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Copper complexation during spring phytoplankton blooms in coastal waters

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ABSTRACT

Cupric ion bioassays were conducted throughout the spring phytoplankton bloom season at two stations—one estuarine, dominated by dinoflagellates, the other coastal, dominated by diatoms. Copper-complexing ligands were detected at both locations throughout this period. Ligand concentrations varied between 0.1 and 0.75 μM, with the estuarine concentrations typically 2–4 times higher than coastal values. Ligands from both locations were destroyed by UV-oxidation and had similar conditional stability constants (range $10^{8.3}$ to $10^{9.2}$) that were significantly correlated with pH, suggesting that the complexing materials are organic chelators with weak acid functional groups. All measured or calculated parameters (DOC, ligand concentration, total copper concentration, salinity, and pH) remained relatively constant at the coastal station through time. The estuarine station was more dynamic, with DOC, total copper, and ligand concentrations varying 2–4 fold during the study. Although ligand concentrations were significantly different between the two locations, concomitant fluctuations in total dissolved copper and conditional stability constants resulted in a relatively constant estimate of the maximum free cupric ion activity at both stations (near 10^{-11} M). This suggests that copper toxicity alone was not responsible for the distinctly different estuarine and nearshore phytoplankton assemblages, although sensitive species might have been inhibited at both locations. Major phytoplankton blooms at both sites were not accompanied by changes in DOC or complexation capacity. A significant inverse correlation between ligand concentration and salinity suggests a terrestrial or sedimentary origin for the copper-complexing compounds.

1. Introduction

Recent laboratory studies document a wide variability in the metal sensitivities of marine phytoplankton species (e.g., Sunda and Guillard, 1976; Anderson and Morel, 1978; Gavis *et al.,* 1981). Since toxicity has generally been linked to free metal ion activities, a logical inference is that variations in the metal speciation of natural waters over time and space could mediate the diversity and abundance of the phytoplankton population. This could include neritic/oceanic separation of species (Gavis *et al.,* 1981; Brand, 1982) as well as the localization of other species within unique chemical environments over smaller distances in the coastal zone (Anderson and Morel, 1978; Murphy and Bellastock, 1980).

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Any quantitative assessment of the link between phytoplankton distribution and natural variations in toxicity must include direct measurements of metal speciation in sea water. While such analysis is not yet possible for certain important metals such as copper, a variety of biological and chemical techniques are under investigation. These include ASV (anodic stripping voltammetry) (Duinker and Kramer, 1977; Batley and Florence, 1976), reverse phase liquid chromatography (Mills and Quinn, 1981; Hanson, 1981), MnO_2 adsorption (Van den Berg, 1982), and bioassay techniques using phytoplankton (Davey *et al.*, 1973), or bacteria (Gillespie and Vaccaro, 1978; Sunda and Gillespie, 1979; Sunda and Ferguson, 1983).

The objective of this study was to use one of these techniques (bacterial bioassay) to quantify the temporal and spatial variations in copper complexation in coastal waters and to compare these variations with the observed composition and distribution of phytoplankton. Two study areas were selected on the basis of their significantly different phytoplankton assemblages during the spring bloom season, with the estuarine location typically dominated by dinoflagellates and the nearshore station by diatoms (Hulburt, 1956; Lillick, 1937). Whereas other investigators have invoked differences in nutrients, temperature or vertical mixing as possible explanations for the temporal or spatial separation of these algal classes (e.g., Gran and Braarud, 1935; Hulburt, 1956; Smayda, 1980), this study searched for a separation mechanism in the factors that control copper speciation in sea water.

2. Methods

a. Study areas. Samples were collected at two stations. The first was in Perch Pond (Falmouth, MA), a shallow salt pond with a narrow inlet channel and a shallow sill which restricts tidal flushing. The second station was 8 km away in Vineyard Sound (70°39′W 40°30′N), approximately 2 km from shore and 15 m deep.

b. Sampling. Water samples were collected at bi-weekly intervals from March through June in 1981. Integrated samples were collected using LPE tubing lowered at a constant rate through the water column. A vacuum was maintained in 1-L wide-mouth CPE bottles using a Nalgene hand pump, such that the water sample could be drawn through the tubing and into the bottle without contacting the pump. Initial samples were discarded and the bottles refilled. The fluid transfer cap (with vacuum and intake ports) was then replaced with a new, clean cap, allowing water to be stored in the same container in which it was collected. All sampling was conducted from the leeward side of small boats, positioned to minimize the possibility of contamination. Although the reliance on small vessels precluded the use of laminar flow hoods or other ultra-clean precautions common to larger operations, these methods were deemed suitable for contamination-free collections in shallow coastal waters.

c. Sample processing. Subsamples (100 ml) were immediately fixed with Utermohls solution for phytoplankton enumeration; others (25 ml) were filtered through combusted glass fiber filters, acidified with phosphoric acid, and stored in combusted Pyrex tubes for DOC analysis; a third set (100 ml) were sealed in glass bottles for salinity determinations. All samples were stored in the dark on ice during transport to the laboratory.

On arrival in the laboratory, DOC samples were frozen. Subsamples (120 ml) of the bulk water sample needed for total copper analysis were immediately poured into a polycarbonate filter-funnel containing a Nuclepore 0.2 μm filter. This water filtered by gravity directly into an LPE bottle which was rinsed with sample once and then refilled. The filtration apparatus remained in a laminar flow hood for several hours during this gradual filtration. The sample was then acidified to pH 1 with Ultrex HCl and stored at 4°C until analysis. One liter of the remaining bulk sample was filtered through a "sandwich" of polycarbonate and glass fiber filters (0.2 μm Nuclepore, and GF/F) using a light vacuum (10–15 cm Hg) and saved for bioassay analysis.

All of the equipment used in sample collection and processing (glassware, plastics, filters, etc.) were routinely soaked for several days in 2N HCl following washing (where appropriate) with Micro detergent. After thorough rinsing with deionized, distilled (DDW) UV-irradiated water, the containers were dried in a laminar flow hood. All laboratory manipulations were conducted in the laminar hood as well.

Phytoplankton were enumerated using an inverted microscope, and phytoplankton carbon (μg C l^{-1}) calculated for each species as the product of cell plasma volume, cell density, and the empirically determined conversion factor, F, given by Edler (1979; F = .13 for armored dinoflagellates; = .11 for diatoms and naked dinoflagellates).

DOC was measured by the wet oxidation method of Menzel and Vaccaro (1964); salinity using a bench salinometer; and pH using an Orion 601A meter and 91–05 combination glass electrode (immediately prior to the bioassay). Rainfall data were provided by R. E. Payne of the Woods Hole Oceanographic Institution. UV-irradiated sea water was prepared by 16 hr exposure to a 1200 watt mercury arc lamp in 100 ml quartz tubes.

Total copper was measured using a modification of the cobalt pyrrolidine dithiocarbamate coprecipitation method of Boyle and Edmond (1975). This modification (Boyle, pers. comm.) used 50 ml Teflon centrifuge tubes containing 40 ml of acidified sample. 0.5 ml of a $10^{-1.9}$ M solution of $CoCl_2 \cdot 6\ H_2O$ was added, followed by 1 ml of a 2% solution of recrystalized ammonium pyrrolidine dithiocarbamate (APDC). The resulting precipitate was allowed to settle overnight and then centrifuged for 45 min at 2500 rpm. The supernatant was removed by aspiration and the pellet resuspended in 40 ml of DDW and centrifuged again. Following aspiration, the tubes were spiked with 1 ml of concentrated HNO_3 (Ultrex) and heated in a sand bath. The remaining pellet was dissolved in 1 ml of 0.1 N HNO_3 and the solution transferred to sample vials for analysis. With this procedure, the copper was concentrated 40-fold without being

removed from the Teflon tubes. Copper standards were made up as standard additions to Vineyard Sound water and concentrated by the above method.

Copper analyses were performed on a Perkin Elmer model 4000 Atomic Absorption Spectrophotometer with an HGA 400 graphite furnace and automatic sampling unit. Cobalt recovery (typically 95–100%) was determined using flame atomic absorption on a Perkin Elmer model 403. Reported values are the mean of two analyses. Replicates of the various water types indicate a precision (expressed as one standard deviation relative to the mean) of 5 to 15%.

d. Bioassay procedure. The bacterial clone used was originally isolated by Gillespie and Vaccaro (1978). Although procedures for growing and harvesting the cultures were similar to those described by these authors, we made two important modifications. First, prior to the start of the sampling season, 25 agar slants were started from one culture and then stored at 4°C. Thus, each experiment could be inoculated without concern for contamination during repeated transfer operations. Our second modification was that a liquid culture was inoculated 36 hours before the experiment, with a transfer to fresh medium made 24 hours thereafter to assure that the bacteria were healthy and actively growing prior to the experiment. Cells were harvested by centrifuging and resuspending in 0.2 μm filtered sea water three times; inoculum volumes were determined from turbidity measurements.

The water to be bioassayed was dispensed by 10 ml aliquots into silicone-coated (SC-87, Pierce Chemical Co.) Pyrex culture tubes. Freshly-made copper stock solutions were then dispensed to give 10 sets of triplicate tubes with added copper concentrations ranging from 0–788 nmol kg^{-1}. Another series of triplicate tubes containing the same water was spiked with a copper-NTA buffer solution which fixed the cupric ion activity and thus provided calibration data (Sunda and Ferguson, 1983). We found that 10^{-5} or 10^{-6} M NTA gave satisfactory results in our relatively organic-rich study areas. In some samples, a different NTA concentration was added to a third set of tubes to test if the bioassayed response to cupric ion activity was independent of the concentration of NTA. These duplicate calibrations generally yielded ligand strengths and concentrations within the precision of the method (see below).

After four hours of equilibration with the copper additions, the bioassay and calibration tubes were inoculated with bacteria at a concentration of approximately 10^6 ml^{-1} and allowed to equilibrate for 2 h. Then 0.1 ml of a 0.9 μCi ml^{-1} solution of glucose (specific activity 302 μCi mol^{-1}, Amersham Corp.) was added to each tube. Uptake was terminated after 30 min by the addition of 1 ml of 10% formaldehyde solution. Bacteria were then filtered onto 0.45 μm Gelman Metricel membrane filters and their glucose incorporation measured by liquid scintillation counting techniques.

e. Copper complexation. Since each bioassay run consisted of one set of copper additions to the sea water sample plus an associated series of CuNTA additions to the

same sample, it was possible to reduce the raw data using the "internal calibration" method of Sunda and Ferguson (1983) and Sunda *et al.* (1984). In most cases, calculated ligand strengths and concentrations were the same as those obtained using "external" calibration (i.e., all bioassay samples are analyzed relative to a standard, predetermined calibration curve where 50% inhibition of glucose uptake occurs at pCu^{2+} 9.1; Sunda and Gillespie, 1979). On a number of occasions, however the response of the bacterium in the tubes with Cu-NTA additions differed from the expected relationship such that 50% uptake inhibition occurred at higher cupric ion activity. As will be discussed later, this shift in apparent sensitivity is related to pH effects on ligand strength and to the concentration of natural ligand in the sample. The advantage of the "internal calibration" method is that these effects are common to the bioassay and calibration tubes alike such that the data can be analyzed using the relative difference between the two treatments.

As described in detail by Sunda and Ferguson (1983) and Sunda *et al.* (1984), for each combination of added copper plus NTA in the calibration series, the concentration of CuNTA complex should be equal to the total concentration of copper in that solution (Cu_T) minus the copper concentration that causes the same inhibition of glucose uptake in the NTA-free bioassay samples. These CuNTA estimates can then be used to compute the cupric ion activity in each calibration tube using the following equation (which includes the effects of calcium and magnesium complexes with NTA):

$$(CuNTA) = \frac{(NTA_T)\{Cu^{2+}\}K_{CuNTA}}{\{Cu^{2+}\}K_{CuNTA} + \{Mg^{2+}\}K_{MgNTA} + \{Ca^{2+}\}K_{CaNTA}} \quad (1)$$

In this equation, (NTA_T) represents the concentration of added NTA, Cu^{2+}, Mg^{2+}, and Ca^{2+} are the activities of copper, magnesium and calcium, and K_{CuNTA}, K_{mgNTA} and K_{CaNTA} are stability constants from Martell and Smith (1974). At 20°C, these constants are $10^{12.96}$, $10^{6.41}$, and $10^{5.41}$ respectively. The activities of Mg^{2+} and Ca^{2+} can be computed from published sea water concentrations (Goldberg, 1965; Wilson, 1973) with salinity adjustments using total ion activity coefficients (Whitfield, 1973).

These manipulations lead to a relationship between cupric ion activity and total copper concentration for each bioassay sample. This in turn can be analyzed using Scatchard analysis (Rosenthal, 1967; Robard and Feldman, 1975) to estimate the concentration (L_T) and conditional stability constant (K_c) for the copper-complexing ligand. Given the sensitivity of our bioassay organism, the data in all cases were most easily modelled assuming that the reactions involve one copper atom and one ligand molecule. K_c is valid at the experimental salinity and pH and is defined as:

$$K_c = \frac{(CuL)}{(Cu^{2+})(L)} \quad (2)$$

where (CuL), (Cu^{2+}), and (L) are the concentrations of copper complex, free cupric ion, and "free" ligand, respectively. The latter term includes all ligand complexes

except those with copper. As in Sunda and Ferguson (1983), K_c is defined in terms of concentration rather than activity. Copper speciation can be described by the equation:

$$Cu_T = \frac{\gamma\,(Cu^{2+})}{R} + \frac{L_T\,(Cu^{2+})\,K_c}{1 + (Cu^{2+})\,K_c} \tag{3}$$

where γ is the activity coefficient, and R is the ratio of cupric ion activity to the concentration of dissolved inorganic copper species (Sunda and Gillespie, 1979). This ratio is independent of Cu_T and varies inversely with pH such that $R = 10^{-2.0}$ at pH 8.0 and $10^{-1.8}$ at pH 7.8. This relationship is based on stability constants for copper-inorganic complexes (Sunda and Hanson, 1979; Zuehlke and Kester, 1983) and the UV-bioassay data in this paper. Since Scatchard analysis yields values for L_T and K_c, and since Cu_T can be measured, Eq. 3 can be used to estimate the cupric ion activity of the original sample.

3. Results

a. Phytoplankton. The two study areas were dominated by significantly different phytoplankton assemblages. In Perch Pond, a dense bloom of the dinoflagellate *Heterocapsa triquetra* accounted for most of the phytoplankton carbon until late April; this was followed by another dinoflagellate, *Dinophysis acuminata,* in May (Fig. 1F). The only diatom of significance, *Chaetoceros simplex,* did account for over 80% of the phytoplankton carbon in June however.

Vineyard Sound was dominated entirely by diatoms, with dinoflagellates comprising a maximum of 3% of the phytoplankton carbon (Fig. 2F). *Chaetoceros compressus* was the dominant species through the end of April, followed by a large bloom of *Rhizoselenia setigera* in early June (2×10^5 cells l^{-1}). *H. triquetra,* present in bloom concentrations 8 km away in Perch Pond, was undetectable in Vineyard Sound samples.

b. Physical and chemical characteristics. The influence of rainfall and groundwater flow can be seen in the Perch Pond salinity and pH measurements, which ranged from 23-28‰ and 7.3–8.0, respectively (Table 1). These variations were reflected in the total copper concentrations, which were generally higher at lower salinities (Figs. 1A, 3A), averaging 9 nmol kg^{-1}. Perch Pond DOC measurements show a large peak at the end of the *H. triquetra* bloom in late April and some indication of an increase thereafter (Fig. 1C).

In contrast, Vineyard Sound exhibited a much more stable environment. Salinity remained nearly constant over time at 32‰ (Fig. 2B), as did total copper at 6–8 nmol kg^{-1} (Fig. 2A, 3A). DOC concentrations were also generally lower and fluctuated less than those in Perch Pond (Fig. 2C).

Table 1. Copper complexation data summary.

Date	Location	Sal. (‰)	Cu_T nmol kg^{-1}	DOC (mg l^{-1})	pH	L_T (μM)	Scatchard log K_c	Calc. pCu^{2+}	CuL (% of Cu_T)
3/03	PP	28.1	7.4	1.3	7.3	0.46	8.3	10.7	95
	VS	32.2	7.6	1.6	7.9	0.18	8.9	10.9	87
3/10	PP	28.0	7.2	1.1	7.8	0.18	8.8	10.8	88
	VS	32.3	6.6	1.4	8.0	0.10	9.0	10.8	81
3/24	PP	27.9	8.9	1.1	8.0	0.26	8.9	10.9	87
	VS	32.1	8.7	1.4	8.0	0.19	9.0	10.9	87
4/07	PP	26.8	8.5	1.2	7.9	0.40	8.8	11.1	92
	VS	32.5	6.3	1.0	8.0	0.16	9.0	11.1	85
4/16	PP	25.0	10.0	0.9	7.8	0.43	8.7	10.9	93
	VS	32.5	8.13	0.9	7.8	0.22	8.8	10.9	89
4/28	PP	28.1	9.3	2.4	7.9	0.43	8.8	11.1	93
	VS	31.9	7.8	1.1	8.1	0.10	9.2	11.0	84
5/11	PP	24.5	15.8	1.3	7.5	0.75	8.6	10.9	98
	VS	32.2	7.8	1.0	7.9	0.15	8.7	10.7	80
5/18	PP	24.4	10.5	1.4	7.6	0.44	8.7	10.9	96
	VS	32.3	7.8*	NM	7.8	0.21*	8.7*	10.8*	87*
6/03	PP	26.8	6.6	1.78	7.8	0.42	8.9	11.3	95
	VS	32.1	8.7	0.9	7.9	0.09	8.8	10.5	70
6/22	PP	22.6	13.2	1.9	7.7	0.55	8.6	10.8	95
	VS	31.9	6.9	1.1	7.9	0.15	8.9	10.9	85

*No Cu_T measurement; 7.8 nmol kg^{-1} assumed.
NM = not measured

c. Copper complexation. The pattern of ^{14}C-glucose incorporation varied over time in Perch Pond and consequently the calculated complexation capacity or concentration of copper-complexing ligand (L_T) fluctuated as well (Fig. 1D). L_T concentrations were relatively constant through time at the coastal Vineyard Sound station. Ligand concentrations were significantly higher in Perch Pond than in Vineyard Sound (Wilcoxons' signed ranks test, $P < 0.01$; Sokal and Rohlf, 1981), with values between 0.18–0.75 and 0.09–0.21 μM respectively (Table 1). Total copper (Cu_T) was also significantly higher in Perch Pond ($P < .01$). In contrast, ligand strength was significantly lower in the estuarine samples ($P < .01$), where the conditional stability constant (K_c) ranged between $10^{8.3}$ and $10^{8.9}$ versus $10^{8.7}$ to $10^{9.2}$ in Vineyard Sound.

If data from the two stations are combined, significant ($P < .01$) inverse relationships between salinity and both Cu_T and L_T become apparent (Fig. 3). Similarly, when log K_c from all samples is plotted as a function of pH (Fig. 4), a significant linear relationship is obtained:

$$\log K_c = (0.9)\,\mathrm{pH} + 1.7 \qquad (R^2 = 0.81;\ P < .001) \tag{4}$$

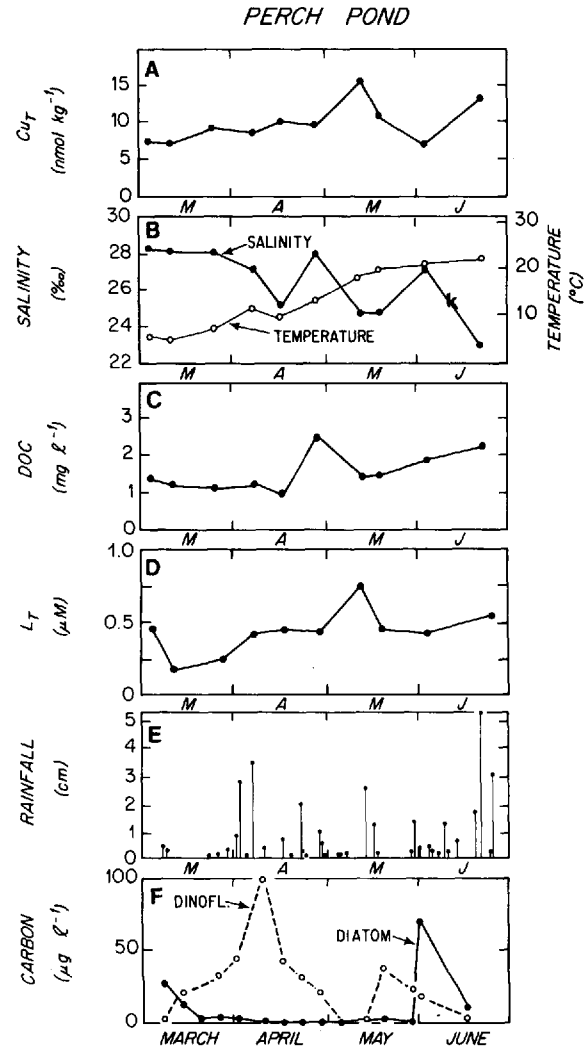

Figure 1. Perch Pond time series. (A) Total dissolved copper; (B) Salinity (●) and temperature (○); (C) Dissolved organic carbon; (D) Copper-complexing ligand concentration; (E) Rainfall; (F) phytoplankton carbon, dinoflagellate (○) and diatom (●).

This suggests that we may be detecting the same ligand in all samples and that copper binding varies with pH. Thus at pH 8 we would expect log K_c = 8.9; at pH 7.5, log K_c = 8.45. Despite the highly significant correlation in Eq. 4, it should be stressed that the observed relationship may be coincidental, as would be the case for example, if natural factors controlling pH also determined the type of ligand present in our samples.

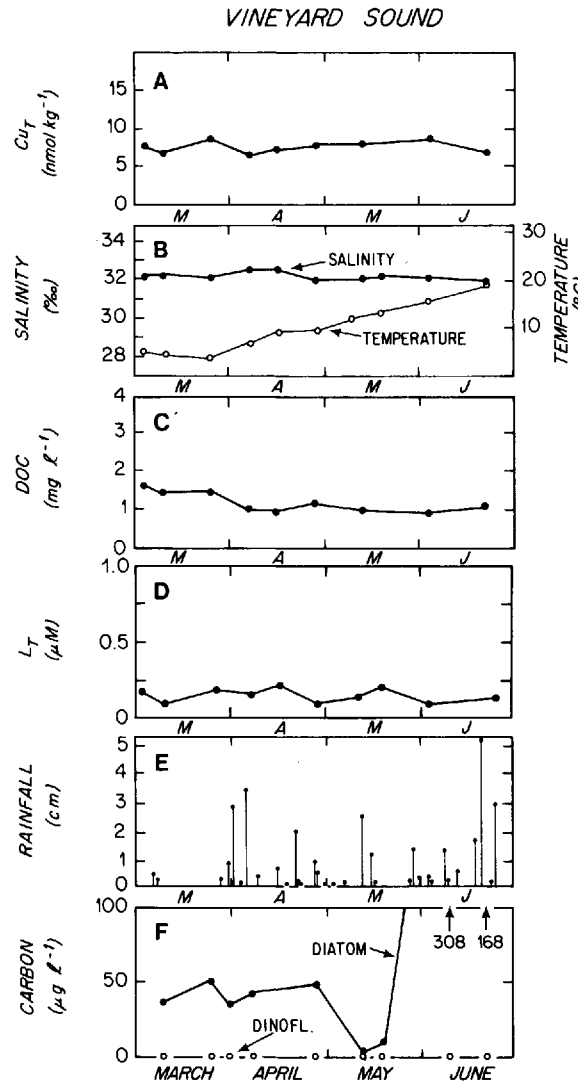

Figure 2. Vineyard Sound time series. Symbols same as in Figure 1.

Since the capacity to complex copper could be removed from both water types by UV-irradiation (Fig. 5), the bioassay data from UV-treated samples provided an opportunity to estimate the value of R in Eq. 3 (the ratio of cupric ion activity to the concentration of dissolved inorganic copper species). Assuming inorganic complexation only, the data in Figure 5 could be modeled with $R = 10^{-2.1}$ for Perch Pond water at pH 8, and $R = 10^{-2.0}$ for Vineyard Sound at pH 8.1. These values are quite close to those obtained using published stability constants for copper inorganic complexes in

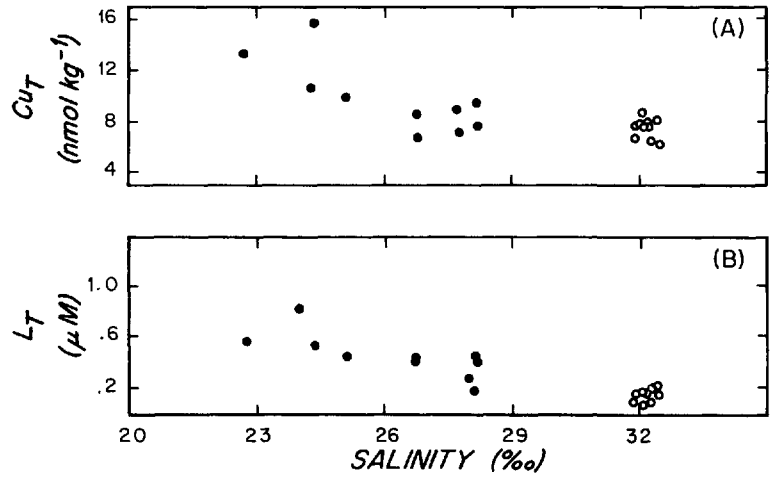

Figure 3. Total dissolved copper (A) and copper complexing ligand concentrations (B) versus salinity. Perch Pond (●), Vineyard Sound (○).

sea water (Sunda and Hanson, 1979; Zuehlke and Kester, 1983), which suggests that the bacterial response to copper in our samples was mediated by naturally-occurring ligands and not by artifacts such as bacterial exudates or copper adsorption, for example. UV-irradiated water containing an added ligand with known concentration

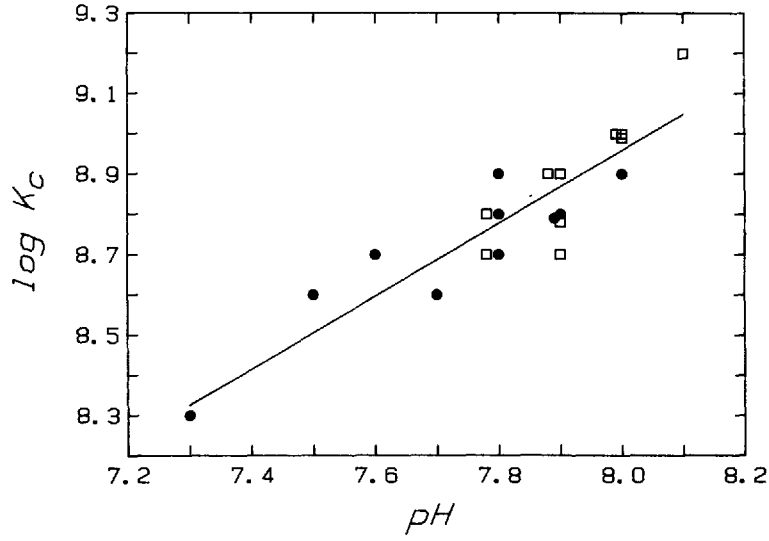

Figure 4. Variations in the conditional stability constant K_c (Eq. 1) with pH; Perch Pond (●), Vineyard Sound (○).

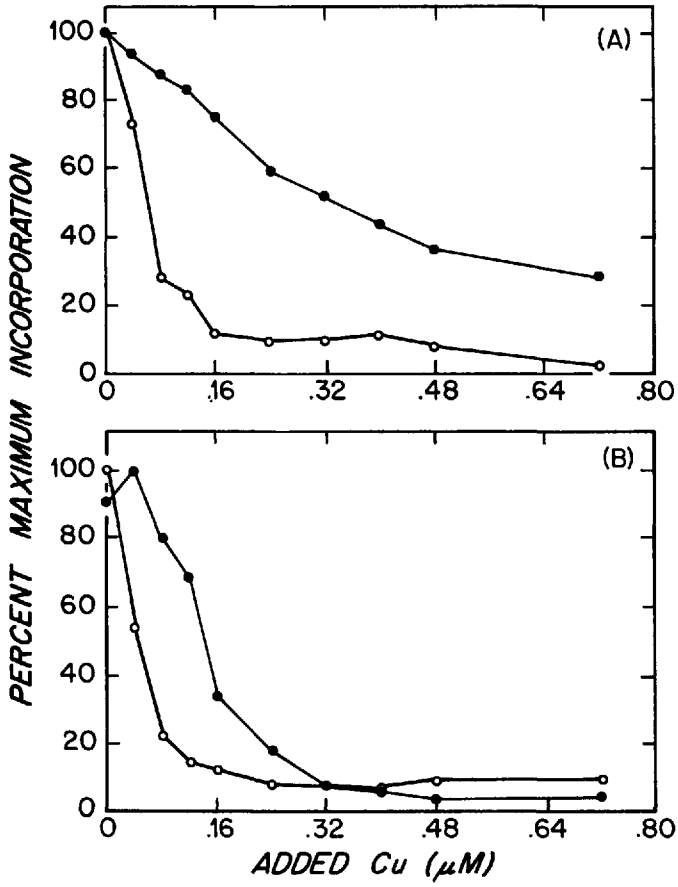

Figure 5. Bioassay results before (●) and after (○) UV-irradiation. (A) Perch Pond, pH = 8.0; (B) Vineyard Sound, pH = 8.1. Note that in both cases, the UV curve can be modelled using inorganic copper complexation only, with R in Eq. 3 equal to $10^{-2.1}$ in A and $10^{-2.0}$ in B.

and strength was also used to test the method. When 0.2 μM of histidine was added, the bioassay results indicated a ligand concentration of 0.18 μM and a conditional stability constant of $10^{9.2}$. The equivalent conditional constant calculated using the chemical equilibrium program MINEQL (Westall *et al.*, 1976) was $10^{9.3}$ at pH 8.1.

A measure of the precision of this method was obtained by running the same Perch Pond water sample through the bioassay procedure five times over a period of 2 months. Between analyses, the filtered sample was stored at 4°C in the dark—conditions that do not alter the complexation characteristics significantly (Sunda and Gillespie, 1979). Replicate estimates of L_T ranged from 0.17 to 0.23 μM and K_c from $10^{8.9}$ to $10^{9.24}$. The coefficients of variation for L_T and K_c were 13 and 32% respectively.

4. Discussion

Several of the factors that control copper speciation in sea water varied significantly between an estuarine and a nearshore coastal station. The coastal site was characterized by relatively low and invariant concentrations of total copper and copper-complexing ligands with high conditional stability constants, whereas the estuarine site had higher and more variable concentrations of copper and ligand but lower ligand strengths. Despite these differences, estimates of the maximum free cupric ion activity were quite similar between the two stations through time ($\sim 10^{-11}$ M), a level inhibitory to only the most sensitive phytoplankton species. These calculations suggest that copper toxicity alone was not responsible for the distinctly different phytoplankton assemblages observed at the two stations. Major phytoplankton blooms at both sites were not accompanied by significant changes in DOC or complexation capacity, although an increase in L_T did occur at the estuarine site several weeks after one bloom ended. A significant inverse correlation between ligand concentration and salinity suggests a terrestrial or sedimentary origin for the copper-complexing compounds. These observations and inferences will be discussed in detail below.

a. Variations over time and space. Looking first at the Perch Pond data, it is evident that most of the measured and calculated parameters fluctuated significantly throughout the study. The observed total copper and pH variations are consistent with variable inputs of freshwater (via streams and groundwater) containing relatively high levels of copper. The ligand concentration increased slightly throughout the study, with a peak in May. The highest and lowest concentrations differed by a factor of 4, well beyond the uncertainty of the bioassay analysis. The Vineyard Sound data, in contrast, were more uniform, with total copper, salinity, DOC, and ligand concentration virtually constant through time.

Any evaluation of the implication of variations in L_T with respect to copper speciation must account for concomitant fluctuations in the total copper concentration and the strength of the ligand (K_c). Eq. 3 incorporates all of these parameters and permits the free cupric ion activity of the original sample to be calculated. It is important to note, however, that the sensitivity of our bacterial clone places limitations on these estimates of pCu^{2+}. When our data are plotted for Scatchard analysis, the best fit is generally a straight line indicative of the presence of one ligand. Using natural bacterial communities as bioassay organisms, Sunda and Ferguson (1983) were able to extend the range of pCu^{2+} over which inhibition could be monitored, with the result that two ligand models were often needed to account for the curvature in their Scatchard plots. In addition to one ligand in relatively high concentrations with $K_c \sim 10^9$ as we found, these authors detected another ligand at lower concentrations but with $K_c \sim 10^{11}$. Since our method could not resolve the latter class of strong ligands at low concentrations, calculations of cupric ion activity in our samples, therefore, yield maximum estimates. Thus the pCu^{2+} values in Table 1 may over-estimate the true

toxicity of copper in our samples, but they do provide an upper bound which is nevertheless informative.

Our calculated pCu^{2+} values ranged between 10.7 and 11.3 in Perch Pond and between 10.5 and 11.1 in Vineyard Sound. In both cases, this represents a 4-fold difference between extremes ($\Delta pCu^{2+} = 0.6$).

Although the calculated strength and concentration of the complexing ligands varied over time and between stations, it is our belief that all ligands detected fall into one general class of compounds. At both stations, copper complexing capacity was removed by UV-irradiation. More importantly, K_c values from both stations were highly correlated with pH, with one regression (Eq. 4) providing an excellent fit to the combined data. This suggests copper binding to ligands with weak acid functional groups, a conclusion consistent with the data of Sunda and Hanson (1979) who observed slopes of $\Delta \log K_c/\Delta pH$ between 1.0 and 1.3 for copper-binding ligands in river water. Our equivalent slope from Eq. 4 is 0.9.

Comparisons between these data and those from other studies that have examined variations in copper complexation in sea water are complicated by differences in analytical technique and the likelihood of contamination in older studies. One commonly reported parameter is the percent of copper that is organically bound (CuL). Foster and Morris (1971) used solvent extraction to show that 9 to 40% of Cu_T in Menai Straits, Wales was organically bound. Using resin chromatography, Montgomery and Santiago (1978) measured CuL as 17 to 48% of Cu_T along a coastal transect near Puerto Rico. Mills and Quinn (1981) and Hanson (1981) used C_{18} reverse phase liquid chromatography to estimate CuL in Narragansett Bay (8 to 56%) and in the Connecticut River estuary (24 to 60%) respectively.

Several techniques yield considerably higher CuL percentages. Duinker and Kramer (1977) and Huizenga (1982) found 80 and 91% of Cu_T in surface waters of the North Sea and North Atlantic slope (respectively) to be non-ASV labile (presumably CuL). With a reasonable assumption about the initial total copper concentration, a MnO_2 adsorption method by Van den Berg (1982) indicated that more than 95% of the copper in sea water from the Irish Sea was organically bound. Sunda and Ferguson (1983) used bioassay techniques similar to those described here and found 82 to 99% organically bound copper in five sea water types, ranging from highly productive coastal to oligotrophic oceanic stations. Finally, Sigimura and Suzuki (1981) reported 90% complexation of copper in North Pacific surface sea water using ligand exchange methods.

For comparative purposes, the percentage of organically-complexed copper observed in our samples is reported in Table 1. With the exception of one value of 70%, the remainder are between 85 and 98%. The data thus agree with those studies reporting relatively high copper complexation in natural waters.

A few studies also report ligand concentrations and/or conditional stability constants. As in the CuL comparisons, our data are similar to the bioassay results of

Sunda and Ferguson (1983), although their analysis indicated the presence of two classes of ligands. The ligand present in the highest concentrations (0.02–0.13 μM) in nearshore waters had K_c values ranging from $10^{8.9}$ to $10^{9.8}$ (pH 8.1–8.2), similar to those we obtained. A much stronger ligand in 5 to 20 nM concentrations had $K_c > 10^{11}$. Similarly, Gachter *et al.* (1978) found two classes of ligands in lake water samples and reported conditional stability constants of $10^{7.4}$ and $10^{10.3}$ at pH 8.1. Ligand concentrations near 0.1 μM and conditional constants of $10^{9.8}$ were reported by Van den Berg (1982) in surface water from the Irish Sea and the Atlantic Ocean. Mantoura *et al.* (1978) extracted organic ligands from coastal sea water and reported overall copper stability constants of $10^{8.9}$ to $10^{10.2}$. If these values are corrected for calcium and magnesium competition, the resulting conditional constants (equivalent to our K_c) would be several orders of magnitude lower, assuming that these cations bind to the same sites as copper. One test of this assumption was made by Sunda and Hanson (1979) who used a cupric ion electrode to study humic materials in river water. Their results indicated that the addition of calcium or magnesium to their samples had little effect on the conditional stability constants calculated for copper. The uncorrected constants of Mantoura *et al.* (1978) may thus be valid representations of the true K_c for copper reacting with organic ligands in sea water, and their values are in excellent agreement with those reported here.

In general, then, the different methodologies used in these studies make comparisons difficult. There does seem to be some consistency in measured ligand concentrations—ranging from 0.1 to 0.5 μM in different water types, but the data on stability constants differ both in the number of ligands present and in the affinity of these ligands for copper. For this reason, it is more appropriate to stress the relative differences between our stations or the variations over time at each station, rather than to place undue emphasis on the absolute concentration of free or complexed copper that we have calculated.

In this context, it seems that the relatively small differences in our estimates of the maximum cupric ion activity in Perch Pond and Vineyard Sound cannot account for the strikingly different phytoplankton assemblages observed. Figure 1F and 2F document a clear dominance of dinoflagellates in Perch Pond and diatoms in Vineyard Sound, but there was no systematic difference in calculated cupric ion activities.

To relate these calculations to phytoplankton sensitivities, it is necessary to refer to laboratory culture studies that measure growth inhibition in well-buffered copper-chelator systems similar to those used in our bioassay calibration. The most comprehensive survey is that of Gavis *et al.* (1981) who showed that only 1 of 24 clones (11 species) tested had an inhibition threshold at cupric ion activities less than 10^{-11}M while 8 of the 24 showed sight inhibition near $10^{-10.8}$. The remaining 15 isolates tolerated cupric ion activities well above the maximum levels we calculated for Perch Pond and Vineyard Sound. Recognizing the uncertainties in the pCu^{2+} calculations for our natural water samples, it is nevertheless clear that diverse phytoplankton popula-

tions could have flourished even if our estimates of the maximum cupric ion activity were correct, with inhibition of only the most sensitive species. The presence of 10–20 nM concentrations of a second ligand with a strong affinity for copper ($K_c > 10^{11}$; Sunda and Ferguson, 1983) would increase our cupric ion activity estimates to $10^{-11.5}$ or 10^{-12}M, a range where copper inhibition of any species is highly unlikely. We are left with the inference that factors such as vertical mixing, grazing, pressure, nutrients, temperature, or other metals and/or metal ratio effects caused the phytoplankton crop differences. In the latter instance, it should be emphasized that Rueter and Morel (1981) and Sunda *et al.* (1981) demonstrated that both zinc and manganese can alter the toxicity of copper to phytoplankton. Examination of these interactions was beyond the scope of this study, however.

b. Ligand source. One possible source for the ligands detected in this study would be terrestrial runoff with its associated humic and fulvic components. The gradual input of this material to coastal waters from groundwater or river flow is consistent with the relatively steady background L_T concentrations observed, the significant inverse correlation between L_T and salinity, and the generally higher ligand concentrations reported here compared to more oceanic regions (Sunda and Ferguson, 1983).

It is noteworthy that DOC concentrations did not change during either the Perch Pond dinoflagellate bloom or the two Vineyard Sound diatom blooms. In Perch Pond, however, the sharp decline of the *Heterocapsa* population was followed by a DOC increase, which was followed in turn several weeks later by a peak in L_T. Sampling was unfortunately terminated before the decline of the large *R. setigera* bloom in Vineyard Sound in late May, so a similar effect, if present, was not observed.

The overall sequence at both stations suggests that the actively growing phytoplankton populations did not change background DOC or L_T concentrations significantly (Sharp, 1977; McKnight and Morel, 1979). The delayed peak in L_T seen in Perch Pond may be linked to restricted tidal flushing and processes associated with bloom decline (cell mortality, bacterial degradation, or excretion and decomposition during grazing; Ogura, 1977; Allen, 1976; Hasle and Abdullah, 1981), but a transformation step would be required to convert relatively unreactive "new" DOC into compounds with high affinities for copper. Such a pathway was recently proposed by Harvey *et al.* (1983), converting biogenic polyunsaturated fatty acids (which would be produced in large quantities by nutrient-limited phytoplankton) to marine fulvic acids through oxidative crosslinking.

The lack of significant complexation during the phytoplankton blooms in our study appears to be at odds with a number of laboratory studies that document the release of micro-molar concentrations of copper-complexing agents in phytoplankton cultures (Fogg and Westlake, 1955; Swallow *et al.*, 1978; McKnight and Morel, 1979; Van den Berg *et al.*, 1979; Fisher and Fabris, 1982). Since the cell densities in the laboratory cultures were typically several orders of magnitude higher than the bloom populations

we observed in natural water samples, however, it is reasonable to expect that phytoplankton-produced complexing ligands would have been in the 10^{-8} M range in our samples (McKnight and Morel, 1979), well below our detection limits and the 0.1–0.75 μM ligand concentrations observed throughout the study. It would appear that the bulk of the copper complexation in our study area was due to refractory compounds of terrestrial origin.

Other explanations for the DOC and L_T increases are possible, however. For example, Hines *et al.* (1982) document a late spring surge in pore water DOC in estuarine surface sediments, presumably linked to increasing temperature and the resulting changes in microbial activity and bioturbation. In shallow areas, this DOC could be introduced to overlying waters by mixing events, leading perhaps to water column increases. In this instance, our observed sequence of bloom decline, DOC increase, and L_T increase at the estuarine location (Fig. 1) would be purely coincidental. Regardless of the source of DOC, however, our data demonstrate that the phytoplankton blooms we monitored were not associated with a direct production of detectable levels of copper-complexing compounds. It is possible that these ligands eventually formed during the bloom decomposition and remineralization process, but such a speculation awaits more detailed studies than those presented here.

c. Overview. Given the differences between reported values of complexation capacity and ligand strength among the many studies on this topic, the validity of any method should be closely scrutinized. The bioassay technique employed here provides reproducible estimates of these parameters upon replicate analyses, fails to detect organic complexation following UV-irradiation of the samples, and correctly estimates the conditional stability constant of well-defined chelators such as histidine. On the negative side, a major concern with our method is whether the NTA added for calibration alters the relationship between cupric ion activity and glucose uptake. Several studies indicate the synergistic effects of other metals such as Zn or Mn on copper toxicity in phytoplankton (Rueter and Morel, 1981; Sunda *et al.*, 1981), so it is reasonable to question whether these metal ratio effects are relevant to bacterial systems and, if so, whether NTA additions to the calibration tubes create ratios sufficiently different from those in the NTA-free tubes to alter the bacterial response to cupric ion activity. NTA does not have a sufficiently high stability constant to alter manganese ion activities at the NTA concentrations used in this study, but concerns over shifts in zinc ion activity are justified. However, duplicate analyses using different NTA concentrations generally give estimates of L_T and K_c, that were the same within the precision of our method. Furthermore, a recent review by Sunda *et al.* (1984) found a good agreement between bioassay results and direct measurements of cupric ion activity in freshwater samples using a cupric ion electrode. We conclude that metal ratio effects, if present, are not sufficiently large to materially affect our results.

Cupric ion bioassays, although cumbersome and time consuming, do provide useful

insight into the parameters that control copper complexation in natural waters. These methods have permitted us to compare two different marine environments and to demonstrate that there were significant differences in complexation capacity between the two stations, but that differences in pH, total copper concentration, and conditional stability constants counter-balance or compensate for the excess ligand.

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