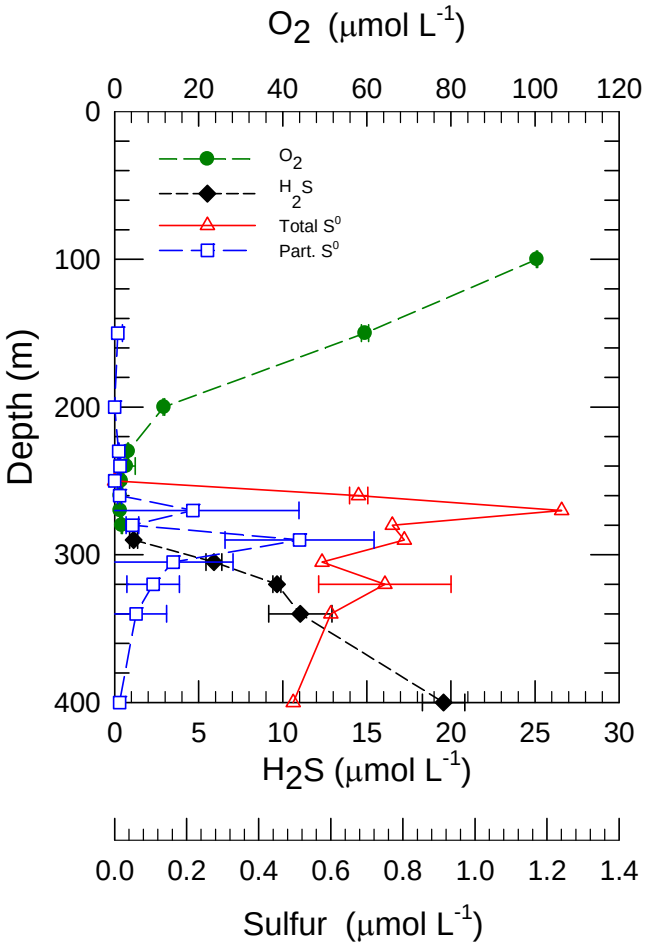

Figure 2.5. Depth distributions of total zero-valent sulfur and particulate elemental sulfur at Cariaco station A during CAR 132. Total sulfur includes particulate elemental sulfur + the sulfane fraction of polysulfides, and colloidal sulfur. Note even at 400m, zero-valent sulfur was still detectable.

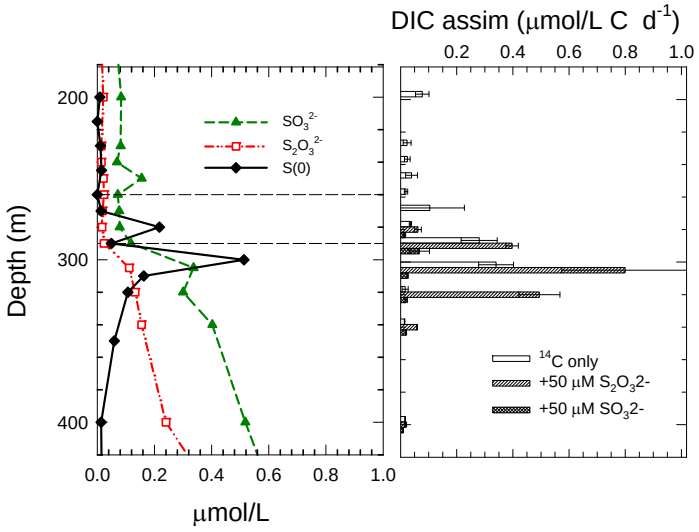

Figure 2.6. Left panel shows profiles of $S_2O_3^{2-}$, SO_3^{2-} and particulate S^0 during CAR 132 station A. Right panel shows chemoautotrophic fixation of carbon without amendments, and with added $S_2O_3^{2-}$ and SO_3^{2-}. Sulfite did not simulate carbon fixation (in fact rates were lower than controls) but thiosulfate simulate carbon fixation at deeper suboxic and upper anoxic zones.

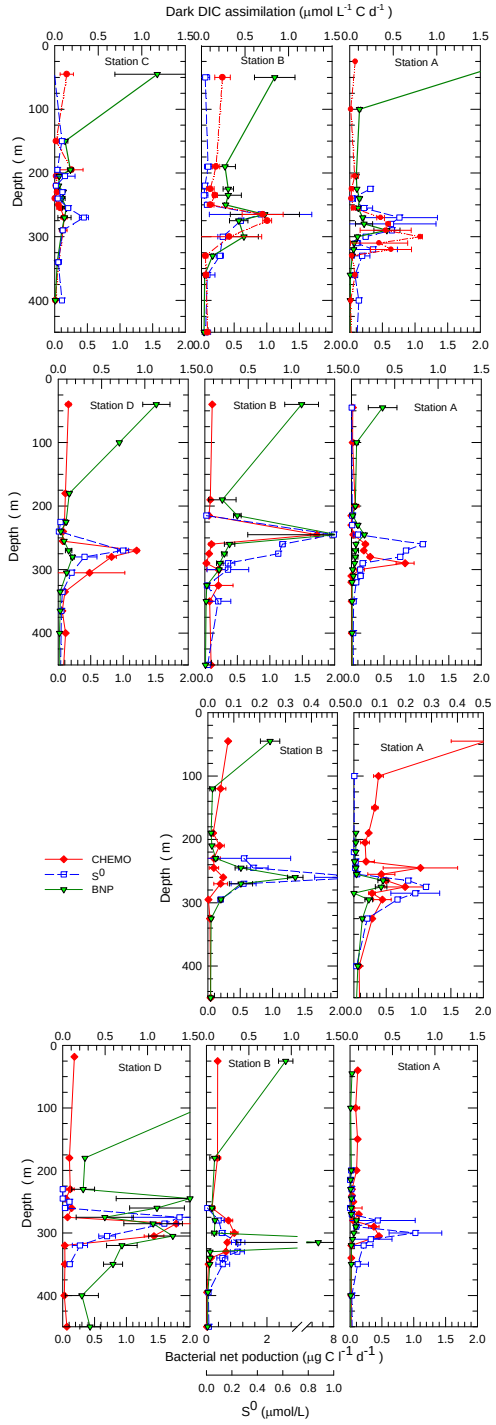

Figure 2.7. Depth profiles of particulate elemental sulfur, dark carbon fixation rate (chemoautotrophic production, CHEMO) and heterotrophic bacterial net production (BNP) for the four cruises. From top to bottom: CAR-118, CAR-122, CAR-128, and CAR-132. Note chemoautotrophic production maximum corresponds with S^0. Note the scale change in CAR-132 station B.

CHAPTER THREE: Stable sulfur isotopes in the water column of the Cariaco Basin

Abstract

Previous geochemical and microbiological studies in the Cariaco Basin off the coast of Venezuela indicate intense elemental cycling and a dynamic microbial loop near the oxic-anoxic interface. We obtained detailed distributions of sulfur isotopes in the Cariaco water column with high depth resolution across the redox transition zone to aid in understanding how sulfur species are involved in these processes. The sulfur isotope composition of total dissolved sulfide and sulfate were measured in the water column as part of the on-going CARIACO (CArbon Retention In A Colored Ocean) time series project. Sulfate ($\delta^{34}S_{SO4}$) and sulfide ($\delta^{34}S_{H2S}$) isotopic patterns were similar to trends observed in the Black Sea water column: $\delta^{34}S_{H2S}$ and $\delta^{34}S_{SO4}$ values were constant in the deep anoxic water (varying within 0.6‰ for sulfide and 0.3‰ for sulfate), with the $\delta^{34}S_{H2S}$ roughly 54‰ depleted in ^{34}S relative to $\delta^{34}S_{SO4}$. Near the oxic-anoxic interface, however, the $\delta^{34}S_{H2S}$ value was ~3‰ heavier than that in the deep water, which we interpret to reflect sulfide oxidation and/or *in situ* sulfide production through rapid sulfate reduction. Observed $\delta^{34}S_{H2S}$ and $\Delta^{33}S_{H2S}$ at the redoxcline in this environmental study did not provide unequivocal evidence to support the important role thought to be played by sulfur intermediate disproportionation by previous studies. An increase in $\delta^{34}S_{SO4}$ values with depth indicates a reservoir effect associated with removal of ^{32}S-enriched

sulfur during sulfide production through bacterial sulfate reduction (BSR) at deeper levels. Repeated observation of minimum $\delta^{34}S_{SO4}$ values near the interface suggests 'addition' of ^{32}S during sulfide oxidation at the chemocline. Our results which do not either establish or disprove a role for sulfur intermediate disproportionation in the deep anoxic water column, may reflect fractionations during sulfate reduction that are larger than those observed to date in culture experiments.

1. **Introduction**

The isotopic composition of sulfur compounds has been used to explore the biogeochemical cycling of sulfur in both modern and ancient marine environments (Canfield and Teske, 1996; Lyons, 1997). Sulfate-reducing bacteria (SRB) produce sulfide depleted in ^{34}S compared to the initial sulfate (Kaplan and Rittenburg, 1964), and the level of ^{34}S depletion of sulfide in sediments and anoxic water in natural systems is commonly 45-70‰ relative to coexisting sulfate. To date, no experiments with pure cultures or natural populations of SRB have yielded fractionations greater than 46‰ (Kemp and Thode, 1968; Habicht and Canfield, 1997; Detmers et al., 2001; Canfield, 2001); however, some studies (Goldhaber and Kaplan, 1980; Wortmann et al., 2001; Rudnicki et al., 2001) have argued that large fractionations in some environments are produced by sulfate reduction acting alone. Recent model work supports that the metabolic pathways of sulfate reduction can produce fractionations as large as 75‰ (Brunner and Bernasconi, 2005; Johnston et al., 2007; Farquhar et al., 2008). Much of the current literature, however, has invoked additional fractionations associated with oxidative sulfur cycling to explain the large sulfur isotope offsets observed in nature between coeval sulfide and sulfate.

Canfield and Thamdrup (1994) argued that sulfide produced via sulfate reduction can be oxidized to elemental sulfur, which can subsequently undergo disproportionation to yield ^{34}S- depleted sulfide and ^{34}S- enriched sulfate. Repeated cycles of oxidation and disproportionation can theoretically produce progressively larger sulfur isotopic offsets between the sulfide and sulfate pools. In this model, fractionation during sulfide oxidation is assumed to be very small, as supported by previous observations of natural and experimental systems (Fry et al., 1986; Fry et al., 1988; Zerkle et al., 2009). Thus, the isotopic composition of sulfide and sulfate records the initial fractionation during sulfate reduction plus the additional fractionations generated through repeated cycles of sulfide oxidation to sulfur intermediates and disproportionation of these compounds to sulfide and sulfate (Canfield and Thamdrup, 1994; Habicht et al., 1998; Böttcher et al., 2005). Such cycling has been used to explain the strongly ^{34}S-depleted sulfur isotope compositions often observed in sedimentary sulfides (Habicht and Canfield, 2001). Typical fractionations during disproportionation of sulfur intermediates in bacterial cultures are shown in equation 1-3 (Habicht and Canfield, 2001):

$$4S^0 + 4H_2O \rightarrow 3HS^- + SO_4^{2-} + 5H^+$$

$$(\delta^{34}S^0 - \delta^{34}HS^- = 7‰;\ \delta^{34}S^0 - \delta^{34}SO_4^{2-} = -21‰) \quad (1)$$

$$S_2O_3^{2-} + H_2O \rightarrow HS^- + SO_4^{2-} + H^+$$

$$(\delta^{34}S_2O_3^{2-} - \delta^{34}HS^- = 3‰–15‰;\ \delta^{34}S_2O_3^{2-} - \delta^{34}SO_4^{2-} = -3‰–-15‰) \quad (2)$$

$$4SO_3^{2-} + H^+ \rightarrow H_2S + 3SO_4^{2-}$$

$$(\delta^{34}SO_3^{2-} - \delta^{34}HS^- = 28‰;\ \delta^{34}SO_3^{2-} - \delta^{34}SO_4^{2-} = -10‰) \quad (3)$$

Disproportionation of sulfur intermediates can generate sulfide highly depleted in ^{34}S; however, the isotope effect associated with chemical and chemoautotrophic sulfide

oxidation is different in magnitude and direction. The resulting contrast in sulfur isotope composition is potentially useful for investigation of the competition between oxidation of sulfide and sulfur intermediate disproportionation. For example, Fry et al. (1988) reported a fractionation factor during chemical (abiotic) sulfide oxidation by O_2 of -5.2‰. It has also been reported that sulfide oxidation mediated by chemoautotrophs would generate ^{34}S enrichments. These fractionations have been reported to range from -18‰ to -1‰ (Kaplan and Rittenberg, 1964). Note that the large value of -18‰ has been questioned by Fry et al. (1986) since this value was produced in experiments that possessed significant sulfur intermediates, and it has not been independently confirmed.

Large isotopic offsets between sulfide and sulfate have been reported in the Cariaco water column (Fry et al., 1991), but the mechanistic controls remain elusive because of the small size of the sulfur pool and rapid turnover of sulfur intermediates (e.g. sulfite, thiosulfate and elemental sulfur). Fry et al. (1991) analyzed the sulfide and sulfate isotopic properties of the Cariaco Basin water column and found that the sulfur isotope offset between sulfide and sulfate was about -52‰, and that the isotopic compositions, and thus offsets, were relatively constant with depth. A gap in this study, however, is that only data below 400 m where sulfide concentrations were higher than 10 μmol/L were reported. Werne et al. (2003) analyzed sulfur isotopes in the Cariaco sediments to investigate the timing and possible pathways of organic matter sulfurization. They found that the $\delta^{34}S$ difference between pore water sulfate and sulfide generally increased from 53‰ near the sediment water interface to 60–63‰ at 4 m depth. Despite this context, no previous effort has been made to investigate the sulfur isotope signal at the redoxcline of

the water column where disproportionation of sulfur intermediate have been suggested to be important (Taylor et al., 2001; Li et al., 2008).

As part of the on-going CARIACO time series project, Li et al. (2008) found maxima in concentrations of $S_2O_3^{2-}$, SO_3^{2-}, particulate S^0, and total zero-valent S^0 at the oxic-anoxic interface. They hypothesized that elemental sulfur and thiosulfate disproportionation could play an important role at the redoxcline in supporting dark carbon fixation of chemoautotrophic bacteria. In the present study, we investigate the temporal sulfur cycle from the perspective of stable isotope compositions of water column sulfate and sulfide. We focused specifically on sulfur chemistry at the oxic-anoxic interface where chemoautotrophic production is always the highest and sulfur disproportionation could be important (Taylor et al., 2001; Ho et al., 2004; Li et al., 2008). The goal was to explore the operative chemical and biological processes that control the sulfide isotope composition in the redoxcline. In addition, we found temporal variability in the sulfur isotope signal, which responded to the physical forcing of oxygen intrusion associated with basin scale water mass circulation.

We also present data from an emerging sulfur isotope technique that involves analysis of isotope ratios $^{33}S/^{32}S$ (and $^{36}S/^{32}S$) in addition to the more conventional $^{34}S/^{32}S$ ratios to shed further light on fractionation effects associated with sulfate reduction, disproportionation of sulfur intermediates, and sulfur oxidation metabolisms. It has been argued that variations among $^{33}S/^{32}S$ and $^{36}S/^{32}S$ ratios provide information that is independent but complementary to that obtained from traditional $^{34}S/^{32}S$ studies (Farquhar et al., 2003, 2007, 2008; Johnston et al., 2005, 2007; Ono et al., 2006; Zerkle et al., 2009).

2. Materials and Methods

2.1 Field sites

The Cariaco Basin is located on the continental shelf of northeastern Venezuela (Fig. 3.1). Seasonal migration of the Intertropical Convergence Zone (ITCZ) greatly influences primary productivity which increases during winter/spring upwelling months (Jan-May) and declines during summer/fall (Jun-Dec) (Muller-Karger and Aparicio-Castro, 1994). CARIACO Station A (10°30′ N 64°40′ W) was sampled on 30 November 2007 and 20 May 2008 aboard the R/V *Hermano Ginés*. Water samples were collected in 8-L Teflon-lined Niskin bottles deployed on a Seabird rosette system equipped with a CTD, a YSI oxygen probe, a SeaTec c-beam transmissometer (660 nm) and a Chelsea profiling fluorometer for chlorophyll-a. The Niskin bottles were slightly pressurized with N_2 during subsampling to minimize chemical oxidation. Peaks in the transmissometer beam attenuation, which were found to be reliable proxies for bacterial maxima near the interface, were used to target the oxic-anoxic interface in the Cariaco Basin.

2.2 Methods

2.2.1 Oxygen and sulfur species analysis

Detailed methods for measurement of oxygen, sulfide, and sulfur intermediates species have been reported in Li et al. (2008). Briefly, oxygen was measured *in situ* with an oxygen probe attached to the CTD and confirmed by Winkler titration. Dissolved sulfide was measured by methylene blue method (Cline et al., 1969). Thiosulfate and sulfite were analyzed with the method of Vairavamurthy and Mopper (1990) as modified by Hayes et al. (2006). Total zero-valent sulfur was analyzed with UV-VIS by HPLC (Li et al., 2008). Sulfate concentrations were measured from aliquots of samples collected for

sulfur isotope analysis on an Agilent 7400 Quadrupole ICP-MS system at the University of California, Riverside. Replicate analyses of SO_4-S concentration agreed within 5% at the 100 ppb range.

2.2.2 Sulfur isotope measurement

Sulfur isotope samples were collected for both of the cruises and analyzed by two methods (continuous flow for the Nov 2007 cruise and a dual-inlet approach for samples collected during the May 2008 cruise). We first describe sampling techniques and then the specific methodologies for each cruise. Inter-calibration of the ^{34}S isotope composition between labs of University of California, Riverside and University of Maryland falls well within analytical error, 0.2‰ (data not shown).

2.2.2.1 Shipboard sample collection

Ten ml of seawater were collected from discrete water column depths for sulfate-S isotope analysis. Exposure of dissolved sulfide to atmospheric oxygen was minimized by sampling water column by placing the tip of a 10 ml all-glass Hamilton Gas-Tight syringe below the surface of water flowing upward through a 60 ml plastic syringe barrel which was attached to the Niskin bottle by Tygon tubing. The sample was immediately injected into a glass serum vial containing 0.55 ml buffered formalin (final conc. 2%) and 1.25 ml 0.05 mM zinc acetate (final conc. 5 μM) and stored frozen until analysis. Zinc acetate-fixed water samples were filtered with a 0.2 μm membrane filter to remove sulfides precipitated as ZnS. The sulfate in the filtrate was then precipitated with 4 ml 25% (w/v) $BaCl_2$. Ten ml of 4 M HCl was used to rinse the filter for 30 seconds to remove any co-precipitated $BaCO_3$. Samples are acidified only at the filtration step to minimize the oxygen isotope exchange between sulfate and water under low pH conditions.

Large volumes of water (1 to10 L) were collected in collapsible LDPE cubitainers for sulfide isotopic analysis. Each container was allowed to overflow at least half of the volume before 8 ml 0.05 M zinc acetate was added. Care was taken to mix the zinc solution throughout the cubitainer to ensure quantitative precipitation of dissolved sulfide as ZnS. Samples were shaken and left for 1 day to form a coarse ZnS precipitate at the bottom of the vessel before filtration. ZnS was collected by filtration on 0.45 μm HA Millipore filters in the lab at Estación de Investigaciones Marinas de Margarita (EDIMAR). The filters were immediately frozen and stored at -20 °C until analysis.

Sulfur distillations were performed at University of California, Riverside. Filters were first acidified with 6N HCl under N_2, and the released sulfide was precipitated in traps containing 30 ml aqueous silver nitrate (3% w/v) with 10% NH_4OH. Precipitated Ag_2S was collected by filtration on a 0.45 μm nitrocellulose filter. We recognize that sulfide precipitation will also collect metal monosulfides present in the water column. Independent measurements confirm that water column acid volatile sulfide concentrations are at least three orders of magnitude lower than that of dissolved sulfide (chapter 4). Furthermore, the small fractionation (-0.5‰ to 1.2‰) typically observed during metal monosulfide formation (Böttcher et al., 1998) suggests minimal isotopic separation between these reduced sulfur pools. As such, we will lump this fraction with the dissolved sulfide in the following discussion.

2.2.2.2 Sulfate and sulfide isotopes, Nov 2007 cruise

Precipitates of sulfate ($BaSO_4$) and sulfide (Ag_2S) were dried at room temperature, homogenized, and weighed with excess V_2O_5 in tin capsules for continuous flow $^{34}S/^{32}S$ isotopic analysis. Sulfur isotope ratios of samples were determined using a Thermo

Finnigan Delta V Advantage isotope ratio mass spectrometer coupled with a Costech ECS elemental analyzer for online sample combustion and analysis. The sulfur isotope results are expressed as per mil (‰) deviations relative to the Vienna Canyon Diablo Troilite (V-CDT) standards using the standard δ notation:

$$\delta^{34}S_{sample} = \left[\frac{^{34}S/^{32}S_{\ sample}}{^{34}S/^{32}S_{\ VCDT}} - 1 \right] \text{ x } 1000 \qquad (4)$$

Sulfate isotope measurements were normalized to international standards NBS-127 (+21.1‰), IAEA SO-5 (+0.49‰) and SO-6 (-34.05‰). Sulfide isotopes were calibrated against IAEA S1 (-0.3‰), S2 (+22.65‰), and S3 (-32.5‰). Reproducibility of standard reference materials and replicate sample analyses were better than ±0.2‰.

2.2.2.3 Multiple sulfur isotopes for sulfate and sulfide, May 2008 cruise

For samples collected during May 2008, $^{33}S/^{32}S$, $^{34}S/^{32}S$ and $^{36}S/^{32}S$ ratios were determined at the University of Maryland. For sulfide sample processing, a similar approach to that described above is used to convert ZnS to Ag_2S. For sulfate isotope analysis, $BaSO_4$ was chemically reduced to H_2S using a heated reducing solution prepared from 125 mL concentrated HI, 205 mL concentrated HCl, and 61 mL concentrated H_3PO4, which was then boiled under a N_2 atmosphere for 3 h (Forrest and Newman, 1977). The evolved sulfide was sparged with nitrogen and captured as ZnS using a zinc acetate trapping solution. The ZnS precipitates were converted to Ag_2S by addition of 1M $AgNO_3$ and rinsed with 250 ml MilliQ water and 50 ml concentrated NH_4OH.

Details of the sulfur isotope fluorination methods have been described previously (Farquhar et al., 2008; Zerkle et al., 2009). Briefly, a 1-3 mg Ag_2S sample (for S in sulfate and sulfide) was wrapped in aluminum foil, and pumped under vacuum (10^{-3} torr)

in a Ni reaction vessel. A 10 times excess of pure F_2 was added at 250 ºC for 8 hours to produce sulfur hexafluoride (SF_6). The fluorinated gas mixture was purified cryogenically (distilled at -110 ºC) and by gas chromatography (TCD GC equipped with a 12' molecular sieve 5/Haysep Q column). SF_6 gas was measured as SF_5^+ (m/e of 127, 128, 129, 131 for $^{32}SF_5^+$, $^{33}SF_5^+$, $^{34}SF_5^+$, and $^{36}SF_5^+$) on a Thermo Finnigan MAT 253 Gas Source Mass Spectrometer. Isotopic data are reported relative to V-CDT.

We report $^{34}S/^{32}S$ data using standard delta notation (δ). The less abundant sulfur isotopes ($^{33}S/^{32}S$ and $^{36}S/^{32}S$) were reported using the capital delta notation (Δ), where

$$\Delta^{33}S = \left[\left(\frac{^{33}S/^{32}S_{\text{sample}}}{^{33}S/^{32}S_{\text{VCDT}}} \right) - \left(\frac{^{34}S/^{32}S_{\text{sample}}}{^{34}S/^{32}S_{\text{VCDT}}} \right)^{0.515} \right] \times 1000 \quad (5) \text{ and}$$

$$\Delta^{36}S = \left[\left(\frac{^{36}S/^{32}S_{\text{sample}}}{^{36}S/^{32}S_{\text{VCDT}}} \right) - \left(\frac{^{34}S/^{32}S_{\text{sample}}}{^{34}S/^{32}S_{\text{VCDT}}} \right)^{1.90} \right] \times 1000. \quad (6)$$

The exponents in these relationships (0.515 and 1.90) define the reference fractionation line (RFL) and approximate single-step thermodynamic equilibrium isotope exchange effects (Hulston and Thode, 1965). An estimate of the uncertainty is provided by the current long-term reproducibility for fluorination and isotopic analysis of reference materials in the Maryland lab (past 2 years), which is (2 σ) 0.28, 0.016, and 0.4‰ for $\delta^{34}S$, $\Delta^{33}S$ and $\Delta^{36}S$, respectively. Measurements of IAEA S1 at the University of Maryland presently yield the $\delta^{34}S$ = -0.30‰, $\Delta^{33}S$ = +0.0944‰ and $\Delta^{36}S$ = 0.69‰. Measurements of $\delta^{34}S$ for IAEA S2 and S3 undertaken at Maryland are reported in Ono et al. (2006) as 22.31‰ and -32.51‰, respectively (note these are direct measurements and no scale compression corrections were applied).

3. Results

3.1 Oxygen and sulfur compounds

Water column oxygen concentation decreased abruptly to below the limit of detection at 230 m and 260 m for Nov 2007 and May 2008, respectively (Figs. 3.2A, C). The concentration of H_2S increased with depth below 260 m (Nov 2007) and 270 m (May 2008), reaching nearly 63 μM at 1300 m. During the Nov 2007 cruise, a comparatively narrow peak for total zero-valent S^0 was found in the redox transition zone, with a maximum of 1.45 μM at a depth of 260 m (Fig. 3.2B). A broader yet smaller peak for total S^0 was observed in May 2008 (Fig. 3.2D). The presence of S^0 at depth during the spring sampling period is thought to be associated with an oxygen intrusion event (Fig. 3.2C), as indicated by the sulfide minimum at about 300 m. Thiosulfate and sulfite concentrations were only slightly above their detection limits (0.3 and 0.1 μM, respectively) at all depths measured during the two cruises. Compared to seawater (SO_4/Cl = 0.14; g/g), sulfate concentration normalized to chloride in the Cariaco water column did not show systematic change within the precision of the method we used (Fig. 3.3).

3.2 Sulfur isotope $\delta^{34}S$

3.2.1 Dissolved sulfide $\delta^{34}S$

The range in isotope compositions of dissolved sulfide in the Cariaco water column varied from -29.9‰ to -32.6‰ and from -28.9‰ to -32.8‰ for Nov 2007 and May 2008, respectively (Table 3.1, Fig. 3.4). In the vertical distribution of $\delta^{34}S_{H2S}$, ^{34}S enrichments are detected in the uppermost and lowest parts of the anoxic water column on both cruises. This pattern has also been observed in vertical patterns of $\delta^{34}S_{H2S}$ in the Black Sea (Sweeney and Kaplan, 1980; Fry et al., 1991; Neretin et al., 2003). ^{34}S enrichment in

sulfide from the upper anoxic zone was seen in Cariaco samples as deep as 400 m (sulfide concentration 19 μmol/L), which is coincident with the maximum depth at which chemoautotrophic production can be detected (Taylor et al., 2001, 2006).

3.2. 2 Sulfate $\delta^{34}S$

$\delta^{34}S_{SO4}$ ranged from 21.0‰ to 21.3‰ (average 21.1‰) in November 2007 and from 21.2‰ to 21.7‰ (average 21.4‰) in May 2008 (Fig. 3.5). Similar variations across the oxic-anoxic interface were observed during Jan 2005 and May 2005 (Percy, 2006). The extent of variation in $\delta^{34}S_{SO4}$ value was only slightly greater than analytical precision ($\pm$ 0.2‰); however the repeated observation of an isotopic minimum at the redoxcline during all four cruises is suggestive of an environmental pattern.

3.3 Multiple sulfur isotopes of sulfate and sulfide

We complemented the May 2008 $\delta^{34}S$ data set with multiple sulfur isotope measurements ($\Delta^{33}S$ and $\Delta^{36}S$) in an attempt to elucidate sulfide oxidation and sulfur intermediate disproportionation effects (Fig. 3.6). We observed $\delta^{34}S$ sulfide enrichments near the oxic-anoxic interface and relatively constant values with greater depth similar to water column patterns observed during Nov 2007 (Fig. 3.4). A minimum in $\Delta^{33}S$ of sulfate was detected at 280 m (0.027‰), while $\Delta^{33}S$ of sulfate does not change significantly in the rest of the oxic and anoxic water column, varying only from 0.034‰ to 0.048‰. The $\Delta^{33}S$ of sulfide was lowest near the chemocline, increasing from 0.123 to 0.155‰ at depth. The $\Delta^{36}S$ was more negative for sulfide than for sulfate. No depth-dependent variation of $\Delta^{36}S$ for sulfide or sulfate was observed that exceeded analytical uncertainty.

4. Discussion

4.1 Sulfate $\delta^{34}S$

The mean $\delta^{34}S_{SO4}$ (21.2‰) in the Cariaco water column is equivalent to sulfate values found in coastal waters and the modern open ocean (Hoefs, 2004). The observed isotope variation for sulfate throughout the water column extends only slightly beyond the precision of the method. This relationship reflects the very small fraction of sulfate converted to sulfide. Therefore, sulfate reduction, sulfide oxidation, and disproportionation of sulfur intermediates do not affect $\delta^{34}S_{SO4}$ significantly. A change in $\delta^{34}S_{SO4}$ values by 0.1‰ in the Cariaco would require addition or removal of 63 μmol/L sulfide for the observed sulfate concentration of 28 mM and a 54‰ isotope offset between sulfide and sulfate. Even so, the slight increase of $\delta^{34}S$ for sulfate with water depth (especially in May 2008) is consistent with net sulfate reduction in the water column, during which preferential reduction of the lighter–sulfate (^{32}S enriched) produces a ^{34}S enriched sulfate residue. This detectable $\delta^{34}S_{SO4}$ increase with depth may also imply a small sink associated with settling of sulfide minerals from the water column, which is consistent with syngenetic pyrite formation in the Cariaco water column (Chapter 4).

We consistently observed small excursions in sulfate isotopic composition proximal to the suboxic zone (Fig. 3.5), suggesting addition of ^{32}S to the sulfate pool from sulfide oxidation. Measurements of sulfate concentrations in the water column were not sensitive enough to reveal small changes with depth (Fig. 3.3). We also saw a slight increase of $\delta^{34}S_{SO4}$ during upwelling season (May 2005 and May 2008, average 21.3‰) compared to non-upwelling samples (Jan 2005 and Nov 2007, average 21.1‰) (Fig. 3.5), which could be explained by higher primary production and presumably increased rates of sulfate reduction in May. This assertion is consistent with observations

in coastal North Sea waters, where upwelling and primary production were thought to change sulfur isotope ratios seasonally, resulting in a positive 0.2‰ shift of sulfate $\delta^{34}S$ from spring to summer (Böttcher et al., 2007). However, these small differences, which approach the limits of precision for the measurements, may also reflect small calibration differences between the two labs and further sampling is required to confirm the seasonal variation in sulfate isotope values.

4.2 Temporal shift in $\delta^{34}S_{H2S}$

An apparent temporal variation was observed in dissolved sulfide $\delta^{34}S$ values from the upper water column compared to invariant values in the deep anoxic water (below 500 m). $\delta^{34}S_{H2S}$ at the oxic-anoxic interface was 1‰ heavier in May 2008 than in Nov 2007 (Fig. 3.4). In May 2008, we observed an oxygen intrusion event as oxygenated water was introduced to below 300 m (Figs. 3.2C, D). We detected free sulfide from 260 m to 290 m when the station was first sampled. However, during our cast to collect water samples for sulfide isotope analysis 12 hours later, we did not detect ZnS precipitate in our samples from 260 m to 290 m (which was confirmed by acid distillation), implying that all of the sulfide above 290 m had been scavenged. Unfortunately, the transient nature of the intrusion event meant that we do not have a concurrent sulfide profile from that specific cast to verify this suggestion. There is considerable evidence for periodic ventilation of Cariaco Basin deep water. Holmen and Rooth (1990) convincingly demonstrated from tritium data that relatively young water must reach deep into the basin on a decadal time scale. They proposed two distinct sources: a high-salinity but low-volume source, originating along the Venezuela coast sinking to the bottom and a larger source from water injected at the sills, providing water that sinks to mid-depths. Since

1995, repeated observations of small maxima in dissolved oxygen in the vicinity of the chemocline have suggested the occurrence of intrusions of oxygenated water into the upper part of the anoxic water column (Scranton et al., 2001). Ventilation events occurring in 1997 and 1998 were associated with eddies near the shelf in the southeastern Caribbean Sea. It was hypothesized that this would result in colder and denser Caribbean Sea water being drawn up over the sill, subsequently sinking within the Cariaco Basin and spreading along isopycnals (Astor et al., 2003). The most ^{34}S enriched sulfide isotope composition in May 2008 was observed at the same depth as the sulfide minimum (Fig. 3.2C). Oxidation is also consistent with an increase in total zero-valent sulfur concentration (Fig. 3.2D) and chemoautotrophic production (Taylor, unpubl. data), and minimum of dissolved Fe^{2+}, Mn^{2+} and methane (data not shown).

Our deep isotope values for dissolved sulfide were 1.4‰ lighter than that reported by Fry et al. (1991). This difference may suggest temporal variation during the past 20 years in the Cariaco Basin. Scranton et al. (2006) reported a sulfide concentration (in deep waters, 1300 m) increase from 35 μmol/L in 1999 to 70 μmol/L at present. However, a portion of the isotope difference between this study and Fry et al. (1991) may be due to a change of recommended standardization of stable sulfur isotope ratios from CDT to VCDT (Coplen and Krouse, 1998; Ding et al., 2001). The data from Fry et al. (1991) was reported relative to the CDT standard, while the data we report in this work were normalized to the VCDT standards. The difference in results using the two standards can be as large as 0.7‰ (Sweeney and Kaplan, 1980; Böttcher et al., 1997) and the inhomogeneity of the CDT material itself can be 0.4‰ (Coplen and Krouse, 1998). As a result, the estimated uncertainty could be as large as 1.1‰. Therefore, the difference

between our recent data and results from Fry et al. (1991) is likely to be a combination of the change of isotope scale and also temporal shift of Cariaco system.

4.3 Enrichment of $\delta^{34}S_{H2S}$ near the chemocline

Our sulfide isotope data in the water column of the Cariaco Basin showed an increase in $\delta^{34}S$ values near the oxic-anoxic interface (Fig. 3.4). A similar trend has been observed at the Black Sea redox interface, where sulfide is enriched in ^{34}S by 3-8‰ compared to dissolved sulfide in the deeper anoxic water (Fry et al., 1991; Neretin et al., 2003). The origin of this trend is presumably related to either the production (from sulfate reduction) or the consumption (oxidation) of sulfide. We explore these possibilities below.

Sulfur isotope fractionations associated with sulfate reduction may be smaller within and near the chemocline compared to deeper waters if there is, for instance, a difference in the way that the sulfate reducers metabolize sulfur in different parts of the water column or if there is a difference in the community structure of sulfate reducers as a function of depth (Detmers et al., 2001). An inverse relationship between sulfate reduction rate and isotope fractionation has been observed within bacterial cultures and in natural populations of sulfate reducing bacteria (Kaplan and Rittenberg, 1964; Canfield, 2001; Aharon and Fu, 2003). Differences in rates and fractionations have also been sometimes observed as a consequence of differences in organic substrate quantity and/or quality (Canfield, 2001; Detmers et al., 2001). Higher concentrations of organic carbon newly fixed by chemoautotrophic bacteria are found close to and within the chemocline (Taylor et al., 2001; Chapter 4 of this thesis), and these could in turn be used as an organic substrate by sulfate reducers, perhaps enhancing reduction rates and resulting in smaller fractionations associated with sulfide production. This observation is consistent

with what has been found in the Black Sea water column where direct measurements using radiotracer techniques found higher sulfate reduction rates near the interface relative to the deeper waters (Albert et al., 1995). We do not have evidence to support or refute the possibility that there may also be a change in the structure of the community of sulfate reducers with depth, but this is also a variable that has the potential to contribute to smaller fractionations associated with sulfide production.

The other possible explanation for slightly heavier sulfide at the interface is sulfide loss via oxidation. There are several different pathways for sulfide oxidation and the direction and magnitude of the associated isotope effect depends on which pathway(s) prevails. Zerkle et al. (2009) and Fry et al. (1988) reported isotope fractionations from 0 to +3‰ for the oxidation of H_2S to S^0 by anoxygenic phototrophic sulfur oxidizing organisms. Oxidation with this fractionation would yield sulfide that is depleted in ^{34}S rather than the enrichments we observe in upper parts of the chemocline, and phototrophic oxidation is not expected at the depths of the Cariaco chemocline. Other relevant oxidation pathways that can produce the $\delta^{34}S$ enrichment trends seen in the Cariaco chemocline include chemical (abiotic) and chemoautotrophic sulfide oxidation. Sulfur intermediates (e.g., S^0, $S_2O_3^{2-}$, SO_3^{2-}) indicative of sulfide oxidation, are always observed near the Cariaco oxic-anoxic interface (Hayes et al., 2006; Percy et al., 2008; Li et al., 2008; Fig. 3.2, herein). We therefore suggest that sulfide oxidation is the more likely explanation for our isotopic observations.

We can evaluate these two possibilities using reasoning similar to those presented by Fry et al. (1991) and Mariotti et al. (1981). Fry et al. (1991) described an approach to evaluate the possibility of mixing of two sources; one source would be a small standing

pool of sulfide with $\delta^{34}S_I$ and another would be added sulfide with $\delta^{34}S_A$. Fry et al. (1991) described the mass balance relationship as:

$$\delta^{34}S_D * c_D = \delta^{34}S_I * c_I + \delta^{34}S_A * (c_D - c_I), \quad (7)$$

where c and $\delta^{34}S$ are the concentration and isotope composition of sulfide, and the subscript D refers to the observed sulfide at depth, I refers to sulfide produced at the oxic-anoxic interface, and A denotes added sulfide at each depth. Rearranging this gives the linear relationship:

$$\delta^{34}S_D = (\delta^{34}S_I - \delta^{34}S_A) * c_I / c_D + \delta^{34}S_A, \quad (8)$$

Our data plot as a line in Figure 3.7A. The intercepts, which range between -32 and -33‰, are quite consistent with a constant fractionation.

The second approach, described by Mariotti et al. (1981), evaluates the possibility that the relationship between $\delta^{34}S_{H2S}$ and concentration can be described by a Rayleigh process with constant fractionation:

$$\delta_t - \delta_0 = 10^3 (\alpha - 1) \ln f \quad (9)$$

where δ_t is the $\delta^{34}S$ value of the residual f (H_2S) fraction; δ_0 is the $\delta^{34}S$ value of the original H_2S. We are aware of the fact that Rayleigh fractionation may not apply to the Cariaco Basin water column since it is not a closed system, but this approach provides a minimum estimate of the fractionation factor and an end-member for comparison with the Black Sea. The enrichment factor, ε, is given by 10^3 (α-1). Using this approach, the fractionation factor during sulfide oxidation near the interface in Nov 2007 is -0.69‰ (excluding 260 m data since the $\delta^{34}S_{H2S}$ value at this depth is off the ^{34}S enrichment trend) and -1.15‰ in May 2008 (Fig. 3.7B). This result is similar to fractionation at the interface of the Black Sea where ε = -1.6‰ (Fry et al., 1991). It is also similar to an

experimental value measured for chemical and chemolithotrophic oxidation of sulfide (Fry et al., 1988). We therefore favor an explanation that attributes the change in $^{34}S/^{32}S$ of sulfide in the upper parts of the water column to sulfide oxidation via chemical or chemoautotrophic pathways rather than the simple mixing model.

4.4 Variation of $\delta^{34}S_{H2S}$ in the deep water

A slight ^{34}S enrichment of dissolved sulfide was also observed in the lowest parts of the anoxic water column (Fig. 3.4). The sulfide produced within the deep anoxic water at 500 m ($\delta^{34}S_{H2S} \approx$ -33‰) is depleted in ^{34}S relative to the $\delta^{34}S_{H2S}$ value of -29‰ reported for the Cariaco surface sediments (Werne et al., 2003). A mixture of sulfide produced within the deep anoxic water and sulfide produced in the sediment may explain bottom water sulfides of intermediate isotopic values ($\delta^{34}S_{H2S} \approx$ -32‰).

The importance of organic matter decomposition by SRB in the water column of the Cariaco Basin has only been estimated by indirect means. Fanning and Pilson (1972) and Scranton et al. (1987) argued that the sulfide profiles in the Cariaco water column could be explained if all sulfate reduction took place in the sediment. Based on molybdate inhibition-acetate turnover experiments, Ho et al. (2004) suggested that sulfate reduction was not a dominant pathway for organic carbon decomposition in the Cariaco water column within the chemocline. Other studies, however, have suggested sulfate reduction does occur in the water column. For example, Hastings and Emerson (1988) argued for sulfate reduction on the basis of alkalinity and total inorganic carbon measurements, and Lin et al. (2006) observed an enrichment of sulfate-reducing δ-proteobacterial cells (SRB385 positive) near the oxic/anoxic interface using Fluorescent *In Situ* Hybridization microscopy. Based on both the concentration profiles of sulfide

reported here that do not show a simple monotonic increase with depth and by the minimum in the $\delta^{34}S_{H2S}$ in the middle of the water column (Fig. 3.4), we argue that the process of sulfate reduction is operative in the Cariaco water column.

Because rates of sulfate reduction in the Cariaco water column and in the sediment have never been determined directly using the ^{35}S radiotracer technique, we attempt to estimate a plausible upper limit for the rate of sulfate reduction in the water based on the particulate organic carbon (POC) flux measured at the same station. Sediment trap data show a decrease in POC from 410 m and 1210 m (Thunell et al., 2000). The most likely pathway for organic matter remineralization between these depths is through dissimilatory sulfate reduction since other oxidants that can yield energy more efficiently have already been depleted well above these depths. In the Black Sea, the integrated rates of sulfate reduction obtained using radiotracer technique and those estimated from particulate carbon flux are in reasonable agreement (Albert et al., 1995).

Thus, we can place a reasonable limit on the amount of sulfate that is reduced for a given POC flux loss based on the following reaction stoichiometry (Froelich et al., 1979):

$$(CH_2O)_{106}(NH_3)_{16}(H_3PO_4) + 53\ SO_4^{2-} = 106HCO_3^- + 53\ H_2S + 16NH_3 + H_3PO_4. \quad (10)$$

This equation yields a C:S ratio of 2:1. Using the sediment trap data from 1995 to 2006 between 410 m to 1210 m (*http://www.imars.usf.edu/CAR*), the mean carbon flux is 2.4 ± 2.4 mmol C m^{-2} d^{-1} (n=199), and therefore an upper limit on the mean areal rate for sulfate reduction is 1.2 ± 1.2 mmol S m^{-2} d^{-1}. If we assume that the sulfate reduction rate from 410 m to 1210 m is constant, the volumetric sulfate reduction rate would be 1.6 ± 1.5 nmol L^{-1} d^{-1}.

In the Black Sea, Albert et al. (1995) found a narrow band of intense sulfate reduction at the oxic-anoxic interface, a constant rate in the deep anoxic waters, and a sub-surface maximum at the sediment-water interface. A similar conclusion was reached in a modeling study by Neretin et al. (2001) in the Black Sea. In the Cariaco sediment, Donahue et al. (2008) calculated the sulfate reduction rates to be 100-1000 nmol L^{-1} d^{-1}. Therefore, the sulfate reduction rate in the deep anoxic water column appears to be 2 to 3 orders of magnitude lower than sulfate reduction in the sediment. This finding is consistent with results found in the Black Sea (Albert et al., 1995; Weber et al., 2001), where sulfate reduction in the surface sediments are one to three orders of magnitude higher than the rates in the anoxic water column. Therefore, consistent with the inverse relationship between sulfate reduction rate and isotope fractionation during sulfate reduction (Canfield, 2001), the mid-depth minimum of $\delta^{34}S_{H2S}$ in Cariaco water column is likely a reflection of low *in situ* sulfate reduction rates combined with comparatively intense microbial sulfate reduction at the sediment-water interface.

4.5 Evaluating sulfate reduction and disproportionation in the deep water

The isotope offset between dissolved sulfide and sulfate in the deep Cariaco anoxic water column is high (-54‰) and comparable to offsets seen in many other euxinic basins (Table 3.2), including the Black Sea (-62‰) (e.g., Fry et al., 1991; Neretin et al., 2003), Mariager Fjord (-42‰) (Sørensen and Canfield, 2004), and Framvaren Fjord (-42‰) (Mandernack et al., 2003). The origin of these offsets has been a topic of considerable debate. Sweeney and Kaplan (1980) argued that isotopic fractionation resulting from biological sulfate reduction controlled the $\delta^{34}S$ of dissolved sulfide in the Black Sea water column, while Neretin et al. (2003) attributed this fractionation to a combination of

sulfate reduction and biological disproportionation of sulfur intermediates. Neretin et al. (2003) argued either that sulfur intermediates formed near the interface are transported to the deep water where they undergo disproportionation, or that sulfur intermediates can be formed *in situ* by oxygen intrusion to the deep anoxic water. The role of disproportionation has been demonstrated for the Mariager Fjord using a combination of incubations and *in situ* measurements (Sørensen and Canfield, 2004). However, comparable fractionation in the Framvaren Fjord has been argued to reflect slow sulfate reduction where low quality organic matter serves as the electron donor (Mandernack et al., 2003). These interpretations have varied and currently, a role for disproportionation of sulfur intermediates is generally sought when offsets exceed ~46‰. The role of disproportionation in environmental settings is not completely clear as it has been pointed out that explanations involving anaerobic sulfide oxidation and bacterial disproportionation of sulfur intermediates under hypersulfidic conditions are also problematic (Wortmann et al., 2001; Werne et al., 2003; Brunner and Bernasconi, 2005).

It also may be possible for sulfate reduction metabolism alone to produce isotopically large fractionations. A model of Brunner and Bernasconi (2005) expanded upon existing models of sulfate reduction (Rees, 1973) using larger fractionation factors for steps in the reduction of sulfite to sulfide and incorporating additional steps involving more sulfur intermediates from the reduction of sulfite to sulfide. This revised model can in principle allow larger maximum sulfur isotope fractionation by SRB (up to ~70‰). Support for this model is provided by evidence from minor isotope data from laboratory culture experiments (Johnston et al., 2007; Farquhar et al., 2008).

A number of approaches can be developed to evaluate whether sulfur isotope fractionations in the Cariaco deep anoxic water are attributable to sulfate reduction alone, or to a combination of sulfate reduction and disproportionation of sulfur intermediates. To date, these approaches have been based on models/arguments that are calibrated using limited observations from experiments with sulfur disproportionators and sulfate reducers. Although these approaches might be valid, interpretations will likely evolve with improved data coverage of isotope effects associated with sulfate reducers and sulfur disproportionators under environmental conditions.

An alternative constraint could be provided by considering the limits of the fields allowed by models of sulfate reduction metabolism. We compare our multiple isotope measurements of fractionations between sulfide and coexisting sulfate from the Cariaco with data for pure culture and natural population studies of SRB (Fig. 3.8). In Cariaco deep water (depths > 320 m), $\Delta^{33}S$ values fall within the range observed for pure cultures and environmental populations of sulfate reducers. However, for similar $\Delta^{33}S$, larger $\delta^{34}S$ fractionations were observed in the Cariaco than those of pure cultures and natural population (Fig. 3.8), which may reflect the relative efficiency for uptake of the natural substrate used as an electron donor compared to the transport of sulfate into and out of the cell (Canfield, 2001).

The minor isotope factionation factor ($^{33}\lambda$) offers an additional constraint on the isotopic offset between Cariaco water column sulfate and sulfide. We have used current convention (Farquhar et al., 2003; Johnston et al., 2005; Farquhar et al., 2008) to describe the relationship between fractionations for $^{33}S/^{32}S$ and $^{34}S/^{32}S$ using the exponent $^{33}\lambda$ in the deeper parts of the water column. Using an approach like that described in equation 9

(Fry et al., 1991) and Farquhar et al. (2003) yields $^{33}\lambda$ values of 0.5127 ± 0.0002 for the fractionation between sulfate and sulfide in the deep water (from 320 m to 1300 m). The experimental range in $^{33}\lambda$ observed in pure cultures of SRB varied from 0.5077 to 0.5125, and 0.5145 to 0.5187 for disproportionation of elemental sulfur and sulfite (Johnston et al., 2005, 2007). The $^{33}\lambda$ exponent of 0.5127 calculated from multi-sulfur isotope measurements of coeval sulfate and sulfide in the anoxic deep waters of the Cariaco basin is most consistent with the range observed for sulfate reduction. Although such a $^{33}\lambda$ remains to be demonstrated in experiments with pure cultures and natural populations of SRB, the role of disproportionation in the middle water column is thought unlikely, given the low concentrations of sulfur intermediates and the inhibitory effect of elevated sulfide concentrations at depth. We suggest that low sulfate reduction rates are the main factor influencing the sulfur isotope composition of sulfate and sulfide and the concentration of sulfide in the water column. The difficulty with this hypothesis is that large fractionations have not been demonstrated in experiments with pure cultures and natural populations of sulfate reducers.

4.6 Revisiting the possibility of disproportionation at the chemocline

In the Cariaco Basin, a pronounced peak in the rate of dark CO_2 fixation has been observed repeatedly in the redoxcline of the water column (Tuttle and Jannasch, 1979; Morris et al., 1995; Taylor et al., 2001). However, the presence of a large chemoautotrophy-based community seems problematic, since vertical fluxes of reductants (H_2S, NH_4^+, Fe^{2+}, Mn^{2+}) and oxidants (O_2, MnO_2, Fe_2O_3, NO_3^-) can only support a few percent of the measured chemoautotrophic production at the CARIACO station (Taylor et al., 2001). Imbalance between chemoautotrophic production and

calculated vertical diffusive fluxes of substrates has also been observed in other anoxic water bodies, such as the Black Sea (Jørgensen et al., 1991; Murray et al., 1995), Mariager Fjord (Fenchel et al., 1995; Zopfi et al., 2001) and the Baltic Sea (Jost et al., 2008). Efficient sulfur cycling, especially through sulfur intermediate disproportionation, has been proposed to explain the high biological production observed at the Cariaco Basin chemocline (Taylor et al., 2001; Ho et al., 2004; Hayes et al., 2006; Li et al., 2008). Our original goal in using sulfur isotope analyses in this study was to look for evidence of sulfur intermediate disproportionation in the isotope signals near the interface, where a peak in chemoautotrophic production is consistently observed. If disproportionation of sulfur intermediates contributes significantly to the isotopic signature of $\delta^{34}S$ and $\Delta^{33}S$ in sulfur species near the interface, direct measurement and modeling work would help to quantify the extent to which sulfur disproportionating bacteria were contributing. In fact, as can be concluded from the above modeling using $\delta^{34}S$, disproportionation of sulfur intermediates is not the dominant process influencing sulfur isotope composition near the chemocline.

The question of whether the process of disproportionation of sulfur intermediates is operative at the chemocline remains to be answered. We observed one data point of an overall decrease of $\delta^{34}S_{H2S}$ (-31.1‰ at 260 m, Fig. 3.4) within the redoxcline in Nov 2007, which is consistent with the isotope effect associated with sulfur intermediate disproportionation. For this cruise, this was the depth where sulfide first started to accumulate (sulfide concentration was below 1 μmol/l), and coincident with maxima in elemental sulfur concentration (Fig. 3.2) and chemoautotrophic bacteria activity (Taylor, unpubl. data), and where we would expect sulfur disproportionation to be an important

process. Thus, the minimum of sulfide isotope at the top of anoxic zone may reflect the activity of sulfur intermediate disproportionators very close to the interface.

The value of $^{33}\lambda$ near the chemocline (290 m) that is extracted from the measurements of multiple sulfur isotopes for May 2008 is 0.506 ± 0.002. Although minor isotopic fractionations during sulfide oxidation have not been measured directly, this value of $^{33}\lambda$ is significantly lower than that anticipated if sulfide oxidation is acting alone (Farquhar et al., 2003). To be fair about all possibilities, the value of λ may also imply that the fractionation for sulfate reduction changed in this part of the water column, but this does not appear to be the simplest explanation for our observations. Therefore, the λ value observed across the interface is not easily reconciled with sulfide oxidation acting alone. While these data may imply a role for disproportionation in the Cariaco redoxcline, it is not straightforward to prove this is the case, and it may also reflect a change in magnitude of the fractionation associated with sulfate reduction in the water column.

In fact, isotope signal from sulfur intermediate disproportionators can be complicated by a number of factors. Zerkle et al. (2009) examined the isotopic consequence of including sulfide oxidation in a multi-sulfur isotope model with sulfate reduction and sulfur intermediate disproportionation. They suggested that during chemoautotrophic and chemical oxidation of sulfide, redistribution of materials diminishes the net $\delta^{34}S$ and $\Delta^{33}S$ isotope effect of sulfate reduction and sulfur-intermediate disproportionation and can mask the isotopic signal for sulfur disproportionation if significant sulfide oxidation occurs. Therefore, following the results of Zerkle et al. (2009), it is still possible that sulfur intermediate disproportionation is

occurring in the redox interface of the Cariaco, but the isotopic signal for disproportionation could be masked by high rates of sulfide oxidation. Furthermore, experimental results of Böttcher and Thamdrup (2001) indicate that sulfur isotope effects during sulfur disproportionation are much smaller when MnO_2 is present due to the superimposed chemical oxidation of H_2S to sulfate by MnO_2. They concluded that the overall influence of sulfur disproportionation on sulfur and oxygen stable isotopes in natural environments depends on the proportion and relative cycling rates of Fe (III) and Mn (IV). Following Habicht and Canfield (2001), highly ^{34}S depleted sulfide will be generated when sulfide oxidation proceeds through elemental sulfur disproportionation; however, if followed by thiosulfate disproportionation, less depleted sulfide would be generated. In the Cariaco Basin, thiosulfate enrichment stimulated the chemoautotrophic production more efficiently than sulfite and elemental sulfur (Li et al., 2008; Taylor, unpubl. data). Therefore, if disproportionation of thiosulfate is more important, the isotope signal from disproportionators might be even more diminished.

Future studies employing fine scale sampling at the very top of the sulfide zone using multiple sulfur isotopes on sulfide, sulfate and even sulfur intermediate pools in the Cariaco Basin will have potential to distinguish between sulfide oxidation and sulfur intermediate disproportionation. A goal would be to further disentangle the proportional contribution of the two processes on the basis of more isotope data from the environment and to better constrain of the fractionation factor and $^{33}\lambda$.

5. Conclusions

We present the sulfur isotope composition of sulfide and sulfate, concentration of sulfur species within the biogeochemical context of the Cariaco Basin to explore how the

sulfur transformations affect the sulfur isotope signal in the water column. Small magnitude variations of the $\delta^{34}S_{SO4}$ in the water column were repeatedly observed at the redox interface. These variations are broadly consistent with a reservoir effect associated with sulfide production and may be slightly larger than that allowed by the standing pool of sulfide (e.g., an unsampled sink for ^{34}S-depleted sulfur such as iron sulfide that settled from the water column may be present). Variability in the isotopic composition and concentrations of dissolved sulfate and sulfide appear to reflect temporal variability associated with carbon supply tied to primary productivity and recirculation of oxygenated water throughout the basin.

The sulfur isotope variations ($\delta^{34}S$) and concentration data for the upper 400 m of the water column appear to be consistent with a fractionation related to sulfide loss consistent with chemoautotrophic or abiological sulfide oxidation, but information provided by $^{33}S/^{32}S$ (λ) implies that additional processes also likely contribute to the sulfur isotope variations. These processes may reflect changes in the magnitude of the fractionation associated with sulfate reduction within the water column, perhaps as a result of variations in quantity and quality of organic matter electron donors. The sulfur isotope fractionations ($\delta^{34}S$ and $^{33}\lambda$) and concentration data for the water column below 500 m are consistent with a significant contribution of sulfate reducers to the sulfur cycle. The fractionations between sulfate and sulfide are very large in this interval and would be above the range of known fractionations from laboratory culture experiments, but are within the limits of current models of sulfate reduction metabolism. Further work should focus on evaluating fractionations in the water column–perhaps by incubations. The fractionations also appear to decrease approaching the sediment–water interface,

suggesting a contribution of sulfide from the sediment, but also suggesting the presence of a source of sulfide even more ^{34}S-depleted in the water column. We speculate that the large fractionations are related to slow rates for sulfate reduction. However, the full extent to which the process of sulfur disproportionation remains to be fully explored.

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Table 3.1. Sulfide concentrations and sulfide isotope composition in the Cariaco water column in Nov 2007 and May 2008

Cruise time	Depth (m)	Sulfide (µmol/L)	$\delta^{34}S_{H2S}$ (‰)	$\Delta^{33}S_{H2S}$ (‰)	$\Delta^{36}S_{H2S}$ (‰)
Nov-07	260	0.9	-31.1		
Nov-07	265	2.2	-29.9		
Nov-07	270	4.1	-30.8		
Nov-07	280	4.1	-30.8		
Nov-07	290	5.4	-31.1		
Nov-07	400	19.0	-31.3		
Nov-07	500	29.0	-32.6		
Nov-07	900	55.6	-32.5		
Nov-07	1300	64.2	-32.4		
8-May	290	3.7	-28.9	0.123	-1.207
8-May	320	8.6	-31.1	0.143	-1.058
8-May	400	19.6	-32.3	0.156	-1.258
8-May	500	34.1	-32.8	0.155	-1.407
8-May	900	56.8	-32.4	0.146	-1.420
8-May	1300	62.2	-32.2	0.147	-1.369

Table 3.2. Sulfur isotope measurements in euxinic water bodies

Site	Sulfide sulfur isotope	Sulfate sulfur isotope	Reference
Black Sea	−38‰ to −40‰ (1)	N/D	Neretin et al., 2003
	−35‰ to −40‰ (1)	N/D	Fry et al., 1991
	−38.7‰ to −40.9‰ (1)	18.2‰ to 20.2‰ (2)	Sweeney and Kaplan, 1980
Cariaco Basin	−30.2‰ to −32.5‰ (1)	21.0‰ to 21.7‰ (4)	this study
	aver −31.2‰ (3) *		Fry et al., 1991
Framvaren Fjord	−33‰ to −10‰ (2)	20.1‰ to 42.5‰ (2)	Mandernack et al., 2003
Mariager Fjord	−14‰ to −22‰ ** (4)	20.5 ± 0.2‰ (3)	Sorensen and Canfield, 2004
Orca Basin		19.3‰ (3)	Sheu et al., 1988

1: decrease with depth; 2 increase with depth; 3 constant; 4 irrespective of depth
*: Below 400 m; ** : the range shows annual cycle instead of change with depth

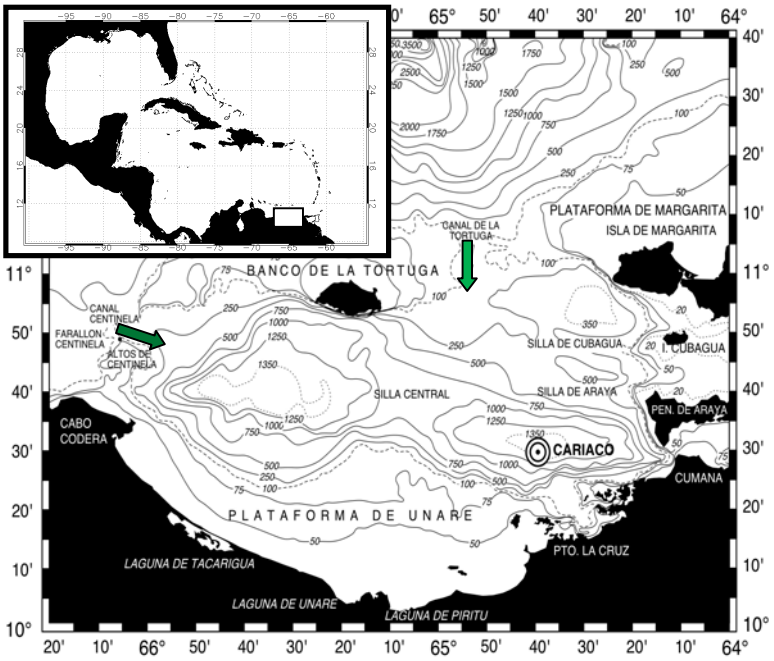

Figure 3.1. Map of the Cariaco Basin with CARIACO time series station indicated by circle. Isobaths are in meters. Arrows indicate pathways for water intrusion from the Caribbean Sea.

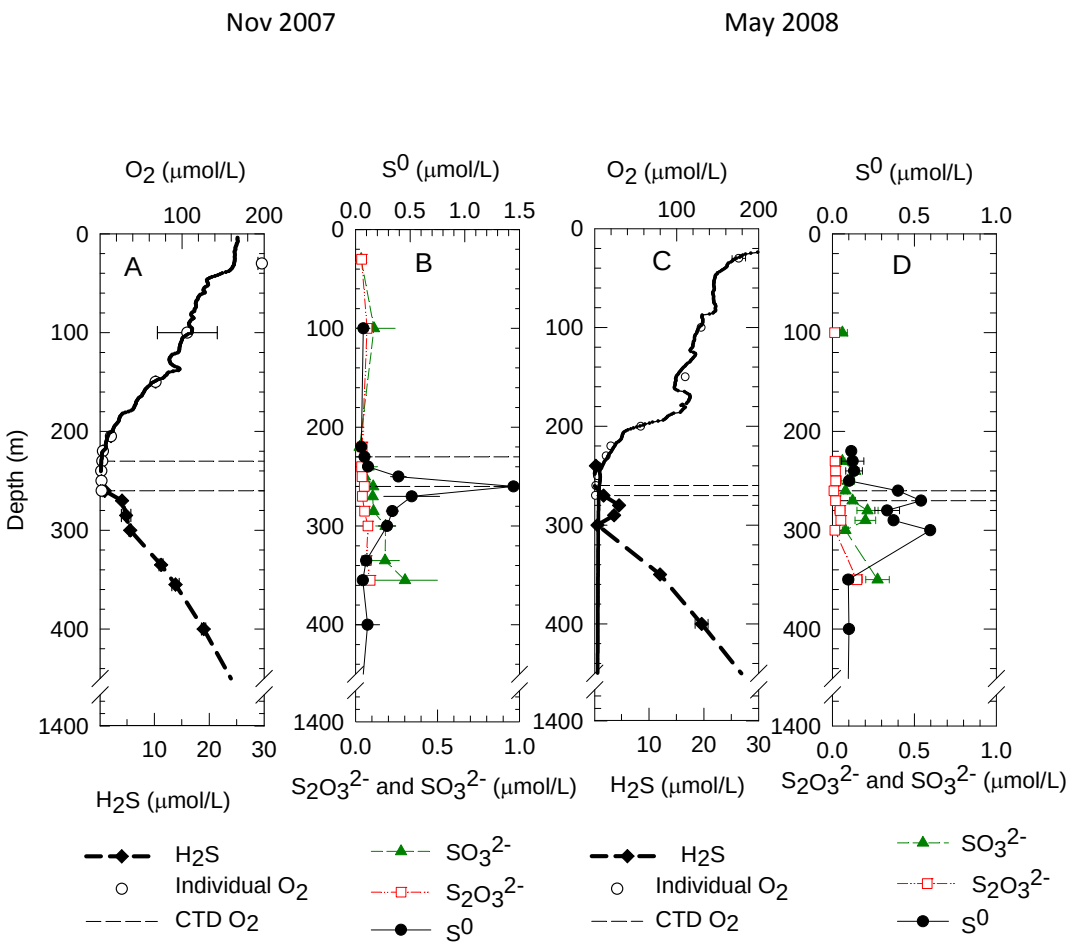

Figure 3.2. Chemical gradients in the water column. (A) Concentrations of oxygen and hydrogen sulfide in Nov 2007 samples. (B) Concentrations of thiosulfate, sulfite and total zero-valent sulfur in Nov 2007 samples. (C) Concentrations of oxygen and hydrogen sulfide in May 2008 samples. (D) Concentrations of thiosulfate, sulfite and total zero-valent sulfur in May 2008 samples.

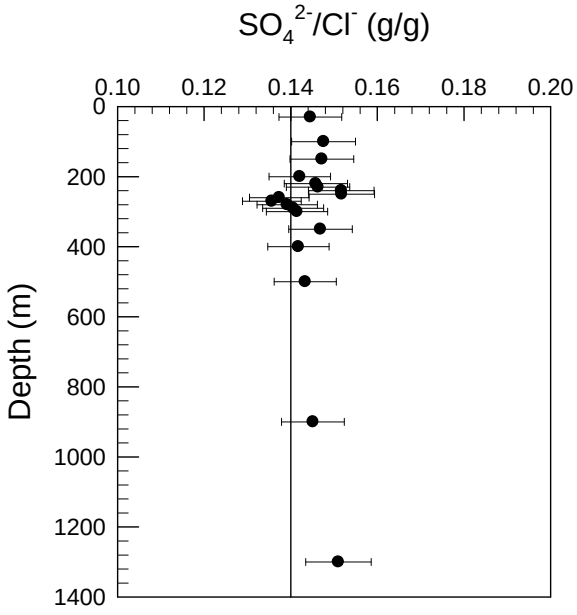

Figure 3.3. Depth profile of sulfate normalized to chloride in Nov 2007. Error bar 5% is used. The line is the value of normal seawater $SO_4/Cl = 0.14$ (g/g).

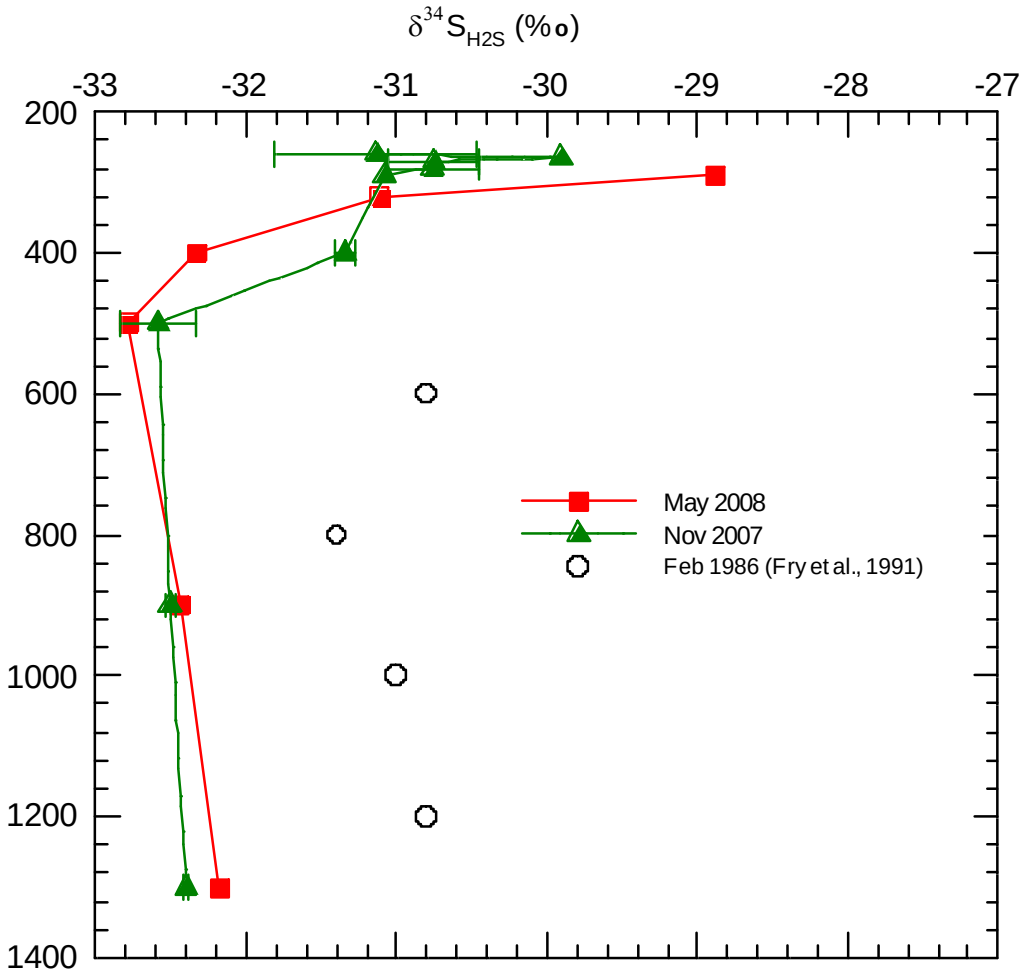

Figure 3.4. The vertical distribution of $\delta^{34}S$ (dissolved H_2S) in the water of the Cariaco in Nov 2007 and May 2008. Plotted together are 1986 data collected from the Cariaco station reported by Fry et al. (1991). Note Fry data were reported using CDT scale, instead of V-CDT scale.

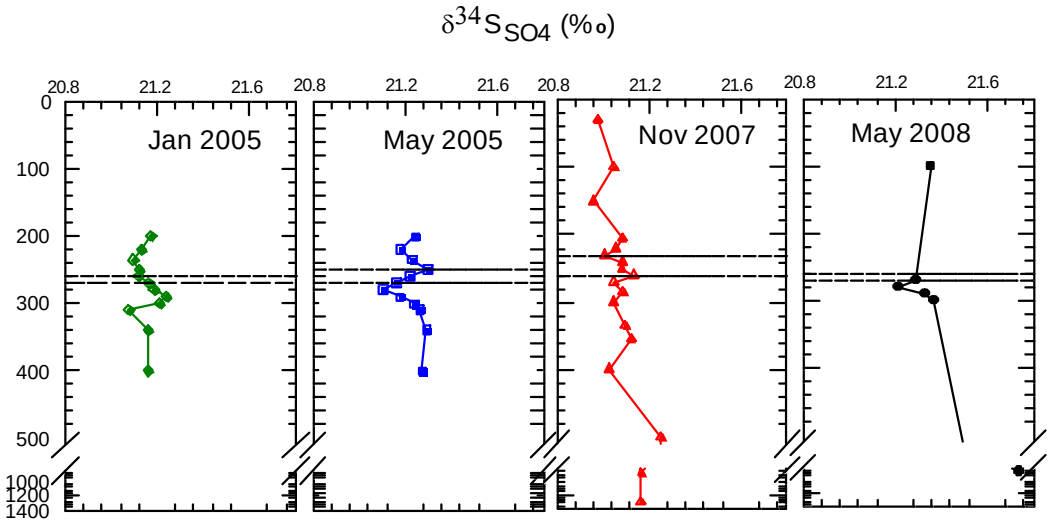

Figure 3.5. The vertical distribution of water column sulfate $\delta^{34}S$ in Nov 2007 and May 2008 (this study) and Jan 2005 and May 2005 (Percy 2006). Note the small scale changes across the chemocline (horizonal dashed lines). Although the variability in sulfate isotope values approaches analytical resolution (± 0.2‰), the consistent pattern of an isotopic minimum near the redox interface suggests active sulfur cycling.

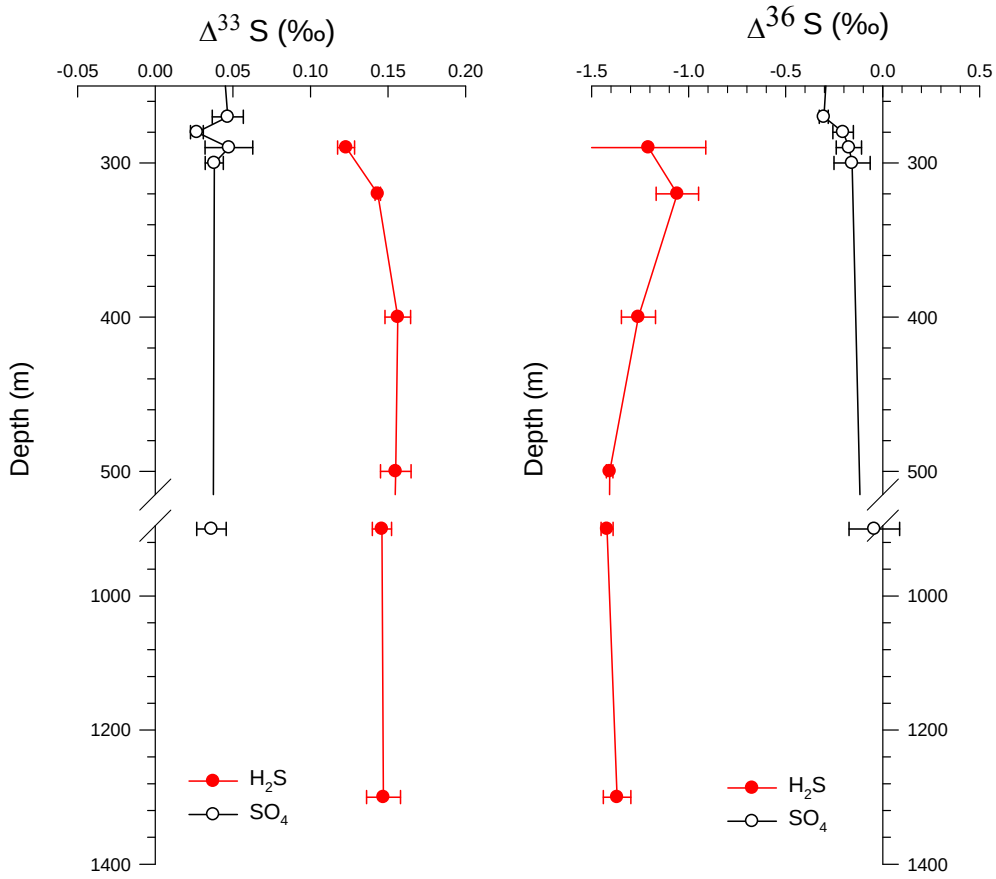

Figure 3.6. Depth profiles of $\Delta^{33}S$ and $\Delta^{36}S$ for sulfide and sulfate observed during May 2008 cruise. The chemocline is located at about 270 m water depth.

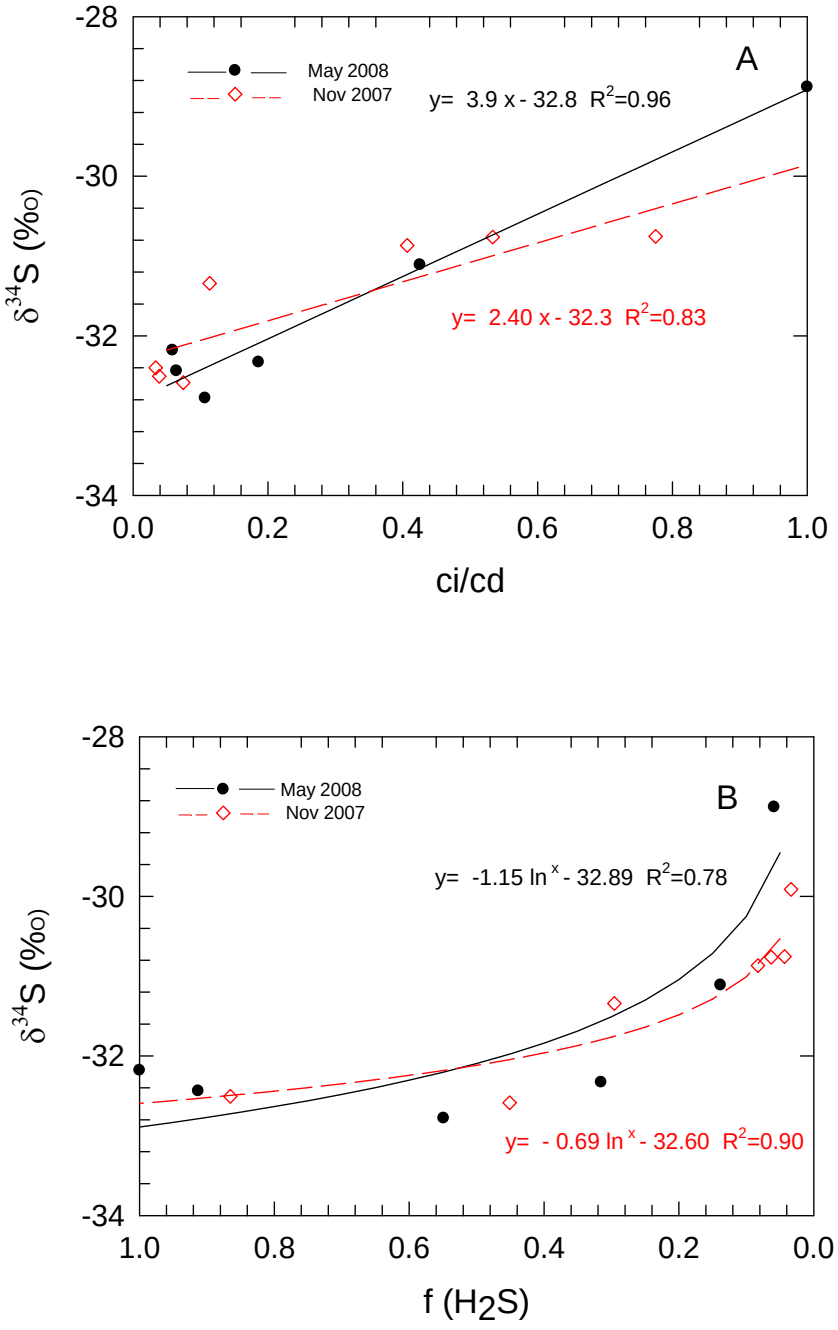

Figure 3.7. Model results to explain dissolved sulfide isotope $\delta^{34}S_{H2S}$ due to (A) mixing with sulfide produced at the interface and sulfide produced in the deep anoxic water; (B) isotopic fractionation during sulfide oxidation.

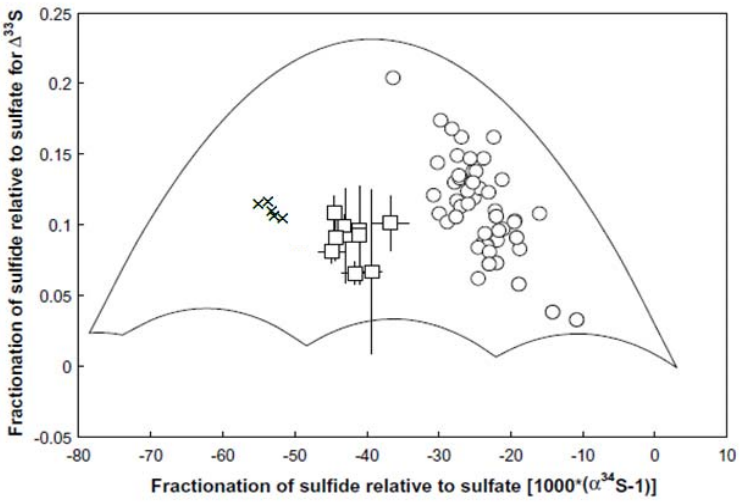

Figure 3.8. The fractionation for $\Delta^{33}S$ [1000*($\alpha^{33}S$ - $\alpha^{34}S^{0.515}$)] vs fractionation for $\delta^{34}S$[1000* ($\alpha^{34}S$ - 1)] of sulfide normalized to sulfate, modified plot after Farquhar et al. (2008). Fields of solution for metabolic network models for sulfate reduction by Brunner and Bernasconi (2005). Crosses are from depths > 300 m in the Cariaco Basin presented herein. Circles are pure cultures of sulfate reducers from Farquhar et al. (2003) and Johnston et al. (2005, 2007). Squares are natural populations of sulfate reducers from Farquhar et al. (2008) (The error bars are estimates for 2 σ uncertainty).

CHAPTER FOUR: Particulate sulfur species in the water column of the Cariaco Basin

Abstract

The biogeochemistry of iron sulfide minerals in the water column of the Cariaco Basin was investigated in Nov 2007 and May 2008 as part of the on-going CARIACO (CArbon Retention In A Colored Ocean) time series project. The concentrations of particulate sulfur species, including acid volatile sulfur, greigite, pyrite and particulate elemental sulfur, were determined with high resolution near the oxic-anoxic interface. In Nov 2007 (the non-upwelling season), acid volatile sulfur was low throughout the water column, with the highest concentration just at the depth where sulfide was first detected (260 m), and with a second peak at 500 m. Greigite, pyrite and particulate elemental sulfur showed distinct maxima near the interface. In May 2008 (the upwelling season), acid volatile sulfur was not detected in the water column. Maxima in greigite, pyrite and particulate elemental sulfur again were observed near the interface. We also studied the iron sulfide mineral flux from the sediment trap materials at the Cariaco station. Reduced sulfur comprised 0.2-0.4 % of the total particulate flux in the anoxic water column, with a pyrite flux of 1.7-6.0 mg m^{-2} d^{-1}. Consistent with the iron sulfide mineral concentration profiles in the water column, the sulfur isotopic composition of particulate sulfur found in deep anoxic traps was similar to that of dissolved sulfide near the chemocline. We conclude that pyrite is mainly formed within the redoxcline where sulfur cycling imparts a distinct isotopic signature compared to dissolved sulfide in the deep anoxic water. This

is consistent with our previous study of sulfur species and chemoautotrophic production, which suggests the reaction of sulfide with FeOOH is an important pathway for sulfide oxidation and sulfur intermediate formation near the interface. Pyrite and elemental sulfur distributions favor a pyrite formation pathway via the reaction of FeS with polysulfides or particulate elemental sulfur near the interface. Comparisons of thermodynamic prediction with the actual concentration profiles of iron sulfide minerals lead us to argue that microbes may mediate this precipitation. A preliminary sulfur budget in the anoxic water column of the Cariaco Basin is constructed. The sulfide loss term is dominated by sulfide oxidation at the chemocline, while the major source of sulfide appears to be the sulfide diffusive flux from the sediment to the water column, although *in situ* sulfide production can't be ignored. The imbalance between the sources and sinks of sulfur in the anoxic zone suggests that either a significant sulfide loss term is missing or our calculation based on 1-D diffusive flux of sulfide to the interface is an underestimation of sulfide loss within the redoxcline.

1. Introduction

The relationship between pyrite and organic carbon has been used broadly to reconstruct the paleo-environments under which sediments have accumulated (Berner, 1982; Raiswell and Berner, 1985; Lyons et al., 2003). In marine anoxic basins, such as the Black Sea and the Cariaco Basin, pyrite in the sediment can be either syngenetic (pyrite formation in the anoxic water column) or diagenetic (formed in the sediment after deposition) (Muramoto et al., 1991; Perry and Pedersen, 1993; Henneke et al., 1997; Lyons, 1997; Werne et al., 2003). Pyrite that is formed in the anoxic water column will

sink to the underlying sediment which complicates the interpretation of past environmental conditions.

Formation of pyrite in the water column across the oxic-anoxic interface has been observed in systems where metabolisable organic matter, elemental sulfur and reactive iron are abundant. Based on isotope data, Muramoto et al. (1991) argued that iron sulfide precipitation in the water column of the Black Sea occured near the oxic anoxic interface. A similar conclusion was reached by Lyons and Berner (1992) who studied S isotopes of pyrite in Black Sea sediment. Calvert et al. (1998) suggested that most of the syngenetic pyrite in the Black Sea was formed in the upper part of the anoxic zone (upper 200 m), while others argued the formation zone was wider and could extend down to 1000-1500 m in the water column (Tambiev and Zhabina, 1998). In the Orca basin, a high density of particulate FeS was found at the brine-seawater interface (Trefry et al., 1984; Hurtgen et al., 1999). In the Framvaren fjord, based on SEM, Skei (1988) concluded that pyrite is most likely to be formed near the redoxcline. In addition, several researchers have used the concentrations of dissolved Fe and sulfide to argue for the production of iron sulfide minerals based on thermodynamics (Jacobs et al., 1985; Landing and Lewis, 1991). In the Cariaco Basin, temporal fluctuations in Fe and sulfide concentrations seem to be an indicator of water column mineral formation (Jacobs et al., 1987; Scranton et al., 2001; Percy et al., 2008). However, due to the requirement for large samples (hundreds of milligrams), only a limited direct quantification measurements of sulfide minerals in anoxic water columns have been made (Perry and Pedersen, 1993; Cutter and Kluckhohn, 1999).

The sulfur isotope composition can be used as a tracer for the origin of particulate sulfide phases or as an indicator of processes. Werne et al. (2003) calculated the contribution of syngenetic pyrite in Cariaco Basin sediment based on the assumption that the syngenetic pyrite isotope composition should be similar to the isotope signal of deep water sulfide, and concluded that pyrite in the surface sediment could all be from the water column. However to date, no direct measurements have been made of pyrite concentration in the water column of the Cariaco Basin, of the magnitude of particulate sulfur flux to the sediment, or of the isotopic composition which pyrite might carry when it sinks from the water to the sediment.

In this study, we measured concentrations of suspended elemental sulfur, acid volatile sulfur (AVS), greigite, and pyrite in the water column of the Cariaco Basin during both upwelling and non-upwelling seasons. We also present dissolved iron and sulfide data from the same cruises in order to evaluate thermodynamic controls. In addition, we examined the magnitude and patterns of iron sulfide fluxes measured by times series sediment traps over the same two cruises. The isotope composition of particulate sulfide flux was measured and used to determine whether it originated mainly from dissolved sulfide near the oxic anoxic interface or in the deeper waters.

2. Materials and Methods

2.1 Field sites

The Cariaco Basin is a 1400 m deep depression located off the northern coast of Venezuela (Fig. 4.1). Exchange with the open Caribbean Sea is restricted by sills located around 90-150 m (Richard, 1975). A strong pycnocline inhibits vertical mixing below about 100 meters. Since both restricted horizontal and vertical exchange limit the flux of

oxygen consumed during organic matter remineralization, the water column is anoxic below around 200-350 m (Scranton et al., 2001). Productivity, which is influenced by the migration of Intertropical Convergence Zone (ITCZ), is high during winter/spring upwelling months and low during summer/fall (Muller-Karger and Aparicio-Castro, 1994).

2.2 Sample collection

2.2.1 Water samples

A single station (station A: 10°30′ N 64°40′ W) has been sampled monthly since 1995 as part of the CARIACO (CArbon Retention In A Colored Ocean) time series project. A general description of the CARIACO program and links to data can be found at the CARIACO web site *(http://www.imars.usf.edu/CAR/).* Water samples were collected in twelve 8-L Teflon-lined Niskin bottles mounted on a Seabird rosette system equipped with a SBE 25 CTD, a SBE 43 oxygen probe, a WetLab profiling fluorometer for chlorophyll-*a*, and a WetLab c-beam transmissometer (660 nm). The Niskin bottles were slightly pressurized with N_2 during sample withdrawal to minimize O_2 contact. Peaks in the transmissometer beam attenuation data have been proven to be reliable proxies for bacterial maxima near the interface in the Cariaco Basin (Taylor et al., 2001), and sampling depths were adjusted accordingly to resolve these features.

2.2.1.1 Oxygen and sulfide

Seawater samples for dissolved oxygen were drawn into standard oxygen bottles in duplicate and Winkler reagents were added immediately. Details of methods are provided in Astor et al. (2003). The oxygen probe on the CTD also provided continuous O_2 profiles which were corrected for response time and calibrated with Winkler O_2 data

(Astor, pers. comm.). Triplicate samples for sulfide were collected and analyzed as described by Cline (1969).

2.2.1.2 Dissolved Fe

Duplicate dissolved iron samples were collected with Air-Tite Norm-Ject 10cc luer-lock plastic syringes and filtered using 0.45 μm Whatman GD/XP 25mm syringe filters. Handling of sample bottles on deck was done by two gloved analysts with considerable care to avoid touching surfaces, and with gloves and vials kept bagged between samples to maintain cleanliness. After returning to Stony Brook, samples were acidified with concentrated quartz-distilled Optima grade HCl (Fisher Scientific) to a pH of 2 and kept in a refrigerator for about a month before analysis. Samples were then analyzed on a Perkin-Elmer AAnalyst 800 Graphite Furnace Atomic Absorption Spectrometer. Replication for dissolved iron analysis was always better than 15% (range for duplicate samples in the anoxic water). We have ignored iron concentration for oxic waters due to low levels and concerns about shipboard contamination. Further details are presented in Percy et al. (2008).

2.2.1.3 Particulate elemental sulfur

Duplicate particulate elemental sulfur samples (c.a 200 ml) were collected by passing water through 47 mm, 0.2 μm polycarbonate membrane filters held in polycarbonate filter holders and analyzed on a Shimadzu HPLC. Details can be found in Li et al. (2008).

2.2.1.4 Particulate C, N, S

In Nov 2007, duplicate particulate carbon, nitrogen and sulfur samples were collected by syringe-filtering 300 ml water samples through 13 mm Gelman A/E glass

fiber filters (1 μm pore size, pretreated following the protocol of Cutter and Radford-Knoery (1991)). In May 2008, 1-2 L samples were filtered for each depth to improve the detection limit. After filtration, 80 ml of 20 ppt NaCl solution was rinsed through the filter to remove sulfate. The CNS filters were then placed in 2.5 ml screw top polypropylene vials, sealed and immediately frozen.

The determination of particulate total C, N, and S was carried out using a pyrolysis/gas chromatography method described in Cutter and Radford-Knoery (1991). The detection limit for C, N, and S were 0.3 μmol C L^{-1}, 0.1 μmol N L^{-1}, and 0.07 μmol S L^{-1}, respectively for the Nov 2007 cruise. The detection limit was lowered to roughly 0.1 μmol C L^{-1}, 0.03 μmol N L^{-1}, and 0.02 μmol S L^{-1} respectively for May 2008 cruise.

2.2.1.5 Particulate AVS, Fe_3S_4 and FeS_2

Duplicate samples for particulate AVS, greigite, and pyrite were acquired by gravity filtering directly from the N_2 pressurized Niskin bottle. Filter holders loaded with 0.2 μm polycarbonate filters were attached to the Niskin bottle by tygon tubing. To determine the filtered volume, filtrate was collected for each filter in a graduated cylinder. We collected 200-300 ml water for each sample. The filters were dried by passing argon gas through the filter and immediately sealed in plastic bags under vacuum with a food sealer and stored at -20 °C until analysis in the lab.

Sulfur distillations were performed at Old Dominion University. We employed a sequential chemical leaching treatment on the suspended materials captured on the filters (Cutter and Kluckhohn, 1999). To keep the system oxygen-free, a glass stripping vessel was used and solution was always injected through an injection port on the stripper. Helium was flowing through the system all the time during the extraction. First, HCl was

added (final concentration: 0.5 M) to liberate H_2S from AVS. In addition to FeS, the AVS fraction may include other metal sulfides (MS), which might make up a significant fraction of the AVS (Luther, 2005). Therefore, AVS represents a maximum estimate of iron monosulfide. The liberated sulfide from AVS was trapped on a simple gas chromatographic column (glass U-tube containing Porapak QS, wrapped with Ni-Cr wire connected to a variable transformer, and immersed in liquid nitrogen). After 15 min stripping, the liquid nitrogen was removed and the column was warmed back to room temperature. The eluted sulfide was then determined with a photoionization detector.

The dilute acid treatment was followed by injection of a solution of potassium iodide (final concentration: 0.25%) and sodium tetrahydridoborate (final concentration: 0.4%) and the sample was stripped for another 15 min. This step yields the concentration of greigite Fe_3S_4 (Cutter and Oatts, 1987). Finally, concentrated HCl (final concentration: 4 M) and $CrCl_2$ (final concentration: 1 M) were added. After 22 min stripping, hydrogen sulfide was determined, yielding chromium reducible sulfur (CRS). We have tested yields for elemental sulfur using these chromium reduction conditions, and obtained a value of 103 ± 7 % (n=6). Since we measured particulate elemental sulfur directly by HPLC as well, we can calculate the concentration of pyrite by subtracting elemental sulfur from CRS. For the volume filtered in this study, the detection limit was roughly 0.5 nmol L^{-1}. More details of the method are available in Cutter and Kluckhohn (1999).

2.2.2 Sediment trap samples

At the Cariaco time series station, a single mooring consisting of four Mark VII automated sediment traps along a single wire has been in operation since 1995 (Thunell et al., 2000). A fifth trap, deployed now in the oxic part of the water column, was added

in 2002. At present, the traps are located at depths of 150 m, 220 m, 400 m, 810 m and 1210 m. The 0.5 m^2 cone shaped traps are baffled to minimize turbulence over the trap. Each trap contains 13 cups which collect falling particulate matter for 2-week intervals before rotating to allow the next cup to collect particles. Formalin solution was added before deployment to each cup to prevent further microbial activity. After retrieval, samples were stored in sealed containers and kept refrigerated. Whole trap samples were split using a precision rotary splitter, then freeze dried and weighed, and one sixteenth of each sample in Nov 2007 and May 2008 was supplied for this study. We chose the sediment trap's collection period to overlap the time of our two cruises. We had enough material for iron sulfide concentration measurement from each cup for the time period that was close to our Nov 2007 cruise, while the period overlapping May 2008, only two cups (150 m and 400 m) had enough material for analysis. Details of sediment trap sampling can be found in Thunell et al. (2000).

2.2.2.1 Reduced sulfur concentration analysis

Duplicate 0.5-1.0 mg samples from the sediment traps were extracted to quantify the concentration of AVS, greigite and CRS. The leaching procedure was identical to that described above for the filters collected from the water column. Additional 40-50 mg splits of sediment trap materials also were extracted using 10 ml of carbon tetrachloride in an ultrasonic bath for 10 min for analysis of pyrite only. The extraction was repeated two times, and then the solids were dried in the oven overnight. Since elemental sulfur was removed by CCl_4, the extracted samples were analyzed using the chromium reduction technique for pyrite only. Elemental sulfur concentrations in the particle flux were subsequently calculated as the difference between chromium reducible sulfur and

pyrite. Although the freeze drying does not cause overall loss of sulfur (Canfield, 1986), AVS may decompose as a result of this process and the sulfur originally associated with iron monosulfide minerals may remain in a form such as elemental sulfur which is analyzed by the chromium reduction procedure (Morse and Cornwell, 1987). This may explain our result (see below) that the measured AVS was very low while the elemental sulfur concentration was relatively high, though similar results were found in sediment cores from the Cariaco (Werne et al., 2003; Lyons et al., 2003).

2.2.2.2 Reduced sulfur isotope composition analysis

Splits of sediment trap materials were extracted at the University of California, Riverside for total reduced sulfur (S_{TRS}) by the chromium reduction method described by Canfield (1986). The evolved H_2S was carried by N_2 gas and trapped by about 30 ml 3% silver nitrate with 10% NH_4OH. The silver sulfide precipitate was concentrated, rinsed, dried and homogenized. Then about 0.3 mg Ag_2S was placed in a silver boat with 1:10 ratio of sample V_2O_5 catalyst and analyzed for its sulfur isotope composition using a Thermo Delta V advantage isotope ratio gas spectrometer fitted with a Costech ECS elemental analyzer for online sample combustion and analysis. All sulfur isotope compositions are reported in standard delta notation as per mil (‰) deviations from Vienna Canyon Diablo Troilite (V-CDT):

$$\delta^{34}\text{Ssample} = \left[\frac{^{34}S/^{32}S_{\text{sample}}}{^{34}S/^{32}S_{\text{VCDT}}} - 1\right] \text{ x } 1000$$

Precision for standards was better than 0.2‰.

2.2.2.3 SEM analysis

Splits of samples from the sediment traps were homogenized roughly and then an environmental Hitachi TM 1000 SEM with EDX (energy-dispersive X-ray analysis) at

the University of Southern California was used to directly observe mineral morphologies, especially framboidal pyrite, in the sediment trap materials. This instrument does not need further coating; therefore the alteration or loss from the sediment trap samples was minimized. Areas judged to be 'typical' on a subjective basis were chosen to identify iron sulfide minerals; therefore, the result was qualitative to semi-quantitative. In addition, the extreme heterogeneity of the distribution of the sulfide minerals precluded making reliable quantitative judgements. The available instrumentation also made it possible to discriminate minerals based on iron sulfur ratios. However, the iron:sulfur concentration ratios in our samples were always too high for any iron sulfide mineral, probably due to contributions from iron oxides and clay mineral-associated iron (data not shown). This phenomenon has also been observed previously in coastal sediment (Morse and Cornwell, 1987).

3. Results

3.1 Oxygen and sulfide profile

In Nov 2007, oxygen concentrations decreased with depth and dropped below the detection limit of Winkler titration (<2 μmol L^{-1}) at 230 m (Fig. 4.2). Sulfide was first detected at 260 m, and increased to 65 μmol L^{-1} at 1300 m. Therefore, we operationally define the suboxic zone for this cruise as occurring between 230 m to 260 m. However in May 2008, the suboxic zone was narrower, from 260m to 270 m. As suggested by the sulfide profile and other measured parameters (data not shown), sulfide was actively scavenged by intruded oxygen at 300 m (Scranton et al., 2008).

3.2 Dissolved Fe

The water column profile of dissolved Fe (II) during Nov 2007 and May 2008 is presented in Figure 4.3. Fe (II) concentrations in the oxic water column were low compared to the concentration in the anoxic water column (Percy et al., 2008), so we didn't begin measurement until the suboxic zone. Vertical dissolved iron profiles in the Cariaco anoxic water column show classic distribution patterns as observed in other anoxic environments, such as the Black Sea (Spencer and Brewer, 1971), reflecting redox-potential and mineral formation controls. In Nov 2007, dissolved Fe concentration started to increase at 260 m and reached a maximum at 500 m (314 ± 28 nmol L^{-1}). In comparison, in May 2008, dissolved iron started to increase at 270 m, showed a minimum at 300 m consistent with an oxygen intrusion, and then increased again, peaking at 400 m (396 ± 77 nmol L^{-1}).

3.3 Particulate elemental sulfur

In Nov 2007, a particulate elemental sulfur maximum (1.15 μmol L^{-1}) was found at the interface, indicating a zone of intense sulfide oxidation (Fig. 4.2A). In May 2008, a smaller sulfur maximum (with the highest value of 0.3 μmol L^{-1}) was found below the suboxic zone at 280 m (Fig. 4.2B).

3.4 Particulate C, N, S in the water column

The vertical profiles of total particulate carbon, total particulate nitrogen, C/N ratio and total particulate sulfur are shown in Figure 4.4. In Nov 2007, the primary maxima of carbon and nitrogen were found to be at the surface (30 m), with secondary maxima at the oxic-anoxic interface. The C and N maxima in the shallow water are due to phytoplankton photosynthesis, while the C, N maxima near the interface likely result from chemoautotrophic microbes (Taylor et al., 2001). These data are in general

agreement with particulate organic C and N values obtained in Jan 2002 (Taylor et al., 2006). The C/N ratio in the upper 200 meters increases as a function of depth. However, near the interface there is a minimum of C/N, consistent with mid-water new production within the redoxcline. The concentration of this newly produced organic matter is comparable to that in the Black Sea, while only 10 % of that in the Framvaren fjord (Cutter and Kluckhohn, 1999).

Total sulfur retained on 1 μm filters in the surface water and the deep anoxic water was below the detection limit in Nov 2007. However, near the interface, particulate sulfur reached a maximum (2.45 μmol L^{-1}). Comparatively large variations in carbon, nitrogen, and especially sulfur concentrations (Fig. 4.4D) was partly due to the fact that we only filtered 300 ml water during this cruise. We therefore increased the volume of filtered water in May 2008 to get a better estimate of the total sulfur concentration. As seen from the lower panel of Fig. 4.4, profiles of C, N and C/N in May 2008 were similar to Nov 2007, with some differences in absolute concentration. Total sulfur showed a much smaller maximum near the interface in May 2008, and also a second maximum was observed in the surface water.

3.5 Particulate AVS, Fe_3S_4 and FeS_2, and organic sulfur

In Nov 2007, AVS was only detectable at 2 depths, with the highest concentration (0.75 nmol S L^{-1}) just at the depth where sulfide was first detected. A second peak may have been present at 500 m (0.16 nmol S L^{-1}) (Fig. 4.5A). The highest concentration of both greigite and pyrite was found near the interface (3.3 nmol S L^{-1} and 33.2 nmol S L^{-1}, respectively) (Figs. 4.5B, C). The inorganic, particulate iron sulfide species in suspended matter were mostly pyrite. While greigite was almost undetectable in the surface oxic

water and deep anoxic water, pyrite in the oxic water (30m: 16 nmol S L^{-1}) and anoxic water (1300 m: 19 nmol S L^{-1}) was well above the detection limit. Previous studies have shown that particulate iron in the deep anoxic part of the Cariaco water column varied from 20 nmol L^{-1} to 68 nmol L^{-1} (Bacon et al., 1980), which is of the same order of magnitude as our pyrite concentration measurement.

Particulate organic sulfur (POS) can be calculated from the difference between total particulate sulfur (Fig. 4.4D) and the sum of elemental sulfur and iron sulfide minerals (Cutter and Kluckhohn, 1999). Although the determined values are probably underestimates as a 1 μm pore size filter was used for total particulate sulfur, the calculated POS was a quantitatively important component of particulate sulfur (Fig. 4.5D). In Nov 2007, sufficient material was not collected during this cruise to determine the total sulfur in the oxic water and deep anoxic zone. However, concentrations of total sulfur were high enough near the interface to show an enrichment of POS near the interface where it comprises 60-90% of total particulate sulfur.

In May 2008, AVS was not detected at any depth (data not shown). As was seen in Nov 2007, greigite and pyrite showed peaks near the interface, with maximum concentrations of 2.1 nmol S L^{-1} and 80.5 nmol S L^{-1} respectively (Figs. 4.5E, F). In the deep anoxic water, greigite and pyrite were low but both were above the detection limit. At the greatest depth sampled, the concentrations of greigite and pyrite increased again, suggesting either additional formation of these iron sulfide minerals in the deep water, or particle resuspension from the sediment. POS during this cruise clearly has a maximum near the interface where it comprises 20-80% of total sulfur (Fig. 4.5G)

3.6 Iron sulfide minerals in sediment trap material

The concentrations of AVS minerals in the sediment trap were less than 0.5 μmol S g^{-1} dry sediment both from traps deployed in oxic water and traps deployed in the anoxic zone (Fig. 4.6A). Greigite varied from 1-6 μmol S g^{-1} in the traps, with higher concentration in the surface trap but similar values from the suboxic zone to anoxic water column (Fig. 4.6B). Chromium reducible sulfur was an order of magnitude higher than greigite and AVS, ranging from 8 to 62 μmol S g^{-1} (Fig. 4.6C). Our CRS data are in general agreement with previous CRS measurements from sediment trap samples collected in the Cariaco Basin from other cruises (12-69 μmol S g^{-1}, Luther and Yucel, pers. comm.). Similar to greigite, CRS was more abundant in the sediment trap deployed in the oxic water at 150 m than in the deeper water column. The concentration of pyrite corrected for elemental sulfur ranged from 8 to 40 μmol S g^{-1} (Fig. 4.6D). As mentioned in the methods section, the difference between CRS and pyrite may be in the form of elemental sulfur (Muramoto et al., 1991). The elemental sulfur concentration calculated by difference was 0-27.3 μmol S g^{-1} dry sediment. Comparison between Fig. 4.6C and Fig. 4.6D suggests that most of the chromium reducible sulfur in the anoxic zone is actually in the form of pyrite, although elemental sulfur is also significant.

The particulate sulfur flux collected in anoxic sediment traps had a $\delta^{34}S$ composition ranging from -29.2 to -30.0 ‰ (Fig. 4.7). Analytically, the isotopic data reported reflect total reduced sulfur. However, given the fact that chromium reducible sulfur is normally 10 times higher than the combination of AVS and greigite, the data are assumed to represent CRS. Chapter 3 measured the sulfur isotope composition of dissolved sulfide in the water column of the Cariaco over a wide range of depths and found two distinct isotopic zones: 1) a deep zone below 400 m, with an average $\delta^{34}S$

composition of -32.4 ‰ and a narrow range from -32. 8 to -32.2 ‰; and 2) a shallow zone, 10-60 m thick, which was situated immediately below the O_2/H_2S interface and where the $\delta^{34}S$ of sulfide varies from -29.0 to -30.0 ‰. Fractionations occurring during the transformations from sulfide to pyrite in sedimentary systems are minor, with values generally less than 1 ‰ (Wilkin and Barnes, 1996). Therefore, the fact that the particulate sulfide flux in deep anoxic water column has an isotopic composition similar to dissolved sulfide near the interface, instead of dissolved sulfide isotope in the deeper anoxic water, suggests a strong link between these two sulfur reservoirs and is consistent with the argument that the interface is the main formation zone for pyrite in the water column of the Cariaco Basin.

SEM observations showed the presence of framboids, including both spherical and irregularly shaped aggregates of submicron-sized pyrite microcrystals (Fig. 4.8). Similar framboidal pyrite has been observed in other euxinic water columns (Skei, 1988; Muramoto et al., 1991). The size of the framboidal pyrite particles was assessed using *image-J* software. In the shallower sediment trap (150 m, 220 m, 400 m and 810 m), the size ranged from 1.8 to 3.3 μm, and in the deep trap (1200 m), it ranged from 1.3 to 6.5 μm. The average size is 3.2 ± 1.8 μm, which is in agreement with the average framboidal pyrite size (5 μm) found in both modern and ancient sediment (Wilkin et al., 1996). Only two pictures of framboidal crystals have been published for Black Sea sediment traps (5.7 and 6.8 μm, Muramoto et al., 1991), so it is hard to make a more complete comparison of the size of framboidal pyrite in the two anoxic basins. Framboidal size has been suggested to be an indicator for whether a depositional environment is euxinic versus oxic (Wilkin et al., 1996; Bottcher and Lepland, 2000), with framboidal pyrite in euxinic

sediment (5.0 ± 1.7 μm) smaller in size than framboids from oxic environment (7.7 ± 4.1 μm).

4. Discussion

4.1 Organic sulfur formation near the interface

In the Cariaco Basin, an enrichment of particulate organic sulfur was observed on both cruises near the oxic-anoxic interface, especially in Nov 2007, which is consistent with the sulfurization of organic matter leading to the formation of POS taking place mainly near the redoxcline. Organic sulfur formation in the anoxic water column has been proposed previously. For examples, Putschew et al. (1996) studied the sulfur-bound compounds in the sediment of meromictic Lake Cadagno, and suggested that the formation of organo-sulfur compounds started in the water column. Werne et al. (2003) studied organic sulfur in Cariaco sediment, and found that the kerogen sulfur was as high as 0.4% at the surface sediment, suggesting either a water column source or very effective formation in the uppermost centimeters of the sediment. Enrichment of organic sulfur was also detected in the water column at the oxic-anoxic interface of the Black Sea and of the Framvaren Fjord (Cutter and Kluckhohn, 1999).

In surface water, primary organic matter has a relatively small concentration of sulfur (Chen et al., 1996). Surface water particulate organic sulfur concentrations were below the detection limit for the first cruise of this study, but for the second cruise, values above blank were measured. When organic matter reaches the sulfidic zone, incorporation of reduced sulfur into organic matter starts during organic matter decomposition. The specific mechanism for organic matter sulfurization require sulfide, either directly or through reactive sulfur intermediates (Vairavamurthy and Mopper, 1987;

Anderson and Pratt, 1995). For example, sulfite can react with organic molecules to produce sulfonates ($R\text{-}SO_3^-$), which have been found to be major organic sulfur compounds in the sediment (Vairavamurthy et al., 1994). H_2S and solid FeS seem to react with a wide range of organic compounds in the sediment to form organic sulfur (Luther, 2005). Since thiosulfate, sulfite and particulate elemental sulfur were always enriched near the Cariaco oxic-anoxic interface (Hayes et al., 2006; Percy et al., 2008; Li et al., 2008), the addition of sulfur to OM during the decomposition through a sulfur intermediates pathway is plausible.

4.2 Comparison between direct concentration measurements and thermodynamic prediction

Determination of dissolved iron and sulfide, together with solubility calculations, has been used to predict precipitation of iron sulfides, such as mackinawite (FeS), greigite (Fe_3S_4) and pyrite (FeS_2). Using this approach, several anoxic marine systems have been estimated to be supersaturated with respect to mackinawite or greigite, including the Black Sea (Landing and Lewis, 1991), the Framvaren Fjord (Landing and Westerlund, 1988), and the Cariaco Basin (Scranton et al., 2001). We have been able to examine how this saturation level varies over time in the Cariaco Basin because of a relatively high sampling frequency. The delivery of continental material by river run off in this area is influenced by rainfall (Martinez et al., 2007). Dissolved iron concentrations from the CARIACO station during the last eight cruises, for which we used one consistent protocol for sampling and analysis, showed that iron concentration was highest in Jan 2005, decreased in May 2005 (Percy et al., 2008), increased again in Nov 2006 and April 2007, and then decreased Nov 2007 and May 2008 (Fig. 4.3). The observation of

fluctuating dissolved Fe concentrations suggests a complicated scenario of non-steady state behavior in this system. This fluctuation of iron concentration is believed to reflect a balance between mineral precipitation and terrestrial input (Scranton et al., 2001; Percy et al., 2008). Dissolved Mn data showed a similar variation with time (data not shown).

To calculate the ion activity product (IAP) from each depth for each cruise (Fig. 4.9), we used our measured dissolved Fe as Fe^{2+} since we don't have sufficient information to accurately calculate speciation of metals, and considered hydrogen sulfide to be HS^- since HS^- is the dominant species of sulfide when pH=7.6. Details can be found in Scranton et al. (2001) and Percy et al. (2008). Briefly, we used γFe^{2+} =0.26, γH^+ = γHS^- =0.7 (Morel, 1983) and the following equation, to calculate IAP for each depth:

$$IAP_{FeS} = \frac{\gamma_{Fe2+}\gamma_{HS-}[Fe^{2+}][HS^-]}{[H^+]\,\gamma_{H+}} \qquad (1)$$

These values can be compared with literature values for apparent solubility constants (K′sp, -3.6 for FeS, -4.4 for greigite, and -16.4 for pyrite (not marked on the graph since it is off scale)) from Davison (1991). For most cruises, the depth where we first observed the ion activity product IAP > K'sp (greigite) is about 50 m-100 m below the interface. Starting at around 400 m, IAP begins to exceed K'sp for Fe_3S_4 (Fig. 4.9). Pyrite was supersaturated even near the interface, while for all cruises, FeS was undersaturated. Comparison of IAP to the solubility product of respective pure metal sulfides suggests that the solubility of the iron sulfide minerals was controlled by Fe_3S_4 and FeS in the deep anoxic water.

From thermodynamic predictions, a higher concentration of iron sulfide minerals would be more likely to be found in the deep water instead of near the oxic-anoxic interface. The increase of greigite and pyrite in the deep anoxic column (deeper than 500

m), especially in May 2008, agrees with this prediction. However, the maxima of iron sulfide minerals do not occur at the points of maximum supersaturation, since we detected the maximum of greigite and pyrite near the interface where IAP was lower.

Maxima in greigite and pyrite near the interface suggest pyrite formation is controlled kinetically rather than thermodynamically. Schoonen and Barnes (1991) suggested that pyrite formation from aqueous solution is inhibited by the energy barrier for pyrite nucleation. They argued that one possible mechanism to overcome this barrier is through precursor iron sulfide which could provide an active surface which would enhance pyrite nucleation kinetics. Another possibility is that the reaction may be mediated by microbes. Freke and Tate (1961) found 'magnetic Fe_4S_5' produced by semi-continuous cultures of sulfate reducing bacteria. Bacterial cell surface layers may be potent metal binding agents that can scavenge metal ions from solution, effectively concentrating them (Beveridge and Fyfe, 1984; Douglas and Douglas, 2001). The concentration of metals may in turn promote the binding of counter-ions such as S^{2-}. For example, small microaerophilic magnetite-producing cocci were present at the top of the chemocline of a meromictic salt pond, while a greigite-producing packet-forming bacterium occurred at the base of the chemocline (Simmons et al., 2004). Magneto-tactic bacteria which exert a great degree of crystallochemical control over the nucleation and growth of the mineral particles are ubiquitous in aquatic habitats (Blakemore, 1982). They are found in the highest numbers at the oxic-anoxic interface and this feature has been proposed to explain magnetic material found at the Paleocene-Eocene boundary (Lippert and Zachos, 2007). In addition, new evidence has revealed that natural communities of sulfate-reducing bacteria (SRB) can generate essentially pure ZnS

deposits from dilute groundwater solutions, providing support for a biogenic origin of many low-temperature metal sulfide ore deposits (Labrenz et al., 2000). The high heterogeneity of the pyrite distribution in the water column and the sediment trap materials also raises the importance of micro-environments where it might be possible to precipitate iron sulfide minerals even when bulk water is undersaturated with respect to a specific mineral phase.

4.3 Pyrite detection in the oxic water column

Pyrite concentrations in the oxic water column of the Cariaco Basin and in the sediment trap deployed in the oxic zone were above the detection limit (Figs. 4.5, 4.6), and the sulfur isotopic composition of particulate inorganic sulfides collected in the oxic water column showed a significantly heavier sulfur isotope composition compared to that seen in the anoxic water column (Fig. 4.7). Isotopically heavier pyrite detected in oxic waters may result from resuspended sedimentary material being horizontally transported to the open basin. During the transport, framboids might escape rapid oxidation due to organic inhibitors and/or surface coatings, as has been suggested to explain pyrite detected in Kau Bay oxic water (Middelburg et al., 1988). In comparison, pyrite was below the detection limit when a direct concentration measurement was made in the oxic water of Framvaren fjord (Cutter and Kluckhohn, 1999). Unfortunately no information on the geographic variation of sulfide isotope composition in the sediment in various parts of the Cariaco Basin is available. In the Black Sea, Lyons (1997) found that the sulfide isotope composition in the shallow shelf sediment was 10 ‰ heavier than that in the deep anoxic basin. In even shallower coastal region, where the sulfate concentration is low, two things can happen (Hurtgen, 2003). First, as sulfate-reducing bacteria preferentially

consume the light ^{32}S in forming pyrite, the residual sulfate reservoir becomes enriched in ^{34}S. Second, as sulfate concentrations drop below a certain level, bacteria lose their ability to discriminate between sulfur isotopes, and therefore fractionations decrease (Hurtgen, 2003). The overall effect of sulfate limitation is a more enriched sulfur isotope signal in pyrite. This heavy isotope pyrite might then be delivered to the Cariaco through river run off (Martinez et al., 2007) and get captured in the sediment traps in the oxic water. High levels of thiosulfate and sulfite have also been observed in the oxic water column of the Cariaco Basin on several occasions and this has been attributed to advective transport from shallower areas (Hayes et al., 2006; Percy et al., 2008; Li et al., 2008). Future research aiming to address the source and the fate of this part of pyrite is strongly encouraged. We need to know where this fraction of pyrite forms, whether *in situ* in the oxic water column or transported to the Cariaco time series station from the resuspended sediment of shallower area. Why is this isotope composition so unique? What is the fate of the heavy pyrite once it sinks through the redoxcline? All these questions are worthy of further investigation.

4.4 Pyrite detection in the redoxcline and the anoxic water column

Significant greigite and pyrite formation is linked to oxic-anoxic transitions and to a requirement for dissolved sulfide, ferrous iron and an oxidant (Goldhaber and Kaplan, 1974; Wilkin et al., 1996). Therefore, maximum production rates are specifically limited to regions immediately adjacent to redox interfaces. This is consistent with the observation in many systems that pyrite is formed mainly near a redox boundary, either in the water column (Muramoto et al., 1991; Cutter and Kluckhohn, 1999) or in the sediment (Canfield, 1989; Rozan et al., 2002). Table 4.1 compares the pyrite

concentration in several anoxic systems. The maximum concentration of pyrite in the Cariaco Basin is similar to the others.

In the current literature, there are three major mechanisms proposed for pyrite formation from precursor FeS at low temperature:

I. Polysulfide and S^0 pathway (Richard, 1975; Luther, 1991)

$$FeS(s) + S^0 \rightarrow FeS_2(s) \quad (2)$$

$$FeS(s) + S_n^{2-} \rightarrow FeS_2(s) + S_{n-1}^{2-} \quad (3)$$

II. Ferrous iron loss pathway (Schoonen and Barnes, 199l)

$$2FeS(s) + 2H^+ \rightarrow FeS_2(s) + Fe^{2+} + H_2 \quad (4)$$

III. H_2S pathway (Drobner et al., 1990; Richard, 1997; Hurtgen et al., 1999)

$$FeS\ (aq) + H_2S \rightarrow FeS_2(s) + H_2 \quad (5)$$

In all three, for pyrite to form, FeS is essential. In the following discussion, we consider FeS as a class of minerals, such as mackinawite (FeS), and greigite (Fe_3S_4) (Berner, 1970). We have speculated before that there might be rapid sulfide scavenging by iron near the Cariaco interface (Li et al., 2008) since the energy yield of the sulfur intermediate disproportionation reactions can become more favorable if sulfide is scavenged by forming FeS (Thamdrup et al., 1993):

$$3S^0 + 2Fe(OH)_3 \Rightarrow SO_4^{2-} + 2FeS + 2H_2O + 2H^+ \quad (6)$$

This suggests a consortium of sulfur intermediate disproportionators and Fe-reducing bacteria. However, such an assumption is currently speculative in view of the experimental data.

It is difficult to pin down a specific mechanism of iron sulfur formation in the water column of the Cariaco Basin using the data we have so far. Conditions in the chemocline

of the Cariaco Basin closely parallel those associated with the preferential formation of pyrite within surficial sediment layers of modern marine settings (Goldhaber and Kaplan, 1974). A pool of sulfur intermediates (particulate elemental sulfur and polysulfides) is available at the interface (Li et al., 2008). Based on the spatial coupling between the distribution of sulfur intermediates and FeS, formation of pyrite may occur through a rapidly formed 'FeS' precursor phase via addition of elemental sulfur and polysulfide ions (Berner, 1984; Schoonen and Barnes, 1991). Since pH is 7.6 near the interface where we detected maximum elemental sulfur, pathway III (the H_2S pathway) is not favored as it requires strict anoxic conditions and low pH (<6.6) (Richard et al., 1997). In addition, if pathway III were the dominant process in our system, we would expect pyrite precipitation to occur during long exposure of iron-containing minerals to dissolved sulfide in the anoxic zone. We have no data to allow us to assess the importance of the second pathway.

4.5 Transport of particulate sulfur flux to the Cariaco sediment

Both concentration and $\delta^{34}S$ of pyrite in surficial Cariaco sediment, together with a relatively constant degree of pyritization downcore, suggest syngenetic pyrite accumulated from the water column is an important fraction in the sediment (Werne et al., 2003). We can calculate the syngenetic pyrite flux to the sediment based on our sediment trap data. Pyrite flux is estimated using our pyrite concentration measurements and the total mass flux from each cup (Thunell et al., data, available on *http://www.imars.usf.edu/CAR*). Results are presented in Table 4.2. Although the concentration of iron sulfide minerals in the sediment traps changed with depth (Fig. 4.6), the total reduced sulfur flux at different depths in the Cariaco Basin is quite constant from

the chemocline to the anoxic water, consistent with the oxic-anoxic interface as the main formation area. On average, 60% of the total reduced sulfur flux measured in the sediment trap was greigite and pyrite, with the remaining portion was present as elemental sulfur (the difference between Fig. 4.6C and 4.6D). AVS was only slightly above the detection limit. As mentioned in the methodology section, an oxidation artifact during subsampling might be one possible explanation of the lack of AVS. However, the sediment from the Cariaco contains only minor amount of FeS (approaching lower detection limit, 0.05%) but high concentrations of sulfur as pyrite (1.2-1.6%) (Lyons et al., 2003; Werne et al., 2003). These authors suggested that the absence of FeS in the sediment might be due to the transformation of FeS to FeS_2 in the water column. The reduced sulfur flux made up 0.2-0.4% by weight of the total particulate flux from the anoxic water column to the sediment, compared to 1.2% by weight pyrite in the surface Cariaco sediment. Therefore, syngenetic pyrite could contribute about one third of the pyrite in the surface sediment of the Cariaco, with the rest possibly being formed *in situ*.

Framboidal pyrite is another clue to syngenetic formation and has been found suspended in the water in a variety of environments (Skei, 1988; Muramoto et al., 1991). Based on lab and field research, the processes that cause pyrite to have a framboidal morphology are generally believed to occur during the formation of progressively more S-rich phases (mackinawite to greigite to pyrite: Sweeney and Kaplan, 1973; Morse et al., 1987; Wilkin et al., 1996) in contrast to euhedral grains that form via 'fast' direct nucleation and subsequent growth (Giblin and Howarth, 1984). The formation of pyrite with framboidal texture is apparently favored when iron monosulfide and greigite rapidly convert to pyrite. The formation and growth of precursor greigite is a key stage of

framboidal development (Morse et al., 1987). Sweeney and Kaplan (1973) proposed that greigite was an important intermediate during transformation of mackinawite to pyrite. In this reaction, mackinawite combines with elemental sulfur to produce greigite and then greigite is converted to pyrite either through dissociation ($Fe_3S_4 \rightarrow 2FeS + FeS_2$) or by further sulfidation ($Fe_3S_4 + 2S_0 \rightarrow 3FeS_2$). The profiles of greigite, pyrite and elemental sulfur in the water column of the Cariaco Basin satisfy the requirement (Fig. 4.5).

Framboidal pyrite has a maximum density of 3.7 g cm^{-3} (Richard, 1969) and therefore readily sinks. The sinking rates of framboids can be estimated using the Stokes's law, and the time needed for pyrite that is formed near the interface to reach the deep water column and the sediment can be estimated. We used an average size of 3.2 μm based on our limited measurements. A sinking rate of 86 cm d^{-1} can be calculated using this size and density of 3.7 g cm^{-3}. This velocity is about half of that estimated in the Framvaren fjord (156 cm d^{-1}, Skei, 1988) due to the size difference. With this velocity, it would take 1390 days (about 3.8 yr) for a framboid formed near the interface (200 m) to settle to the basin floor (1400 m). We can also estimate the turnover time of pyrite by calculating the inventory of pyrite between 200 m and 1400 m and then dividing by our pyrite flux measurement. We used the pyrite concentration and flux data collected from Nov 2007 since we have the most complete data set during that cruise. The integrated pyrite inventory from Nov 2007 is 15 mmol S m^{-2}, and the average flux of pyrite from the four sediment traps deployed in the anoxic water is 0.017± 0.002 mmol S m^{-2} day^{-1}. This gives a turnover rate of pyrite in the Cariaco anoxic water column of 906 days (2.5 years), which is similar to the sinking time of pyrite from the interface to the bottom.

4.6 Preliminary sulfur budget

A simple budget for sulfide cycling is summarized to estimate the importance and relative magnitudes of different processes in the anoxic Cariaco water column (Fig. 4.10). The oxic-anoxic interface is defined as the depth where sulfide is higher than our detection limit (0.5 μmol L^{-1}) and the anoxic zone extends from that depth to the sediment-water interface. We have estimated sulfide flux across the oxic- anoxic interface, and production of metal sulfide minerals as the main sinks, and sulfide flux from the sediment and sulfide production in the water column as the main sources.

1) Sulfide at the oxic-anoxic interface is consumed by oxidation. We assume all sulfide is supplied from below and that all sulfide is rapidly oxidized. We can estimate the upward flux of sulfide by calculating a 1-D vertical eddy diffusive flux from the deep anoxic water column:

$$J = -K_z \frac{\Delta c}{\Delta z} \qquad (7)$$

where K_z is the vertical eddy diffusion coefficient and $\frac{\Delta c}{\Delta z}$ is the sulfide concentration gradient for depths between oxic-anoxic interface and 400 m. A value for the vertical eddy diffusion coefficient, K_z for any depth z, can be estimated from the density gradient (Gargett, 1984):

$$K_z = a_0 \left(\frac{1}{N^2} \right)^{1/2}, \text{ where } N = \left(-\frac{g}{\rho} \frac{\partial \rho}{\partial z} \right)^{1/2} \qquad (8)$$

where a_0 is related to the input of energy to the basin via internal waves, ρ is the density at a given depth, g is the gravitational constant, and $\frac{\partial \rho}{\partial z}$ is the density gradient at that depth. A value of 0.0004 $cm^2\ s^{-2}$ was chosen here for a_0, as it falls between values calculated for restricted fjords (0.0001 $cm^2\ s^{-2}$) and the open ocean (0.001 $cm^2\ s^{-2}$)

(Gargett, 1984). Our value is similar to that used in other studies in the Black Sea (a_0=0.0004 cm^2 s^{-2}, Landing and Lewis, 1991), and Mariager Fjord (a_0=0.0002 cm^2 s^{-2}, Ramsing et al., 1996; a_0=0.0005 cm^2 s^{-2}, Zopfi et al., 2001) but is lower than used by Scranton et al. (1987). Using equation 10 and data from Cariaco time series station, the upward sulfide flux calculated based on data from 24 cruises ranged from 0.09 to 0.52 mmol m^{-2} d^{-1}, with an average of 0.25 ± 0.12 mmol m^{-2} d^{-1}.

2) Iron sulfides, and perhaps elemental sulfur, precipitate rapidly near the oxic-anoxic interface. The calculated particulate sulfur formation rate (AVS + greigite + CRS) based on sediment trap data ranges from 0.03 to 0.10 mmol m^{-2} d^{-1}, with an average flux of 0.04 ± 0.01 mmol m^{-2} d^{-1}.

3) No direct measurement of sulfate reduction rate either in the Cariaco water column or in the sediment has been carried out. In the water column, an indirect approach can be taken by estimating how much organic carbon is lost below the suboxic zone in the deep anoxic water. To get an upper limit of sulfate reduction rate, we assume that once organic aggregates sink below the suboxic zone, they will be decomposed through sulfate reduction according to the following equation (Froelich et al., 1979):

$$(CH_2O)_{106}(NH_3)_{16}(H_3PO_4) + 53\ SO_4^{2-} = 106HCO_3^- + 53\ H_2S + 16NH_3 + H_3PO_4 \quad (9)$$

Here the C:S ratio is 2:1. Using sediment trap data from 410 m to 1210 m, between 1995 and 2006 (*http://www.imars.usf.edu/CAR*), we estimate a maximum areal sulfate reduction rate of 1.2 ± 1.2 mmol S m^{-2} d^{-1} (n=199).

We can also estimate the *in situ* sulfide production rate in the water column using modeling software. PROFILE was used to calculate the depth distribution of sulfide consumption/production rates in the Cariaco water column. This model has been used by

Neretin et al. (2001) to estimate the sulfide loss/production rate in the Black Sea. Details of this model can be found in Berg et al. (1998). Briefly, a numerical procedure is used to select the simplest net production-consumption profile that simulates the measured concentrations of the modeled solutes based on F-testing (Kleinbaum and Kupper, 1978). We used the vertical diffusion coefficient K_z calculated above for the Nov 2007 and May 2008 cruises, together with the measured hydrogen sulfide profile for the simulation (Fig. 4.11). We divide the water column into 9 layers initially as input and measured sulfide concentrations at the top and the bottom of the model are used as our boundary conditions. Statistically, the model will determine the lowest number of equally spaced zones that provides a good explanation of the measured concentration profile. Once the least number of zones is found, the output of the model will give rates of net production/consumption of sulfide. After the simulation, depth-integrated sulfide production rates at the deep anoxic zone can be calculated. Based on results from the Nov 2007 and May 2008 cruises, the net water column sulfide production term in the anoxic water column was 0.63 mmol S m^{-2} d^{-1} for Nov 2007 and 1.4 mmol S m^{-2} d^{-1} for May 2008, respectively, which is very similar to the 1.2 ± 1.2 mmol S m^{-2} d^{-1} estimated from decrease in flux of organic carbon.

4) The estimated sulfide flux from the sediment to the water column was taken from Scranton et al. (1987), where they estimated the sulfide flux based on sulfide pore water profiles from the sediment. The average sulfide flux was 2.3 ± 1.1 mmol S m^{-2} d^{-1} (n=6).

Our sulfur budget calculations (Fig. 4. 10) provide some clues for the interpretation of the present state of sulfur cycling in the Cariaco system. The model predicts two

processes are important for sulfide losses near the interface and it is apparent that the loss rate through sulfide oxidation is an order of magnitude higher than sulfide loss through particulate sulfur flux. *In situ* sulfide production is about half of the diffusive flux from the sediment, in contrast with the conclusion of Scranton et al. (1987), who argued that the sulfide flux from the sediment was the dominant source for sulfide in the water column. When the sum of sulfide losses (oxidation and particulate sulfide flux) is compared to the sum of sulfide sources (*in situ* sulfide production and sulfide diffusive flux from the sediment), the loss rate of sulfide (0.28 ± 0.14 mmol m^{-2} d^{-1}) is an order of magnitude lower than the sulfide source (4.1 ± 1.9 mmol m^{-2} d^{-1}). Use of a higher value of K_z would improve the comparison. The importance of sulfide loss by lateral ventilation processes in the Cariaco is not known. Based on our estimate of the sulfide source (4.1 ± 1.9 mmol m^{-2} d^{-1}) and sulfide inventory in the anoxic water (5×10^4 mmol m^{-2}), the residence time of sulfide in deep water of the Cariaco Basin is about 40 years, consistent with previous Cariaco anoxic water residence times predicted by Redfield et al. (1963).

5. Conclusion

In this study, active pyrite formation in the anoxic Cariaco water column was demonstrated. Greigite and pyrite showed concentration maxima near the oxic-anoxic interface, while iron monosulfide was almost undetectable. Elemental sulfur plus iron sulfides comprised only 10-40 % of total particulate sulfur, with the rest likely composed of particulate organic sulfur. Comparison of the IAP for iron sulfide minerals with the actual concentration profile suggest that thermodynamic control is not the only factor affecting mineral precipitation. Rather, iron sulfide mineral formation near the interface

may be kinetically controlled, possibly mediated by microbes. Higher levels of metal sulfides at intermediate depths rather than in the deep water of Cariaco indicate that a mechanism of pyrite formation requiring sulfur intermediates is important in the Cariaco Basin. As measured by times series sediment traps, pyrite is the dominant iron sulfide mineral that is transported to the sediment. Reduced sulfur flux composed 0.2-0.4% of the total particulate flux from the anoxic water column to the sediment, compared to surface sediment which contain 1.2 % pyrite. We also observed that particulate sulfur flux in the sediment traps was similar in isotopic composition to the ambient sulfide around the chemocline region and obviously different from the narrow range of $\delta^{34}S$ values observed for the deep reservoir, suggesting formation of particulate sulfur near the redox interface.

The importance and relative magnitudes of different processes in the anoxic Cariaco water column can be estimated using a preliminary sulfur budget. Based on our calculation, which assumes a 1-D system, the source and loss terms of sulfur in the water column are not well balanced. We argue it could be caused by the underestimate of sulfide loss due to oxidation process caused by lateral injection or by underestimate of vertical mixing rate. Further investigations, including using radiotracers to directly measure sulfide oxidation and sulfate reduction rates, especially near the chemocline, would provide a better understanding of the whole sulfur budget.

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Table 4.1. Maximum concentration of pyrite in the water column

Location	Pyrite maximum (nmol L^{-1})	Position maximum detected	Reference
Powell lake	435	middle of the anoxic zone	Perry and Pedersen, 1993
Sakinaw lake	80	middle of the anoxic zone	Perry and Pedersen, 1993
Framvaren Fjord	3000	oxic-anoxic interface	Cutter and Kluckhohn 1999
the Black Sea	90	oxic-anoxic interface	Cutter and Kluckhohn 1999
the Cariaco Basin	80	oxic-anoxic interface	this study

Table 4.2. Pyrite concentration and flux from different depths in the Cariaco Basin

Sediment trap ID	Total Mass Flux $g/m^2/day$	Average FeS_2 (μmol S/g)	FeS_2 flux (mg $S/m^2/day$)
Nov 2007: 150 m	0.70	30.2	2.5
Nov 2007: 220 m	1.15	13.5	1.9
Nov 2007: 400 m	0.94	17.5	2.0
Nov 2007: 810 m	0.69	22.4	1.8
Nov 2007: 1210 m	0.64	32.1	2.5
May 2008: 150 m	2.09	23.7	6.0
May 2008: 400 m	0.87	16.3	1.7

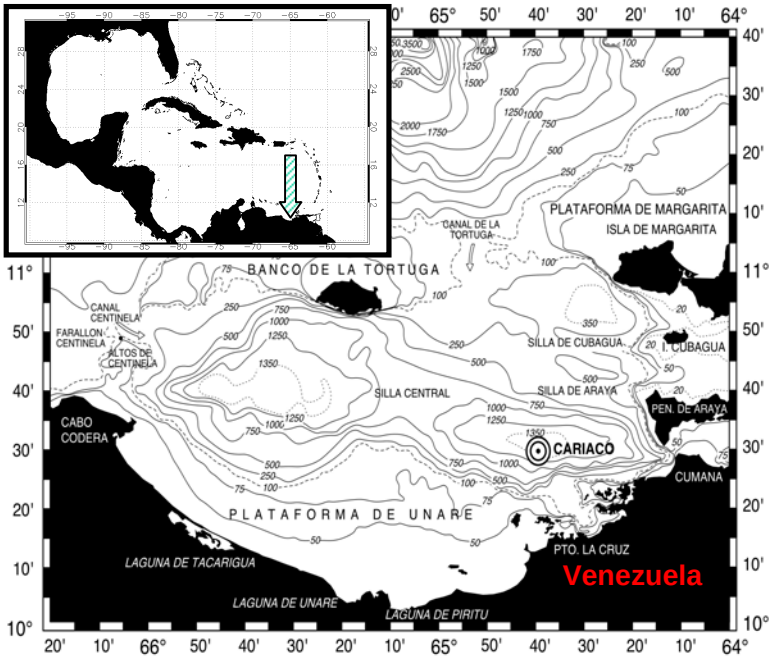


Figure 4.1. Station locations in the Cariaco Basin. Sampling was conducted during both upwelling (May 2008) and relaxation periods (Nov 2007).

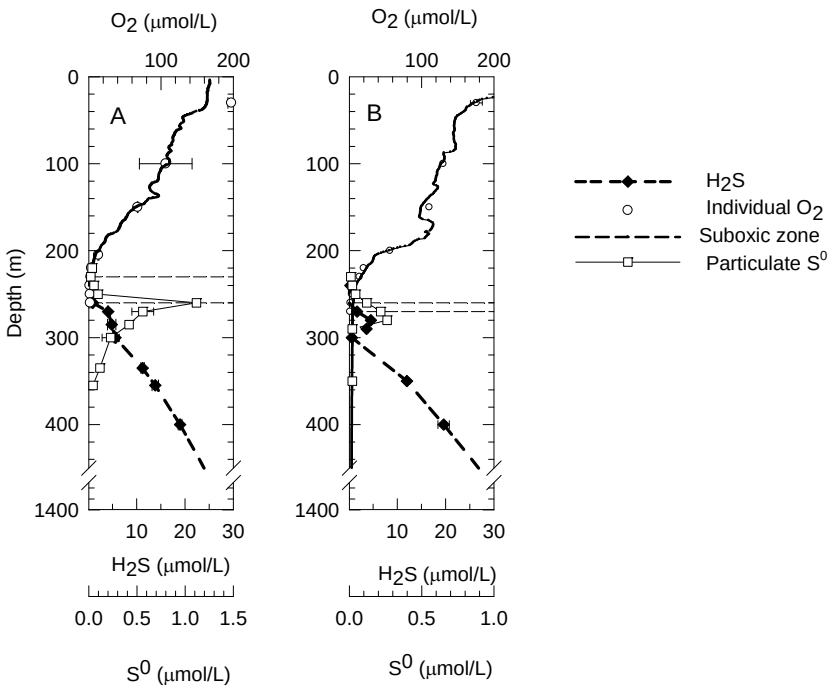


Figure 4.2. Chemical gradients in the water column. Concentrations of oxygen, sulfide and particulate elemental sulfur in samples from date Nov 2007 (A) and May 2008 (B).

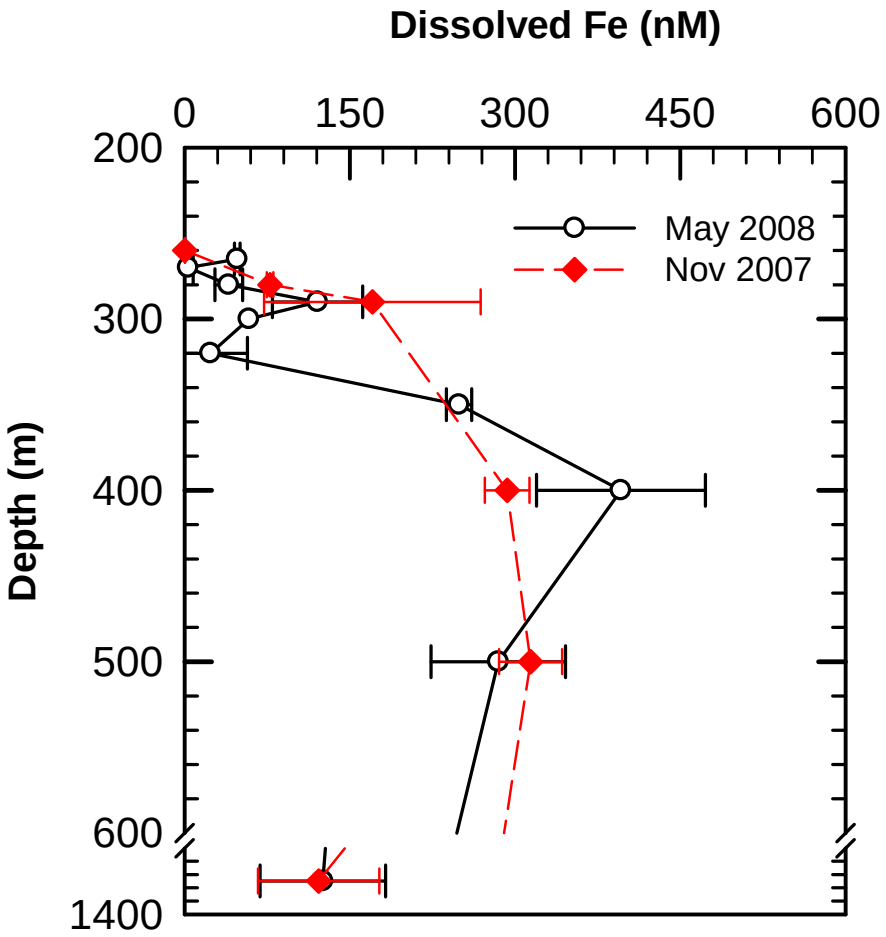


Figure 4.3. Dissolved iron profile in suboxic and anoxic water column during the two cruises, date Nov 2007 and date May 2008.

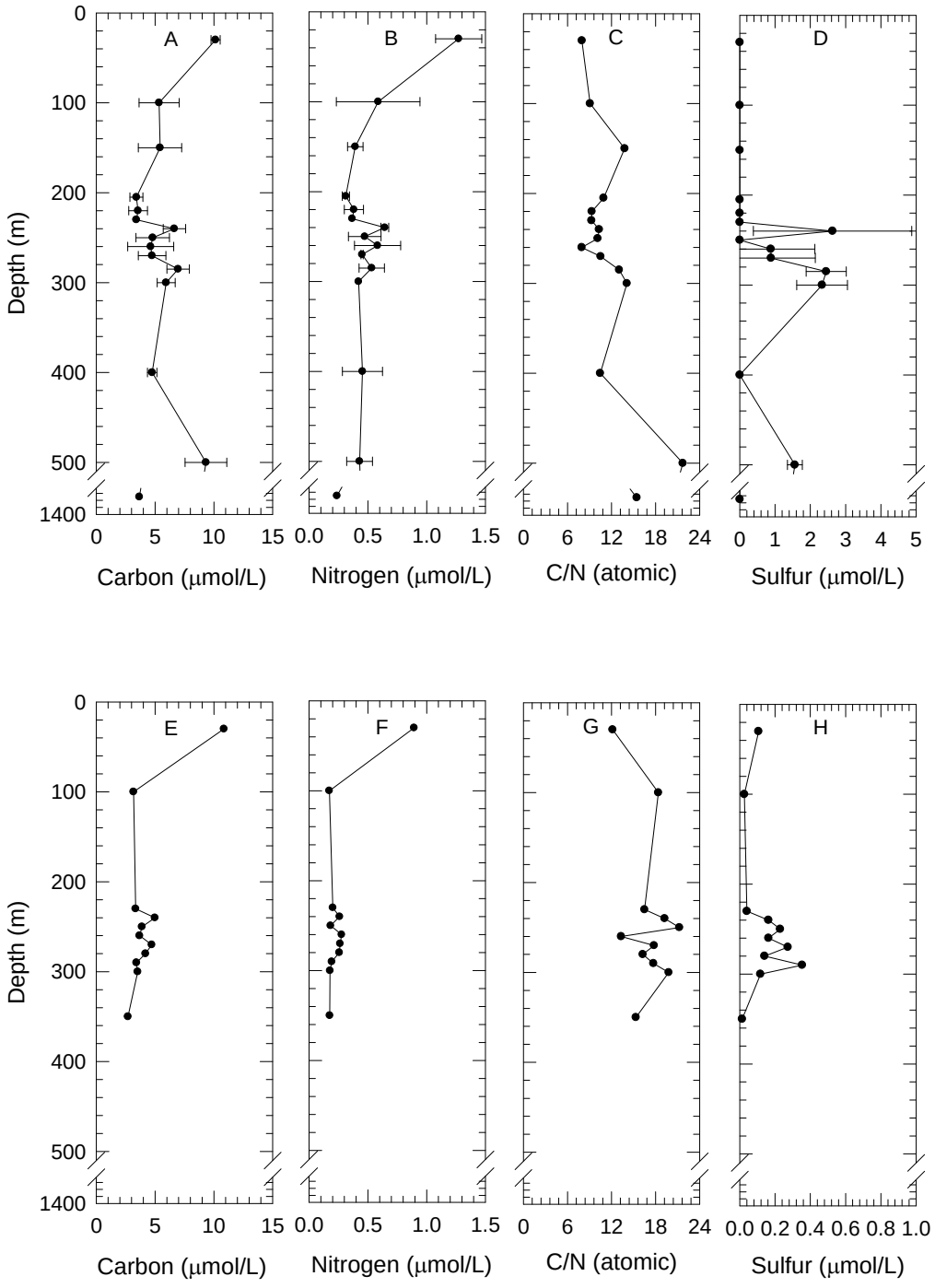


Figure 4.4. Depth profiles for total carbon, total nitrogen, atomic C/N ratio, and total sulfur on suspended particles in the Cariaco Basin. The upper panel (A, B, C, D) is the data from date Nov 2007 and the lower panel (E, F, G, H) is the data from date May 2008.

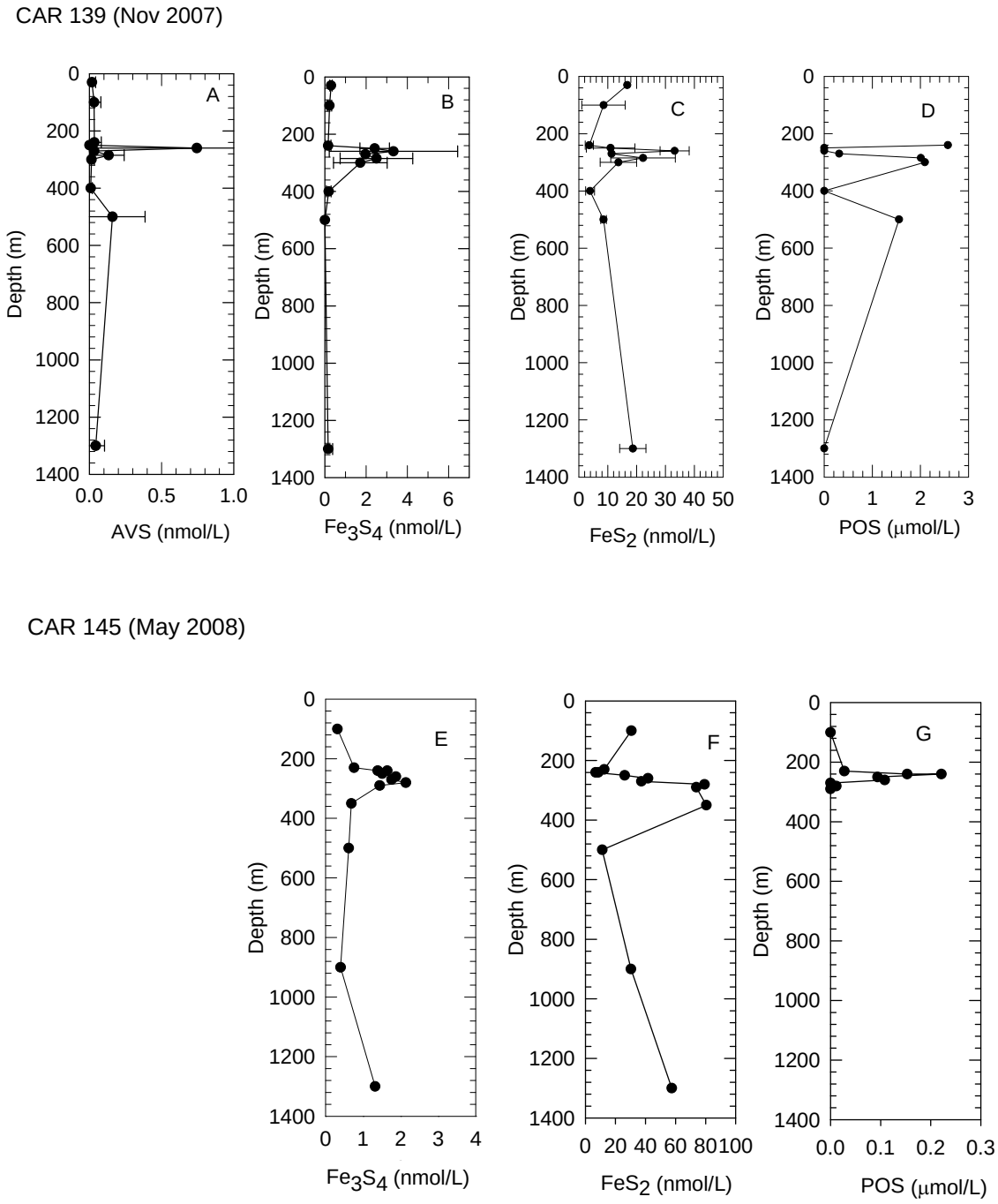


Figure 4.5. Depth profiles for AVS, greigite (Fe_3S_4), pyrite (FeS_2), POS (particulate organic sulfur) in Cariaco Basin water column. The upper panels (A, B, C, D) are date Nov 2007 and the lower panels (E, F, G) are for samples from date May 2008. During this cruise, AVS was not detected.

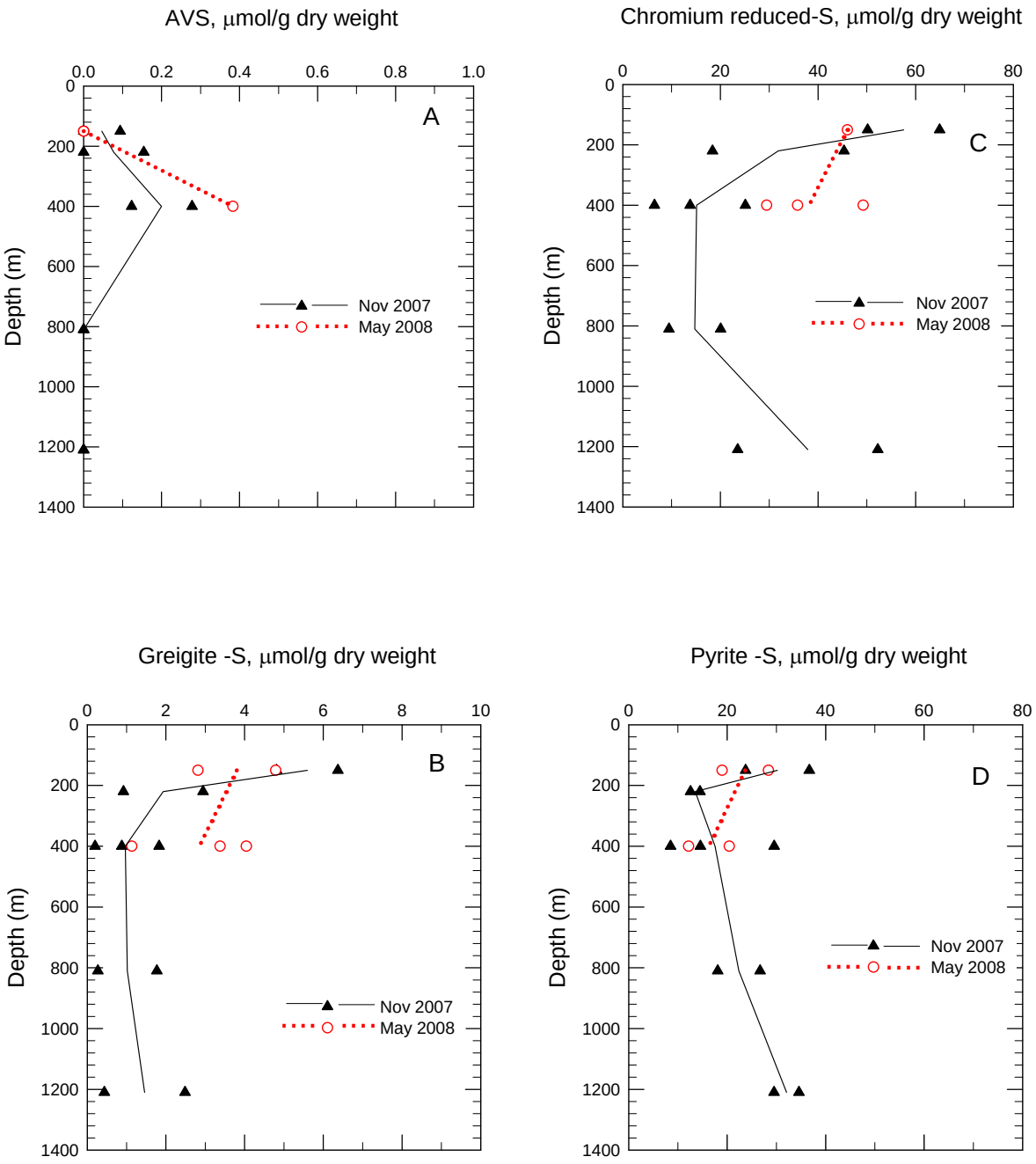


Figure 4.6. Concentrations of particulate iron sulfur (AVS, greigite, CRS, pyrite) in sediment trap materials at CARIACO time series station A.

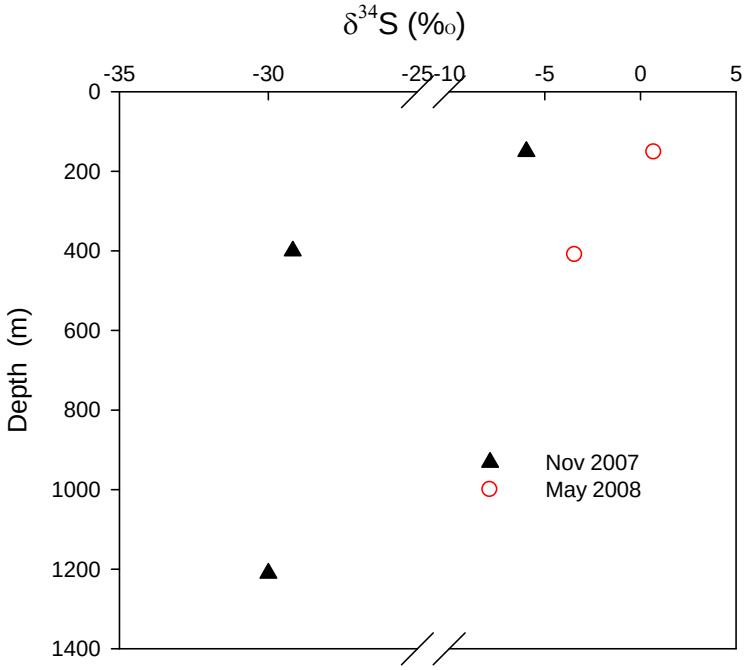
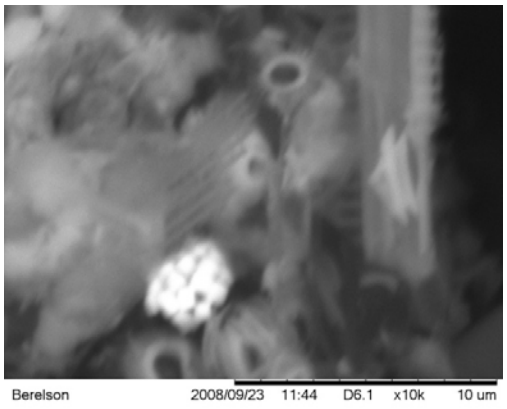
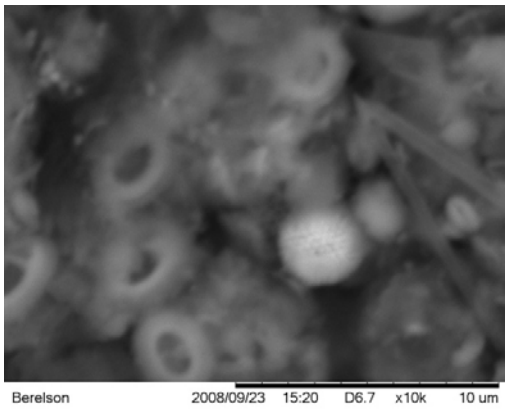


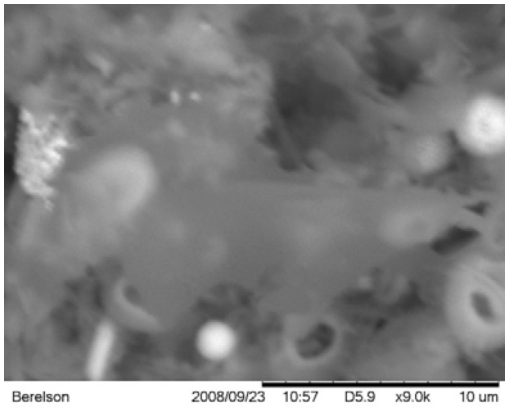
Figure 4.7. Sulfur isotope ($\delta^{34}S$) composition of particulate sulfur flux collected in sediment traps deployed at different depths at station A in the Cariaco Basin.



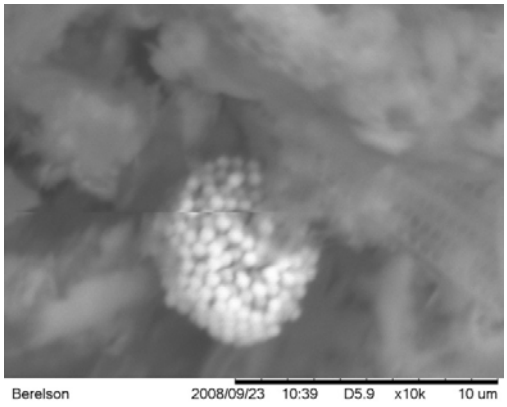
225 m



810 m



1210 m



1210 m

Figure 4.8. SEM photographs of pyrite framboids in sediment trap materials collected at the Cariaco time series station.

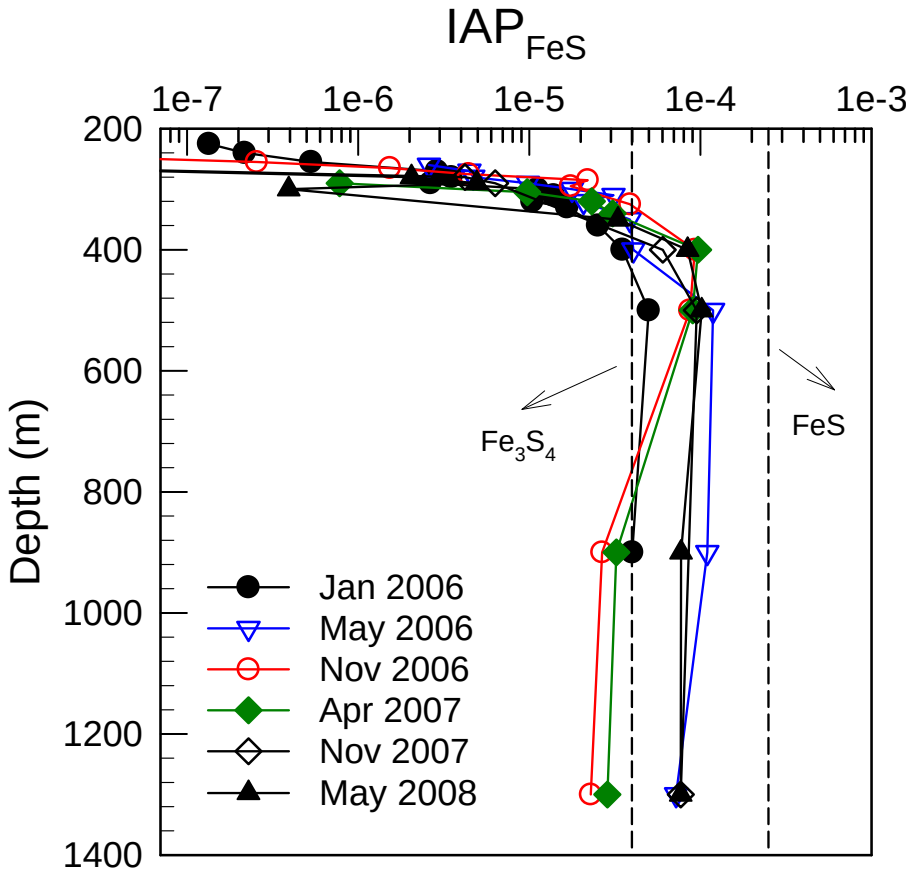


Figure 4.9. Calculations of ion activity products for FeS for six cruises. The vertical lines represent literature values for solubility product constants for the two mineral forms.

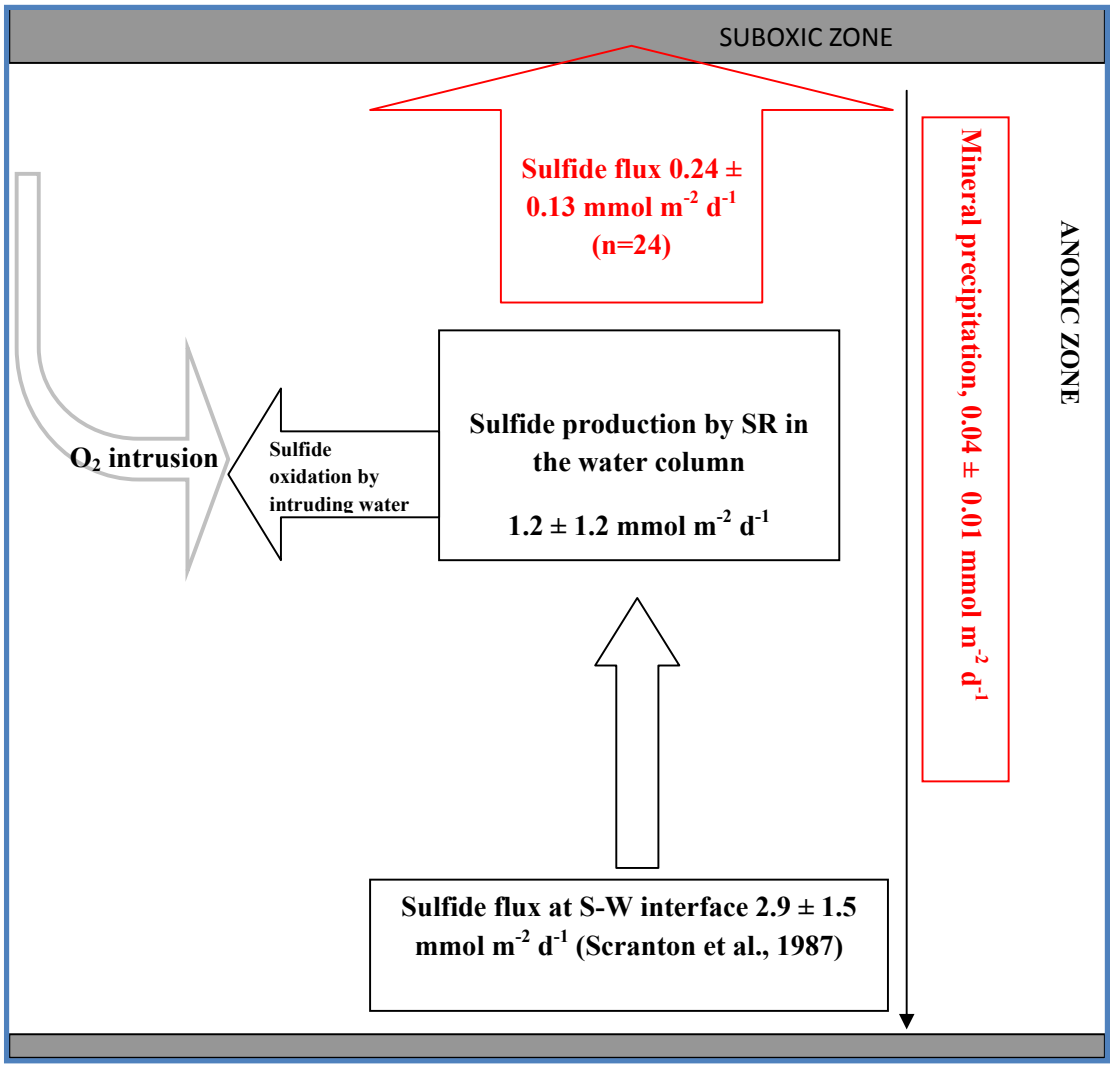


Figure 4.10. Sulfur budget for the Cariaco anoxic zone. Process rates are in unit of mmol m^{-2} d^{-1}.

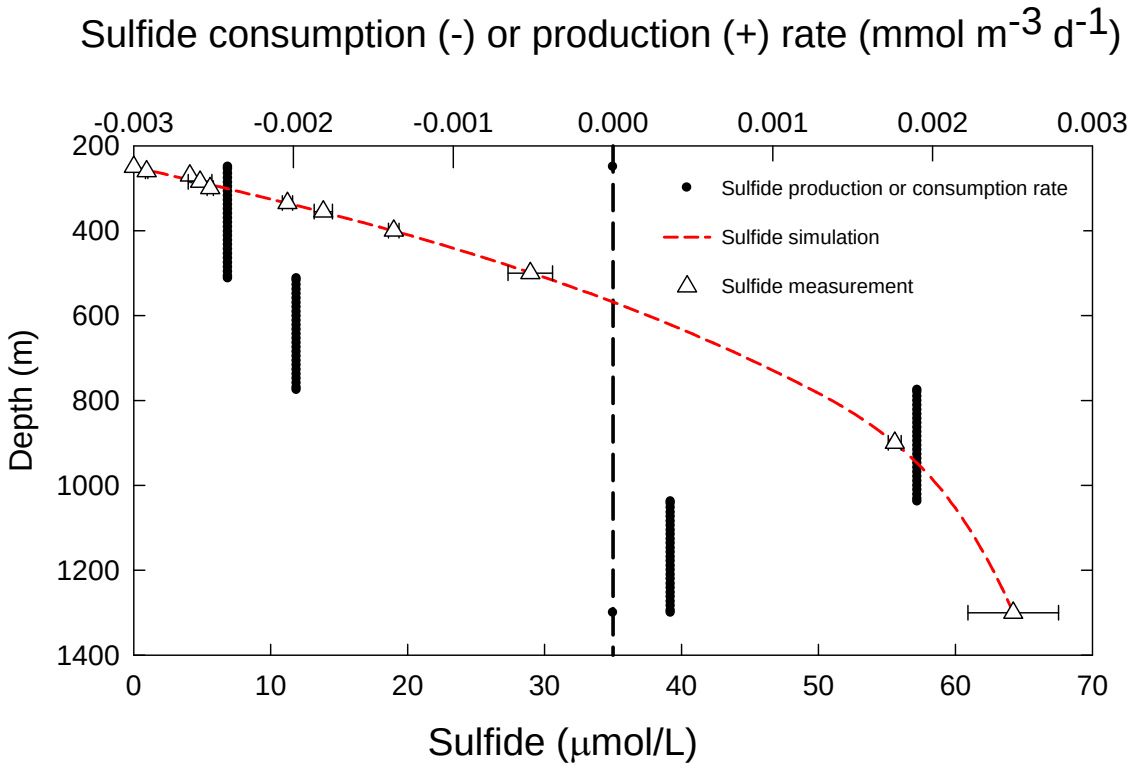


Figure 4.11. PROFILE simulation of hydrogen sulfide and modeling result of net sulfide production and consumption rates calculated by Nov 2007 H_2S distribution (triangles) in the Cariaco water column.

CHAPTER FIVE: Imbalance of supply of oxidants and reductants to the redoxcline and chemoautotrophic production in the Cariaco Basin

Abstract

Since 1995, the Cariaco participants have obtained two or three profiles per year of chemoautotrophic production, oxygen, nitrate, ammonium, sulfide and methane at the CARIACO time series station and less frequently, at three other stations in the Cariaco Basin. Rates of chemoautotrophy are consistently elevated in the suboxic and upper anoxic zones. In this paper we present estimates for the past twelve years of vertical fluxes of oxidants and reductants using a simple one dimensional model, and compare them to chemoautotrophic production rates. In general, downward oxidant fluxes (in electron-equivalents) from major species balanced well with upward electron fluxes from inorganic reductants. However, vertical fluxes of electron donors/acceptors calculated using typical mixing rate constants only explained a small fraction (less than 10%) of the measured chemoautotrophic production if we assume a theoretical stoichiometry of $1H_2S$: 1 CO_2: 1 O_2. In exploring this imbalance, we present estimates of the energy yield of different redox reactions under *in situ* conditions. Our results suggest that aerobic oxidation of methane, sulfide and hydrogen are energy effective at the Cariaco interface. Higher (energy capture) efficiencies of chemoautotrophic bacteria together with an improved understanding of the physics and chemistry of the Cariaco system may explain the apparent imbalance between biological demand and chemical supply.

1. Introduction

In the Cariaco Basin, a pronounced peak in the rate of dark CO_2 fixation has been repeatedly observed in the redoxcline of the water column (Tuttle and Jannasch 1979; Morris et al. 1985; Taylor et al. 2001). However, the presence of a large, active chemoautotrophy-based community seems problematic, since vertical fluxes of reductants (H_2S, NH_4^+, Fe^{2+}, Mn^{2+}) and oxidants (O_2, MnO_2, Fe_2O_3, NO_3^-) calculated from the first seven cruises at the Cariaco time series station only supported a small fraction of the measured chemoautotrophic production (Taylor et al. 2001). Significant imbalances between chemoautotrophic production and diffusive fluxes of substrates have been observed in other anoxic water bodies, such as the Black Sea (Jørgensen et al. 1991; Murray et al. 1995), the Mariager Fjord (Fenchel et al. 1995; Zopfi et al. 2001) and anoxic deeps in the Baltic Sea (Jost et al. 2008). To explain these imbalances, Zopfi et al. (2001) suggested that bacterial fixation of carbon was not necessarily a measure of lithoautotrophic biomass formation but rather could be attributed to anaplerotic fixation of CO_2 by heterotrophic bacteria. However, anaplerotic reactions have been found to account for only 1-8 % of heterotrophic biomass production (Romanenko, 1964; Li et al. 1982). Also measured heterotrophic activity using ^{3}H-leucine is consistently much lower than that of chemolithotrophic activity in the Cariaco redoxcline, averaging 7% (Taylor et al. 2006).

Other processes have been proposed to explain this imbalance, such as *in situ* sulfide production and phototrophic sulfide oxidation (Jørgensen et al. 1991), lateral intrusion which could supply additional substrates (Taylor et al. 2001), symbiotic association of chemoautotrophs with protozoa (Taylor et al. 2001), positive chemotaxis

of sulfur- oxidizing bacteria toward the oxic-anoxic interface (Sorokin 1972), and high cell-specific CO_2 uptake (Jost et al. 2008). Of these processes, only phototrophic production can be excluded from consideration for the Cariaco because irradiance is generally attenuated to <0.01% of incident light at the depth of peak carbon fixation (Taylor et al. 2006).

In this study, we analyzed data from the beginning of the CARIACO project to the present. The data set include information for 24 cruises for the time series station (A), 6 cruises for a station southeast of La Tortuga Channel (B), 3 cruises for a station in the western basin (C), and 2 cruises for a station in the northern section of the eastern sub-basin (D). We use a one dimensional vertical diffusion model to explore the relationship between chemoautotrophic production and inorganic reactants in the water column. Specifically, we have concentrated on several issues: 1) whether the fluxes of electron donors and acceptors to the Cariaco interface are balanced; 2) how fluxes of these compounds and integrated chemoautotrophic production vary through time and space, and 3) whether there are predictable relationships between fluxes of oxidants and reductants with chemoautotrophic production. In addition to the possible underestimates of the vertical eddy diffusion coefficients, we explore other explanations for the apparently high chemoautotrophic production in this specific environment, such as high *in situ* sulfide oxidation rates, as well as constraints on energy capture for carbon fixation by chemoautotrophs.

2. Data

The data employed in this study are from water samples collected in the Cariaco Basin as part of the international CARIACO (CArbon Retention in a Colored Ocean)

sampling program. In addition to the routine monthly productivity/hydrography cruises at station A carried out by EDIMAR scientists, we have conducted two or three additional cruises each year to perform more detailed chemical and microbiological measurements, some of which include additional stations. Cruise dates and station locations are listed in Table 5.1. Since Jan 2004 we have also collected nutrient samples during our detailed redoxcline study. Data links can be found at our project website *http://www.imars.usf.edu/CAR* and all data are available from the authors. More detailed description of general biogeochemical features of the Cariaco Basin are given in Taylor et al. (2001, 2006), Scranton et al. (2001), Hayes et al. (2006), Percy et al. (2008), and Li et al. (2008) and references therein.

The biogeochemical parameters considered in our flux calculations include dissolved oxygen, nitrate, sulfide, ammonium, and methane, with typical profiles shown in Fig. 5.1. Dissolved oxygen and nitrate decrease to the detection limit at the top of the suboxic zone. At the base of the suboxic zone, dissolved sulfide, ammonium, and methane begin to increase downwards. Nitrite is present only at trace levels (factor of 10 lower than other parameters) and is ignored. Particulate and dissolved Fe and Mn concentrations (tens of nmol/L) are also relatively low and the data quality of our measurements of oxidized Fe and Mn varies (Scranton et al. 2001; Percy et al. 2008). Therefore we have not considered them further.

3. Flux model

As a first order approximation of major dissolved oxidants and reactants fluxes to the interface and to assess their relative importance for chemoautotrophic production, we use a simple one dimensional (1-D) model, assuming transport exclusively occurs by

vertical eddy diffusion. At steady-state, the vertical flux (J) is calculated with Fick's first law using the concentration gradients of the various compounds:

$$J = -K_z \frac{\Delta c}{\Delta z} \qquad (1)$$

where K_z is the vertical eddy diffusion coefficient and $\frac{\Delta c}{\Delta z}$ is the concentration gradient.

To calculate the concentration gradient of oxygen (Fig. 5.1), we visually inspected the CTD O_2 profile for every cruise. We identified the depth interval immediately above the oxic- anoxic interface where there was a significant change in the vertical concentration gradient. Thus, the depth interval over which approximations are made varies with cruise. A linear regression was fit through the points over the depth interval that yielded the highest r^2 (coefficient of determination). Only regressions with r^2 higher than 0.98 and $\geq$ 30 data points were used.

For the nitrate flux, we had far fewer data points so we fit linear regressions from the nitrate maximum to the depth where concentrations reached the detection limit. Coefficients of determination for these regressions were always higher than 0.97. For both oxygen and nitrate, linear regression slopes were used as the concentration gradient.

For sulfide, ammonium and methane, two ways of estimating concentration gradients were employed. Using sulfide from CAR 132 as an example (Fig. 5.2), sulfide distributions can be described by the exponential function:

$$C_{HS^-} = C_0 (1-e^{-bz}) \qquad (2)$$

where the oxic-anoxic interface was set as zero depth, C_0=71.4 μmol L^{-1}, b=0.0028 m^{-1}

For this station, the concentration gradient would be $\frac{\partial c}{\partial z} = C_0 \, b \, e^{-bz}$ (3)

So at the oxic anoxic interface (Z=0), $\frac{\partial c}{\partial z} = C_0\, b$ (4)

Another way to estimate concentration gradients is to fit the data for reduced species between first appearance of sulfide and 400 m with a linear regression, since the profiles are typically relatively linear above that depth (Fig. 5.1). Tests using the two approaches with data from four recent cruises (CAR 118 to CAR 132) gave similar results. For simplicity, all the sulfide, ammonium and methane gradients were calculated using a linear fit to the data in the upper part of the anoxic zone.

One of the most problematic aspects of a 1-D model is selection of K_z. A value for the vertical eddy diffusion coefficient, K_z for any depth z, can be estimated from the density gradient (Gargett 1984):

$$K_z = a_0 \left(\frac{1}{N^2} \right)^{1/2} \quad (5)$$

$$\text{where } N = \left(-\frac{g}{\rho} \frac{\partial \rho}{\partial z} \right)^{1/2} \quad (6)$$

a_0 is related to the input of energy to the basin via internal waves and potentially other processes, ρ is the density at a given depth, g is the gravitational acceleration constant, and $\frac{\partial \rho}{\partial z}$ is water density gradient over a finite depth interval. A value of 0.0004 $cm^2\ s^{-2}$ was chosen for a_0, as it falls between values calculated for restricted fjords 0.0001 $cm^2\ s^{-2}$ and the open ocean 0.001 $cm^2\ s^{-2}$ (Gargett 1984). The chosen value also falls within the range used for similar systems such as the Black Sea (a_0=0.0004 $cm^2\ s^{-2}$, Lewis and Landing 1991), and Mariager Fjord (a_0=0.0002 $cm^2\ s^{-2}$, Ramsing et al. 1996; a_0=0.0005 $cm^2\ s^{-2}$, Zopfi et al. 2001). However, previous models of the Cariaco Basin used much higher values of a_0 to fit temporal variability in hydrographic parameters (Scranton et al. 1987). Using the observed density gradient in the water column and a_0 as above, we

obtained an average K_z value of 0.15 cm^2 s^{-1} for O_2 and nitrate from 200 to 250 m, the depth for which we calculated the concentration gradients. For sulfide, ammonium and methane, an average K_z value of 0.30 cm^2 s^{-1} from 300 to 400 m was chosen. This is about a factor of 3-6 lower than used by Scranton et al. (1987). Since this 1-D diffusion flux model is very sensitive to values of K_z, factors influencing K_z or choice of a_0 are very important and will be considered in more detail below.

4. Results

4.1 Flux of chemical substrates to the interface

The results of flux calculations for the five major electron acceptors and electron donors are presented in Table 5.1 and Fig. 5.3. For station A, the calculated downward flux of oxygen to the interface varied between 0.18 mmol O_2 m^{-2} d^{-1} and 1.83 mmol O_2 m^{-2} d^{-1} with the two highest values during CAR 112 (1.78 mmol O_2 m^{-2} d^{-1}) and CAR 128 (1.83 mmol O_2 m^{-2} d^{-1}). The variation in downward nitrate flux was less, with a maximum of 0.23 mmol N m^{-2} d^{-1} and a minimum of 0.13 mmol N m^{-2} d^{-1}. The upward sulfide flux varied from 0.065 to 0.52 mmol S m^{-2} d^{-1}. Sulfide fluxes, especially for the last four years, showed seasonal variation between upwelling (Jan- May, higher flux) and non-upwelling seasons (Jun- Dec, lower flux). Upward ammonium and methane fluxes varied the least among the five components we analyzed in this work, from 0.11 to 0.14 mmol N m^{-2} d^{-1} and from 0.035 to 0.074 mmol C m^{-2} d^{-1}, respectively, with no apparent seasonal trend.

For station B, C and D, fluxes of electron donors and acceptors were similar to those found at Sta. A (Fig. 5.3). One-way ANOVA suggests that fluxes of sulfide ($p<=0.001$), ammonium ($p=0.004$) and nitrate ($p=0.048$) varied significantly among the

four stations. This outcome is largely influenced by the higher fluxes observed at station B than the other three stations. Average fluxes of sulfide, ammonium, and nitrate at station B (and frequently at station D) were twice those of station A. In contrast, fluxes of methane ($p=0.45$), oxygen ($p=0.13$) and integrated chemoautotrophic production ($p=0.42$) did not show significant variability among the four stations.

Under most circumstances, oxygen flux to the interface was much higher than the nitrate flux, although the nitrate flux was comparable to the oxygen flux in a few cases (such as CAR 122). On average, oxygen contributed 60-80 % of the oxidizing potential in electron equivalents. The sulfide flux was always more important than the flux of ammonium and methane, ranging from 50 to 70% of the reducing equivalents.

The fluxes of chemical species have been converted to millimoles of electron equivalents being transferred so that the total electron balance of the system could be evaluated (Fig. 5.4). The number of electrons that can be donated or accepted by a given compound is based on an assumption of complete oxidation or reduction, respectively (Table 5.2). As shown in Fig. 5.4, the total downward oxidizing flux of oxygen and nitrate was between 50 and 200% of the total upward reducing flux from ammonium, sulfide and methane. Given the approximations made in the calculation, the pooled electron flux potentials balanced well between oxidants and reductants.

4.2 Comparison of biological demand and calculated vertical flux of substrates

All parameters in Table 5.1 were subjected to Pearson product-moment correlation analysis. No significant relationship was found among any of the parameters either with all of the data at four stations or within a single station. Since we have the longest records at station A (24 cruises), we focus the rest of our exploration of the relationship between

chemoautotrophic production and the substrate fluxes on results from this station. The chemoautotrophic rates from the first several cruises at station A are very high (Fig. 5.3). Early incubations were performed in septa vials with Teflon/butyl rubber septa. Beginning in 1998, incubations were carried out in glass bottles with ground glass stoppers. Although we have no evidence invalidating the high chemoautotrophy values for the early cruises, due to the change of methodology, data prior to Nov 1998 will not be included in the following discussion.

Fig. 5.5 shows the relationship between oxygen flux and chemoautotrophic production. Data can be classified into three groups: Group I are the data from Nov 1998 to Jan 2004. Most of the data in group II are from cruises after Jan 2004. Group III is made up of the two extremely high oxygen flux estimates from CAR 112 and CAR 128. In both group I and II, chemoautotrophic production increases with increasing oxygen flux as expected. To explain the difference between group I and group II, we hypothesize that there may have been a regime shift in the system resulting in variation in reductant or oxidant supply or in microbial community.

In the Cariaco, sulfide concentrations in the deep water have changed dramatically over time (Fig. 5.6). The maximum sulfide in the deep water was about 75.7 μmol L^{-1} at the start of CARIACO project (Nov 1995), reached a minimum value in 1999, and has risen again after 2002. Since 2004, sulfide in the deep water has stabilized around 64 μmol/L. Data in group I were collected during the period of low bottom water sulfide concentration, which appears to have been caused by transport of oxidants like oxidized Mn and Fe into the deep basin after an earthquake, as suggested by Scranton et al. (2001). During this period, the potential sulfide oxidation rate could have been much higher than

that supported by vertical diffusive flux of O_2, since metal oxidants can play an important role in oxidizing the sulfide. Therefore, higher chemoautotrophic production would be possible.

In addition to changes in sulfide concentration in the deep water with time, there has been a distinct temporal shift in the depth of oxygen disappearance and of sulfide appearance. The gap between these two horizons is the suboxic zone (Fig. 5.7), which we have operationally defined as the layer where both oxygen and sulfide concentrations are below 1–2 μmol/L (Li et al. 2008). The depth of sulfide appearance has varied, but beginning in 2004, it shoaled from 350 m to 255 m. The shoaling of the sulfidic zone could be explained by either 1) higher primary production and thus higher POC flux and higher oxygen demand or 2) less supply, either vertical or lateral, of oxidants into the anoxic zone. So far, there is no evidence for consistent trends of increasing surface primary production (*http://www.imars.usf.edu/CAR*), but lower oxidant supply is consistent with decreased chemoautotrophic production since 2004.

Chemoautotrophic production not only requires oxidants but also reductants. The relationship between sulfide flux and chemoautotrophic production tends to be negative, especially after Jan 2004 (Inner panel in Fig. 5.8, p=0.064, statistically significant). Periods when the measured sulfide concentrations near the interface are low, yielding low fluxes, may represent times when more sulfide had been oxidized to produce sulfur intermediates near the interface, resulting in more active chemoautotrophic production (Percy et al. 2008; Li et al. 2008). Thiosulfate and sulfite inventories were higher when calculated sulfide fluxes were low (Fig. 5.9). Unfortunately we do not have data for sulfur intermediates prior to Jan 2004. We did observe seasonal differences in sulfide flux

between upwelling (Jan- May, higher flux) and non-upwelling seasons (Jun- Dec, lower flux). During non-upwelling, it is possible that intrusions of oxygenated waters are more common due to the increased circulation. In this case, more sulfide may get oxidized to produce the sulfur intermediates. Thus when the sulfide flux is lower, the concentrations of sulfur intermediates and chemoautotrophic production could be higher.

If the theoretical stoichiometry for chemoautotrophy is 1 H_2S: 1 CO_2: 1 O_2 for aerobic sulfide oxidation (Taylor et al. 2001) and if transport of chemical substrates is primarily vertical, the flux calculations from all the stations, are consistent with previous reports that vertical fluxes of oxidants and reductants to the interface are inadequate to satisfy chemoautotrophic demand (Fig. 5.3). Vertical eddy diffusive flux can only support a few percent of the chemoautotrophic production, with sulfide supplying 0.2 -4.2 % of the required reductants and oxygen supplying 0.4%-12% of required oxidants.

5. Discussion

Dark carbon fixation within redoxclines can represent a significant contribution to total carbon production for both marine and freshwater environments. Our Cariaco Basin data collected on 24 cruises at station A from 1998 to 2008 show that integrated chemoautotrophy rates have varied from 13 to 68 mmol C m^{-2} d^{-1}, and can be comparable to primary production rates at times. However, we have not been able to balance vertical oxidant/reductant flux with biological demand using a 1-D eddy diffusion model. The fact that the calculated vertical electron flux of oxidants and reductants agreed within a factor of 2 at all four stations during 17 cruises (Fig. 5.4), suggests that our understanding of the controls on energy supply and chemoautotrophic production in anoxic

environments is incomplete. In the following, we will discuss some possible solutions to this problem.

5.1 Potential importance for fast sulfide oxidation *in situ*

Sulfide oxidation results in the formation of sulfur intermediates, such as polysulfides (S_n^{2-}), elemental sulfur (S_8^0), thiosulfate ($S_2O_3^{2-}$) and sulfite (SO_3^{2-}), and their distributions have been recently studied in the Cariaco Basin (Hayes et al. 2006; Percy et al. 2008; Li et al. 2008).

The stoichiometric relationship between sulfide and ammonium is also consistent with production of sulfur intermediate through sulfide oxidation. The theoretical ratios of the production of the two chemical species in anoxic environment can be calculated from the average stoichiometry of biomass remineralization by sulfate reduction using the following equation (Froelich et al. 1979):

$$(CH_2O)_{106}(NH_3)_{16}(H_3PO_4) + 53\ SO_4^{2-} = 106HCO_3^- + 53\ H_2S + 16NH_3 + H_3PO_4 \qquad (7)$$

This yields a relative release ratio of 3.3 for H_2S:NH_4^+. In the Cariaco Basin, the H_2S:NH_4^+ ratio near the interface is considerably lower, averaging 0.9 (Fig. 5.10a), while the ratio in the deeper part of the basin is 2.5. Since concentrations of sulfur intermediates have been measured beginning with CAR 96, we can correct the sulfide balance as follows:

$$[\text{Reduced sulfur}]_{total} = H_2S + S^0 + 2\ S_2O_3^{2-} + SO_3^{2-} \qquad (8)$$

This correction yields a ratio of total reduced S: NH_4^+ of 2.3 near the interface, approximating the deep water value (Fig. 5.10b). This calculation suggests that about 60% of the sulfide is oxidized near the interface forming sulfur intermediates. Ramsing et al. (1996) observed a similar pattern in the upper layers of Mariager Fjord and they suggested that faster reoxidation of sulfide than ammonium most likely explained the difference. The low H_2S/NH_4 ratio around the interface is also consistent with results shown below that nitrification is less energy effective than sulfide oxidation. This would tend to mean ammonium isn't removed near the interface.

The isotopic compositions of sulfide and sulfate near the interface also are consistent with sulfide oxidation (Chapter 3 of this thesis). If we assume aerobic sulfide oxidation is the dominant process across the 60-80 m thick redoxcline, that the reaction occurs with a 1C: 1S stoichiometry, and that the integrated chemoautotrophic production is 13-68 mmol m^{-2} d^{-1} at station A, then the *in situ* oxidation of sulfide that is required to satisfy biological demand is roughly 0.3 –2.2 μmol H_2S L^{-1} d^{-1}. This is similar to the rate of sulfide oxidation measured at the Black Sea oxic-anoxic interface (1.5-3.6 μmol H_2S L^{-1} d^{-1}, Jorgensen et al. 1991). Unfortunately, we don't have direct measurements of the sulfide oxidation rate in the Cariaco. A potential sulfide oxidation rate was measured in CAR 139 by monitoring the increase of thiosulfate and sulfite during onboard incubations. Sulfide stock solution (final concentration: 30 μmol L^{-1}) was spiked into sealed bottles at two depths: 205m (40 m above the interface, where oxygen was about 14 μmol L^{-1}) and 285m (where there was no dissolved oxygen). Then thiosulfate and sulfite concentration were monitored during a 24-hour incubation. No significant sulfide oxidation took place at 285 m in the anoxic zone (data not shown). However, at 205 m, both thiosulfate and

sulfite were produced at a rate of 2.8 μmol L^{-1} d^{-1} (Fig. 5.11). This gives a potential sulfide oxidation rate of 8.4 μmol L^{-1} d^{-1} if we assume that thiosulfate and sulfite are the main sulfide oxidation products (Zhang and Millero 1993). Since this is the only experiment we have carried out to measure sulfide oxidation rate in the Cariaco Basin and it was not done in a strictly anoxic environment, it is likely that the rate was an overestimate, but it does suggest that these products are readily formed.

The sources of sulfide to the redoxcline remain problematic. Diffusive flux from the deep anoxic water calculated from our 1-D model is too low to support the required oxidation rate. However, as mentioned earlier, the calculated flux is controlled largely by K_z. A larger value of K_z (such as the 0.6-1.8 cm^2 s^{-1} used in the model of Scranton et al. (1987)) would increase the vertical diffusive flux of sulfide to the interface 4-6 times. Chapter 4 of this thesis showed that sulfide oxidation near the interface would have to be ca. 10 times higher than the vertical flux calculated with a K_z of 0.3 cm^2 s^{-1} if sulfide sinks and sources were to balance with each other. Therefore, it seems likely that the vertical fluxes estimated here are too low and suffer because of our inadequate knowledge of the physics of the system.

Another possible explanation for the apparent discrepancy between vertical fluxes of chemical species and biological demand is that horizontal supply of these species is very important (Taylor et al. 2001). Transient lateral intrusions of oxic water or high wind events that circulate and introduce oxygenated water into the suboxic or anoxic zone may supply electron acceptors, and these processes could deliver oxygen to the interface at much higher rates than vertical eddy diffusion (Scranton et al. 2001; Astor et al. 2003). Hayes et al. (2006) suggested that enough O_2 may be able to enter the basin

through intrusions to produce sufficient thiosulfate to support the chemoautotrophic growth of bacteria. In the Black Sea, these redox species (and especially oxidants) are thought to be provided in part by Ekman transport, and more than 50% of sulfide production appears to be oxidized by the lateral supply of oxygen (Konovalov and Murray 2001). Unfortunately, the magnitude, frequency, and velocity of intrusions in the Cariaco Basin are still not clear.

Sulfide might also be supplied to the Sta. A interface through horizontal transport from shallower, more productive area. However, spatial variability of sulfide inventory in the four stations we have sampled so far was relatively low and in the shallowest station we sampled (sta. B), the sulfide inventory was only twice of that in the time series station A (Percy et al. 2008). Our direct flux calculation from different stations also suggests that the diffusive flux variation among stations was not high enough to explain the shortage of sulfide at time series station A (Table 5.1).

In addition to vertical and horizontal diffusive sulfide flux, *in situ* sulfide production by sulfate reduction might play an important role. There is considerable evidence suggesting that sulfate reduction can occur in microenvironments. The maximum sulfate reduction measured near the interface of the Black Sea was about 35 nmol L^{-1} d^{-1} (Jorgensen et al. 1991). However, no direct measurement of the sulfate reduction rate in the Cariaco water column has been carried out. Hayes et al. (2006) argued that the particulate organic carbon sinking flux was too low to support the required H_2S flux. However, they only considered the carbon flux to the 400 m sediment traps. Therefore the carbon delivered to the interface and produced by chemoautotrophs at the redoxcline were not included. Based on sediment trap data collected between 1995

and 2008 (*http://www.imars.usf.edu/CAR*), the mean organic flux to 225 m was 6 ± 3.8 mmol C m^{-2} d^{-1} compared to a mean organic flux to 400 m of 4.4 ± 3.4 mmol C m^{-2} d^{-1} (n=232). On some occasions, the sediment trap flux at 400 m was higher than that at 225 m (65 out of 232), which is consistent with mid-water source of organic matter (Taylor et al. 2001). The integrated chemoautotrophic production between 250 m to 400 m ranged from 13 to 68 mmol C m^{-2} d^{-1} (Table 5.1). According to flux model of Pace et al. (1987), about 50 % of the organic carbon settling from 225 m would be decomposed between 225 m and 400 m. However, carbon removal must be lower than this estimate due to the fact that most of the new carbon is fixed from 300-400 m instead of from 225-300 m. Even if only half of the carbon fixed by chemoautotrophs was transported to 400 m, the organic flux at 400 m would be always much higher than that in the 225 m trap (12.5-40 mmol C m^{-2} d^{-1} as opposed to 6 mmol C m^{-2} d^{-1}) . Since that is not what we observed, we argue that most of the carbon fixed near the interface by chemoautotrophs must be recycled, possibly by sulfate reducers, within the upper anoxic zone and without much flux to the deep anoxic water.

Another piece of supporting evidence for high sulfide production rates in the water column comes from the change of sulfide concentration in the anoxic Cariaco water column with time (Fig. 5.6). Between May 2001 and Jan 2004, sulfide concentrations in the deep anoxic water increased from 30 μmol L^{-1} to 65 μmol L^{-1}. *In situ* production of sulfide can be estimated from the sulfide inventory changes divided by time, resulting in a minimum (assuming no oxidation) rate of 27.6 mmol m^{-2} d^{-1}, 50-100 times higher than the sulfide production rate estimated using the 1-D flux model.

5.2 Chemoautotrophic bacterial yield efficiencies

In order to discuss the discrepancy of biological demand and substrates supply, we define η-O for oxygen as the ratio between integrated chemoautotrophic production and calculated oxygen flux. η-S can be similarly defined using sulfide flux. Higher values of η indicate higher unmet demand for chemical reactants. As shown in Table 5.1, η-O and η-S are always higher than 50 at station A, meaning demand is always fifty times supply.

Since the average η-S of sulfide is as high as 212 ± 167 at station A, there may be other explanations for the lack of reduced energy than an underestimate of vertical diffusive flux and underestimated *in situ* sulfide oxidation. Chemoautotrophs are thought to be able to adopt the most energetically efficient CO_2 fixation pathway available given the thermodynamic constraints of their environments and the highly constrained cost of biomass synthesis (McCollom and Amend 2005). Most estimates (including the one discussed so far in this chapter) attempting to reconcile biological demand and substrate flux are based on a stoichiometry for carbon fixation by sulfide-oxidizing bacteria of 1C: $1O_2$: $1H_2S$. The standard free energy requirement for carbon dioxide fixation by oxygenic phototrophs (reaction 9) is +470.6 kJ (mol CO_2)$^{-1}$.

$$6\ CO_2 + 6\ H_2O \rightarrow C_6H_{12}O_6 + 6\ O_2 \qquad (9)$$

However, this pathway has been challenged when applied to chemoautotrophic bacteria (Nelson and Kagen 1995; Kelly 1999). The major energy spent during photosynthesis is associated with the breakage of the thermodynamically stable H-O bond in water to gain electrons (Overmann and Garcia-Pichel 2000), while for chemoautotrophs using sulfide as electron donors, the energy requirement should be much lower. The process whereby CO_2 is fixed in chemolithoautotrophic bacteria potentially might instead more correctly

resemble equation 10. The ΔG value for this reaction is +114 kJ (mol CO_2)$^{-1}$ (Stryer 1988; Nelson and Kagen 1995; Kelly 1999), only a quarter of that of equation 9.

$$6CO_2 + 18ATP + 12NADH + 12H^+ + 12H_2O \rightarrow C_6H_{12}O_6 + 18ADP + 12NAD^+ + 18Pi \quad (10)$$

Calculation of the free energy change (ΔG) of redox reactions using *in situ* substrate concentrations may be helpful in identifying key factors controlling chemoautotrophic microbial community structures and their activities (McCollom and Shock 1997). For example, one may be able to predict the types of energy metabolism in a given habitat if physical and chemical conditions are measured and energy yields of different reactions are subsequently calculated (Shock and Holland 2004).

As mentioned above, the theoretical energy requirement for chemoautotrophs to fix 1 mol of carbon is about 114 kJ (mol CO_2)$^{-1}$. It must be remembered, however, that there is no direct chemical coupling between reduced substrate oxidation and the process of CO_2 reduction. The former serves only to generate the NAD(P)H and ATP required for the carboxylation of ribulose bisphosphate and reduction to hexose (Kelly 1999).

The relationship between the free-energy change (energy output) from the oxidation of reduced substrates by chemolithotrophs, and the free-energy requirement (energy input) for carbon dioxide fixation to support their autotrophic growth has long been used to estimate the efficiency of energy conservation from reduced substrate oxidation (Baas Becking and Parks 1927; Kelly 1990). In this thesis, we calculated the free energies of different reactions using environmental data (concentrations, pH, temperature) to explore which reactions would be able to support the growth of

chemoautotrophs using reaction 10. The amount of chemical energy available from each chemical reaction under ambient conditions (ΔG_r) was calculated using the Gibbs free energy equation: $\Delta G_r = \Delta G_r^o + RT \ln Q$, where ΔG_r^o is the free energy change under standard conditions and Q is the activity quotient of reactants involved calculated under in situ conditions in the Cariaco.

The types of chemoautotrophic redox processes occurring within redoxclines are controlled by the diversity of substrates available for conversion to biochemical energy. In this study, we mainly focus on the reactions involving sulfur, methane, hydrogen, Fe/Mn and ammonium across the oxic-anoxic interface. We assume a H_2 concentration of 2 nM at the interface as reported by Scranton et al. (1984) and 1 μM for HS^-, S^0, $S_2O_3^{2-}$, SO_3^{2-}, NH_4^+ and CH_4, representing typical conditions at depths where chemoautotrophic production is highest (Scranton et al. 2001; Li et al. 2008). Values of ΔG_r were calculated with the computer program THERMODYN and the thermodynamic data contained therein (Damgaard and Hanselmann 1997). In these calculations, we assume the activity coefficient of all dissolved compounds to be 1 since we do not have sufficient information to accurately calculate the detailed speciation of the substrates in the system. The ΔG_r value calculated also would be more meaningful if we could use the intracellular substrate concentrations; however, this information is also unavailable. Therefore, the calculation we present is an approximation and is designed to provide a broad indication of the relative amount of energy available from different reactions within redoxcline conditions. From this we can decide whether the reactions involving sulfur species are most energy yielding and whether our previous assumption of 1C:1S is reasonable for chemoautotrophs.

We investigated 34 possible reactions combining different electron donors and acceptors under *in situ* Cariaco conditions (Table 5.3). All reactions are written with 1 as coefficient for reduced substrates. Also included in Table 5.3 is an estimate of Gibbs free energy for each reaction using the most reasonable environmental conditions. Twenty one out of the 34 reactions can release more than 114 KJ for each mol of substrate, which is the amount of energy that is required to fix 1 mol of CO_2.

Oxidation of HS-, CH_4 and H_2 with O_2 yields comparable energy. In contrast, NH_4^+ oxidation yields less than half of the energy released by other three substrates (Table 5.3). In cultured strains, ammonia oxidizers have been shown to fix lower amounts of carbon per mole substrate than sulfur oxidizers (Kuenen and Bos 1987; Horrigan and Springer 1990). For natural populations, Ward (1984) argued that sulfur oxidizers in marine redoxclines would easily outcompete ammonia oxidizers. This is also the case in the Baltic Sea (Labrenz et al. 2005) where oxidation of reduced sulfur compounds instead of ammonium oxidation is the main process responsible for high CO_2 fixation. In the Cariaco Basin, 56 out of 60 sequences in the 16S rDNA library at the redoxcline belonged to the subdivision of the ε-proteobacteria that is closely related to clones using H_2S and H_2 as a major energy source (Madrid et al. 2001). Energy release during aerobic oxidation of sulfur intermediates is comparable to that of methane, H_2 and HS^- oxidation. The energy yield of disproportionation of sulfur intermediates is lower but these reactions are still exergonic (equation 24, 25 in Table 5.3).

The low to undetectable concentrations of oxygen and nitrate at the CO_2 fixation maximum have suggested the possibility of other electron acceptors. Disproportionation of sulfur intermediates should be energy favorable under *in situ* conditions at the Cariaco

chemocline (Li et al. 2008). Metal oxidants could also be used by chemoautotrophic bacteria. Under *in situ* conditions, energy yields of reactions involving metal oxidants, such as equation 6, 15, 21, and 30, are also reasonably high (Table 5.3).

According to Table 5.3 (using equation 3) and Fig. 5.12, each mol of O_2 and H_2S consumed near the interface could release roughly 743 kJ, or 6 times the 114 KJ required to fix 1 mol CO_2 by chemoautotrophic bacteria. Thus, sulfide oxidation has the possibility of a maximum possible growth yield approaching 6.6 mol CO_2 assimilated (mol sulfide oxidized for energy-coupled processes)$^{-1}$. Heijnen and van Dijken (1992) argued that the total amount of energy required to synthesize major cell components for anaerobes is 3-10 times lower than for aerobes. Therefore, the problem of insufficient supply of oxygen and sulfide would be significantly relieved if the energy released oxidizing 1 mol of sulfide with 2 mol O_2 could fix 6-8 mol CO_2, rather than the classic stoichiometry for carbon fixation by sulfide-oxidizing bacteria of 1C: $1O_2$: $1H_2S$.

There are four major pathways for CO_2 fixation: the Calvin–Benson–Bassham (CBB) cycle, reverse tricarboxylic acid (rTCA) cycle, 3-hydroxypropionate (3-HP) cycle and reductive acetyl coenzyme A (acetyl-CoA) pathway. The CBB cycle requires three molecules of ATP for the fixation of one CO_2, while the rTCA cycle requires only one ATP for one CO_2. In the Cariaco redoxcline, key enzymes in CBB cycle (RuBisCo gene I and II) have been amplified, and several ε-proteobacteria have been found in the Cariaco Basin which utilize the reverse TCA cycle (Chistoserdov et al. unpubl. data). Carbon isotopic studies and mRNA-based surveys have shown that the rTCA cycle may represent the principal carbon fixation pathway in deep-sea vent ecosystems, and ε-proteobacteria are proposed to operate mainly by the rTCA pathway (Takai et al. 2005). We consistently

observe enrichments of ε-proteobacteria within the redoxcline of the Cariaco Basin (Lin et al. 2006). If the reductive acetyl-CoA pathway, used by chemoautotrophs inhabiting H_2-rich environments, were important, energy requirements would be lower than estimation using equation 10 since CO_2 is directly reduced by H_2 and thus ATP input is not required. However, little is known about the acetyl-CoA cycle in the environment due to the lack of a robust primer set.

As shown in Table 5.3, sulfide oxidation (such as reaction 3) is one of the most energy efficient mechanisms available to organisms in the redoxcline. The mechanisms controlling oxidation of hydrogen sulfide and sulfur intermediates are not well known. However, a general sulfide oxidation (Sox) enzymatic system is present in several different sulfur-oxidizing bacteria (Friedrich et al. 2001). As many as 15 genes encode for Sox and this pathway can use different forms of reduced inorganic sulfur compounds (Friedrich et al. 2001; Rother et al. 2001; Meyer et al. 2007). Catalytic activity with a single gene alone or in combination with other genes was detected, greatly improving electron and energy released from oxidation of one mol substrate. Rother et al. (2001) showed that, through the Sox gene system, 1 mol of thiosulfate yielded about 8 mols of electrons, which is in accordance with the theoretical yield.

6 Conclusions and future work

The present study clearly demonstrated that under the assumption of steady-state conditions, downward fluxes from the major oxidants balanced well with upward fluxes of reductants in electron equivalents. However vertical fluxes of these compounds can not explain observed high chemoautotrophic production at any of the four stations unless

the eddy diffusion coefficient is much higher. There is no systematic and significant relationship between oxygen/sulfide fluxes and chemoautotrophic production.

Efficient cycling of sulfur intermediates might play an important role in meeting the biological demand. Isotope evidence suggests that sulfide oxidation may be very rapid. Even then we are left with the question of where and how sufficient sulfide can be supplied. Sulfide oxidation rates using radiotracer ^{35}S should be measured in the near future in parallel to chemoautotrophic production measurements, but with relative low sulfide concentration (no more than 30 μmol L^{-1}) since high concentration of sulfide might be toxic to the bacterial community. If oxidation rates match the high chemoautotrophic production, there are several possibilities to explain experimental data: 1) the eddy diffusion coefficient that I use to calculate sulfide flux is significantly underestimated; or 2) the chemoautotrophic production and the sulfide oxidation rate are both overestimated due to methodological problems; or 3) *in situ* sulfide production near the chemocline is underestimated.

If the energy requirement of chemoautotrophs to fix CO_2 is lower than previously thought, the imbalance between the chemical fluxes and biological demand would be less. Also a far better understanding of the physical regime of the system is needed. The magnitude, frequency, and velocity of intrusions remain unclear.

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Table 5.1. Fluxes of electron donors and acceptors to the interface of the Cariaco Basin

Station	Date M/D/Y	Cruise ID	Oxygen flux (mmol m^{-2} d^{-1})	Nitrate flux (mmol m^{-2} d^{-1})	Sulfide flux (mmol m^{-2} d^{-1})	Ammonium flux (mmol m^{-2} d^{-1})	Methane flux (mmol m^{-2} d^{-1})	Integrated chemo production (mmol c m^{-2} d^{-1})	η -R (sulfide)	η -O (oxygen)
A	11/10/96	013					0.047	278		
A	5/10/97	019			0.50			128	257	
A	11/13/97	025			0.25		0.053	264	1073	
A	3/12/98	029	0.91		0.43		0.072			
A	7/9/98	032			0.19		0.080	122	647	
A	11/10/98	036	0.25		0.25		0.067	49	198	200
A	5/6/99	042	0.80		0.19		0.040	37	190	45
A	11/4/99	048					0.038	20		
A	5/11/00	054	0.34		0.33		0.055	52	155	153
A	11/3/00	060	0.49		0.21		0.060	68	321	139
A	5/5/01	066	0.36		0.19		0.074	56	298	155
A	1/10/02	074	0.22		0.18		0.037	20	108	90
A	5/7/02	078	0.19		0.09		0.035	41	456	213
A	5/13/03	089	0.53		0.10		0.062	64	637	121
A	1/13/04	096	0.60	0.16	0.06	0.11	0.067	28	428	47
A	5/13/04	100	0.18	0.13	0.23	0.11	0.068	14	59	75
A	1/11/05	108	0.80	0.23	0.13	0.14		35	266	43
A	5/10/05	112	1.78	0.23	0.26	0.14		20	76	11
A	1/12/06	118	0.91	0.18	0.13	0.12	0.044	33	247	37
A	5/10/06	122	0.25	0.22	0.20	0.12	0.059	13	66	54
A	11/2/06	128	1.83	0.22	0.33	0.14	0.058	13	41	7
A	4/10/07	132	0.32	0.16	0.52	0.13	0.044	13	24	39
A	11/30/07	139	0.70	0.14	0.31	0.13	0.054	53	169	75
A	5/30/08	145	1.08	0.23	0.35	0.17	0.061	27	76	25
A		**average**	**0.66 ± 0.49 (n=19)**	**0.19 ± 0.04 (n=10)**	**0.25 ± 0.12 (n=22)**	**0.13 ± 0.02 (n=10)**	**0.056 ± 0.013 (n=21)**	**35 ± 18 (n=19)**	**212 ± 167 (n=18)**	**85 ± 64 (n=18)**

B	1/11/05	108	0.87	0.60	0.37	0.25	0.028	33	89	38
B	5/10/05	112	1.51	0.27	0.73	0.15		25	34	17
B	1/12/06	118	0.50	0.30	0.38	0.19	0.046	39	102	79
B	5/10/06	122	3.41	0.25	0.65	0.24	0.053	20	31	6
B	11/2/06	128	0.43	0.31	0.75	0.29	0.096	3	3	6
B	4/10/07	132	0.87	0.15	0.34	0.16	0.051	20	59	23
B		**average**	**1.26 ± 1.12 (n=6)**	**0.31 ± 0.15 (n=6)**	**0.54 ± 0.19 (n=6)**	**0.21 ± 0.05 (n=6)**	**0.055 ± 0.025 (n=6)**	**23 ± 13 (n=6)**	**54 ± 38 (n=6)**	**28 ± 27 (n=6)**
C	1/11/05	108	0.98	0.25	0.17	0.14	0.040	101	589	102
C	5/10/05	112	3.05	0.19	0.16	0.10		59	380	19
C	1/12/06	118	0.54	0.26	0.32	0.14	0.040	9	29	17
C		**average**	**1.53 ± 1.34 (n=3)**	**0.24 ± 0.04 (n=3)**	**0.22 ± 0.09 (n=3)**	**0.13 ± 0.02 (n=3)**	**0.040 ± 0.000 (n=3)**	**56 ± 46 (n=3)**	**333 ± 283 (n=3)**	**46 ± 49 (n=3)**
D	5/10/06	122	0.62	0.19	0.45	0.17	0.063	42	93	68
D	4/10/07	132	0.39	0.17	0.44	0.19	0.023	38	88	99
D		**average**	**0.50 ± 0.16 (n=2)**	**0.18 ± 0.01 (n=2)**	**0.44 ± 0.01 (n=2)**	**0.18 ± 0.01 (n=2)**	**0.043 ± 0.028 (n=2)**	**40 ± 2 (n=2)**	**90 ± 3 (n=2)**	**83 ± 21 (n=2)**

Table 5.2. Number of electrons that can be transferred by a given compound

	Sulfide	Ammonium	Methane	Oxygen	Nitrate*
Transferable e^- (n)	8	8	8	4	8

*: ammonification to NH_4 is assumed

Table 5.3. Free energy changes for combined electron donors/acceptors under environmental conditions. Free energy of formation is calculated using Damgaard and Hanselmann (1997). For elemental sulfur, the free energy formation of rhombic sulfur ($\Delta G_f^0=0$) is used. The values for O_2, N_2 and H^+ in aqueous solution are also taker as $\Delta G_f^0=0$. Free energy of amorphous FeO(OH) (am) and also g-MnO_2 were used when metal oxidants are involved in the reaction.

#	*Reaction*	ΔG^0 *[KJ]*
1	$CH_4 + 2O_2 \rightarrow CO_2 + 2H_2O$	-774
2	$S_2O_3^{2-} + H_2O + 2\ O_2 \rightarrow 2\ SO_4^{2-} + 2\ H^+$	-766
3	$HS^- + 2\ O_2 \rightarrow SO_4^{2-} + H^+$	-743
4	$S_2O_3^{2-} + 1.6\ NO_3^- + 0.2\ H_2O \rightarrow 2\ SO_4^{2-} + 0.4\ H^+ + 0.8\ N_2$	-688
5	$HS^- + 1.6\ NO_3^- + 0.6\ H^+ \rightarrow SO_4^{2-} + 0.8\ N_2 + 0.8\ H_2O$	-663
6	$S_2O_3^{2-} + 4\ MnO_2 + 6H^+ \rightarrow 4\ Mn^{2+} + 2SO_4^{2-} + 3H_2O$	-636
7	$S^0 + H_2O + 1.5\ O_2 \rightarrow SO_4^{2-} + 2\ H^+$	-576
8	$HS^- + 1.5\ O_2 \rightarrow SO_3^{2-} + H^+$	-518
9	$HS^- + H^+ + H_2O + NO_3^- \rightarrow SO_4^{2-} + NH_4^+$	-419
10	$HS^- + O_2 \rightarrow 0.5\ S_2O_3^{2-} + 0.5\ H_2O$	-359
11	$S^0 + O_2 + H_2O \rightarrow SO_3^{2-} + 2H^+$	-352
12	$NH_4^+ + 2\ O_2 \rightarrow NO_3^- + 2\ H^+ + H_2O$	-321
13	$NH_4^+ + NO_2^- \rightarrow N_2 + 2H_2O$	-279
14	$NH_4^+ + 1.5\ O_2 \rightarrow NO_2^- + 2\ H^+ + H_2O$	-254
15	$S_2O_3^{2-} + 8\ FeOOH + 14H^+ \rightarrow 8Fe^{2+} + 2SO_4^{2-} + 11H_2O$	-253
16	$NH_4^+ + 0.6\ NO_3^- \rightarrow 0.8\ N_2 + 0.4\ H^+ + 1.8\ H_2O$	-230
17	$SO_3^{2-} + 0.5\ O_2 \rightarrow SO_4^{2-}$	-225
18	$H_2 + 0.5\ O_2 \rightarrow 2H_2O$	-199
19	$NH_4^+ + 4\ MnO_2 + 6\ H^+ \rightarrow NO_3^- + 4\ Mn^{2+} + 5H_2O$	-195
20	$S^0 + 0.5\ O_2 + 0.5\ H_2O \rightarrow 0.5\ S_2O_3^{2-} + H^+$	-194
21	$HS- + MnO_2 + 3H^+ \rightarrow Mn^{2+} + S^0 + 2H_2O$	-136
22	$HS^- + 0.25\ O_2 \rightarrow S^0 + 0.5\ H_2O$	-83
23	$NH_4^+ + 3\ FeOOH + 5\ H^+ \rightarrow 0.5\ N_2 + 3\ Fe^{2+} + 6\ H_2O$	-81
24	$S_2O_3^{2-} + H_2O \rightarrow SO_4^{2-} + HS^- + H^+$	-73
25	$S^0 + H_2O \rightarrow 0.25\ SO_4^{2-} + 0.75\ HS^- + 1.25\ H^+$	-70
26	$NO_2^- + 0.5\ O_2 \rightarrow NO_3^-$	-67
27	$Fe^{2+} + 0.25\ O_2 + 1.5\ H_2O \rightarrow FeOOH + 2H^+$	-64
28	$Fe^{2+} + 0.2\ NO_3^- + 1.4\ H_2O \rightarrow FeOOH + 1.8\ H^+ + 0.2\ N_2$	-54
29	$Fe^{2+} + 0.5\ MnO_2 + H_2O \rightarrow 0.5\ Mn^{2+} + FeOOH + H^+$	-54
30	$HS^- + 2FeOOH + 5H^+ \rightarrow 2Fe^{2+} + S^0 + 4H_2O$	-46
31	$S^0 + 0.67\ FeOOH \rightarrow 0.33\ SO_4^{2-} + 0.67FeS + 0.67H$	-39
32	$Mn^{2+} + 0.5\ O_2 + H_2O \rightarrow MnO_2 + 2H^+$	-32
33	$SO_3^{2-} + 0.25\ H^+ \rightarrow 0.75\ SO_4^{2-} + 0.25\ HS^-$	-30
34	$Mn^{2+} + 0.4\ NO_3^- + 0.8\ H_2O \rightarrow 0.2\ N_2 + 1\ MnO_2 + 1.6\ H^+$	-13

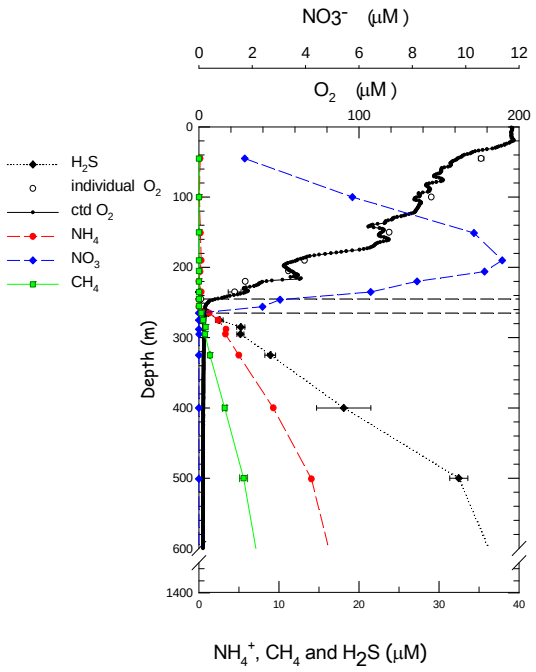

Figure 5.1. Redox biogeochemical structure of the Cariaco Basin (data from CAR 128 station A). Dash line marks the suboxic zone.

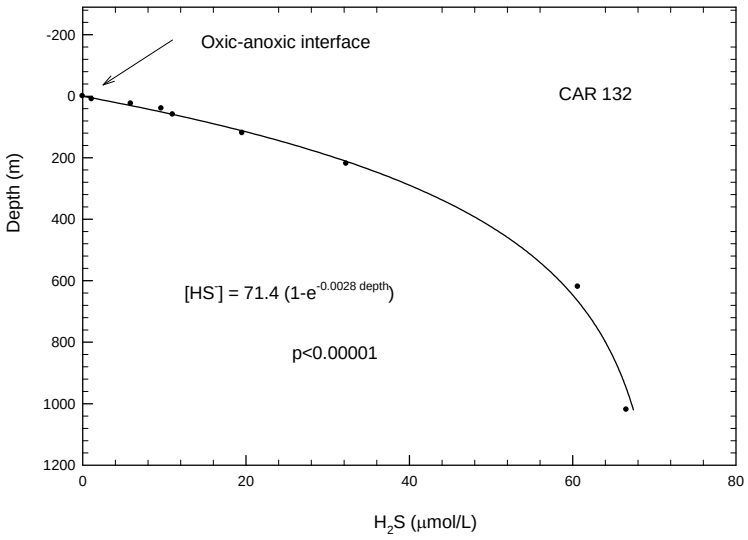

Figure 5.2. Depth profile of sulfide concentration in the Cariaco (CAR 132), simulated by exponential equation to calculate the flux of sulfide.

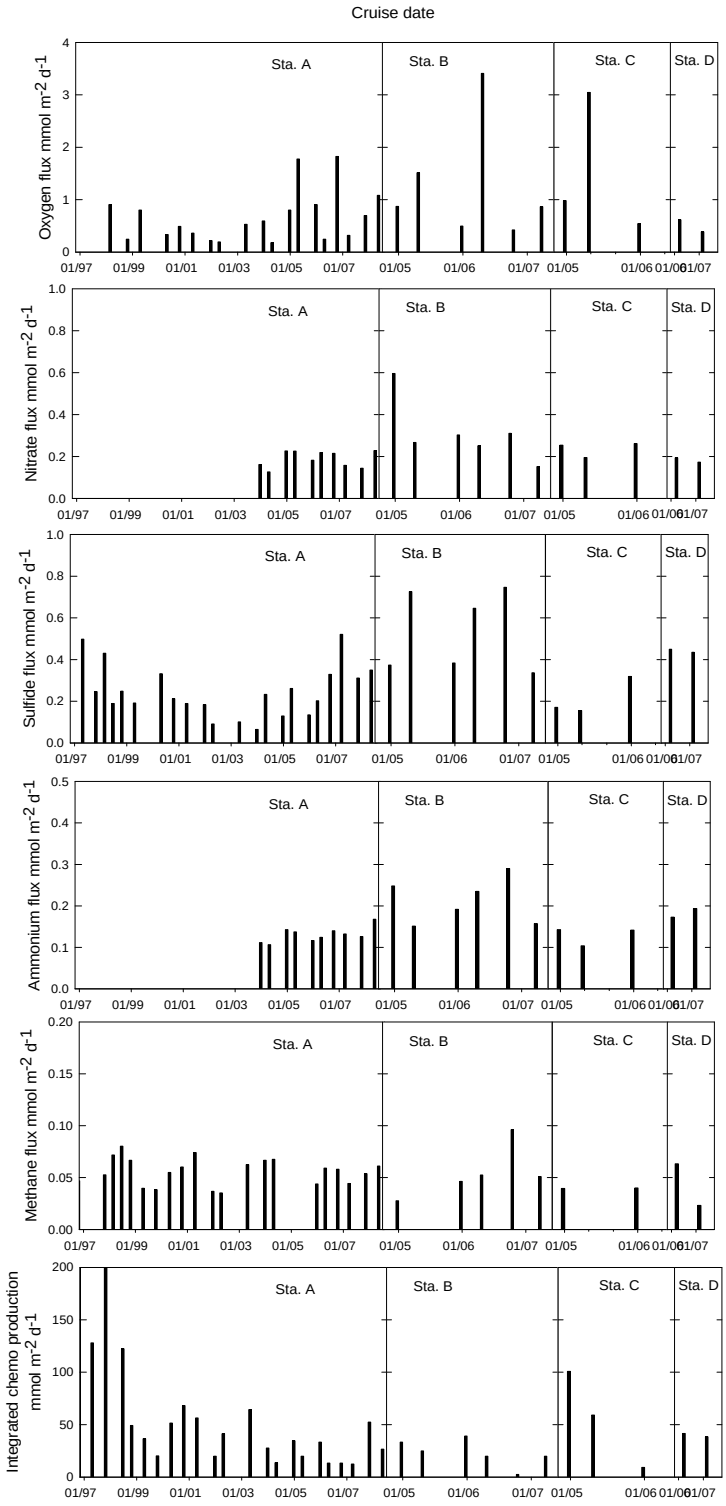

Figure 5.3. Temporal and spatial variation of the fluxes (normalized to per mol substrate) of different substrates to the interface of the Cariaco Basin and integrated chemoautotrophic production.

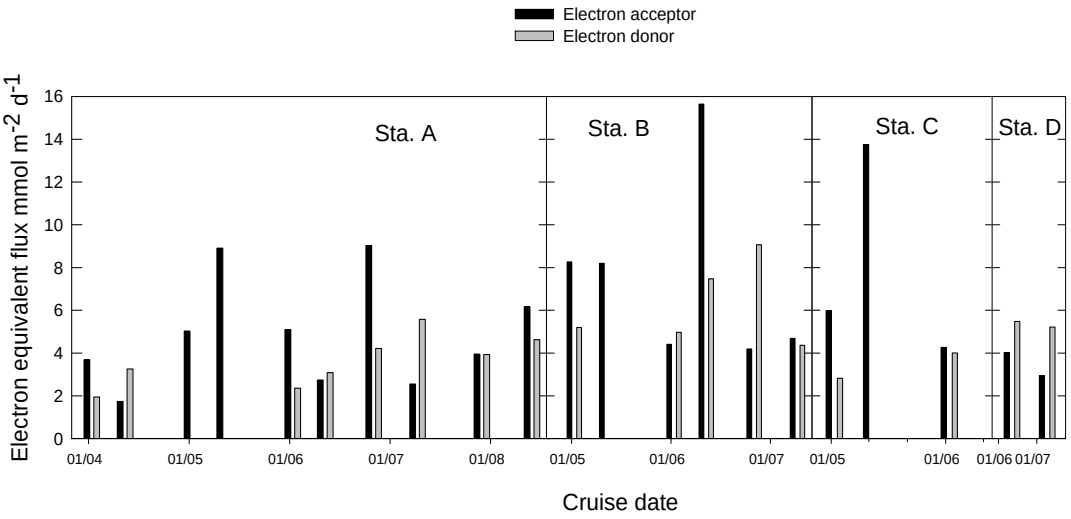

Figure 5.4. Balance of the sum of electron equivalents from different electron donors and acceptors. Only cruises when we have the fluxes calculated from all the five parameters are included.

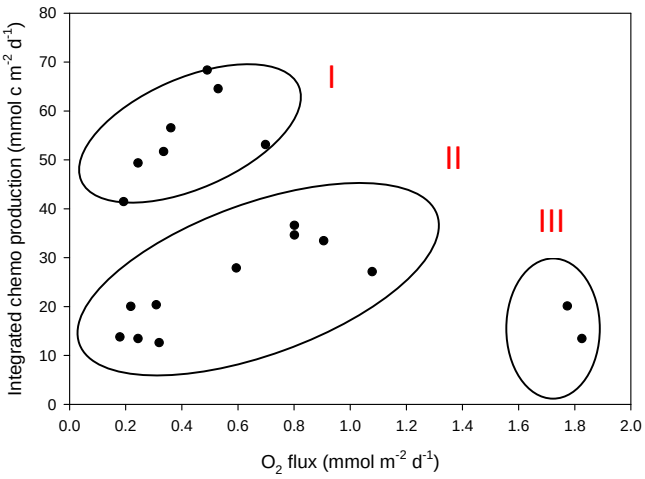

Figure 5.5. Relationship between integrated chemoautotrophic production (from 250 m to 450 m) and oxygen flux at station A (I, II, III stand for different time period).

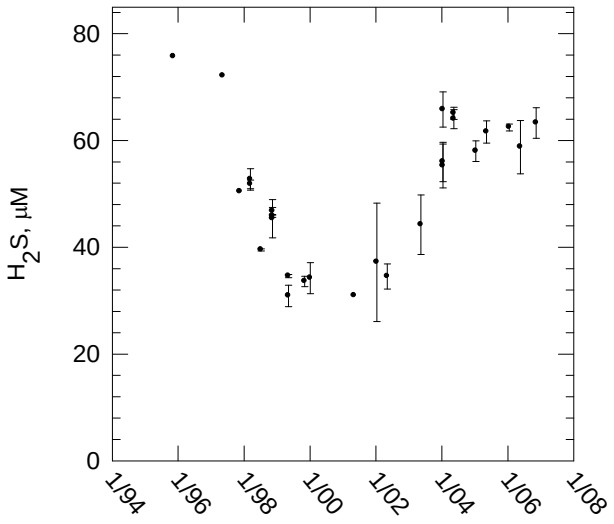

Figure 5.6. Temporal changes in sulfide concentrations in the Cariaco bottom waters (1300 m) over the last 15 years.

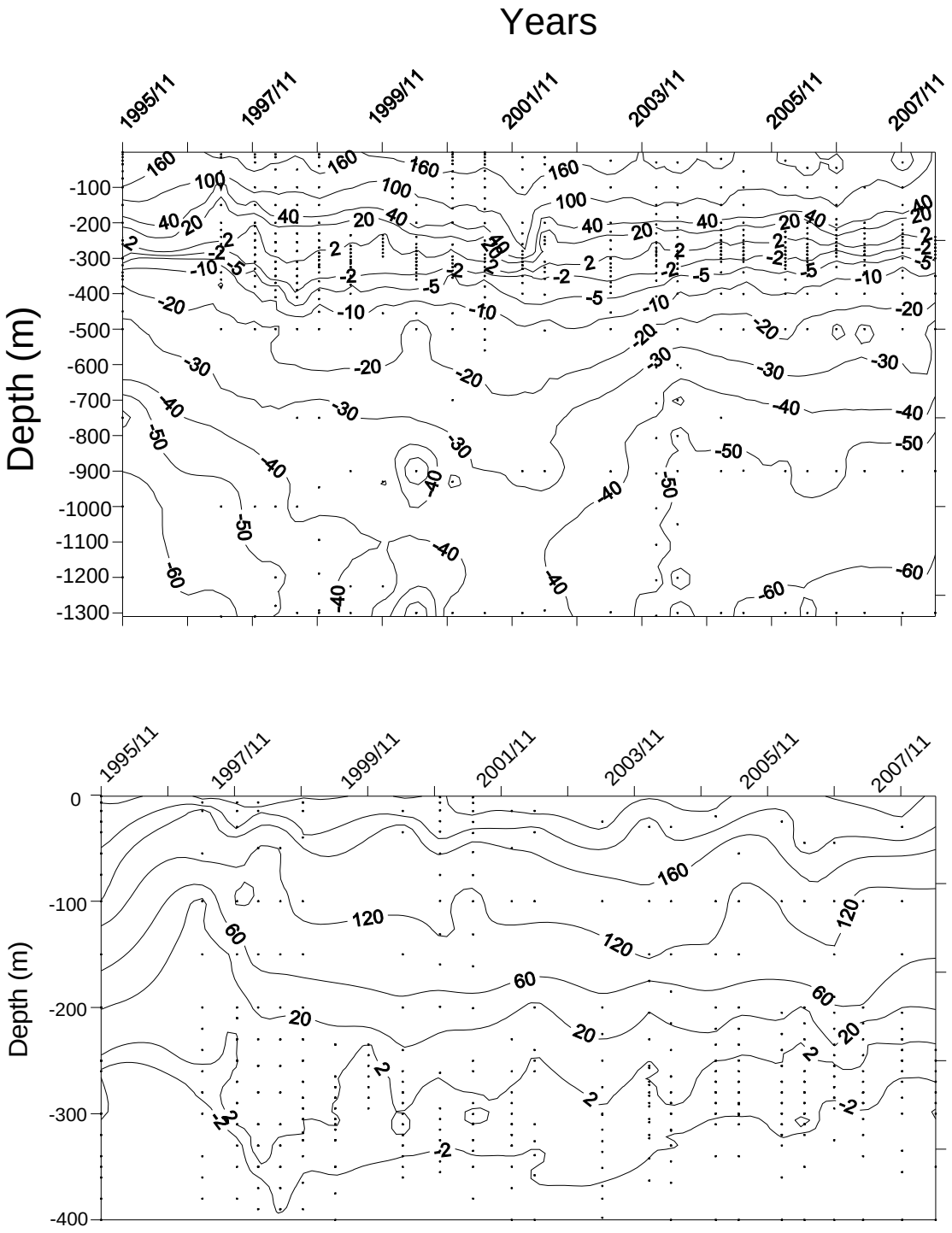

Figure 5.7. Time series of oxygen extinction and sulfide onset depth the positive value (μM) is oxygen, and the negative value is sulfide. In the lower panel, note the shoaling trend of suboxic zone (O_2=2 μM, H_2S=-2 μM) since 2004.

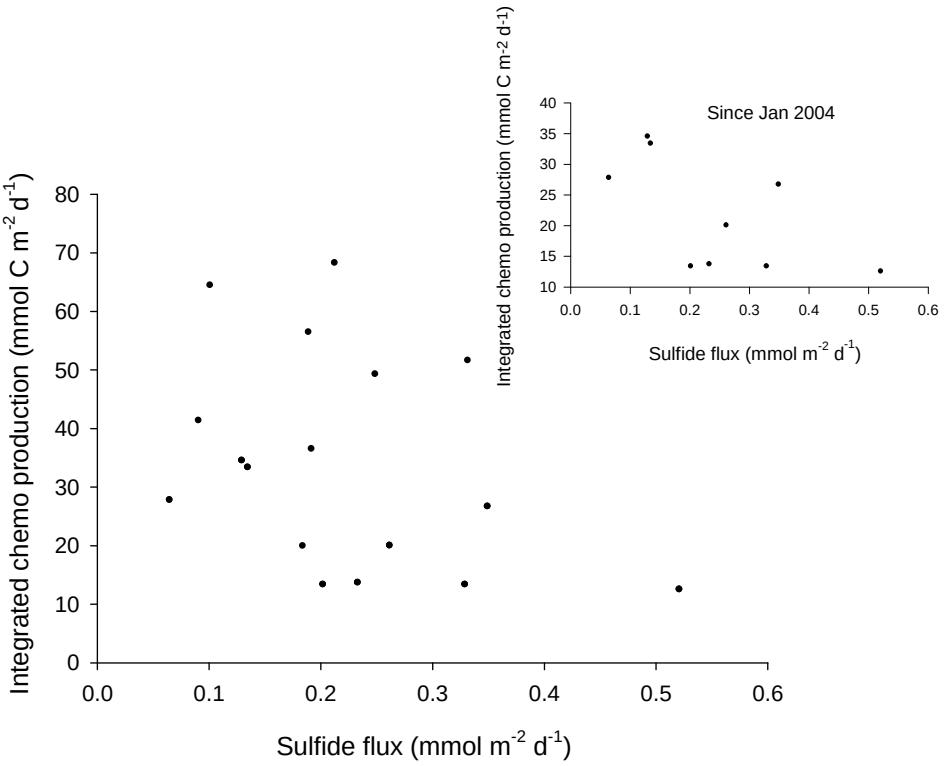

Figure 5.8. Relationship between sulfide flux and chemoautotrophic production.

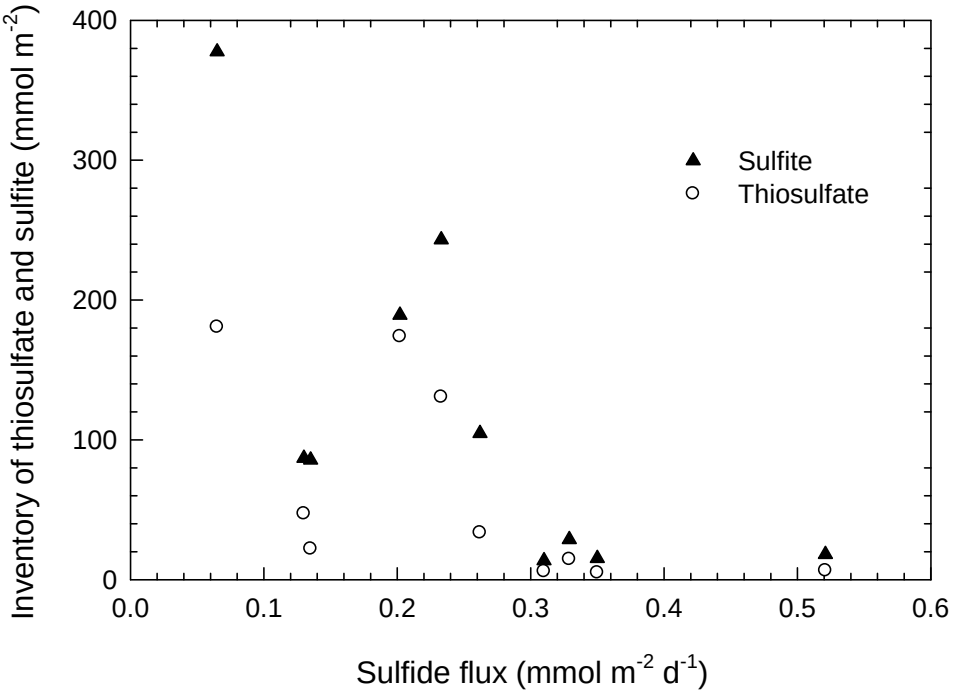

Figure 5.9. Relationship between sulfide flux to the interface and sulfur intermediates inventories around the interface (240 m-340m) from CAR 96 to CAR 145 at station A.

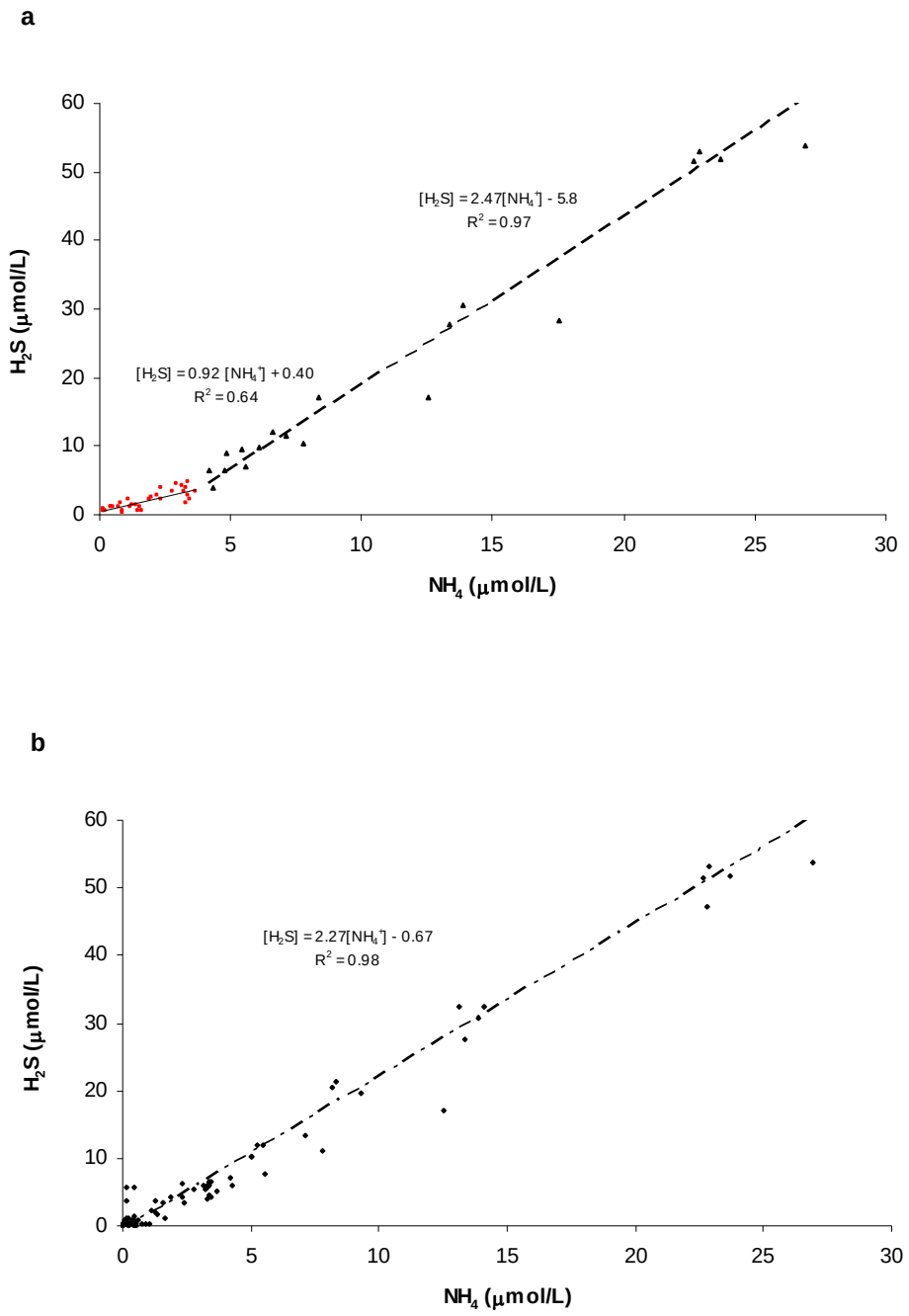

Figure 5.10. (a) Sulfide concentration plotted against ammonium concentration; (b) sulfide: ammonium ratio after corrected for sulfur intermediates.

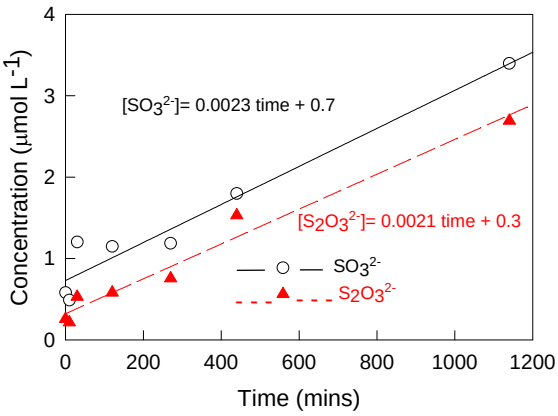

Figure 5.11. Thiosulfate and sulfite production rate determined in closed bottle experiments with water samples at 205 m during CAR 139.

CHAPTER SIX: Conclusions and future directions

1. SUMMARY OF FINDINGS

The focus of this thesis has been sulfur biogeochemical cycling in the Cariaco Basin. Detailed conclusions have been given at the end of each chapter. Here, I summarize them and provide some thoughts about the significance of my findings.

In this thesis, the concentrations of several sulfur intermediates have been determined in the Cariaco water column, including particulate elemental sulfur, total zero-valent sulfur, thiosulfate and sulfite. Results showed that concentrations of sulfur intermediates near the interface vary with space and time. In particular, thiosulfate and sulfite profiles near the interface lack a reproducible distribution pattern, likely reflecting the dynamic and complicated nature of the Cariaco Basin. In contrast, a particulate elemental sulfur peak was consistently observed near the interface. Based on the distribution profiles, and correlation analysis of the relationship between sulfur species and chemoautotrophic production, elemental sulfur seems to be important in supporting the chemoautotrophic bacterial populations near the interface and upper anoxic zone.

The sulfur isotope composition of sulfide and sulfate was also measured and compared to the concentration of sulfur species to explore how different sulfur transformation processes affect the sulfur isotope signal in the Cariaco water column, especially near the redoxcline. Small variations of the $\delta^{34}S_{SO4}$ in the water column were repeatedly observed near the redox interface. These variations are broadly consistent with

a reservoir effect associated with sulfide production and may be slightly larger than that allowed by the standing pool of sulfide (e.g., an unsampled sink may exist for ^{34}S-depleted sulfur such as iron sulfide that settled from the water column). Variation in the isotopic composition and concentrations of dissolved sulfate and sulfide appear to reflect temporal variability associated with carbon supply tied to primary productivity and recirculation of oxygenated water throughout the basin.

The sulfur isotope variations ($\delta^{34}S_{H2S}$) and concentrations of sulfur species in the upper 400 m of the water column appear to be consistent with fractionation related to sulfide loss by chemoautotrophic or abiological sulfide oxidation, but information provided by $^{33}S/^{32}S$ ($^{33}\lambda$) implies that additional processes also likely contribute to sulfur isotope variations. These processes may reflect changes in the magnitude of the fractionation associated with sulfate reduction within the water column, perhaps as a result of variations in quantity and quality of organic matter electron donors. The sulfur isotope fractionations ($\delta^{34}S$ and $\Delta^{33}S$) and concentration data for the water column below 500 m are consistent with a significant contribution of sulfate reducers to the sulfur cycle. The fractionations between sulfate and sulfide are very large in this interval (54‰) and would be greater than the known fractionations from laboratory culture experiments, but are within the limits of current models of sulfate reduction metabolism (Brunner and Bernasconi 2005; Farquhar et al. 2007). We speculate that these large fractionations are related to slow rates for sulfate reduction. The fractionations also appear to decrease approaching the sediment–water interface, suggesting a contribution of sulfide from the sediment, but also the presence of a source of sulfide in the water column that is even more ^{34}S-depleted.

Data from this study also demonstrated active pyrite formation in the Cariaco water column. Greigite and pyrite showed concentration maxima near the oxic-anoxic interface, while AVS was almost undetectable. Particulate elemental sulfur and iron sulfides only accounted for 10-40% of total particulate sulfur, with the rest likely composed of particulate organic sulfur. Comparison of the IAP for iron sulfide minerals with the actual concentration profiles suggests that thermodynamic control is not the only factor affecting mineral precipitation. Rather, iron sulfide mineral formation near the interface may be mediated by microbes. Higher levels of iron sulfides at intermediate depths rather than in the deep water of Cariaco is consistent with the idea that a mechanism of pyrite formation requiring sulfur intermediates was important in the Cariaco Basin. As measured by times series sediment traps, pyrite was the dominant iron sulfide mineral that is transported to the sediment. The reduced sulfur flux represented 0.2-0.4% of the total particulate flux from the anoxic water column to the sediment, compared to surface Cariaco sediment which contained 1.2% pyrite (Werne et al. 2003). We also observed that particulate sulfur flux in the sediment traps was similar in isotopic composition to the ambient sulfide around the chemocline region and obviously different from the narrow range of $\delta^{34}S$ values observed in the deep sulfide reservoir, consistent with formation of particulate iron sulfide minerals near the redox interface.

The importance and relative magnitude of different processes affecting sulfur cycling in the Cariaco anoxic water column were estimated by constructing a preliminary sulfur budget. Based on my calculation, which assumes a 1-D system, the source and loss terms of sulfur in the water column are not well balanced. This imbalance could be caused by underestimation of the vertical mixing coefficient that I used to calculate

sulfide flux to the interface, or by underestimation of sulfide loss due to oxidation processes caused by lateral injection of oxidants.

The present study clearly demonstrated that under the assumption of steady-state conditions and a 1-D system, downward fluxes from the major oxidants balanced well with upward fluxes of reductants in electron equivalents. However, vertical fluxes of these compounds can not explain observed high chemoautotrophic production at any of the four stations unless the eddy diffusion coefficient is much higher (50-100 times), or advection is significant, or the chemoautotrophic production estimates are incorrect. There was no systematic and significant relationship between oxygen/sulfide fluxes and chemoautotrophic production. Efficient cycling of sulfur intermediates might play a role in explaining the discrepancy. On the other hand, isotopic evidence leads me to speculate that sulfide oxidation may be very rapid (much faster than calculated diffusive flux would imply). Then the question is where and how sufficient sulfide can be supplied to the redoxcline. Other than an underestimate of vertical diffusive flux and fast *in situ* sulfide oxidation, high energy yield efficiency of chemoautotrophic bacteria is a possible explanation.

2. FUTURE DIRECTIONS

Although my thesis provides a greatly improved understanding of sulfur cycling in the water column of the Cariaco Basin, it is always important to ask what the next steps are. The following paragraphs outline possible future directions.

Particulate elemental sulfur may be an important substrate for chemoautotrophic bacteria. Being able to demonstrate whether the bacterial production rates and the bacterial community structure respond to the addition of biologically available elemental

sulfur is an obvious next step to provide direct evidence for this hypothesis. Since elemental sulfur is not soluble in water, it is important to find a solvent, which is not toxic to microorganisms, to dissolve sulfur powder to make the stock solution. An elemental sulfur amendment experiment using EGME (Ethylene glycol monomethyl ether) is underway. Alternatively, polysulfides synthesized following Kamyshny et al. (2003) could be used for the stimulation experiment since this might be the form of elemental sulfur that microorganisms are metabolizing in the natural environment. It may be important to add the right form of elemental sulfur to the incubations for further investigation of the relationship between particulate elemental sulfur and the chemoautotrophic production within the redoxcline.

This study strongly suggested that sulfide oxidation and sulfate reduction are the two main processes controlling the sulfur isotope composition in the Cariaco water column. The full extent to which the process of sulfur intermediate disproportionation impacts the system remains to be fully demonstrated, both within the redoxcline and in the deep anoxic water column. Future studies employing fine scale sampling at the very top of the sulfide zone measuring multiple sulfur isotopes inn sulfide, sulfate and even sulfur intermediate pools in the Cariaco Basin would have the potential to distinguish between sulfide oxidation and sulfur intermediate disproportionation. It would be valuable to disentangle the relative contribution of the two processes on the basis of more isotope data from the environment and to better constrain the fractionation factor and the $^{33}\lambda$ of different processes.

In this study, only the sulfur isotope composition of sulfide at the Cariaco time series station was measured, and information on spatial variability of sulfide isotope

composition at different stations (both in the water column and in the sediment) is lacking. Also, the oxygen isotope compositions of sulfate, which is complementary to the sulfur isotopic signal of sulfate, should be investigated in the future.

Pyrite is far above the detection limit both in the oxic and anoxic water column. The finding that the isotopic composition of the particulate sulfur flux in the deep anoxic sediment trap (-30‰) is similar to the sulfide isotope composition immediately below the O_2/H_2S interface is consistent with the argument that pyrite is mainly formed near the interface. However, the isotope content of the particulate sulfur flux measured in the oxic sediment trap is more positive (-6.0‰). This finding is intriguing. Where is this fraction of pyrite from? Has it formed *in situ* in the oxic water column or is it transported to the Cariaco time series station from the resuspended sediment in shallower areas? Why is this isotope composition so unique? What is its fate once it sinks through the redoxcline? All these questions are worthy of further investigation. Approaches to address these questions include more analysis of isotope composition of sedimentary flux from different time periods of sediment traps to test whether this phenomenon is reproducible. Isotope compositions of sedimentary pyrite at different locations in the Cariaco Basin should be measured to evaluate the horizontal transport argument. Large volume filtration of suspended particulate samples at different water depths should also be carried out to yield enough materials for sulfur isotope composition analysis. Analysis should target pyrite both in the oxic and anoxic water column, since it could provide complementary but independent information on pyrite sources and even the formation mechanism in different layers of the water column. It is not unreasonable to suspect that the sulfur isotope composition of suspended pyrite is different from that of the settling pyrite. Moreover,

species- specific (at least separating greigite and pyrite) sulfur isotope composition analysis of both the sediment trap materials and the suspended particulate samples would also be interesting topics to pursue. Overall, these techniques are promising for further investigating the source and sink of pyrite detected in the oxic sediment traps and also to help understand the importance of syngenetic pyrite formation in the water column of anoxic water bodies.

My thesis results suggest that the sulfur loss term might be underestimated near the redoxcline. Further investigations, including using radiotracer techniques to directly measure sulfide oxidation rates and sulfate reduction rates, especially within the redoxcline, would provide a better understanding of the whole sulfur budget. To reconcile the imbalance between the chemical fluxes and biological demand, sulfide oxidation measurements should be carried out in the near future in a way similar to the manner in which chemoautotrophic production is measured (incubation conditions), but with a relatively low sulfide concentration amendments (no more than 10 μmol L^{-1} to best mimic the environmental conditions of the Cariaco redoxcline) since high concentrations of sulfide might be toxic to the microbial community. If we can get the high sulfide oxidation rate to agree with high chemoautotrophic production, there are several possibilities: 1) the diffusive coefficient that I used to calculate sulfide flux is significantly underestimated; or 2) the chemoautotrophic production and the sulfide oxidation rate are both overestimated for methodological reasons; or 3) *in situ* sulfide production within the redoxcline is underestimated.

If the chemoautotrophic microbial community has evoled a higher energy yield to fix CO_2 than previously thought, the imbalance between the chemical fluxes and

biological demand would be relieved. More microbiological incubations should be strongly encouraged to further explore the uniqueness of chemoautotrophic microorganisms. Also a far better understanding of the physical regime of the Cariaco system and other anoxic water bodies is needed. The magnitude, frequency, and velocity of intrusions are still not clear. Modeling and experimental work to better constrain horizontal transport regime will definitely help us further understand the system.

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