Distribution of thiols in the northwest Atlantic Ocean

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Submitted in partial fulfillment of the requirements for the degree of

Master of Science

at the

MASSACHUSETTS INSTITUTE OF TECHNOLOGY

and the

WOODS HOLE OCEANOGRAPHIC INSTITUTION

February 2013

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Massachusetts Institute of Technology
and Woods Hole Oceanographic Institution
December 10th, 2012

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Acknowledgements

The work in this thesis was funded by NSF OCE grant 1132515 and NSF EAR grant 1119373. Funding was also provided by an internal source at WHOI for the first year of this work. Thank you Carl Lamborg, my committee, and Kate Mackey for supporting me intellectually as I progressed through this thing. Thanks to Rob Mason, Chad Hammerschmidt, and Bill Fitzgerald for the opportunity to participate in the cruises. Thank to Melissa Tabatchnick, Prentiss Balcom, Michael Finiguerra, Kati Gosnell, Kathleen Munson, and Susan Gichuki for help sampling. Thanks to my family, Shahandeh Owrang, Molly Burtis, and Mike Horrigan for emotional support.

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Abstract

Thiol substances can form stable complexes with metals (especially copper and mercury) in the surface ocean that can impact cycling and bioavailability of those elements. In this study, I present seven concentration profiles of cysteine and glutathione, two low-molecular weight thiols, from the coastal northwest Atlantic Ocean and the Bermuda Atlantic Time Series (BATS) sampling site in the Sargasso Sea, a first for these regions. These two thiols were found in the upper 200 meters of the ocean at all sites, and the total thiol concentration varied from 0.2 to 3.2 nM. The highest concentration of both thiols was found at the deep chlorophyll maximum in most samples. Thiol concentrations were higher on the continental shelf than in the open ocean. The observed distribution of cysteine and glutathione and thermodynamic stability of copper complexes suggests that Cu(I)-dithiol complexes may be the dominant surface ocean copper and thiol species. Mercury-thiol complexes were also present in thermodynamically modeled seawater, which may provide a vector for mercury uptake in the surface ocean.

1. Introduction

1.1 Thiol Biochemistry

The primary role of thiols (organic compounds possessing the R-SH functional group, the sulfur analog to alcohols) in life processes is defending against oxidative stress (**Figure 1**; Rabenstein, 1989; Ercal et al., 2001). Oxidative stress is caused by reactive oxidizing species (ROS) which damage lipids, proteins and nucleic acids leading to membrane destruction, protein dysfunction, and DNA destruction (Ercal et al., 2001). This damage is mitigated by reaction of ROS with non-enzymatic anti-oxidants, like glutathione and ascorbic acid, and proteins, like superoxide dismutase and catalase (Apel and Hirt, 2004). This process leads to the formation of glutathione dithiols which can be reduced and reused by the cell (Bertini et al., 2007). In many phytoplankton, ROS are a byproduct of photosynthesis and a defense mechanism against photoinhibition (Apel and Hirt, 2004). Thus, thiol production in response to light stress has been observed in phytoplankton cultures subjected to natural light level induced oxidative stress over a diurnal cycle (Dupont et al., 2004).

Thiols also decrease the toxicity of metals by complexing them. Intracellular LMW thiol concentrations increase in phytoplankton, plants, and fungi as a result of metal exposure (Steffens, 1990; Ahner and Morel, 1995; Ahner et al., 2002; Courbot et al., 2004; Dupont et al., 2004; Dupont and Ahner, 2005). Specialized higher molecular weight thiolic peptides, called phytochelatins and metallothioneins, are produced specifically to chelate toxic metals as a result of exposure to metals in phytoplankton

(Ahner et al., 1995; Ahner and Morel, 1995; Bertini et al., 2007). The assemblage of thiols produced varies between phytoplankton species and upon exposure to different metals (Ahner et al., 2002; Dupont and Ahner, 2005).

1.2 Thiol distribution and cycling

Thiols have been found in lake, estuary, and ocean waters (Mopper and Kieber, 1991; Tang et al., 2000; Al-Farawati and van den Berg, 2001; Dupont et al., 2006; Hu et al., 2006; Kawakami and Achterberg, 2012). Greater than 500 nM of dissolved thiols are found in regions where permanently sulfidic conditions are present, such as the Black Sea (Mopper and Kieber, 1991) and porewaters (Chapman et al., 2008). Dissolved thiol concentrations of greater than 10 nM have been observed in regions of high productivity, such as the North Pacific (Dupont et al., 2006), ponds (Hu et al., 2006), and some estuaries (Laglera and van den Berg, 2003). The lowest thiol concentrations (<10 nM) have been found in low productivity estuarine, coastal, and ocean waters (Tang et al., 2000; Al-Farawati and van den Berg, 2001; Dupont et al., 2006).

There are many different thiol compounds present in the environment. Compound specific analysis of dissolved thiols has identified cysteine, glutathione (GSH), and γ-glutamylcysteine as common thiols in most aquatic systems (Mopper and Kieber, 1991; Tang et al., 2000; Hu et al., 2006; Dupont et al., 2006). Some thiols are found in almost all natural waters, such as GSH, but others are either not detectable by the method used or not present. For example, cysteinyl-argine and cysteinyl-glutamine were detected in the North Pacific (Dupont et al., 2006), while GSH and phytochelatin-2 (PC-2) were the dominant thiols in Galveston Bay estuary (Tang et al., 2000). This

difference results from changes in system specific plankton community and type of metal to which the creatures are exposed (Ahner et al., 2002; Dupont and Ahner, 2005). The presence of unidentifiable peaks in some chromatograms suggests that as yet unidentified thiols are present in many environments (Tang et al., 2000).

The primary source of thiols is biogenic production in response to environmental stressors, like metals and light, rather than other potential sources like advective transport and porewater diffusion. Co-variation between dissolved thiols and chlorophyll (Chl *a*) concentrations have been measured in the surface coastal ocean using high throughput sampling (n = 7030; Al-Farawati and Van den Berg, 2001). However, thiols were not found to correlate with Chl *a* in surface ocean profiles from the northwest Pacific (Dupont et al., 2006). This observation suggests biological exudation rather than cell lysis is the primary source of dissolved thiols, because a direct relationship between a proxy of primary production (Chl *a*) and thiols does not exist (Dupont et al., 2006). Many culture studies have shown increases in intracellular and dissolved thiols in response to metal and light stress, which suggests thiol production is dependent on environmental conditions rather than total productivity (Ahner et al., 1995; Ahner and Morel, 1995; Ahner et al., 2002; Dupont et al., 2004[1]; Dupont et al., 2004[2]; Dupont and Ahner, 2005). Field studies have also found increased particulate thiol content in regions of an estuary impacted by copper and zinc pollution (Kawakami and Achterbeg, 2012). Higher concentrations of dissolved thiols are seen in estuaries, yet little to no correlation of thiols with salinity has been found in the coastal ocean (Tang et al., 2000; Al-Farawati and Van den Berg, 2001; Kawakami and Achterberg, 2012). This suggests rivers are not

a major source of thiols to the ocean because these compounds are so labile. Porewater diffusion may be a source of thiols to the water column, but thiol concentrations are often observed to decrease with depth, which suggests this source may not be very important relative to local production (Al-Farawati and van den Berg, 2001; Chapman et al., 2008).

Sinks for thiols include photo-oxidation and biological uptake. Photochemical destruction of the surface ocean thiols has been demonstrated to occur on the order of hours (Moffett, 1995; Laglera et al., 2006; Moingt et al., 2010). Photo-oxidation is likely responsible for the observed increase in thiol content from the surface to a maximum at the thermocline, where primary productivity is high but light levels are low (Dupont et al., 2006). Cysteine has been shown to be taken up in varying degrees by SAR11 and other α-proteobaceria, which are thought to be the dominant heterotrophic bacteria in the ocean (Kiene et al., 1999; Tripp et al., 2008). Thiols may be an important source of fixed sulfur to reduced genome bacteria that dominate deep ocean bacterioplankton communities, and explains the general decrease in thiol concentration with depth seen in the ocean (Al-Farawati and van den Berg, 2001; Dupont et al., 2006).

Dissolved thiols have the potential to exist in both reduced (RS^-) and oxidized (RSSR) forms, which impacts their ability to behave as metal ligands. Cupric ions oxidize cysteine while reducing copper in laboratory experiments, so it is likely this occurs with other LMW thiols and in the surface ocean (Rigo et al., 2004). Spontaneous oxidation of LMW thiols by superoxide and H_2O_2 has been observed in laboratory experiments (Winterbourn and Metodiewa, 1999). Ultraviolet radiation has been shown to catalyze the aforementioned processes in natural waters (Moingt et al., 2010). The

presence of Hg and Ag at levels comparable to those of thiols greatly reduces the rate of GSH oxidation (Hsu-Kim, 2007). A single measurement of dissolved thiols in the surface ocean suggests that approximately half exist in the oxidized form (Dupont et al., 2006).

1.3 Copper in the surface ocean

Copper is an essential nutrient to many organisms, including phytoplankton, and is actively taken up from the environment as a thiol complex (Bertini et al., 2007; Semeniuk et al., 2009). Some copper-bearing proteins, such as metallothioneins, regulate intracellular metal levels and others reduce ROS, such as CuZn superoxide dismutase (Bertini et al., 2007). Membrane proteins have been found in picophytoplankton that actively transport copper into the cell from the environment by ligand exchange with copper-thiol complexes (Van Ho et al., 2002; Semeniuk et al., 2009). Yeast culture studies have suggested that Cu(I)-$(GSH)_2$ are available for uptake by specific membrane transport proteins (Van Ho et al., 2002; Bertini et al., 2007; Semeniuk et al., 2007).

Copper is also a toxin at natural concentrations to some phytoplankton, such as cyanobacteria (Mann et al., 2002; Paytan et al., 2009; Mackey et al, 2012). The toxicity threshold of inorganic dissolved copper for high light adapted oceanic cyanobacteria is ~100 pM, while it is as low as 2 pM for low light adapted cyanobacteria (Mann et al., 2002). The concentration of dissolved copper found in the ocean is typically 1 – 2 nM, which would be considered a lethal level for cyanobacteria if entirely copper existed primarily in its inorganic form (Mann et al., 2002; Sohrin and Bruland, 2011). Thus, the exudation of ligands by phytoplankton that bind copper strongly, such as thiols, is

thought to prevent toxicity (Coale and Bruland, 1988; Moffett et al., 1990; Ahner and Morel, 1995; Dupont et al., 2004; Dupont and Ahner; 2005).

The exact structure of the organic compounds acting as copper ligands in the ocean is unknown, as many studies have relied on indiscriminate quantification methods that determine only concentration (Moffett et al., 1990; Moffett, 1995; Leal and van den Berg, 1998; Al-Farawati and van den Berg, 1999; Leal and van den berg, 2003). These methods have revealed the copper binding ligands to be of biological origin (Moffett et al., 1995; Moffett and Brand, 1996). Recent advances in technology have allowed compound specific thiol determination and direct detection of copper-thiol complexes in cultures (Dupont et al., 2004). Surface ocean extracellular thiols are found at sufficient concentrations to allow significant chelation of copper and in turn impact its speciation and oxidation state (Al-Farawati and van den Berg, 2001; Dupont et al., 2006). Compound specific identification of copper binding ligands has identified thiols as a likely component of the uncharacterized copper ligand pool.

1.4 The role of thiols in mercury methylation and bioavailability

Methylmercury exposure through oceanic fish consumption is a public health concern because of the damage it can cause to the developing central nervous system of unborn and young children (EPA, 2012). However, the exact means by which mercury is methylated and enters the food chain in the open ocean is not definitively understood (Mason et al., 2012). A recent observation of a methylmercury maximum coincident with the oxygen minimum in profiles from the SAFe station suggests that water column mercury methylation is occurring (Hammerschmidt and Bowman, 2012). Upper ocean

methylation may couple anthropogenic mercury emissions to the mercury content of fish in the ocean, as atmospheric deposition is the primary source of mercury to the ocean (Lindberg et al, 2007). Atmospheric mercury deposition, even at remote sites, has increased by at least a factor of three over the last century (Lamborg et al., 2002; Fitzgerald et al., 2005). This connection suggests that decreasing anthropogenic mercury emissions would lead to decreased mercury in fish and subsequently less human exposure to this toxic element (Mason et al., 2012).

The mechanism of oceanic water column mercury methylation is not known, but it may be that biotic conversion is occurring as is observed in lake and marsh sediments and that the presence of thiols enhances mercury bioavailability (Gilmour et al., 1992; Fleming et al., 2006; Hammerschmidt and Bowman, 2012). Culture experiments with SRB and FeRB exposed to inorganic mercury in the presence of LMW thiols have shown increased rates of methylation compared to controls, which upsets the previously held hypothesis that passive diffusion of neutrally charged mercury species was the primary means of transport into the cell and that Hg complexation by organic ligands should lower its bioavailability (Mason et al., 1996; Schaefer and Morel, 2009; Schaefer et al., 2011). Mercury-organic ligand complexes are thought to be the dominant dissolved mercury species, but the identity and distribution of these compounds is not well known (Lamborg et al., 2003; Black et al., 2007). In the context of these recent discoveries, further examination of mercury speciation, particularly under conditions found in the upper ocean, may lead to a better mechanistic understanding of water column mercury methylation.

2. Methods

2.1 Study Area

Samples were collected during two cruises and represent distinct oceanographic regimes **(Figure 2)**. The samples from the New England continental shelf (samples designated by prefix NWA) were collected in July 2010 on *RV Oceanus* cruise # 466 under chief scientists William Fitzgerald and Chad Hammerschmidt (see inset map; *figure 1*). The Bermuda Atlantic Time Series (BATS prefix) samples were collected in August 2010 on UNOLS cruise # 10713 aboard the *RV Atlantic Explorer* under chief scientist Robert Mason **(Table 1)**.

2.2 Sampling Protocol

All samples were collected using General Oceanics trace metal clean GO-Flo water samplers handled following standard metal contamination minimization procedures (Lamborg et al., 2012). Following collection, samples were 0.2 micron filtered in a class 100 clean van and frozen in 250 mL HDPE bottles that had previously been soaked sequentially in Citranox and 10% hydrochloric acid and rinsed with MilliQ water. Hydrographic properties (temperature, salinity, O_2, and others) were measured by *in situ* instrumentation on the sample collection rosette or by separate CTD casts at the same site.

2.3 Derivitization and Chromatography

Thiol derivitization and analysis were done following the method of Tang et al. (2003). Derivitization with the fluorescent tag monobromobimane (mBrB) specifically targets the sulfhydryl site and allows compound specific quantification by HPLC

separation. Samples were thawed and 250 μL of 6 M methanesulfonic acid (MSA) was added to each 100 mL sample, which brought the [MSA] to 15 mM, as this matrix was demonstrated to stabilize refrigerated samples (Tang et al., 2003). Samples were then held at 4°C for up to 2 weeks. Less than 50% of glutathione and no cysteine were lost in a sample of water from Martha's Vineyard Sound that was stored refrigerated in 15 mM MSA for 6 months, which suggests any loss during the two weeks of storage would fall within the range of analytical duplicates (**Figure 4**). Duplicate measurements were made of all samples in 50 mL aliquots. Each sample was neutralized by adding 140 μL of 6 M NaOH as TCEP reduction requires a near neutral pH. Next, 40 μL of 20 mM *tris*(2-carboxyethyl)phosphine (TCEP) was added to reduce the disulfide bonds of oxidized thiols. Samples were kept at room temperature for 30 minutes to allow complete reaction with TCEP. As a result of TCEP reduction, the concentrations presented here represent total thiols rather than just the reduced pool. Next, samples were buffered by the addition of 1 mL of a solution of 2 M boric acid, 0.8 M NaOH to adjust pH to the optimum for mBrB derivitization. Metals that might complete for MBrB sulfhydryl site bonding were chelated by the addition 10 mM EDTA. Next, 80 μL of the derivitizing agent mBrB was added. The derivitization was allowed to occur over 120 minutes at 60°C. Finally, the derivitized solution was acidified with 800 mL of 6 M MSA which brought the final [MSA] to 63 mM (Tang et al., 2003).

The derivitized samples were pre-concentrated on Waters 60 mg OASIS HLB 30 μm resin in 3 mL columns that were conditioned by addition of 3 mL of methanol followed by 5 mL of 15 mM MSA. Samples were loaded onto the columns at ~2 ml

min^{-1} through a low pressure vacuum manifold and then rinsed with 2 mL of 15 mM MSA containing 2% methanol. Finally, samples were eluted into 2 mL amber vials in 1 mL of 100% methanol. The methanol was evaporated overnight by blowing down with air and the resulting residue dissolved in 1 mL of 15 mM MSA.

The derivitized thiols were quantified using HPLC separation and fluorescence detection on an Agilent Technologies 1200 series HPLC with a 4.6 x 250 mm Waters Spherisorb 5 μm column with attached 4.6 x 10 mm guard column. The column was conditioned by pumping a 0.1% trifluoroacetic acid (TFA) solution containing 8% acetonitrile for one hour at 1 mL min^{-1} prior to analysis. A 100 μL aliquot of the sample was injected onto the column and the compounds separated using an eluent which changed from 8 % acetonitrile in 0.1% TFA to 35 % acetonitrile linearly over 47 minutes and then rinsed for 5 minutes in 80% acetonitrile in 0.1% TFA. The column was conditioned for 30 minutes with 92% 0.1% trifluoroacetic acid (TFA) and 8% acetonitrile at 1 mL min^{-1} between each sample analyzed. Following analysis, the column was rinsed in 100% acetonitrile for 1 hour at 1 mL min^{-1} and stored in pure acetonitrile when not in use. The retention time and response for the thiols β-mercaptoethanol, cysteine, homocysteine, cysteinyl-glycine, glutathione, γ-glutamylcysteine, N-acetylcysteine, and PC-2 were established for this elution gradient by standard additions, but detection limits were not established for these thiols. A number of unidentified peaks were observed that may correspond to thiols or other organic compounds for which we lacked standards. Cysteine and glutathione data are presented in this work (**Figure 5**). The detection limits, as determined by the point below which no definable peak was present (0.1 area units),

for cysteine and glutathione were 0.1 and 0.05 nM respectively, as no peak above background was detectable in blanks. The difference in peak area of samples run twice on was <10%. The coefficient of variation for all cysteine MVS standard concentration measurements using the calibration curve of each sample run was 28%, while that for glutathione was 31%. No values differed from the mean with a $p > 0.02$, so it is unlikely the concentration of the MVS standard changed during the course of analyses. Statistical analysis suggests that the primary source of error was in derivitization efficiency and the resulting change in the calibration curve and not in HPLC separation and peak area determination. I hypothesize that the cause of this error is in the column pre-concentration step, where it was difficult to maintain a constant flow rate, columns were re-used, and the temperature of the room varied. Error in all tables presented in this study reflects the range of measured values and not the coefficient of variation.

2.4 Thermodynamic Equilibrium Modeling

In this work, the speciation of copper and mercury is examined in the presence of inorganic seawater components, thiols, and sulfide at typical surface ocean concentrations (**Table 2**). Thiol and trace metal speciation was determined using the thermodynamic equilibrium modeling software SpecE8, which is included in the Geochemist's Workbench software package (Bethke, 2010). The Visual MINTEQ release 2.40 thermodynamic database was used for this work, but modified to include the thiol and sulfide complexes (**Table 3**). The stability constants available for many trace metal-thiol complexes show several orders of magnitude difference between experiments as a result in variation of the temperature, media, and method of detection used in making these

determinations (Berthon, 1995). Conditional stability constants determined for Cu(I)-cysteine and Cu(I)-glutathione determined by thiol titration in seawater were only separated by one order of magnitude (Leal and van den Berg, 1998) so the constants presented for metal-cysteine complexes in Berthon (1995) are considered representative of the combined cysteine and glutathione (RS^-). All thermodynamic modeling was conducted assuming a temperature of 25°C as almost all of these complexes lacked ΔH values necessary for temperature correction to those found at sampling sites.

3. Results

3.1 Northwest Atlantic

The physical parameters for the northwest Atlantic stations suggest a high temperature, low salinity lens above the thermocline transitioning to a sub-thermocline (50 – 150 m) salinity maximum (**Figure 6)**. At all of the northwest Atlantic stations, a deep Chl *a* maximum (DCM) was found 10 – 20 meters below the thermocline. A substantial decrease in $[O_2]$ was seen above the thermocline at all stations except NWA-22. The DCM, thermocline and O_2 maximum were found at deeper depths at stations farther offshore.

Detectable thiols were observed throughout the water column for stations on the shelf, but only to a depth of 300 m at the shelf break and slope stations. The observed concentration of both thiols ranged from below to detection (0.1 nM) to 1.7 nM of glutathione and 1.5 nM of cysteine. The highest concentrations were found at the shallow near shore station (NWA-18) and the lowest at the open ocean site (NWA-22).

3.2 Sargasso Sea

The temperature and salinity of the samples taken at BATS changed slightly between August 4th and 6th, and the greatest change was seen in the upper 100 meters (**Figure 7**). The thermocline on both August 4th and 6th was found at 24 meters depth. The surface water was between 28 and 28.4 °C above the thermocline on both days. Salinity varied between 36.79 and 36.88 on August 4th, but was uniformly 36.84 above the thermocline on the 6th. The near constant temperature of 18 °C between 100 and 500 meters suggests a transition to Northwest Atlantic subtropical gyre mode water, which is characterized by a temperature of close to 18 °C (Worthington, 1959). The Chl *a* and oxygen concentration profiles also showed variation between the 4th and 6th of August. The Chl *a* maximum was found at 96 meters depth on the 4th but deepened to 103 meters the 6th. The oxygen maximum, on the other hand, decreased from 54 meters depth on the 4th to 27 meters depth on the 6th. Oxygen concentrations are relatively constant from 100 to 500 meters depth, and reach a minimum at ~800 meters depth.

Cysteine and glutathione were detected at all depths above 150 meters, and cysteine was found at 300 meters depth on August 4th. Cysteine concentrations were always higher than glutathione concentrations. The concentration of both thiols at 20 meters depth was less than that observed at the DCM, and decreased below the DCM.

4. Discussion

4.1 Distribution of Thiols

Glutathione and cysteine were the only identifiable thiols found in this study, and these were detected in the upper 100 – 300 meters of every station. We have established, through the analysis of standards, the elution times for additional thiols including

homocysteine, γ-glutamylcysteine, cysteinylglycine, N-acetylcysteine, and phytochelatin-2, but did not observe detectable peaks for these compounds. As noted in Figure 5, however, there were several peaks in our sample chromatograms that did not correspond to compounds that we have tested but did apparently react with mBrB. These compounds may have been thiols, though there are other compounds that will react with mBrB and generate fluorescent derivatives (e.g., sulfides, thiosulfates, and sulfites). Thus, there may well have been detectable thiolic compounds present in the samples other than cysteine and glutathione, but they were unidentified. Therefore, we will further refer to the sum of cysteine and glutathione as "total thiol." The total thiol concentration ranged from <0.1 nM to 3.2 nM, which is similar to the range observed in many other environments (**Table 4**). No evidence was found for the presence of γ-glutamylcysteine, but its detection is complicated by the presence of multiple interfering peaks. This contrasts the >10 nM of γ-glutamylcysteine observed in the surface ocean of the Pacific subarctic upwelling (Dupont et al., 2006). However, standard addition experiment conducted for this work showed that 10 nM of γ-glutamylcysteine would have been easily discernible. Thus, the thiol speciation in the NW Atlantic appears to be substantially different than the subarctic Pacific, which may be due to differences in phytoplankton communities and degree of metal stress (Ahner et al., 2002). Although the plankton community was not determined in this study, differences in thiol assemblage between the sites could be due to differences in the species present. Exposure to different metals may also play a role in the observed thiol assemblage. The production of γ-glutamylcysteine has been linked to zinc exposure

in *Emiliani huxley* culture studies, while copper and cadmium exposure led to the production of cysteine and glutathione, respectively (Dupont and Ahner, 2005).

Total thiol concentrations in the coastal and open ocean are less than 15 nM, as observed using both derivitization and voltammetric titration techniques (Al-Farawati and van den Berg, 2001; Dupont et al., 2006). The observed thiol concentrations in this study were similar to those reported in the English Channel, Galveston Bay estuary, and the subarctic North Pacific (**Table 4**). Profiles of both cysteine and glutathione typically showed intermediate thiol concentrations above the DCM, the highest levels of thiols at the DCM, and a decrease below 200 – 300 meters depth. This profile structure is similar to that observed in the North Pacific (Dupont et al., 2006).

The northwest Atlantic stations represent a gradient in oceanographic conditions from highly productive shelf waters to less productive slope waters (Antoine et al., 1996). However, no correlation exists between total thiol content and Chl *a* (**Figure 8**), which is similar to findings in the North Pacific (Dupont et al., 2006). Higher peak concentrations of thiols were found in samples from the continental shelf and slope stations than open ocean sites (**Figure 8**). The lack of co-variation between total thiols and chl *a* gives further support to the hypothesis of Dupont et al. (2006) that the observed profiles are a result of dominantly thiol exudation rather than cell lysis because thiol content does not vary with primary productivity. Station NWA-21-up is located near the shelf break, and shows the greatest concentration of thiols at the Chl *a* maximum. This station also shows a peak at depth that may be the result of porewater thiol source and advection offshore, as

interstitial waters have been found to contain greater than a factor of 1000 higher thiol concentrations than is found in surface waters (Chapman et al., 2009).

4.2 Interpretation of thiol profiles

The thiol concentration increased with depth to the DCM and then decreased once more with depth to below 0.1 nM by 300 meters in stations off the continental shelf. The observed near surface thiol concentration decrease is likely due to photo-oxidation. Glutathione degrades with a pseudo-first order rate constant of 0.1 d^{-1} in UV irradiation chambers designed to replicate sunlight levels found in the surface waters of the Scheldt Estuary (51°N; Laglera et al., 2006). In the Sargasso Sea profiles, *in situ* production of thiols by phytoplankton is the likely source of thiols to the mixed layer and DCM because eddy driven advection has been shown to be slow (0.1 cm^2 s^{-1}) and downward (Ledwell et al., 1993). The peak in thiols found at the DCM is the result of production occurring at low light levels and upward mixing inhibited by the thermocline. Thiols that are found below the DCM are likely derived from transport by particulates, advection, and production by microbes other than phytoplankton. These sources cannot be quantified in this study given the data available. The absence of glutathione and cysteine in water below 300 meters could be due to uptake by bacterioplankton, which have been demonstrated take up cysteine and other thiols quantitatively on the order of hours in culture studies (Kiene et al., 1999; Tripp et al., 2008).

4.3 Thiols as a component of surface ocean dissolved organic carbon

The exact compounds that make up oceanic dissolved organic carbon (DOC) are not well known and particularly difficult to describe in the seawater matrix (Hansell et

al., 2009). This study found up to 0.02 – 0.025 μM DOC as the thiols cysteine and glutathione in the upper 300 meters of the water column, which represents a small fraction of total DOC in the upper ocean, which typically ranges between 50 and 80 μM. However, the labile dissolved organic carbon fraction is thought to have a concentration of < 0.1 μM throughout the open ocean (Hansell et al., 2009). Thus, thiols can represent an important component (~25%) of the labile carbon in the ocean. The labile fraction is thought to turnover on the order of hours to days (Carlson and Ducklow, 1995). A maximum turnover time of months was estimated by Dupont et al. (2006) for surface ocean γ-glutamylcysteine, which is similar to that observed for phytochelatin-2 (Wei and Ahner, 2005). However, this is likely a significant overestimate of actual rates as the calculation is based on the assumption that cell lysis is the only source. Exudation of thiols by living cells is also likely an important source, as has been demonstrated by culture studies (Dupont and Ahner, 2005; Wei and Ahner, 2005). Additionally, incubation studies have found glutathione to have a half-life of 2 – 7 hours in natural seawater (Moing et al., 2006). Thus, thiols represent an important and highly dynamic organic carbon pool in the ocean.

4.4 Copper Concentration Buffering by Thiols

It has been proposed that eukaryotic and prokaryotic phytoplankton produce specific ligands in order to reduce the toxicity of copper by decreasing the concentration of inorganic and free ion species (Bruland and Lohan, 2003). Copper addition experiments have shown that inorganic copper concentrations of those normally observed at sea (~1 nM) would inhibit cyanobacteria growth without the presence of an organic

ligand (Brand et al., 1986). Copper titrations of seawater samples have found copper binding ligands that often have water column distribution, concentrations, stability constants, and sources/sinks identical to those of thiols, so thiols likely make up a portion of the uncharacterized copper complexing ligand pool (Moffett et al., 1990; Moffett, 1995; Leal and van den Berg, 1998; Laglera and van den Berg, 2003).

The concentration of copper binding ligands in the ocean varies from less than one to over ten nanomolar (Coale and Bruland, 1988; Moffett et al., 1990; Moffett, 1995; Leal and van den Berg, 1998; Laglera and van den Berg, 2003; Mackey et al., 2012), which is similar to the range observed for thiols (Tang et al., 2000; Al-Farawati and van den Berg, 2001; Dupont et al., 2006; This work). Voltammetric copper titrations suggest the presence of two classes of copper ligand made up of a stronger binding pool called L1, and a weaker binding pool, that is referred to as L2 (Coale and Bruland, 1988; Moffett et al., 1990; Moffett, 1995; Leal and van den Berg, 1998; Laglera and van den Berg, 2003; Mackey et al., 2012). The first class of ligand, L1, is generally considered to form more stable copper complexes but is only found in the surface ocean (Coale and Bruland, 1988; Moffett et al., 1990). The second ligand class, L2, is found throughout the water column and is found at high concentrations in freshwater (Coale and Bruland, 1988; Laglera and van den Berg, 2003). The ligand L1 was found at identical concentrations to thiols in the surface ocean when both were measured simultaneously (Laglera and van den Berg, 2003). In this work, ~1 nM of total thiols was found in the surface ocean at BATS in August, and a study conducted at BATS in April found ~2 nM of the L1 strong ligand (Mackey et al., 2012). A similar profile of L1 ligands to that

observed in the offshore profiles presented here was found in the Northeast Pacific. The concentration ranged from 0.6 to 2.4 nM, and no L1 ligands were detected below 150 meters depth (Coale and Bruland, 1988). These examples of similarities in concentration and water column distribution of thiols and ligand L1 suggest thiols make up a fraction of the strong binding ligand pool, L1.

Copper complexing ligands L1 and L2 have similar conditional stability constants in seawater to those determined for thiols in seawater. The conditional stability constant of copper-ligand complexes in seawater ranges from $10^{11} - 10^{14}$ (Coale and Bruland, 1988; Moffett et al., 1990; Moffett, 1995; Moffett and Brand, 1996; Leal and van den Berg, 1998; Mackey et al., 2012). Cupric dithiol complex stability constants determined in seawater were found to range from $10^{11} - 10^{12}$, while cuprous dithiol complex stability constants ranged from 10^{13} - 10^{18} (Leal and van den Berg, 1998). The high stability of Cu(I)-dithiol may stabilize a fraction of thiols, and also impact the bioavailability of copper to phytoplankton (Dupont et al., 2004).

A fraction of uncharacterized copper binding ligands have been found to have a biogenic origin (Coale and Bruland, 1988; Moffett, 1990; Moffett and Brand, 1996), and production of these ligands is enhanced in response to metal exposure (Moffett and Brand, 1996; Mackey et al., 2012). The production and recycling of glutathione is a common genomic feature to both cyanobacteria and eukaryotic algae (Courturier et al., 2009), suggesting that the widespread observation of dissolved thiols in the ocean should be expected. Similarly to putative copper ligands L1 and L2, culture and field experiments have shown thiol production to increase with metal exposure (Ahner and

Morel, 1995; Ahner et al., 2002; Dupont and Ahner, 2005). Thiols are exuded by many phytoplankton in response to increases in dissolved metal concentration. Incubation studies have found dissolved L1 and L2 concentrations to double in response to metal exposure from aerosols (Dupont and Ahner, 2004; Mackey et al., 2012). Thus, some fraction of copper ligands is of biological origin.

The sinks of uncharacterized copper ligands are similar to those of thiols. Photo-oxidation has been proposed as a primary sink for putative copper ligands L1 and L2 (Moffett et al., 1990). Thiols degrade in the presence of sunlight as well, but so do many other organic compounds (Laglera and van den Berg, 2006; Moing et al., 2007). The disappearance of ligand L1 with depth (Coale and Bruland, 1988) suggests a similar deepwater sink, likely biological uptake, between these pools. These sinks, however, require more study in order to determine their relative impact on surface ocean thiol cycling.

Together, this evidence suggests that thiols form a fraction of copper binding ligands in the surface ocean, as has been suggested by other authors (Leal and van den Berg, 1998; Al-Farawati and van den Berg, 2001; Dupont et al., 2004). The similarities in concentration, distribution, and conditional stability constant suggest that LMW thiols are part of ligand class L1. The concentrations of cysteine and glutathione observed in this study could make up ~50% of strong copper ligands (L1) in the surface Sargasso Sea (Mackey et al., 2012). Other studies have shown thiols to be found at identical concentrations to L1 in the surface ocean, which may suggest that this is an underestimate due to seasonal changes in thiol concentration at BATS, and thiols make

up the entire strong binding ligand pool (Laglera and van den Berg, 2003). The metals that may compete with copper for thiol bonding, such as Hg and Ag, are found at such low concentrations that they are unlikely to compete for a significant fraction of the thiol pool (Bruland and Lohan, 2003), so copper is given primary attention in this work.

4.5 Implications of thiols as a copper ligand

The presence of LMW thiols as a copper-complexing ligand has implications for transition of copper between ligands with changes in oceanographic regions, the redox chemistry of copper in the surface ocean, and the bioavailability of mercury as a thiol complex. The conditional stability constants determined by Mackey et al. (2012) Combining the observed ligand concentrations with conditional stability constants allows modeling of the distribution of copper between organic ligands in a variety of oceanographic conditions (**Figure 9).** The transition between CuL2 as the primary organic complex and CuL1 commonly occurs between coastal and open ocean, as the concentration of L2 becomes low enough that L1 can compete. CuL1 complexes are dominant in the euphotic zone, with a transition to CuL2 with depth. This distribution suggests that if L1 is a thiol pool, then copper is found mostly as a copper-thiol complex in the surface ocean.

Copper in the surface ocean is typically considered to be dominated by ligand bound Cu(II) complexes because of rapid photo-oxidation of cuprous to cupric copper (Moffett et al., 1990). Free Cu(I) ions are oxidized by O_2 in minutes in surface ocean conditions, but the study used to determine this rate relied on NaCl simulated seawater rather than a more realistic system that includes organic ligands (Sharma and Millero,

1988). The most rapid sink for thiols in the surface ocean is photo-oxidation (Moffett, 1995; Laglera et al., 2006; Moingt et al., 2007). The lowest concentrations of both thiols and copper are found in the samples collected from near surface waters (Dupont et al., 2006; Boye et al., 2012), which may suggest destruction of thiols and availability of copper for particulate scavenging following oxidation.

The potential for Cu(II)-thiol complexes to be part of the ligand pool was assessed by thermodynamically modeling a generic thiol titration in competition with sulfide and inorganic species in the presence of other metals (**Table 5**). This leads to $Hg(RS)_2$ being the dominant species out of total thiols when the concentration is less than $2x\ 10^{-12}$ M, and $NiRS^+$ as the dominant thiol species when higher concentrations are present. These calculations suggest that Cu(II) complexes are not the primary thiol species in the surface ocean, but rather serve as a step in the formation of Cu(I).

Cupric ions are reduced to cuprous ions in the presence of thiols. Rapid reduction of Cu(II) to Cu(I) by thiols has been observed in the laboratory and simulated seawater experiments (Leal and van den Berg, 1998; Rigo et al, 2004). Cuprous ions then form polythiol complexes with high stability constants (Osterberg et al., 1997; Corazza et al., 1996; Leal and van den Berg, 1998). It has also been proposed that Cu(II) is reduced to Cu(I) - dithiol via a membrane bound Cu(II) reductase (Semeniuk et al., 2009). Accurate measurements of Cu(II)-thiol complex stability are complicated by the rapid reduction of cupric ions by cysteine (Berthon, 1995; Stumm and Morgan, 1996). This has implications for the oxidation state of copper in the surface ocean, as laboratory experiments using seawater and neutral pH solutions with thiol additions have shown

near complete reduction of Cu(II) to Cu(I) in under an hour when the ratio of Cu(II) to thiols is less than ~0.5:1 (Corazza et al., 1996; Laglera and van den Berg, 2001; Rigo et al., 2004). This stoichiometry is similar to the observed Cu:ligand ratio observed at some sites in the ocean (Leal and van den Berg, 1998), and the general presence of excess ligand relative to copper (Coale and Bruland, 1988; Moffet,1995; Mackey et al., 2012). Therefore, it seems reasonable that Cu(II) would be unstable in the presence of reduced thiols in the surface ocean.

The Cu(I)-dithiol complexes are so thermodynamically stable that they preclude the formation of any other metal-thiol complexes until the concentration of thiols is equal to or greater than the Cu(I) concentration (**Table 6**). This is surprising given the high stability of mercury-thiol complexes (**Table 6**), so Cu(I)-dithiol complexes should be considered in mercury speciation modeling. As a caveat, limited spectroscopic evidence suggests Hg(II) displacement of Cu(I) in dithiol complexes at intracellular conditions (Aliaga et al., 2010). However, these conditions still suggest the presence of bioavailable Hg-thiol complexes in the surface ocean given the observed excess of thiols and other LMW copper ligands relative to copper in the surface ocean (Mackey et al., 2012).

5. Conclusion

The concentration of the thiolic compounds cysteine and glutathione were measured in the surface ocean in the coastal northwest Atlantic Ocean and Sargasso Sea oligotrophic gyre. Cysteine and glutathione were the only thiols detected, in contrast to other studies, although several unidentified peaks also were observed but not quantified in the samples. Thiols were found in the upper 200 meters at all open ocean stations and

at all depths on the continental shelf, with a range of 0.2 – 3.2 nM. Thiol concentrations were higher in coastal waters, which may reflect the productivity gradient that exists between near shore and open ocean regimes. The highest thiol concentrations were typically found at the DCM. The concentrations of thiols found in the surface ocean suggest they may be an important and identifiable fraction of labile DOC.

The distribution of thiols found in this study agrees with widely published copper ligand distributions. The presence of thiols likely plays an important role in the euphotic zone cycling and bioavailability of copper. The levels of thiols observed in this work could support reduction of copper in the surface ocean and formation of highly stable and bioavailable Cu(I)-dithiol complexes. Mercury-thiol complexes are also likely to form in the surface ocean, which enhances the bioavailability of mercury.

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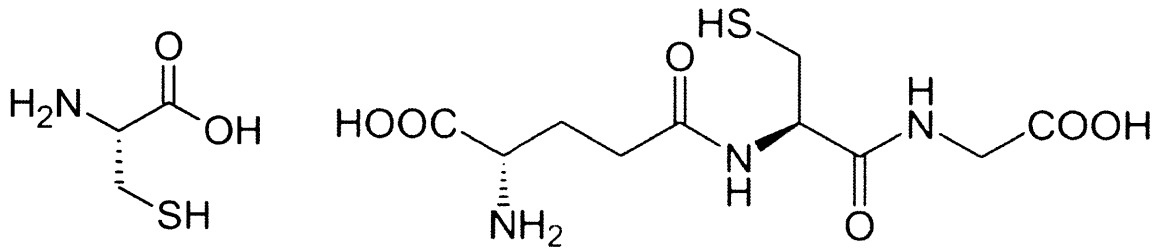

Cysteine Glutathione

Figure 1. The structure of cysteine and glutathione, the two thiol compounds this work focuses on. Note the presence of a protonated sulfhydryl site where metal and dithiol bonding occurs.

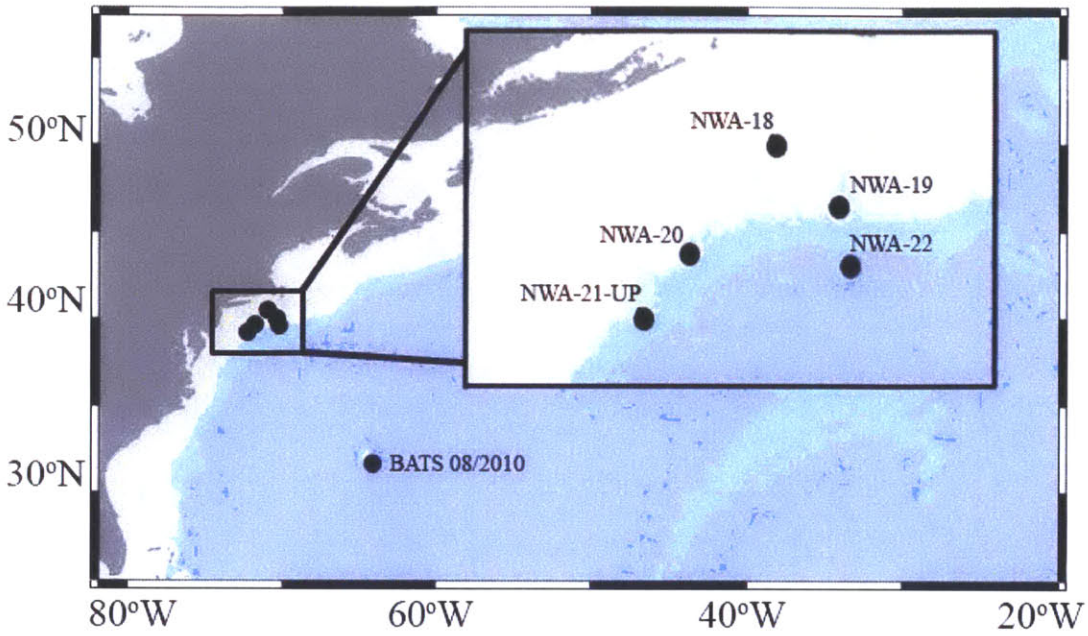

Figure 2. Map of North Atlantic showing station location and names. Samples were also collected as part of GEOTRACES but those data are not included here.

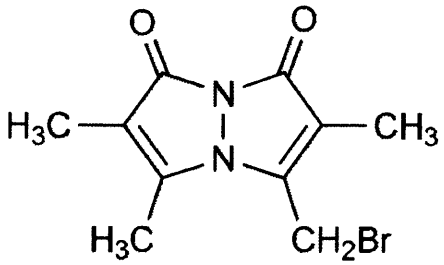

Figure 3. Structure of mBrB which was used as a derivitizing agent in this study.

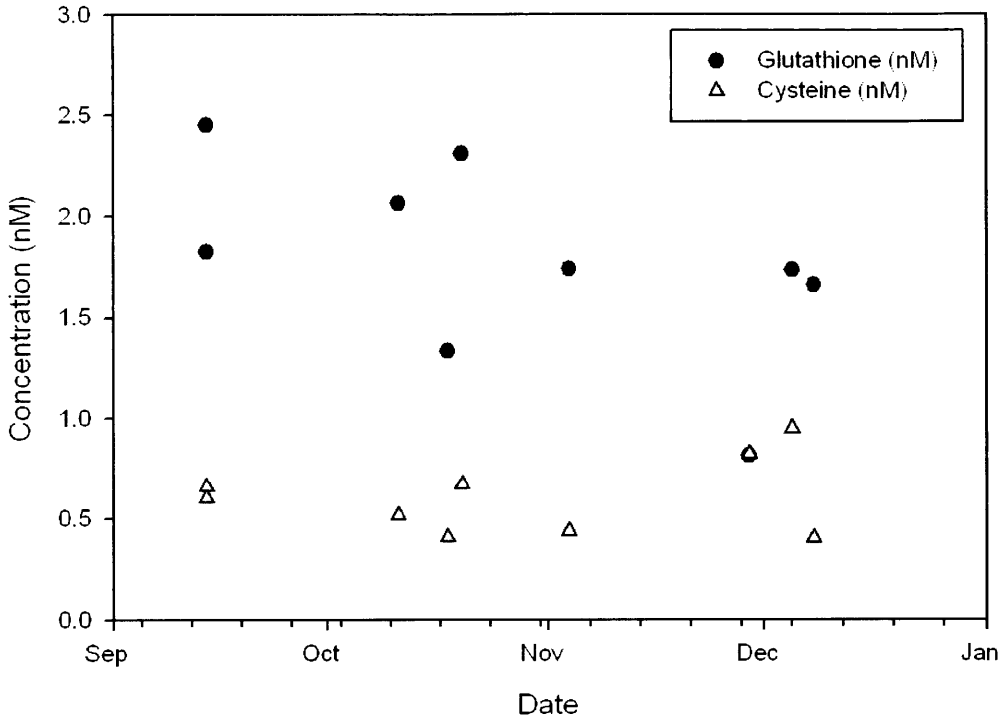

Figure 4. Changes in concentration of glutathione and cysteine in a sample of water from Martha's Vineyard Sound stored in 15 mM MSA over 3 months.

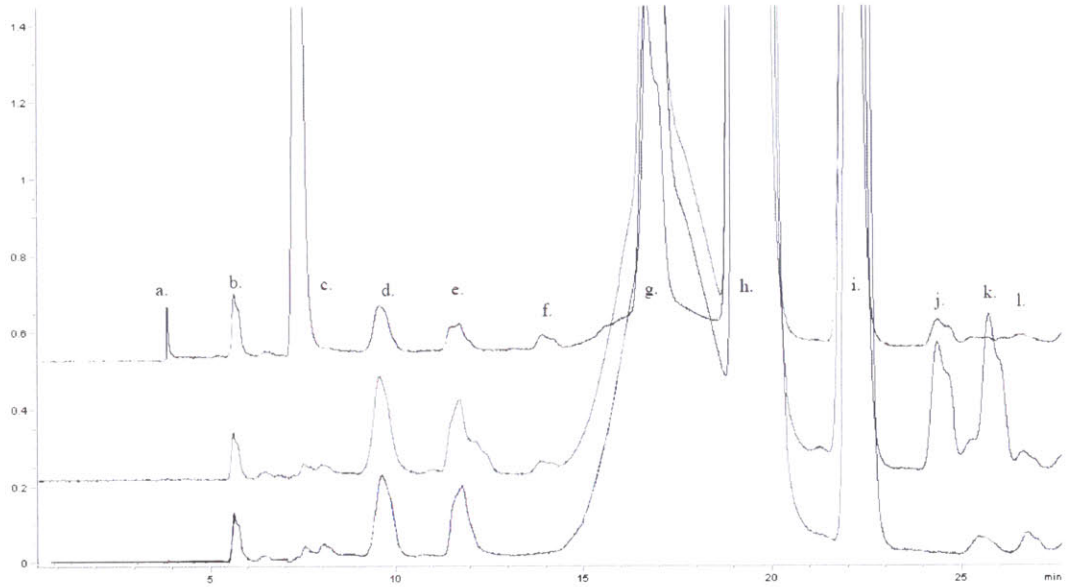

Figure 5. Typical elution gradients of a surface seawater sample (top chromatogram), MilliQ water spiked with cysteine, glutathione, γ-glutamylcysteine standards (middle chromatogram), and MilliQ water with no added thiols (bottom chromatogram). Peaks b., d., e., g., h., i., and l. are peaks associated with mBrB found in all analyses. Peaks a. and c. were unidentified compounds that were common in natural water samples. Peak f. is cysteine, peak j. is glutathione, and peak k. is γ-glutamylcysteine.

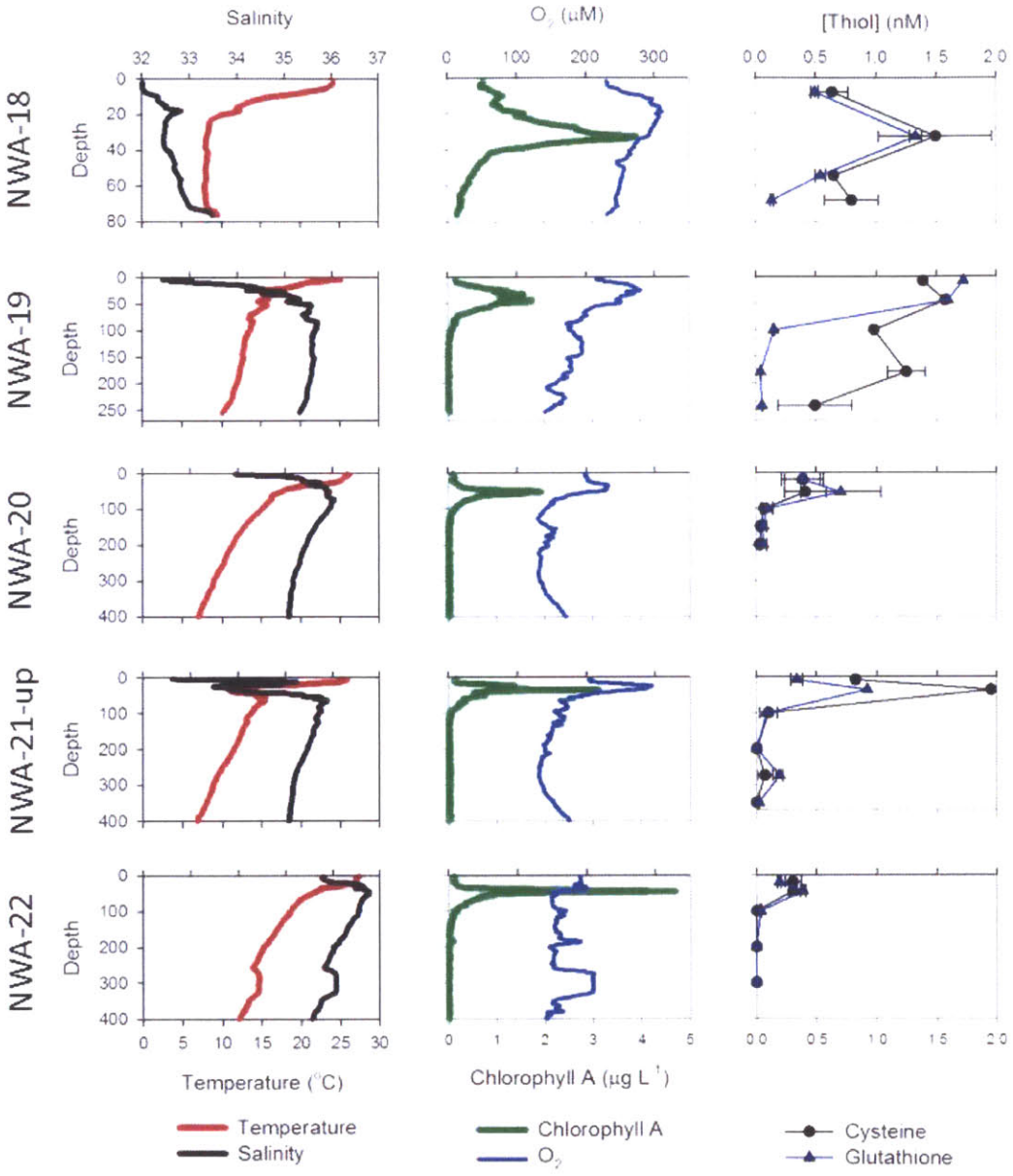

Figure 6. Temperature, salinity, Chl a, [O_2], [Cysteine], and [Glutathione] in the northwest Atlantic and New England continental shelf.

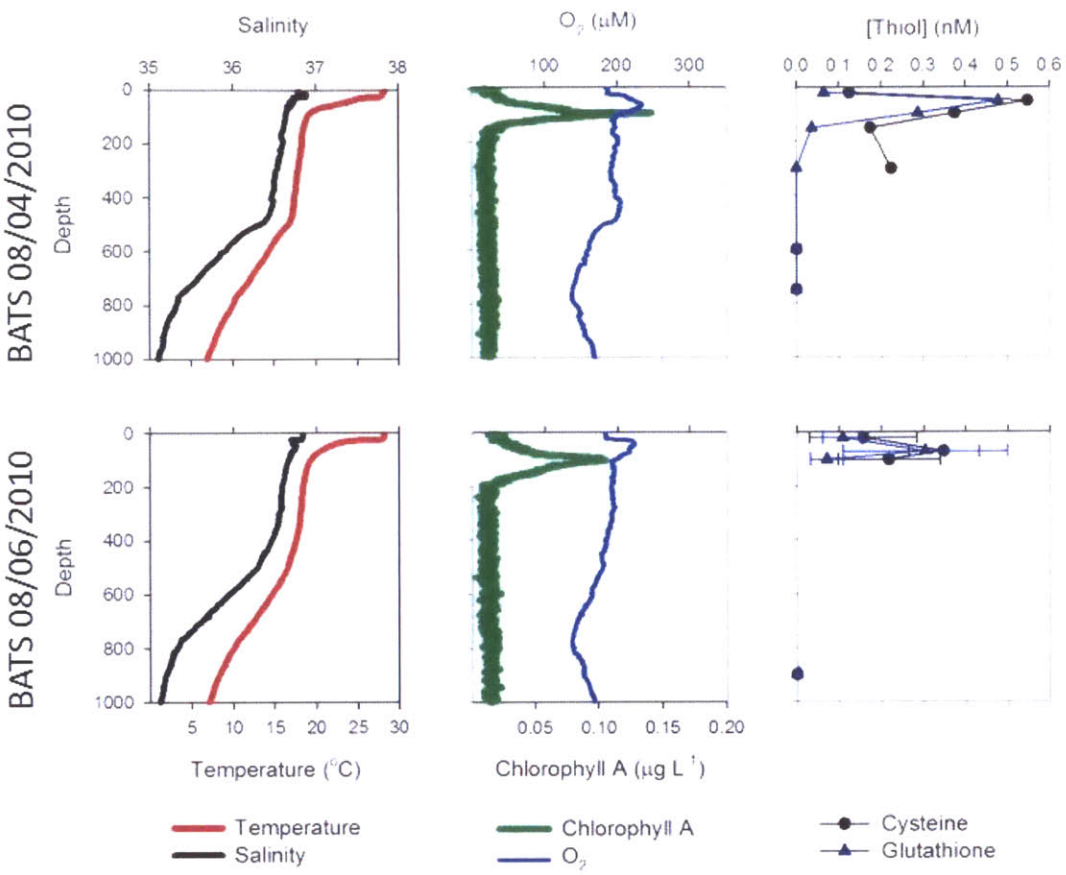

Figure 7. Temperature, salinity, Chl a, [O_2], [Cysteine], and [Glutathione] at the BATS sampling site southeast of Bermuda in the Sargasso sea, North Atlantic.

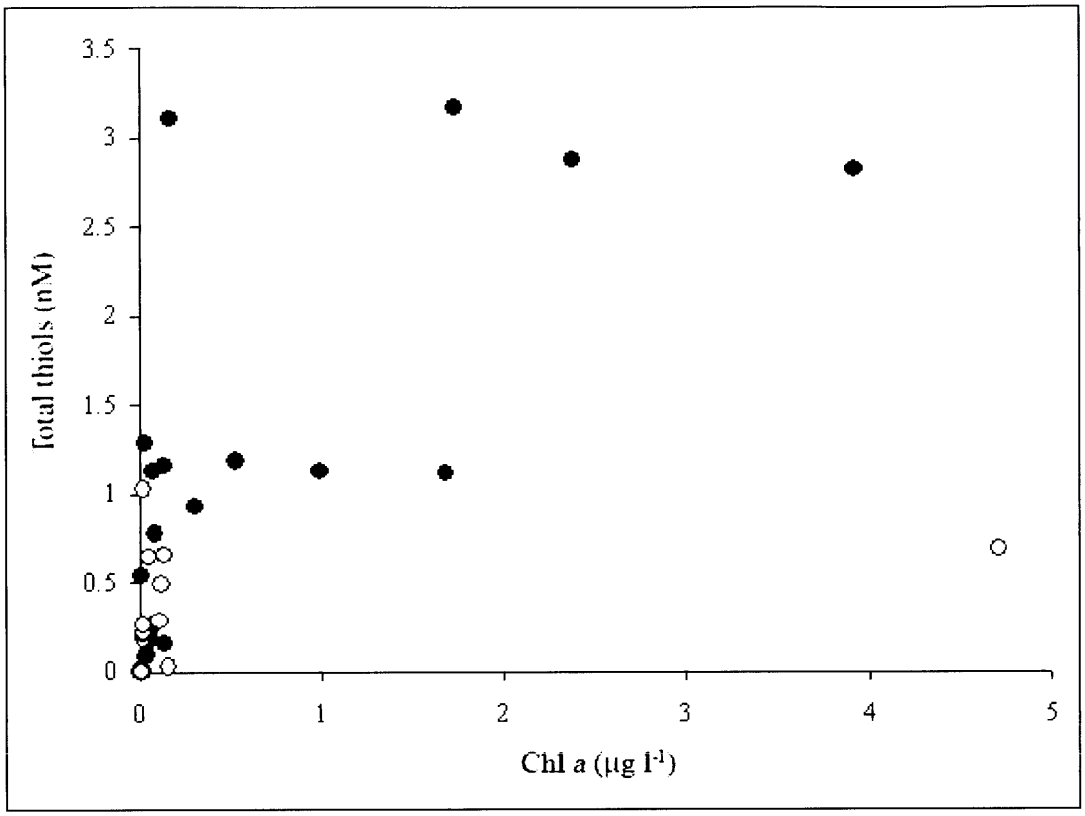

Figure 8. Total thiol concentration versus Chl a content. Filled circles are from continental shelf and slope stations (NWA-18, NWA-19, NWA-20, NWA-21-up), while open circles represent the open ocean stations (NWA-22 and BATS samples).

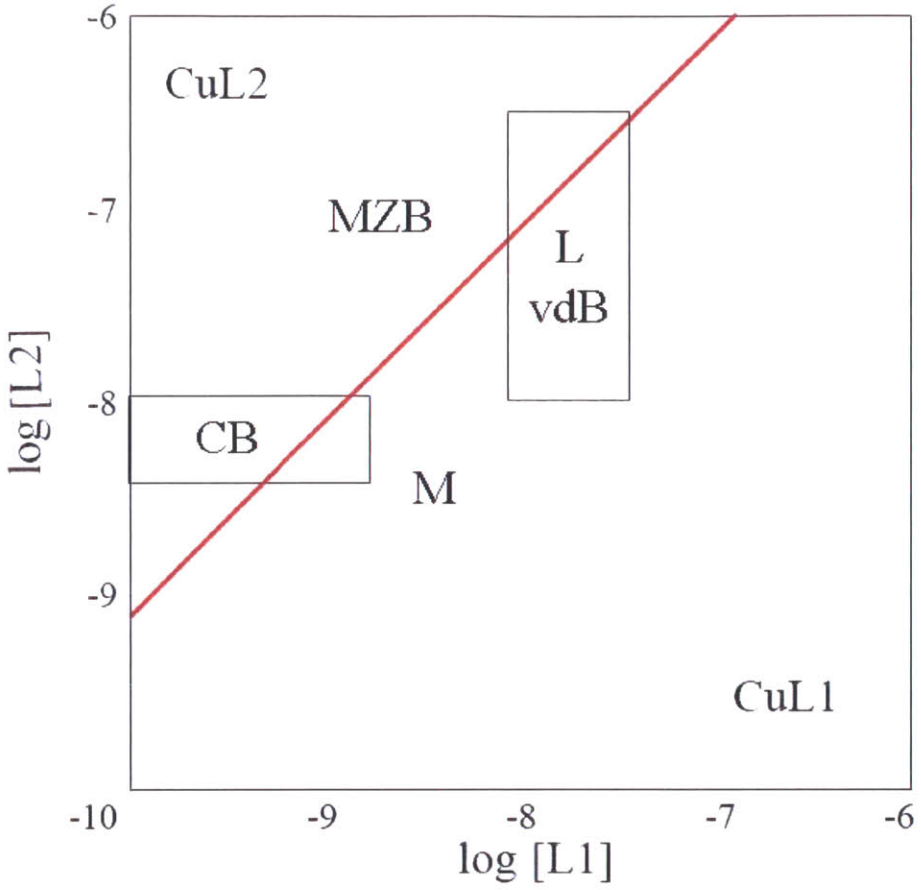

Figure 9. Copper – ligand complex speciation (CuL1 and CuL2) based on the most recent measurement of CuL1 and CuL2 conditional stability constants ($10^{14.38}$ and $10^{13.52}$ respectively; Mackey et al., 2012). The box labeled CB represents the range of values measured over a depth profile by Coale and Bruland (1988). The box labeled L vdB is the range of samples taken over a transect of an estuary (Laglera and van den Berg, 2003). The "M" symbol is the surface water collected at BATS in April 2010 (Mackey et al., 2012). The MZB symbol represents samples taken from 16 and 140 m in the southwest Sargasso Sea (Moffett et al., 1990).

Table 1. Sampling site date, time, location, and depth.

Site	Date	Time	Latitude	Longitude	Depth (m)
NWA-18	07/11/2010	02:00	40° 30'	70° 35'	76
NWA-19	07/12/2010	18:50	40° 00'	70° 25'	260
NWA-20	07/13/2010	08:45	39°40'	71°50'	620
NWA-21-up	07/14/2010	08:40	39°14'	72°19'	480
NWA-22	07/15/2010	22:30	39°35'	70°15'	2280
BATS-1	08/04/2010	17:26	31° 40'	64°10'	4680
BATS-2	08/06/2010	11:40	31°40'	31°40'	4680

Table 2. Total concentrations of elements used in thermodynamic equilibrium model

Component	Concentration (M)
Ag^{+}	20×10^{-12}
Al^{+++}	2×10^{-9}
Br^{-}	0.84×10^{-3}
CO_3^{--}	2.25×10^{-3}
Ca^{++}	10.1×10^{-3}
Cd^{++}	0.6×10^{-9}
Cl^{-}	0.546
$Cu^{+/++}$	0.5×10^{-9}
F^{-}	68×10^{-6}
Fe^{+++}	0.5×10^{-9}
Hg^{++}	1×10^{-12}
HS^{-a}	10×10^{-12}
K^{+}	10.2×10^{-3}
Li^{+}	25.9×10^{-6}
Mg^{++}	53×10^{-3}
Mn^{++}	0.3×10^{-9}
Na^{+}	0.468
Ni^{++}	8×10^{-9}
Pb^{++}	10×10^{-12}
SO_4^{--}	28×10^{-3}
Sr^{++}	90×10^{-6}
Zn^{++}	5×10^{-9}

Concentrations used are the oceanic mean given in Bruland and Lohan (2003)
[a]Sulfide concentration based on the measurement of less than 50 pM in the North Atlantic surface waters (Cutter et al., 1999)

Table 3. Reactions added to standard thermodynamic database

Reaction	Log K	Reference
$AgRS = Ag^{+} + RS^{-}$	-14.0	1
$AlRS^{++} = Al^{+++} + RS^{-}$	-6.4	1
$CaRS^{+} = Ca^{++} + RS^{-}$	-2	1
$CdRS^{+} = Cd^{++} + RS^{-}$	-10.3	1
$Cd(RS)_2 = Cd^{++} + 2RS^{-}$	-16.9	1
$Cu(RS)2 = Cu^{+} + 2RS^{-}$	-32.6	2
$Cu(RS)2 = Cu^{++} + 2RS^{-}$	-16	1
$FeRS^{++} = Fe^{+++} + RS^{-}$	-11	1
$Fe(RS)_2^{+} = Fe^{+++} + 2RS^{-}$	-14	1
$Fe(RS)_3 = Fe^{+++} + 3RS^{-}$	-32	1
$HgSHOH + H_2O = Hg(OH)_2 + HS^{-} + H^{+}$	-21.7	3,4
$HgSHCl + 2H_2O = Hg(OH)_2 + Cl^{-} + HS^{-}$	-31.0	3
$HgOHRS + H_2O = Hg(OH)_2 + RS^{-} + H^{+}$	-23.7	3
$Hg(RS)_2 + 2H_2O = Hg(OH)_2 + 2RS^{-} + 2H^{+}$	-47.2	3,4
$HgRS^{+} + 2H_2O = Hg(OH)_2 + RS^{-} + 2H^{+}$	-27.3	3
$MgRS^{+} = Mg^{++} + RS^{-}$	-2.8	1
$MnRS^{+} = Mn^{++} + RS^{-}$	-4.5	1
$Mn(RS)_2 = Mn^{++} + 2RS^{-}$	-8.65	1
$NiRS^{+} = Ni^{++} + RS^{-}$	-9	1
$Ni(RS)_2 = Ni^{++} + 2RS^{-}$	-20.16	1
$PbRS^{+} = Pb^{++} + RS^{-}$	-13.1	1
$Pb(RS)_2 = Pb^{++} + 2RS^{-}$	-19.2	1
$Zn(RS)_2 = Zn^{++} + 2RS^{-}$	-17.9	1

Table 3: Reactions, dissociation constants (K), and references for species added to the Visual MINTEQ release 2.40 database. Numbers in reference column refer to the following: (1) Berthon, 1995 (2)Leal and van den Berg, 1998 (3) Dyrssen and Wedborg, 1991 (4) Skyllberg, 2008

Table 4. Dissolved thiol concentrations in different regions (surface 300 m)

Location	Concentration range (nM)	Reference
Northwest Pacific	1.6 – 19.5 (0.3 – 2.25[a])	1
North Sea and English Channel	0.77 – 3.54	2
Galveston Bay, TX	0.23 – 8.88	3
Northwest Atlantic Ocean	<0.1 – 3.2	This Study
BATS, Sargasso Sea	0.2 – 1.1	This Study

a) Only the sum of cysteine and glutathione is presented in parentheses. γ-glutamylcysteine was the dominant thiol at all sampling sites. (1) Dupont et al., 2006 (2) Al-Farawati and van den Berg, 2001 (3) Tang et al., 2000

Table 5. Speciation of thiols, cupric ion, and mercury in the surface ocean

[RS] (M)	1×10^{-12}	1×10^{-11}	1×10^{-10}	1×10^{-9}	1×10^{-8}
$[RS^-]$ (M)	6.5×10^{-14}	9.0×10^{-13}	9.0×10^{-12}	5.0×10^{-11}	2.6×10^{-10}
% Total Cu as thiol complex	5.8×10^{-10}	1.1×10^{-7}	1.1×10^{-5}	3.4×10^{-4}	9.2×10^{-3}
%Total Hg as thiol complex	21.8	97.9	100	100	100
$Cu(RS)_2$ (M)	$2.9\ 10^{-21}$	5.6×10^{-19}	5.6×10^{-17}	1.7×10^{-15}	4.6×10^{-14}
$Hg(RS)_2$ (M)	2.2×10^{-13}	9.8×10^{-13}	1.0×10^{-12}	1.0×10^{-12}	1.0×10^{-12}

Table 5. Speciation of thiols, cuprous ion, and mercury in the surface ocean

[RS] (M)	1×10^{-12}	1×10^{-11}	1×10^{-10}	1×10^{-9}	1×10^{-8}
$[RS^-]$ (M)	3.9×10^{-18}	1.2×10^{-17}	4.1×10^{-17}	5.8×10^{-13}	2.3×10^{-10}
% Total Cu as thiol complex	0.1	1	10	100	100
%Total Hg as thiol complex	2.6×10^{-7}	2.6×10^{-6}	1.9×10^{-5}	5.0	100
$Cu(RS)_2$ (M)	5.0×10^{-13}	5.0×10^{-12}	5×10^{-11}	5×10^{-10}	5×10^{-10}
$Hg(RS)_2$ (M)	2.6×10^{-21}	2.6×10^{-20}	2.9×10^{-19}	5×10^{-14}	1×10^{-12}