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Distribution of the thiols glutathione and 3-mercaptopropionic acid in Connecticut lakes

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Abstract

The spatiotemporal chemical characteristics of the water column in Linsley Pond, a freshwater lake in Connecticut, were studied from just before its stratification in April through the fall turnover in December. Two low-molecular-weight thiols, glutathione and 3-mercaptopropionic acid (MPA), were detected at nanomolar concentrations in the water column. GSH was detected (average concentrations were ca. 5 nmol L^{-1} in the particulate phase and ca. 3 nmol L^{-1} in the dissolved phase) in surface and near-surface waters only and covaried with chlorophyll *a*. Both particulate and dissolved MPA were measured in the oxic and anoxic regions. In the metalimnion, MPA concentrations were greater than in the oxic water layers above. At times, the MPA concentrations in the metalimnion were as high as those found in the anoxic hypolimnion. The MPA present at different depths in the Linsley Pond water column is most likely produced through dissimilar mechanisms. Throughout the water column, MPA may be a product of the metabolic degradation of sulfur-containing organic compounds; however, in the hypolimnetic waters, dissolved sulfide may play an important role in MPA formation through abiotic nucleophillic addition to unsaturated functionalities in dissolved organic matter. Parallel laboratory experiments were performed to assess the importance of MPA in copper speciation. The results confirmed that despite nanomolar levels in Linsley Pond, MPA does not play an important role in copper speciation. A survey of six additional lakes in Connecticut was made for detectable thiols. Only MPA was detected in four of these lakes, and its distribution was similar to that in Linsley Pond.

Thiols play many important biochemical roles. The pKa of the sulfhydryl group generally ranges between 9–11, and is therefore of relevance to intercellular pH. Additionally the sulfhydryl group can be easily oxidized to form disulfide bonds that can be converted back to corresponding thiols through enzymatic activities (Meister and Anderson 1983). Such properties make thiols very useful as structural elements in the disulfide bridges of proteins, as part of oxidization defense systems, and as regulatory messengers via addition of small molecular weight thiols to proteins (Gilbert 1984). Additionally, the sulfhydryl group in thiol molecules has an extremely high affinity for some transition metal cations (Boulegue et al. 1982). Through the sequestration of these transition metals, thiols may also serve as detoxifying agents under conditions of high metal loading (Meister and Anderson 1983; Grill et al. 1985).

The important role that thiols play in the biogeochemical cycling of sulfur in a natural aquatic system is perhaps the biggest reason they have been widely studied (Mopper and Taylor 1986; Tang et al. 2000; Al-Farawati and Van Den Berg 2001). Thiols also serve as intermediates in the formation of many sulfur compounds. For example, cysteine (Cys) and glutathione (γ-glutamylcysteinylglycine; GSH) can serve as precursors for environmentally important sulfur gases, such as carbonyl sulfide (Flock et al. 1997). In sediments, thiols readily react to form disulfide and polysulfide bridges (Boulegue et al. 1982). These processes may enhance the cross-linking between organic molecules, serving as a key step in the formation of high-molecular-weight organic matter fractions (Aizenshtat et al. 1995). As strong ligands for transition metals, thiols have been shown to promote the mobilization of metals in aquatic environments through complexation reactions (Cullen et al. 1984). However, because of their extremely reactive nature, the steady state concentrations of thiols in natural waters tend to be low, and therefore their detection has been difficult. Based on a review of the available literature, Cys and GSH, two sulfur-containing amino acids, and 3-mercaptopropionic acid (MPA) have been among the most frequently detected thiols in natural aquatic environments (Mopper and Taylor 1986; Tang et al. 2000; Al-Farawati and Van Den Berg 2001).

Previous work on naturally occurring thiols has concentrated on their roles in marine environments. Studies have shown the presence of significant amounts of thiols in anoxic marine waters and sediments (Boulegue et al. 1982; Dyrssen et al. 1985; Mopper and Taylor 1986). Thiols at submicromolar levels have been measured in the Black Sea

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water column as well (Luther et al. 1991). In their effort to assess thiol concentrations in coastal surface waters, Tang et al. (2000) measured thiols in estuarine waters of Galveston Bay, Texas, and found that GSH was the most abundant species detected (0.23–6.23 nmol L^{-1} in the dissolved phase and 0.094–0.72 nmol L^{-1} in the particulate phase). Another study by Al-Farawati and Van Den Berg (2001) demonstrated the distribution of thiols in the coastal waters of the western North Sea and English Channel, where thiol concentrations ranged from 0.70 to 3.60 nmol L^{-1} (thiourea equivalents), a much greater abundance than that of dissolved sulfide (HS^-) (Al-Farawati and Van Den Berg 2001). The authors also reported a similarity between the depth profile of thiols and that of chlorophyll in their field sites, suggesting an important role of marine phytoplankton in thiol distribution (Al-Farawati and Van Den Berg 2001).

By derivatizing thiols using ammonium 7-flurorobenzo-2-oxa-1,3-diazole-4-sulfonate (SBD-F) (Toyooka and Imai 1983), we found that high-performance liquid chromatography (HPLC) separation coupled with fluorescence detection was an effectual technique to both identify and quantify some naturally occurring thiols. The SBD-F derivatization method has been reported to be superior to other precolumn dervitization methods such as *o*-phthaldialdehyde (OPA) (Mopper and Delmas 1984) or monobromobimane (Fahey and Newton 1987), in that SBD-F is highly specific to thiol groups, and there are no interferences from alcohols, phenols, or amino groups (Shimada and Mitamura 1994). Also, unreacted SBD-F is not fluorescent at the excitation wavelength of choice, thus eliminating any interferences attributable to the reagents (Toyooka and Imai 1983).

Unlike the case for marine waters, thiols have not been studied in freshwater environments where they could compete with other dissolved organic substances in the speciation of trace elements. The aim of this work was to identify and quantify thiols in one seasonally stratified lake and to study both spatial and temporal variations during the course of stratification. Our goal was to identify as many measurable thiol species as possible to establish their sources and their potential as complexants for dissolved copper. Six other Connecticut freshwater lakes were sampled less intensively for a cursory understanding of how widely distributed thiols are in local freshwaters.

Materials and methods

Study site—Our study site was Linsley Pond, which has been studied extensively by Hutchinson (Hutchinson 1975). The lake is located in the town of North Branford, Connecticut (41.3°N, 72.8°W). Fed primarily from a stream that drains from Cedar Pond to the northeast, Linsley Pond has a surface area of 0.09 km^2, a maximum depth of 14.8 m, and an average depth of 6 m. Pisgah Brook serves as the one outlet (Canavan and Siver 1995). Linsley Pond stratifies from April through November each year. During stratification, the hypolimnion becomes anoxic, and substantial concentrations of HS^- build up (Canavan and Siver 1995).

Sample collection—Sampling was carried out from April to December 2003, generally on a biweekly basis with more intensive sampling during mid-summer. Water samples were collected at 1-m intervals from surface to bottom (13 m) at the point of maximum depth in the pond. A peristaltic pump and a teflon hose were used for sampling. After lowering the hose to the appropriate depth, enough water was pumped to flush the hose a few times. Filtration was completed in the field by pumping water through acid-cleaned, 0.45-μm-pore-size filter membranes (Durapore, Millipore) set in teflon filter holders. To minimize filtration artifacts, all water samples were collected before the build-up of significant backpressure in our system. Both filtered and nonfiltered water samples were collected in 30-mL high-density polyethylene bottles (Nalgene) with no headspace. The bottles were tightly capped and stored on ice in coolers. Samples were kept at or below 5°C (generally 1–2 h) until analysis. Subsamples used for thiol measurement were treated with the derivatization reagent promptly, and the HPLC analysis of the derivatives was completed within 48 h of sampling.

Thiol determination—Before chromatographic separation, thiols were derivatized with SBD-F. Briefly, each 2.5-mL sample was treated with 270 μL of the derivatizing reagent mixture containing 0.1 mol L^{-1} sodium borate buffer, 0.1 mol L^{-1} NaOH, and 0.15 mg L^{-1} SBD-F (pH 10.5), and then incubated for 60 min at 60°C in the dark. After this reaction, 100 μL of 1 mol L^{-1} methanesulfonic acid (MSA) was added to the samples to stop the derivatization reaction. MSA also helps stabilize the derivatization products (Tang et al. 2000). After derivatization the treated samples were refrigerated (4°C) and kept in the dark until HPLC analysis.

The thiol-SBD adducts possess high quantum yields of fluorescence and are stable for >1 week when stored at 4°C, thus being particularly suited for HPLC analysis (Toyooka and Imai 1983). Thiol derivatives were analyzed on a Dionex DX-500 system (Dionex) equipped with a reversed-phase column (Vydac, 250 mm × 4.6 mm column containing 5 μm C_8 packing material) and a fluorescence detector (Dionex RF 2000). Chromatographic separation was carried out isocratically. The eluent consisted of 0.1% trifluoroacetic acid/5% acetonitrile/95% H_2O (v/v/v). The volume of the injection loop was 200 μL, and the flow rate was 1.0 mL min^{-1}. For fluorescence detection, an excitation wavelength of 385 nm and an emission wavelength of 515 nm were chosen based on our own preliminary steady-state fluorescence measurements.

Calibration curves were constructed by plotting the chromatographic peak area of each analyte against its concentration in the standard solution and fitting the data by linear least-squares regression analysis. Thiols in natural water samples were identified and quantified based on the elution time and peak area of known thiol standards. Measurements made from field samples were performed on both filtered (0.45 μm) and unfiltered lake water samples. We define thiols in the particulate phase as the difference of those labile to SBD-F derivatization between unfiltered

samples and those filtered through 0.45-μm filters. Because not all particle-bound thiols may be liberated under the derivatization conditions, the calculated particulate fraction thiols may underestimate the actual concentration of thiols present in that phase. All reported thiol concentrations in this study are presented as the mean of duplicate measurements.

In this study, we used the low-molecular-weight thiols, Cys, GSH, and MPA, which have been most frequently found in natural waters as model compounds to test the applicability of SBD-F derivatization for freshwater samples. Using these compounds, we produced linear calibration curves across the range of 0.4–60 pmol of injected thiols. The detection limits (calculated by using three standard deviations of the mean noise level) for Cys, GSH, and MPA were 0.3, 0.2, and 0.07 pmol, respectively, corresponding to 1.4 nmol L^{-1} Cys, 1.0 nmol L^{-1} GSH, and 0.4 nmol L^{-1} MPA in the original solutions based on a 200-μL sample injection. Our analytical error was less than 10% (C.V.). Based on these low detection limits and reasonable analytical error, we concluded that the SBD-F method was appropriate to measure the trace levels of thiols expected in freshwaters. We did observe a strong fluorescent signal from natural organic matter present in Linsley Pond, which interfered with that of Cys because of the similarity of their elution times. We were unsuccessful in removing this interference, and therefore we are not able to verify the existence of cysteine in the lake water studied. Phytochelatin-2 (PC_2, $(-Glu-Cys)_n-Gly$, n = 2) was also analyzed with the SBD-F method, but no detectable PC_2 was found in the lake water.

The thiol derivatives were found to be stable over 1 week when stored at 4°C. This time period is much longer than that required for the complete chromatographic analysis of all the samples, which we generally completed within 2 days of sampling. Additional laboratory experiments showed that model thiols spiked into natural unfiltered and filtered samples could be recovered with >95% efficiency (data not shown).

Methionine measurement—Methionine was measured with the OPA derivatization method coupled with HPLC analysis (Lindroth and Mopper 1979; Mopper and Lindroth 1982). For the derivatization, a 2-mL water sample was treated with 20 μL OPA reagent solution that contained 2.5 mg mL^{-1} OPA, 10% (v/v) ethanol, and 1% (v/v) 2-mercaptoethanol (ME) mixed in 0.1 mol L^{-1} borate buffer (pH 10.5). The water sample was allowed to react with the derivatizing reagent at room temperature (~ 20°C) for 2 min. The derivative was then separated on a Vydac C_8 column (250 mm × 4.6 mm, 5-μm C_8 packing material) with a mobile phase consisting of 20% of 12.5 mmol L^{-1} NaH_2PO_4 and 20% of 90% acetonitrile/10% H_2O at a flow rate of 1 mL min^{-1} and detected on a fluorescence detector with the excitation wavelength and emission wavelength set at 330 nm and 458 nm, respectively. Using this configuration, we achieved a detection limit of 0.1 nmol L^{-1} of methionine.

Dissolved organic carbon and chlorophyll a—A field fluorometer/turbidimeter (Aquafluor, Turner Designs) was used to measure in situ chlorophyll *a* (Chl *a*) and turbidity. Later, we confirmed the vertical patterns of chlorophyll abundance by extraction of Chl *a* and laboratory analysis (Parsons and Strickland 1963). We do note that field analyses underestimated total chlorophyll concentration (in some cases by as much as a factor of 10). However, we only require Chl *a* data as an indicator of biological activity; therefore, the fluorometer measurements made in the field provide sufficient relative Chl *a* data needed in this study.

Dissolved organic carbon (DOC) was analyzed by catalytic high-temperature oxidation (Shimadzu TOC 5000) following acidification and purging to remove inorganic carbon. For each sample a minimum of five replicate measurements were made. Measurements continued until the coefficient of variation was <2% or a maximum of 10 measurements were made. Blanks were run often to ensure there was no memory from sample to sample. Based on our experience with this instrument, the precision of these measurements is 0.005 mmol L^{-1}.

Ancillary parameters—Temperature, pH, and conductivity of the lake water were recorded in the field on a YSI 556 MPS (YSI). The reliability of dissolved oxygen measurements was compromised by a defective sensor, so data are not shown. Chloride, nitrate, and sulfate were measured by ion chromatography with an IonPac AS14 anion-exchange column (Dionex, Sunnyvale, CA) and a bicarbonate exchange medium. Sulfide was measured by the methylene blue technique (Cline 1969). Redox conditions are clearly indicated by loss of other electron acceptors, such as nitrate and sulfate. In further discussions, our use of the term "anoxic waters" refers to strata where sulfide was measurable. Subsets of water samples were acidified with 5% HNO_3 (SeaStar) and heated before analysis for major cations measurements by inductively coupled plasma atomic emission spectroscopy. Major cations include: sodium (Na^+), calcium (Ca^{2+}), magnesium (Mg^{2+}), potassium (K^+), iron (Fe^{2+}/Fe^{3+}), and manganese ($Mn^{4+/2+}$).

Samples for total dissolved copper (Cu) measurement were acidified with ultrapure HNO_3 (SeaStar) to a final concentration of 2% HNO_3 and preconcentrated by subboiling evaporation in Teflon tubes on a block heater. A concentration factor of 20:1 was used. After concentration, samples were analyzed with graphite furnace atomic absorption spectrometry (Perkin Elmer 3300 AAS).

Reagents—Thiols and other reagents used in this study were obtained from Sigma (MSA and L-Cysteine), Fluka (SBD-F, OPA, and ME), Aldrich (GSH, MPA, and methionine), EM Science (trifluoroacetic acid), and Fisher Scientific (HPLC grade acetonitrile). All thiols were stored in the dark at 4°C.

Distilled deionized water (18.3 mΩ) produced by a Barnstead ultrapure water system (E-Pure) was used in all experiments. Before use, distilled deionized water used to prepare thiol standard solutions was purged with ultra-high purity nitrogen for at least 60 min at room temperature to purge dissolved oxygen.

Results

Water column characteristics of Linsley Pond—Thermal stratification of Linsley Pond began in April 2003 (Fig. 1A). At this stage, the temperature gradient in the metalimnion (3–6 m) was relatively small and a significant amount of sulfide (>1 μmol L^{-1}) was measurable only below 11 m (Fig. 1C). Stratification was fully established in July, and there was little additional change in the temperature profiles until cooling of the surface water in early October. During complete stratification the lower boundary of the metalimnion was located near 6 m, whereas the upper boundary fluctuated as a result of major weather events (e.g., strong wind and rain storms). The anoxic hypolimnion was below 6 m. Following gradual erosion of the hypolimnion, complete mixing of the lake waters occurred in early December. Similar timing of the turnover has also been observed in previous years (Mylon and Benoit 2001).

Nitrate concentrations in the Linsley Pond epilimnion were ca. 20 μmol L^{-1} in the spring when the sampling began and gradually decreased until July when nitrate levels were below our detection limits (<0.5 μmol L^{-1}). In the hypolimnion, nitrate was already depleted when sampling began. No significant loss of nitrate, however, occurred in the metalimnion even during the period of high productivity. As the hypolimnium began to erode in October, nitrate concentrations in the metalimnion declined, probably because of dilution from mixing with low-nitrate waters from shallower depths (Fig. 1B).

Dissolved sulfide [S(-II)] concentrations in the epilimnion and the metalimnion were always below the detection limit of the standard methylene blue method used in this study (<0.5 μmol L^{-1}). In previous study, S(-II) concentrations in the epilimnion of Linsley Pond were measured to be a few nanomolar using a more sensitive and time-consuming method (Mylon and Benoit 2001). Sulfide concentrations measured in the anoxic hypolimnetic water increased with increasing depth in this region. Sulfide concentrations at the bottom reached ca. 250 μmol L^{-1} during complete stratification. In the winter, sulfide concentrations were undetectable after mixing and aeration of the water column (Fig. 1C).

During stratification, sulfate concentrations decreased rapidly from a relatively uniform level of ca. 350 μmol L^{-1} in the epilimnion and metalimnion to ca. 150 μmol L^{-1} below the thermocline and continued to decrease to as low as ca. 110 μmol L^{-1} at the maximum depth of the lake (Fig. 1D). Before stratification, bottom waters contained ca. 400 μmol L^{-1} sulfate, and therefore generation of ca. 300 μmol L^{-1} sulfide was responsible for the decrease of sulfate to ca. 100 μmol L^{-1}. Water column total S (sulfate plus sulfide) remained constant, suggesting that sulfate reduction occurred in situ.

DOC in Linsley Pond averaged approximately 0.3 mmol L^{-1} and varied subtly but consistently with depth and time. As stratification began, DOC concentrations were nearly the same throughout the water column (ca. 0.28 mmol L^{-1}) (Fig. 1E). As stratification progressed, by early June, DOC profiles exhibited clear spatiotemporal structures. DOC levels in epilimnetic waters were vertically uniform, but absolute concentrations varied throughout the duration of this study. We observed an increase in DOC from ca. 0.28 mmol L^{-1} in early June to 0.35 mmol L^{-1} in the late summer followed by a decrease to 0.30 mmol L^{-1} by December (Fig. 1E). Metalimnetic waters had the lowest DOC levels (ca. 0.21 mmol L^{-1}), and below the thermocline DOC increased gradually with depth to the bottom. This DOC minimum in the metalimnium remained constant during the period of stratification. Water mixing beginning in October disturbed the vertical pattern of DOC. After mixing, the DOC became evenly distributed throughout the water column.

An algal bloom was recorded in the subsurface water of Linsley Pond in April characterized by a peak in Chl *a* concentrations (ca. 100 μg L^{-1}) at 3 m (Fig. 1F). In the surface water of Linsley Pond, we measured an average annual concentration of Chl *a* to be ca. 28 μg L^{-1}. Slight changes resulted from sampling time and weather (e.g., our highest measurements were recorded when sampling was done in the morning of a sunny day). During stratification, the maximum Chl *a* concentrations (50–200 μg L^{-1}) were frequently recorded in the redox transition region (6–8 m). As water mixing proceeded in the fall, the mid-depth maximum declined and Chl *a* content in the water column became uniformly low (ca. 10 μg L^{-1}) by December.

Thiols in Linsley Pond—Two thiol species, GSH and MPA, were detected at nanomolar concentrations in the water column of Linsley Pond (as noted earlier, the fluorescent nature of DOC made it impossible to measure Cys). No peaks other than those for GSH and MPA were discernable on the chromatograms. Both thiols displayed distinctive temporal and spatial distribution patterns. We found GSH mainly in oxic, epilimnetic water although we did detect GSH sporadically at depth. Dissolved GSH (GSH in 0.45-μm filtrate) was <3 nmol L^{-1}, and particulate GSH, calculated as the difference between total GSH (unfiltered) and dissolved GSH, had a depth-averaged concentration of 5 nmol L^{-1} (Fig. 2A,B). GSH concentrations changed considerably over time. Elevated concentrations of GSH were measured from June to August 2003. After August, no GSH was measured in the dissolved phase, and all GSH measured during this period was associated with the particulate phase. In early winter, GSH was absent from the entire water column. High GSH concentrations (11–26 nmol L^{-1}), almost 5–10-fold greater than the average level, were measured in the unfiltered metalimnetic water samples collected on 31 July 2003 (Fig. 2).

In contrast to GSH, MPA was detected in the entire water column of Linsley Pond. MPA was also found in greater abundance than GSH. Commonly, we measured higher concentrations of MPA in the dissolved phase (<0.45 μm) than in the particulate phase. Figure 3 shows MPA profiles from May to December 2003. In the profiles, two MPA maxima were notable: a local maximum in the metalimnion (4–5 m) throughout the stratification period and another MPA maximum in the lower part of the anoxic hypolimnion close to the sediment–water interface. Dis-

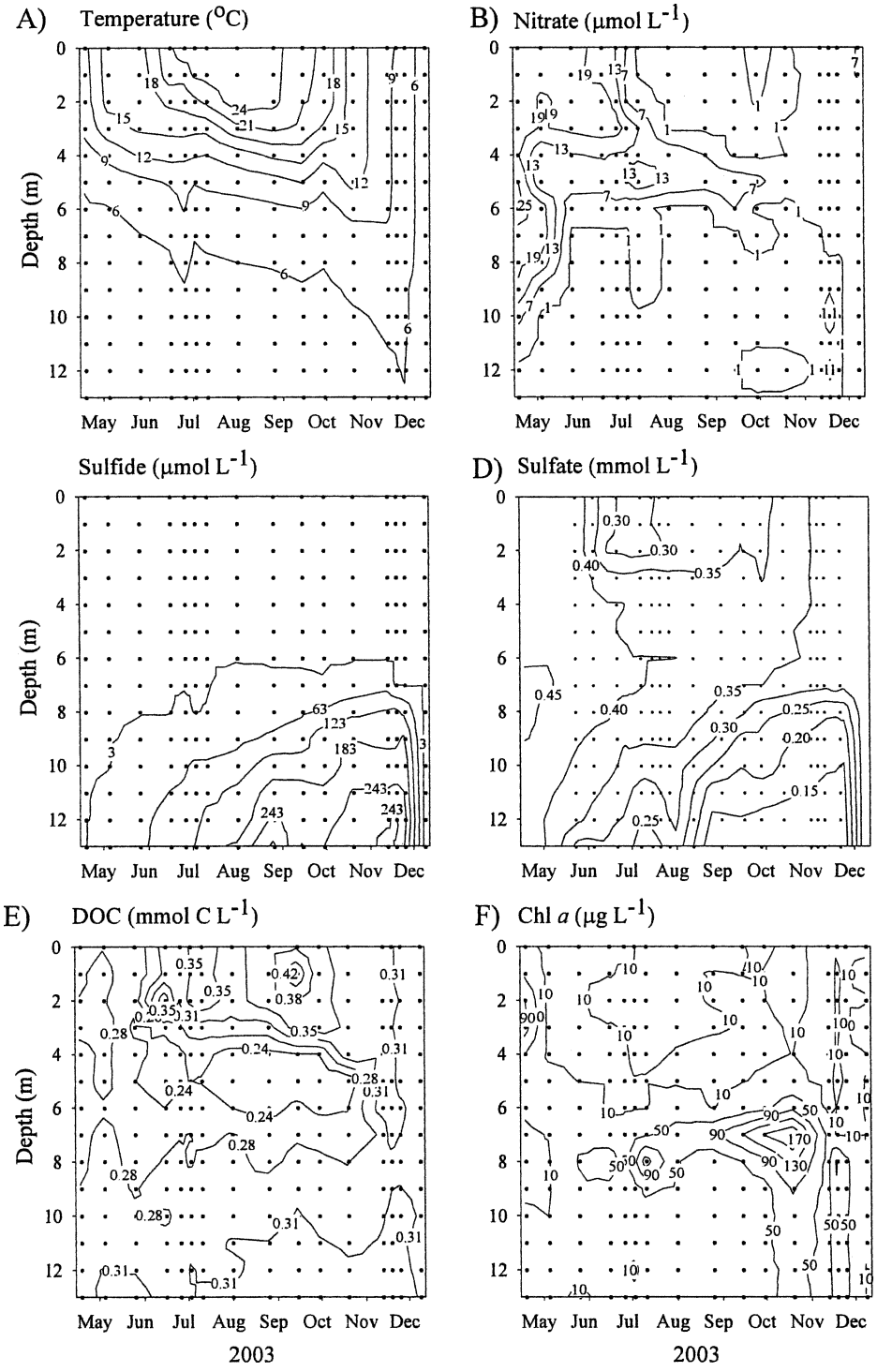

Fig. 1. Spatiotemporal variations in (A) temperature, (B) nitrate, (C) sulfide, (D) sulfate, (E) DOC, and (F) Chl *a* in the water column of Linsley Pond during the period from mid-April to early December 2003 (dots represent sampling times and depths).

solved and particulate MPA in the metalimnetic maximum were ca. 13 nmol L^{-1} and 4 nmol L^{-1}, respectively, whereas in the hypolimnion their respective maximum concentrations were typically 50 nmol L^{-1} and 3 nmol L^{-1}. A surprisingly high dissolved MPA concentration (100 nmol L^{-1}) was measured in the bottom water on 31 July 2003 (79 nmol L^{-1} of MPA was in the particulate phase), generating a hotspot on the isopleths (Fig. 3). This hotspot, however, did not affect the boundaries of the metalimnetic MPA maximum. In the surface waters, MPA concentrations were near 2–3 nmol L^{-1}, about five-fold lower than measured in the

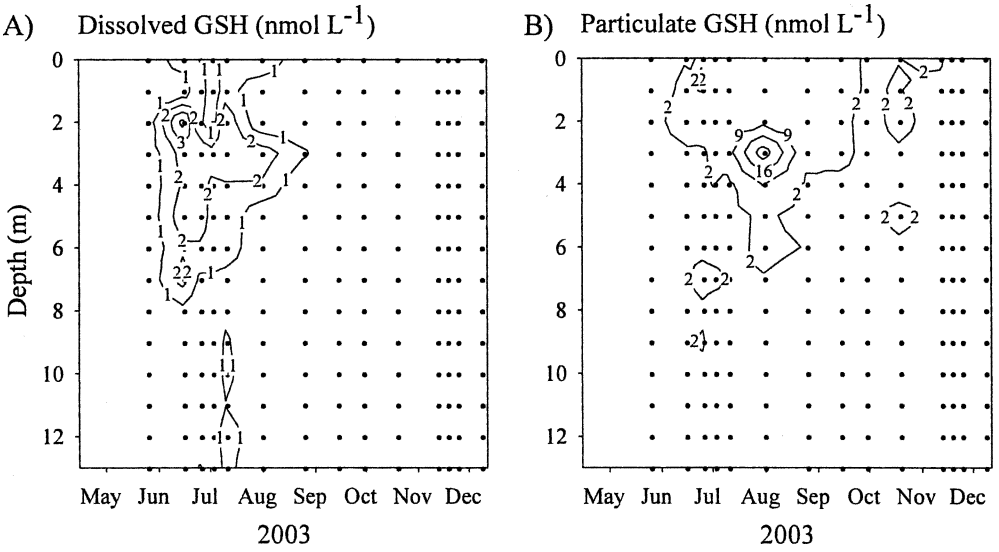

Fig. 2. Isopleths of (A) dissolved GSH and (B) particulate GSH in the water column of Linsley Pond during the period from mid-May to early December 2003 (dots represent sampling times and depths).

metalimnetic maximum, and remained fairly constant during stratification. There was no detectable MPA in the surface water during the fall turnover. Below the metalimnetic maximum, MPA levels declined, and the decreasing trend continued through 8-m depth, where dissolved MPA concentrations were near 5 nmol L^{-1} (about 3 nmol L^{-1} in the particulate phase). Below 8 m, MPA concentrations increased with depth. The rate of this increase was greatest from late spring to late summer and became less pronounced thereafter. Water mixing in the fall affected the MPA vertical distribution pattern, resulting in the loss of the MPA maximum in the metalimnion near the beginning of this process. The deep-water MPA maximum persisted until the fall mixing was complete in December. In December, we were unable to detect MPA in any portion of the water column.

Distribution of thiols in selected Connecticut lakes—To obtain information about the commonality of thiol occurrence in lakes, a survey was carried out in six other Connecticut lakes in the fall of 2004 (13–14 October 2004). Sampled sites included Amos Lake (41.52°N, 71.98°W), Bashan Lake (41.49°N, 72.41°W), Beseck Lake (41.52°N, 72.73°W), Black Pond (41.52°N, 72.74°W), Cedar Lake (41.40°N, 72.49°W), and Uncas Pond (41.37°N, 72.32°W). We sampled these six lakes to cover a variety of biogeochemical properties (Table 1). Beseck Lake and Black Pond are two shallow lakes, each with a maximum depth of 7 m. Beseck Lake is eutrophic whereas Black Lake is mesotrophic, and neither lake stratifies in the summer. The remaining four lakes are deeper (12–14 m) and became thermally stratified in the summer (Canavan and Siver 1995). Although we hoped to capture profiles during the complete stratification of each lake, by the time our sampling was performed, many of these lakes had begun to experience their fall turnover. Mixing of the entire water column had taken place in Black Pond, Beseck Lake, and Bashan Lake, whereas a slight temperature gradient was still observable in waters below 7-m depth in Amos Lake. Cedar Lake and Uncas Pond were still well stratified (Fig. 4A). In each lake, thiol analysis was conducted only on unfiltered water samples.

Among all six lakes surveyed, MPA was the only thiol species detected. Vertical concentration profiles of MPA are shown in Fig. 4B. In the entire water column of Cedar Lake and Bashan Lake (except at 1 m), MPA was below the detection limit (<0.2 nmol L^{-1}). In the other four lakes, including Uncas Pond (stratified), the distribution of MPA concentrations was fairly uniform throughout the water column, ca. 2 nmol L^{-1}. This MPA level was similar to that in the oxic water of Linsley Pond during the period of fall turnover.

Discussion

Background biogeochemistry—Linsley Pond exhibits classic patterns of production and removal of a number of redox-active and biologically important substances. Nitrate is consumed in surface waters by planktonic uptake and in bottom waters through its use as a terminal electron acceptor in anaerobic bacterial respiration. In the metalimnion (~5 m), neither of these processes is very active. With few nitrate sinks in the metalimnion combined with a possible source from the oxidation of NH_4^+ during nitrification, we observe relatively high nitrate concentrations in this region.

Sulfate, in its turn, is reduced, producing sulfide on a one-to-one molar basis. The highest sulfide concentrations were measured closest to the sediment–water interface, which most likely reflects the combination of a longer time that this zone was reducing and sulfide contributions from sediments.

DOC undergoes subtle increases in both surface and deep waters during stratification, presumably as a conse-

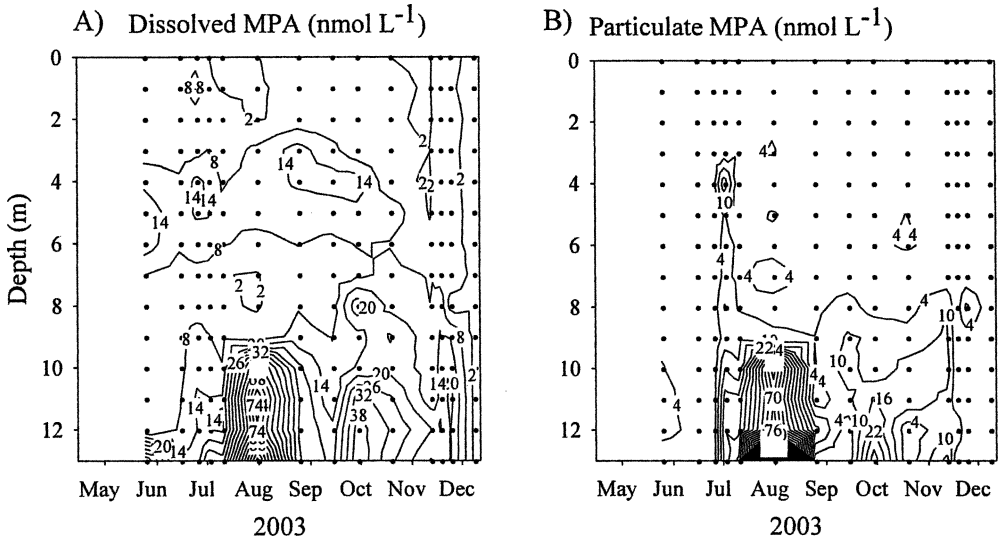

Fig. 3. Isopleths of (A) dissolved MPA and (B) particulate MPA in the water column of Linsley Pond during the period from mid-May to early December 2003 (dots represent sampling times and depths).

Table 1. Hydrographic characteristics and trophic status of the six lakes surveyed. Data from the Connecticut Department of Environmental Protection.

	Maximum depth (m)	Surface area (km^2)	Trophic status
Black Pond	7.0	0.30	mesotrophic
Beseck Lake	7.6	0.49	mesotrophic
Uncas Pond	12	0.28	oligotrophic
Cedar Lake	13	0.30	mesotrophic
Amos Lake	14	0.45	eutrophic
Bashan Lake	14	1.1	oligotrophic

quence of primary productivity in surface waters and decomposition of organic particles in the deeper waters. The lower DOC concentrations in the metalimnion during stratification could be caused by respiration of organic matter in this region. This might be characterized by a greater ratio of more recalcitrant forms or organic carbon to the overall pool of DOC in that region. Hence, we observe lower DOC concentrations in this region.

During stratification, Chl *a* concentrations were low in the epilimnion and high in the hypolimnion with a maximum located at mid-depth (Fig. 1F). The location of this maximum, however, migrated from near the bottom to about 7 m as stratification progressed, coinciding with the rise in the upper boundary of the high sulfide zone (>1 μmol L^{-1}). This pattern seems to reflect the development of green sulfur bacteria (GSB) in the upper hypolimnion of Linsley Pond. GSB have been found to occupy environments where sulfide and light levels are low (Gorlenko et al. 1983). During the period of stratification, the Secchi depth of Linsley Pond was ca. 2.5 m. The light intensity in the upper hypolimnion of Linsley Pond may satisfy the low light requirement of GSB. The upper-hypolimnion Chl *a* maximum observed in the water column of Linsley Pond was similar to that occurring in some other stratified lakes (Chapin et al. 2004).

Glutathione—GSH is the most important and most abundant non-protein thiol in living cells, and its intracellular concentration can be as high as 12 mmol L^{-1} (Meister and Anderson 1983; Giovanelli 1987), almost six orders of magnitude greater than the concentrations of GSH found in natural waters. GSH serves important biological functions such as acting as a redox buffer to prevent oxidative damage and as a detoxifying agent inhibiting the toxicity of some trace metals through complexation reactions (Meister 1983; Meister and Anderson 1983; Cooper and Kristal 1997).

Low nanomolar concentrations of GSH were detected in the oxic surface waters of Linsley Pond. Similar concentrations of GSH have been detected in coastal surface waters, and it is among the most frequently detected thiols in coastal environments (Tang et al. 2000; Al-Farawati and Van Den Berg 2001). In the open ocean, GSH was found to be present throughout the water column, typically at total concentrations of 2–10 nmol L^{-1} in the upper water column and subnanomolar levels in deeper waters (Le Gall and Van Den Berg 1998).

The presence of GSH in coastal waters is related to its release from phytoplankton as demonstrated by the close correspondence between GSH distribution profiles with that of Chl *a*. Matrai and Vetter (1988) observed that particulate GSH in Saanich Inlet, British Columbia, Canada, showed a subsurface maximum and decreased with depth in close correlation to chlorophyll profiles. In the western North Sea and English Channel, the GSH distribution in surface water from different sites was also found to co-vary with chlorophyll concentrations (Al-Farawati and Van Den Berg 2001). In Linsley Pond, the correlation between total GSH (unfiltered) and Chl *a*

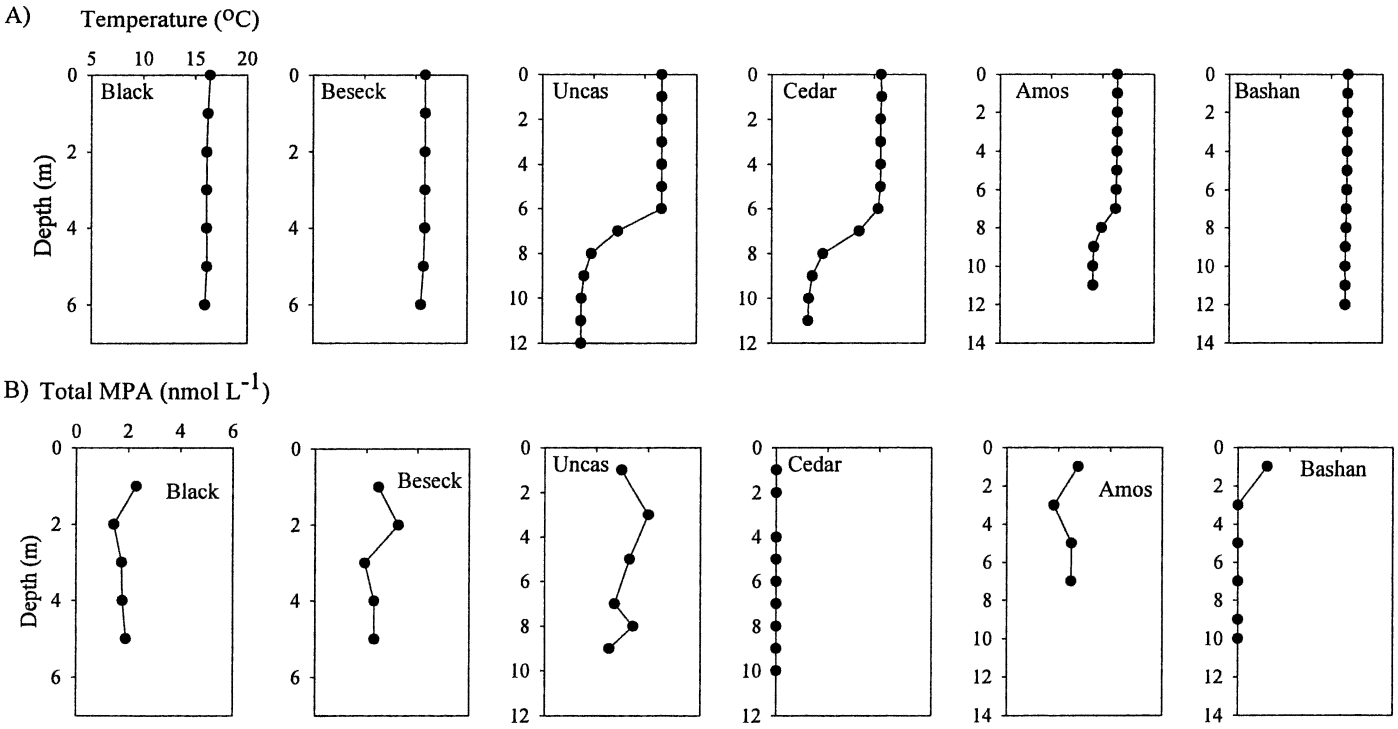

Fig. 4. (A) Temperature gradient and (B) vertical distribution of MPA in the water column of six lakes in Connecticut (sampled on 13 and 14 October 2004).

abundance was weak but statistically significant (Fig. 5). GSH and Chl *a* data only from oxic water layers (above 6 m) were compared in Fig. 5 to eliminate the interference from the upper-hypolimnion Chl *a* maximum. Similar to the case in coastal environments, GSH in the surface waters of Linsley Pond may also have a phytoplanktonic origin. How GSH was released into surrounding environment from phytoplankton is not clear. The release of GSH may be associated with the normal metabolic processes of phytoplankton or as a response to enhanced Cu exposure (Leal et al. 1999). It was suggested that GSH may be released from dead cells as they degrade (Leal et al. 1999). Natural biological activities, such as grazing, are more likely to be responsible for the occurrence of GSH in the oxic water of Linsley Pond than Cu-triggered biological excretion, given the relatively low Cu level (~10 nmol L^{-1}) in the water column of Linsley Pond and the even lower concentration of free copper (Cu^{2+}), based on our own measurements of Cu-complexing ligands (Hu et al. unpubl. data).

GSH production could be dependent on phytoplankton species, light level, and nutrient availability (Matrai and Vetter 1988). The relatively low correlation coefficient between GSH and Chl *a* ($r^2 = 0.16$, or 0.24 after excluding the highest GSH point), however, suggests that there are sources other than phytoplankton for GSH in Linsley Pond. The existence of other sources has been documented in some marine systems. Another possible source of GSH in the oxic water is bacteria. Bacteria contain GSH and have been postulated to contribute to the observed GSH distributions in Saanich Inlet (Matrai and Vetter 1988).

We occasionally detected GSH in anoxic waters from the hypolimnion of Linsley Pond. It is not surprising to find GSH in anoxic waters because GSH has been measured in anoxic coastal sediment pore waters at concentrations as high as 2 μmol L^{-1} (Mopper and Taylor 1986; Kiene et al. 1990). In that study, bacteria were believed to play an important role in GSH production (Mopper and Taylor 1986; Kiene et al. 1990). In light of the scattered detection of GSH in the anoxic waters of Linsley Pond, we suspect that bacterially mediated GSH production most likely was not its significant source.

3-Mercaptopropionic acid—We measured MPA in both oxic and anoxic waters of Linsley Pond, with MPA levels in oxic regions (2–3 nmol L^{-1}) much lower than in anoxic regions (up to 340 nmol L^{-1}). A noticeable bimodal pattern was displayed in MPA depth profiles during stratification, which consisted of a peak in the metalimnion and a generally larger maximum near the sediment–water interface (Fig. 3A). The lower MPA concentrations at the intervening oxic–anoxic interface indicated that diffusion of MPA to the overlying oxic water from the deeper anoxic region did not occur, and thus, the MPA found in the metalimnion most likely resulted from in situ production. To our knowledge, this is the first report of the presence of MPA in oxic waters. Phytoplankton could be a source of MPA, however no correlation was found between MPA and Chl *a* in the oxic waters of Linsley Pond. This argues against phytoplankton as a source for MPA in the metalimnion. Comparison of profiles of MPA and other parameters revealed that the MPA maximum in the

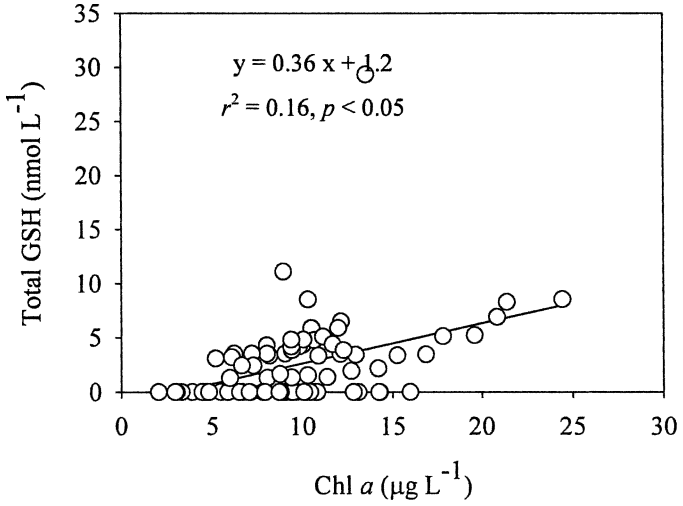

Fig. 5. Relationship of total GSH concentration and Chl *a* in oxic waters (depth <6 m) of Linsley Pond.

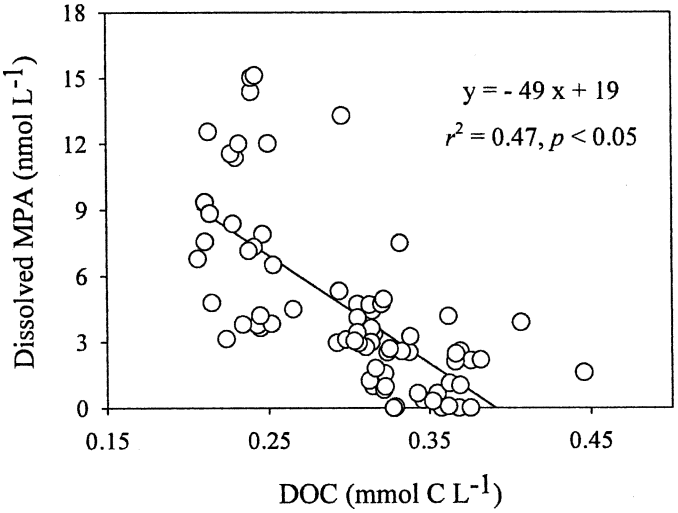

Fig. 6. Relationship of dissolved MPA and DOC in oxic waters (depth <6 m) of Linsley Pond.

metalimnion coincided with the DOC minimum in the same region (Fig. 3A,E). The negative correlation between MPA and DOC in the epilimnion and metalimnion (depth <6 m) is statistically significant (Fig. 6, $r^2 = 0.47$, $p = 0.05$). The co-occurrence of MPA accumulation and DOC depletion at the metalimnion suggests that the mechanisms for MPA production and DOC consumption may be related. The decrease of DOC in the metalimnion of Linsley Pond may be the result of heterotrophic respiration. Without a more complete understanding of the organismal composition and population in the metalimnion of Linsley Pond, we were not able to evaluate the role of bacterial activity on DOC consumption in the metalimnion. It has been shown that MPA may be produced through the metabolism of methionine and homocysteine in anaerobic sediments (Mopper and Taylor 1986; Kiene et al. 1990). As methionine and homocysteine are both commonly occurring amino acids, degradation of settling phytoplankton in the metalimnion is a possible source of these two compounds. In turn, metabolism of these amino acids could serve as a source for MPA in this region. We did not, however, detect methionine with the OPA derivatization method coupled with HPLC analysis (detection limit: ~0.1 nmol L^{-1}) throughout the water column of Linsley Pond. This was not surprising because of the rapid turnover of methionine that is expected for biological amino acids in natural waters (Wetzel 2001). It is unclear whether aerobic bacteria convert sulfur-containing amino acids to MPA, but laboratory incubation experiments indicate that the addition of methionine to metalimnetic waters from Linsley Pond stimulated MPA production (data not shown). This suggests the mechanism where the metabolism of methionine is possibly responsible for the production of MPA in this water. The role of aerobic bacteria in reduced sulfur transformations requires further investigation.

In addition to amino acids, dimethylsulfoniopropionate (DMSP) has been postulated as the precursor of MPA in coastal marine habitats (Kiene and Taylor 1988*b*; Kiene 1996). Although a freshwater dinoflagellate (*Peridinium gatunense*) was found to be able to store DMSP (Ginzburg et al. 1998), to our knowledge, the presence of DMSP in freshwater algae or the water column has not yet been reported in other freshwater lakes. Therefore, DMSP is unlikely to be the precursor of the MPA detected in Linsley Pond.

The upper-hypolimnion Chl *a* maximum in Linsley Pond suggests the possible presence of a GSB community in the thermocline. Although GSB are known to use reduced sulfur as an electron donor during photosynthesis and their ability to use organic reduced sulfur in the same manner has been demonstrated by incubation experiments (Visscher and Taylor 1993), whether GSB could act as a sink for MPA in Linsley Pond remains an interesting question. Given the ease of MPA oxidation under conditions existing in the metalimnion of Linsley Pond, it is plausible to infer from the observed MPA profile that the in situ production of MPA must be quite active to support the MPA peak observed near 4 m. The metalimnetic MPA maximum decreased as the fall turnover began and disappeared completely when mixing proceeded throughout the thermocline in November. The depletion of metalimnetic MPA could be the result of the increased oxygen level in the metalimnion and concomitant disturbance of the bacterial community responsible for MPA production as ventilation by oxygenated surface waters took place.

Higher concentrations of MPA were detected in the anoxic hypolimnion than in the oxic region of Linsley Pond. In the hypolimnion, MPA concentrations increased with depth from an average of 4 nmol L^{-1} at the thermocline to typically 40 nmol L^{-1} close to the sediment–water interface. The occurrence of MPA reported in previous studies has been exclusively associated with anaerobic marine environments, including anaerobic deeper coastal water and anaerobic seawater sediment (Vairavamurthy and Mopper 1987; Sinninghe Damste et

al. 1989). In such environments, MPA was produced by bacterial demethylation of DMSP (Kiene and Taylor 1988*a*) or by an abiotic nucleophilic addition of bisulfide to unsaturated organic compounds (Vairavamurthy and Mopper 1987; Sinninghe Damste et al. 1989). Additionally, MPA might also be produced from the metabolism of methionine and homocysteine in anaerobic sediments. This process involves demethylation, decarboxylation, and deamination of the amino acid molecules (Mopper and Taylor 1986; Kiene et al. 1990). The MPA concentrations measured in the hypolimnion of Linsley Pond were significantly lower than those found in some anaerobic coastal pore waters, which have MPA concentrations as high as 12 μmol L^{-1} (MacCrehan and Shea 1995). Such a discrepancy could be attributed to the absence in Linsley Pond of DMSP, a major MPA precursor in marine and estuarine environments. Considering the fairly abundant quantities of organic materials and sulfide in the hypolimnion of the lake, it is quite likely that the biodegradation of sulfur-containing organic compounds or reactions involving sulfide contribute to the formation of MPA in the anoxic water of Linsley Pond. Sulfur-containing amino acids could be derived from algal cells settling into the hypolimnion. Profiles of hypolimnetic MPA in Linsley Pond exhibited similar vertical and seasonal distribution patterns to that of sulfide (Figs. 1C, 3A), and both profiles showed the appearance of these two species at the very bottom in the early stage of stratification. As anaerobic conditions developed from bottom to upper water, the profiles of sulfide and MPA expanded upward in a similar fashion. The upper boundaries of both the hypolimnetic sulfide zone and the hypolimnetic MPA zones were stabilized at 7 m in midsummer when the stratification was fully established, and both persisted until early winter when surface water ventilated the bottom water. The strong similarity between MPA and sulfide profiles links MPA production possibly through the reaction of sulfide with organic compounds as suggested by previous studies (Vairavamurthy and Mopper 1987; Damste et al. 1989). Currently, it is difficult to distinguish between these two possible mechanisms responsible for the production of MPA in the hypolimnion of Linsley Pond. However, the observation that high levels of MPA were not detected in the bottom portion of the lake in early summer when the water was rich in DOC but not as rich in reduced sulfur favors the abiotic mechanism of MPA production over the metabolic pathway. In this case, the mechanisms for MPA production in both metalimnion and hypolimnion of Linsley Pond must be different because of the vanishingly low sulfide concentrations in the metalimnion.

The presence of abundant MPA in coastal sediments suggested that, in Linsley Pond, a sedimentary source of MPA was possible. More data are needed to evaluate the contribution of sediments to MPA in the overlying water in Linsley Pond.

Occurrence of MPA in additional Connecticut lakes—In spite of the variation in their biological and chemical characteristics, four of the six Connecticut lakes sampled (Black Pond, Beseck Lake, Uncas Pond, and Amos Lake) contained MPA at concentrations similar to those in the oxic water of Linsley Pond. Although no MPA was detected with the SBD-F derivatization method in the other two lakes (Cedar and Bashan), MPA at concentrations between 7 nmol L^{-1} and 10 nmol L^{-1} was measured when after treating samples with TBP, a reductant used to cleave S–S bonds and convert thiols from the oxidized to fully reduced form (Ruegg and Rudinger 1977) (data not shown). The recovery of MPA with tributylphosphine (TBP) indicated that there were sources of MPA in these two lakes, but at the time of sampling the majority had been oxidized. These findings suggest that MPA may be a thiol species ubiquitous in freshwater lakes at very low, but measurable, concentrations. Differences in MPA profiles, however, were observed for these lakes. Cedar Lake and Uncas Pond were the only two lakes that remained stratified at the time of our sampling, but their MPA profiles were quite different from that in Linsley Pond during a similar stratification status. No MPA was found in either the oxic surface water or the anoxic bottom water of Cedar Lake. MPA in the anoxic hypolimnion of Uncas Pond was detected, but at the low levels similar to those found in the oxic water. Variations in chemical and biological characteristics of these lakes are most likely the factors responsible for these differences.

Implication to Cu speciation—Some thiols are strong complexing ligands for trace metals (Krezel and Bal 1999). Detection of thiols at significant concentrations in natural waters suggests that they may play an important role in controlling the speciation of some trace metals (Tang et al. 2000; Al-Farawati and Van Den Berg 2001). However, the interactions between thiols and trace metals go beyond complexation alone. Studies have revealed that trace metals, such as Ni(II), can catalyze the oxidation of thiols by molecular oxygen (Krezel et al. 2003). Trace metals with variable oxidation states, such as Cu and Fe, in addition to catalyzing the oxidation of thiols by oxygen, can anaerobically oxidize thiols (Jameson et al. 1988; Ehrenberg et al. 1989). An important result of our ongoing laboratory experiments show that >95% of MPA (initial concentration = 300 nM) was oxidized in the presence of 10^{-14} mol L^{-1} labile copper (Hu et. al. unpubl. data). Other metals such as Fe may also play a role in the oxidation of some thiols. In light of the large differences in Fe and Cu concentrations in Linsley pond, Fe may be more important in controlling the loss of MPA. The susceptibility of thiols to oxidation, particularly in the presence of trace metals, greatly complicates their potential role in influencing trace metal speciation in oxic waters such as the metalimnetic water of Linsley Pond. In light of this, we postulate that as MPA is produced in the metalimnion of Linsley Pond, it (and likely other thiols as well) undergoes fairly rapid oxidation. Associated with this would be the loss of a potential strong trace metal complexant. The presence of significant concentrations of MPA that we measured in the metalimnion of Linsley Pond would reflect a steady state between the sources and sinks of MPA described above.

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