

The complexation of ‘dissolved’ Cu, Zn, Cd and Pb by soluble and colloidal organic matter in Narragansett Bay, RI

Mark L. Wells^{*}, Peter B. Kozelka, Kenneth W. Bruland

Institute of Marine Sciences, University of California, Santa Cruz, CA 95064, USA

Abstract

It is widely accepted that the speciation of most bioactive metals in seawater is regulated by natural organic ligands, but the nature of these molecules has remained a mystery. We used a combination of physical and chemical separation schemes to better characterize organic molecules complexing Cu, Zn, Cd, and Pb in Narragansett Bay, RI. Conventionally filtered (< 0.2 μm) surface water samples were partitioned by cross-flow ultrafiltration into < 8 kDa and < 1 kDa size fractions and the chemical speciation of metals was determined in each fraction. The results show that organic molecules which complex bioactive metals have markedly different size distributions. The majority (~ 90%) of chelated Zn and Cd, along with their respective unbound ligands, resided in the operationally-defined soluble (< 1 kDa) phase. In contrast, ~ 50% of the chelated Cu was colloidal (> 1 kDa), with the bulk of it found in the 1–8 kDa colloidal size range. Of the three Cu-binding ligand classes measured, the strongest class occurred mainly in the soluble fraction while the weaker Cu-binding ligand classes were predominantly colloidal (> 1 kDa). Approximately 40% of chelated Pb was colloidal but, in contrast to Cu, the bulk of these ligands resided in the larger colloidal size range (8 kDa–0.2 μm). Thus, a continuum of metal complexing ligand size exists, spanning from truly soluble to colloidal, the nature of which differs for individual bioactive metals. These findings support the hypothesis that metal complexation in seawater is dominated by distinct, metal-specific ligand molecules. A central question that now emerges is whether these ligand molecules function predominantly to buffer metal ion activities in seawater, thereby decreasing metal sorption to particulates, or to facilitate metal removal by sweeping organically bound metals into particulate phases via colloid aggregation. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: metals; speciation; ultrafiltration; seawater; ligands; colloidal organic matter; Narragansett Bay

1. Introduction

It is well known that organic complexation dominates the chemical speciation of many dissolved trace metals in seawater, thereby regulating their availability to the biota (Buffle, 1988; Bruland et al., 1991; Donat and Bruland, 1995). It has been presumed that these ligands are soluble molecules that

serve to maintain free metal ion activities within the physiological tolerance range for microorganisms, as well as limit the scavenging loss of bioactive metals to sinking particles. While there is now evidence that some phytoplankton species can be a direct source of Cu-binding ligands (Gonzalez-Davila et al., 1995; Moffett and Brand, 1996), little has been discerned about the direct source of other metal-binding ligands or their physical and chemical natures. Given that a major fraction of marine dissolved organic matter occurs in sub-micron (colloidal) particles

^{*} Corresponding author. Fax: +1-408-459-4882; e-mail: mwells@cats.ucsc.edu

(Wells and Goldberg, 1991; Benner et al., 1992; Guo et al., 1994; Niven et al., 1995), it is possible that some component of metal complexing ligands may not reside in true solution. This distinction is important because aggregation of colloidal organic matter is proposed to be an important mechanism for removing carbon and metals from ocean surface waters (e.g., Honeyman and Santschi, 1989). If so, high affinity metal-complexing moieties in the colloidal phase would greatly improve colloidal scavenging efficiencies, but this view is at odds with the conceptual paradigm that dissolved organic ligands retard the scavenging loss of metals from surface waters (Bruland et al., 1991; Johnson et al., 1997).

Colloidal particles that occur within conventionally filtered (< 0.2 to 0.5 μm) waters are extremely important in the removal of 'dissolved' metals in low salinity estuarine environments (e.g., Sholkovitz and Copland, 1981). Recent improvements in analytical and physical separation methods have verified the importance of colloid aggregation in high salinity estuarine and coastal waters (Whitehouse et al., 1989; Sigleo and Means, 1990; Benoit et al., 1994; Dai et al., 1995; Martin et al., 1995; Sanudo-Wilhelmy et al., 1997; Wells et al., submitted). Although there are few reports of colloidal bioactive metals in oceanic waters (Moran and Moore, 1989; Greenamoyer and Moran, 1997; Wells, in preparation; Powell and Landing, submitted), theoretical arguments based upon total particle mass measurements suggest that colloidal phases are a significant component of dissolved bioactive metals throughout the world's oceans (Moran et al., 1996).

The effect of colloid aggregation on the biogeochemical cycling of bioactive metals in ocean waters increasingly is being probed using ^{234}Th (Baskaran et al., 1992; Moran and Buesseler, 1992; Moran and Buesseler, 1993; Moore and Hunter, 1995; Niven et al., 1995; Moran et al., 1996). Sorption of this highly surface-active tracer to colloids is expected to be controlled largely by non-specific surface interactions. However, if bioactive metals instead become associated with colloids via highly-specific metal chelation, whereby 'sorption' is analogous to specific metal complexation reactions occurring in true solution, then it would be difficult to predict these metal-colloid associations from Th sorption data. Indeed, recent evidence of metal complexing ligands

in the marine colloidal phase (Muller, 1996) raises questions about how effective Th isotopes are for tracing bioactive metal-colloid cycling.

In this study we used a combination of physical and chemical separation schemes to better characterize the nature of organic ligands responsible for complexing Cu, Zn, Cd and Pb in Narragansett Bay, RI. Conventionally filtered surface water samples were processed by cross-flow filtration (CFF) to partition metals and ligands into soluble (< 1 kDa), small colloid (1–8 kDa) and larger colloidal (8 kDa–0.2 μm) size fractions. Our goal was to determine where metal-specific organic ligands resided in this size continuum. Chemical speciation analyses were performed by differential pulse anodic stripping voltammetry (DPASV) using a thin mercury film electrode (Nurnberg, 1977; Coale and Bruland, 1988). We report the (metal-specific) ligand concentrations [L_i] and their conditional stability constants, $K_{ML_i,M}^{\text{cond}}$ for all four metals within the dissolved (< 0.2 μm), < 8 kDa permeate and < 1 kDa permeate fractions. These data are a small subset of a larger study of dissolved metal complexation in Narragansett Bay reported by Kozelka and Bruland (1998).

2. Materials and methods

2.1. Study site

We collected surface waters in the high salinity regions of Narragansett Bay, RI (Fig. 1) during June 1994. Three sites were sampled; one in the Providence River region of upper Narragansett Bay and one each in the central and lower regions of the embayment. Only saline ($S = 24$ –31) portions of the bay were sampled to avoid ionic strength driven physicochemical processes which strongly affect metal and organic geochemistry in low salinity, estuarine environments (e.g., the coagulation and subsequent removal of river-borne Fe-humic colloids—Sholkovitz, 1976; Sholkovitz et al., 1978). The Upper Bay site, closest to the Providence municipal wastewater discharge, was chosen because elevated levels of both nutrients and trace metals were expected. The Middle Bay site characteristically hosts persistent phytoplankton blooms during summer

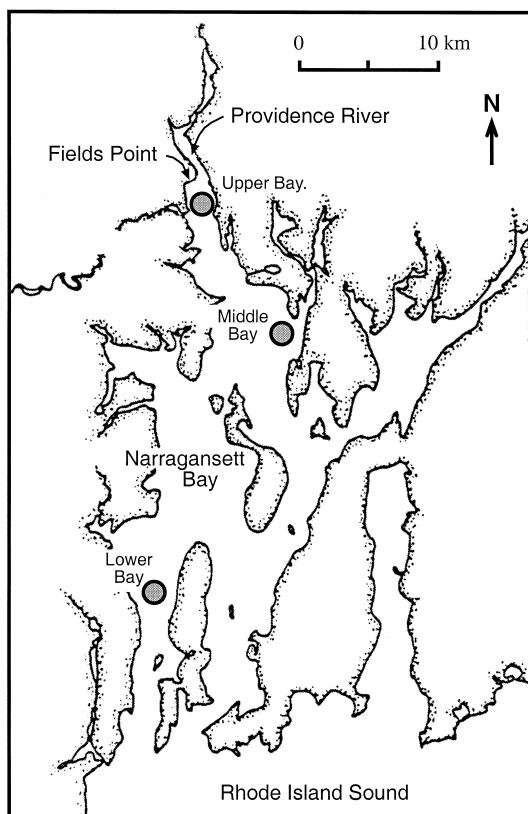


Fig. 1. Station locations in Narragansett bay, RI. The upper bay station (off Sabin Point) is downstream from the primary wastewater discharge of the city of Providence, the Mid-bay station (off Rumstick Point) is the bloom site characterized by high phytoplankton biomass during summer months, and the lower bay site (off General Rocks) is typical of coastal water conditions. Figure adapted from Pilson and Hunt (1989).

months (Sunda, personal communication), apparently fueled by the resupply of nutrient-rich surface waters advecting from the upper bay. In contrast, the Lower Bay site near the mouth of Narragansett bay has lower metal, nutrient and phytoplankton concentrations during summer months and is representative of typical coastal seawater conditions.

2.2. Sample collection

Samples were collected from 1 m depth using acid-cleaned Teflon[®] tubing and a battery operated peristaltic pump. Dissolved samples (2 l) for total metal and chemical speciation analyses were conven-

tionally filtered at the time of collection by attaching an in-line, acid-cleaned 0.2 μm polypropylene cartridge filter (MSI). Samples for DPASV analyses were collected in 1 or 2 l acid-cleaned Teflon (FEP) bottles, triple bagged in plastic and kept on ice in the dark at 4°C until analysis. Cross-flow filtration (CFF) samples were similarly filtered into 20 l fluorinated polyethylene carboys, and immediately transported back to shore for processing.

Cross-flow filtrations and electrochemical analyses of these samples (see below) were performed in a HEPA (class 100) laminar flow clean air bench inside a portable analytical van situated at the University of Rhode Island Narragansett Bay campus. Cross-flow processing was completed within 8 h of sample collection. All conventional filtrate, CFF retentate and permeate samples retained for total metal determinations were acidified with quartz-distilled 6 N HCl to pH 2 and stored several months before analysis. Electrochemical analyses were performed at room temperature on either fresh samples or thawed samples which had been freshly frozen upon collection; freezing is the only method we have found which preserves the speciation of trace metals (Bruland, unpublished data).

2.3. Cross-flow filtration

Fresh dissolved (<0.2 μm) samples were processed with a trace metal clean cross-flow filtration (CFF) system consisting of two polycarbonate leaves fitted with Teflon[®] connectors, a Teflon[®] double-diaphragm pump, and a Teflon[®] backpressure valve, control valves and tubing. Two silicone encapsulated Filtron[®] membranes were used; a 1 kDa or 8 kDa nominal molecular weight cutoff. During CFF processing, concentration polarization effects, which greatly slow filtration rates, are minimized by recirculating sample water from the 20 l reservoir tangentially across the membrane surface and back to the sample reservoir. With each pass, a small portion of the solution and solutes smaller than the molecular weight cutoff permeates through the membrane, while larger substances become concentrated in the decreasing volume of sample retentate.

Although this field study was completed before the recent Colloid Intercomparison Workshop (Bues-

seler et al., 1996), we were aware of the potential problems associated with CFF of seawater and took rigorous steps to optimize the reliability of our results. The cleaned CFF system and membrane (see below) was first flushed by recirculating a fresh sub-sample for 10 min to condition the membrane, pumped dry and briefly flushed again with a fresh sub-sample before beginning to process the remaining sample. Sample recirculation rates were 4–6 l min⁻¹ with total over pressures of ≤ 20 psi and transmembrane pressures of ≤ 5 psi. Permeate flow rates were kept below 200 ml min⁻¹ so that recirculation rates were sufficiently high to maintain optimal membrane performance. Permeate samples were collected once roughly halfway through CFF processing. Although the later Colloid Intercomparison Workshop found that permeate DOC concentrations increased with the concentration factor in some CFF systems (Buesseler et al., 1996), this problem did not occur with the Filtron membranes used in the Narragansett Bay study. Subsequent work has shown that permeate metal concentrations also remain uniform over the small concentration factors (initial sample vol./final retentate vol. = 3–8 \times) used in this study (Wells, unpublished data).

On completing CFF processing, the permeate valve was closed and the final retentate sample recirculated freely across the membrane for 10 min before subsampling for total metal determination. This step improves colloid recovery from the CFF system. After completing the 8 kDa CFF separation, the membrane and system were cleaned by recirculating 25% methanol (Fisher Optima) in 10% HCl (Trace Metal Grade) for 15 min followed by thorough rinsing with deionized (Milli-Q) water. The membrane was then exchanged for the 1 kDa membrane and the process repeated on a replicate 20 l sample.

Equally size-rated CFF membranes from different manufacturers have different effective molecular weight cutoffs in seawater (Buesseler et al., 1996). Given that no filtration method provides either accurate or precise size separations in seawater (Stockner et al., 1989), and that size calibration compounds yield different effective molecular cutoffs under different operating conditions (Buesseler et al., 1996), we broadly class the size fractions obtained here as nominally < 8 kDa and < 1 kDa.

Determination of metal mass balances are important to establish whether significant metal sorption or contamination occurred during CFF processing. Mass balances, indicated by comparing the sum of the measured permeate plus colloid concentrations against the total dissolved ($< 0.2 \mu\text{m}$) concentrations, were generally better than 95% in the Narragansett Bay study (Wells et al., submitted). Unfortunately, initial attempts to determine metal speciation in the CFF retentates proved unreliable. We believe these problems were due to organic fouling of the thin mercury film surface. We therefore restricted our measurements to the permeate fractions and thus cannot verify mass balances for unbound organic ligand concentrations (colloidal ligand concentrations were therefore determined by difference between $< 0.2 \mu\text{m}$ and permeate fractions). However, well leached Filtron[®] membranes (as was the case for this study) display excellent DOC mass balances (Buesseler et al., 1996; Powell et al., 1996), suggesting that any sorption of free metal-binding ligands to the CFF system was minimal.

2.4. Total metal analyses

Total metal concentrations in the dissolved and ultrafiltered samples were analyzed back at UCSC by two analytical methods. The first method, used for 'dissolved' ($< 0.2 \mu\text{m}$) metal determinations, involved chelation with PDC/DDC followed by extraction into chloroform and determination by graphite furnace atomic absorption spectrometry (Bruland et al., 1979, 1985). CFF samples were concentrated before analysis using a modified solid-phase extraction procedure (Wells and Bruland, in press). In short, hydroxyethylthiocarbamate was added to the acidified samples, the pH raised to 8.5 and the sample pumped across a C-18 column which retained the metal–ligand complexes. Metal complexes were eluted from the column with acidic methanol, evaporated to dryness, and the residues re-dissolved in nitric acid. Sample extracts from both methods were analyzed on a Perkin Elmer 4100ZL graphite furnace AA spectrophotometer using Zeeman background correction. Intercomparisons on these Narragansett Bay samples showed excellent agreement between the two analytical methods (within $\sim 5\%$).

2.5. Chemical speciation analyses

Details on the electrochemical instrumentation used in this study have been reported previously (Bruland et al., 1985; Coale and Bruland, 1988; Donat et al., 1994). The instrumentation includes a modified Polarographic analyzer (Princeton Applied Research model 174A), a chart recorder (Houston Instruments) and a 50 ml Teflon® (TFE) electrochemical cell that comprised a Pt wire counter electrode, an Ag/AgCl reference electrode, and a rotating glassy carbon disk (RGCD) working electrode plated with a thin mercury film (TMF).

DPASV/TMF-RGCDE measures chemically labile metal complexes (organic and inorganic) as distinct from non-labile metal complexes. The theory of the titration methods used in electrochemical speciation analyses has been explained in detail (Donat et al., 1994). Operationally, the RGCD electrode surface was polished with a 0.05 μm Al_2O_3 sol at 900 rpm and then rinsed with dilute, quartz-distilled HCl. The TMF was formed by depositing (-1.0 V for 10 min; 4000 rpm) from a degassed (N_2 for 10 min) Hg solution containing 50 ml of deionized water (Milli-Q), 200 μl of saturated KCl (Merck Supra-pur) and 100 μl of 5000 ppm Hg^{2+} . To check for contamination, the TMF was scanned after a 30 s quiescent period to -0.15 V and the resulting voltammogram examined for metal peaks. A new TMF was formed for each titration and was rinsed and conditioned with degassed (N_2) sample water prior to speciation analysis.

To determine metal speciation, increasing additions of the metal of interest were made to 50 ml sub-samples and, after sufficient time for the added metal to equilibrate among the natural species, the labile concentrations of metal were measured. The analytical equilibration times used ranged from 15–40 min, which experience shows greatly exceeds the time required to obtain stable readings with seawaters from coastal and offshore environments.

Separate titrations were performed for each metal because unique deposition potentials (Cu, -0.65 V; Pb, -0.75 V; Cd, -0.85 V; Zn, -1.2 V) were required to avoid electroreduction of labile organic species (Plavsic et al., 1982; Donat et al., 1994). Ten minutes deposition times typically were used although longer times were occasionally needed to

obtain a measurable signal for the non-spiked samples. The quiescent and stripping steps for Pb, Cd, and Zn were performed as previously described (Bruland et al., 1985). To help reduce interferences affecting Cu determinations due to sorption of organics to the TMF, the potential was briefly adjusted to -1.65 V during the initial 5 s of the 30 s quiescent period (Scarano and Bramanti, 1993). However, this additional step did not help in our attempts to analyze CFF retentate samples directly.

Metal complexing ligand concentrations and conditional stability constants were calculated from the titration data after transformation using both Langmuir and Scatchard linearization methods. Langmuir linearization (van den Berg and Kramer, 1979; Ruzic, 1982; van den Berg, 1982) consists of plotting the ratio of the summed inorganic metal species concentration to the metal–ligand complex species concentration ($[\text{M}']/[\text{ML}]$) against $[\text{M}']$ ($[\text{M}']$ determined electrochemically and $[\text{ML}] = [\text{M}_\text{T}] - [\text{M}']$). If the transformation results are linear it is assumed that the natural chelators are within a class of ligands having one representative conditional stability constant ($K_{\text{ML}_i, \text{M}'}^{\text{cond}} = [\text{ML}_i]/[\text{M}'][\text{L}_i]$). The x -intercept of these linearized data gives the reciprocal product of $K_{\text{ML}_i, \text{M}'}^{\text{cond}}$ and the total ligand concentration $[\text{L}_\text{T}]$ while the reciprocal slope of the line gives $[\text{L}_\text{T}]$. These values were verified with Scatchard linearizations, where the ratio of $[\text{ML}]/[\text{M}']$ is plotted against $[\text{ML}]$; the y -intercept is $K_{\text{ML}_i, \text{M}'}^{\text{cond}} \times [\text{L}_\text{T}]$ and the x -intercept is $[\text{L}_\text{T}]$.

Distinctively separate linear regions within a transformation curve indicates that more than one class of ligands exist; for example, a low concentration of ligands having a high conditional stability constant and a higher concentration of ligands with a lower binding strength. With Langmuir plots, one linear region may exist at high $[\text{M}']$ and another at low $[\text{M}']$. In this case, assessing values for $[\text{L}_1]$ (the stronger ligand class) and $K_{\text{ML}_1, \text{M}'}^{\text{cond}}$ is not accurate due to the high curvature in this region of the Langmuir plot. On the other hand, two or more ligand classes are clearly distinct in Scatchard linearizations (see Mantoura et al., 1978) and one can accurately determine concentrations of the separate ligand classes ($[\text{L}_1]$ and $[\text{L}_2]$) and their respective conditional stability constants $K_{\text{ML}_1, \text{M}'}^{\text{cond}}$ and $K_{\text{ML}_2, \text{M}'}^{\text{cond}}$. The determined values of $[\text{L}_i]$ and $K_{\text{ML}_i, \text{M}'}^{\text{cond}}$ are then

Table 1

Copper concentrations and linearization results for conventional and permeate samples collected at three sites in Narragansett Bay in June 1994

| Site and salinity | Size fraction | [Cu _T] (nM) | [Cu'] (nM) | L ₁ (nM) | L ₂ (nM) | log K ^{cond} _{CuL₂,Cu'} | L ₃ (nM) | log K ^{cond} _{CuL₃,Cu'} | Organic Cu |
|----------------------------|---------------|-------------------------|------------|---------------------|---------------------|--|---------------------|--|------------|
| Upper bay <i>S</i> = 24.9 | < 0.2 μm | 27.9 ± 0.5 | n.d. | ~ 38 | 40 ± 10 | 8.8 ± 0.4 | 100 ± 15 | 7.7 ± 0.4 | 100% |
| | < 8 kDa | 23.5 ± 0.5 | n.d. | ~ 22 | 110* | 8.0 | — | — | — |
| | < 1 kDa | 12.0 ± 1.3 | n.d. | ~ 17 | 107* | 8.2 | — | — | — |
| Middle bay <i>S</i> = 28.6 | < 0.2 μm | 16.1 | n.d. | ~ 16 | 20 ± 2 | 8.8 ± 0.1 | 54 ± 4 | 7.7 ± 0.05 | 100% |
| | < 8 kDa | 15.0 | — | — | — | — | — | — | — |
| | < 1 kDa | 9.5 | n.d. | ~ 16 | 22 | 8.6 | 40 | 7.7 | 100% |
| Lower bay <i>S</i> = 30.3 | < 0.2 μm | 12.7 | n.d. | ~ 16 | 15 ± 3 | 9.2 ± 0.1 | 57 ± 17 | 7.5 ± 0.3 | 100% |
| | < 8 kDa | 10.9 | n.d. | ~ 16 | 45* | 8.0 | — | — | — |
| | < 1 kDa | 7.4 | n.d. | ~ 16 | 40* | 7.9 | — | — | — |

[Cu'] is the concentration of free and inorganically complexed species.

The conditional stability constant of the L₁ class of Cu binding ligands exceeded the analytical window of the DPASV method ($K_{CuL_1,Cu'}^{cond} > 10^{11} M^{-1}$).

The log conditional stability constants for the weaker L₂ and L₃ ligand classes are shown.

Values below detection (< 0.2 nM) are shown as n.d. while (—) denotes not measured.

The sum of [L₂] and [L₃] (*) is given where Scatchard linearization results prevented accurate assessment of individual concentrations.

used to calculate the ambient speciation of metals in the different size fractions.

3. Results

Organic metal-complexing ligands exerted a significant to dominant control of the dissolved speciation of Cu (100%), Pb (67–94%), Zn (44–97%) and Cd (73–83%) in Narragansett Bay (Tables 1–4). When detectable, the sum of free and inorganically bound metal concentrations (M') in the dissolved

(< 0.2 μm) and permeate (< 1 kDa or < 8 kDa) samples were analytically indistinguishable (Tables 3 and 4). Total dissolved metal concentrations generally decreased from upper to lower Narragansett Bay (Tables 1–4), yet there were distinct differences in the patterns of metal complexation. Dissolved Cu was entirely complexed by organics at all three sites while the percentage of complexed Pb decreased from ~ 94% in the upper bay to ~ 67% in the lower bay. By contrast, a greater percentage of dissolved Zn and Cd were complexed at the Middle Bay station where phytoplankton biomass was highest (Kozelka and Bruland, 1998).

Table 2

Lead concentrations and linearization results for conventional and permeate samples collected at three sites in Narragansett Bay in June 1994

| Site and salinity | Size fraction | [Pb _T] (nM) | [Pb'] (nM) | L ₁ (nM) | log K ^{cond} _{PbL₁,Pb'} | L ₂ (nM) | log K ^{cond} _{PbL₂,Pb'} | Organic Pb |
|----------------------------|---------------|-------------------------|------------|---------------------|--|---------------------|--|------------|
| Upper bay <i>S</i> = 24.9 | < 0.2 μm | 0.32 ± 0.0 | 0.03 | 0.8 ± 0.2 | 10.0 ± 0.4 | 5.1 ± 0.8 | 8.8 ± 0.3 | 93.7% |
| | < 8 kDa | 0.19 | — | — | — | — | — | — |
| | < 1 kDa | 0.19 | n.d. | n.d. | — | 6.6 | 8.6 | — |
| Middle Bay <i>S</i> = 28.6 | < 0.2 μm | 0.13 | n.d. | 0.6 | 10.2 | 6.0 | 8.6 | 81% |
| | < 8 kDa | 0.079 | — | — | — | — | — | — |
| | < 1 kDa | 0.085 | n.d. | n.d. | — | 4.0 | 8.7 | — |
| Lower bay <i>S</i> = 30.3 | < 0.2 μm | 0.15 | 0.01 | 1.0 | 9.9 | 8.2 | 8.6 | 66.7% |
| | < 8 kDa | 0.095 | — | — | — | — | — | — |
| | < 1 kDa | 0.096 | n.d. | n.d. | — | 7.4 | 8.7 | — |

Results are interpreted as having a stronger (L₁) and weaker (L₂) ligand class.

Values below detection (< 0.01 nM) are shown as n.d. while (—) denotes not measured.

Table 3

Zinc concentrations and linearization results for conventional and permeate samples collected at three sites in Narragansett Bay in June 1994

| Site and salinity | Size fraction | [Zn _T] (nM) | [Zn'] (nM) | [L _T] (nM) | log $K_{ZnL_T, Zn'}^{cond}$ | Organic Zn |
|-----------------------|---------------------|-------------------------|----------------|------------------------|-----------------------------|------------|
| Upper bay $S = 24.9$ | < 0.2 μm | 71.5 | 26.6 | 48.2 | ≥ 9 | 63% |
| | < 8 kDa | 67.8 | 26.6 | 40 | ≥ 9 | 61% |
| | < 1 kDa | 69.5 | — | — | — | — |
| Middle bay $S = 28.6$ | < 0.2 μm | 23.7 ± 0.8 | 0.6 ± 0.03 | 38.4 ± 0.9 | 9.4 ± 0.04 | 97% |
| | < 8 kDa | 21.7 | 1.0 | — | — | 95% |
| | < 1 kDa | 20.6 | 0.7 | 35 | 9.4 | 97% |
| Lower bay $S = 30.3$ | < 0.2 μm | 16.3 ± 1.7 | 8.0 ± 0.8 | 10.6 ± 0.7 | 9.0 ± 0.04 | 51% |
| | < 8 kDa | 16.1 | — | — | — | — |
| | < 1 kDa | 16.1 | 9.0 | 11.1 | 9.3 | 44% |

Titration data suggest only one class of Zn complexing ligands (L_T) was present.

Values below detection (< 0.01 nM) are shown as n.d. while (—) denotes not measured.

Despite the high degree of metal complexation, total ligand concentrations (the sum of each ligand class) did not vary in accordance with dissolved metal concentrations. Overall, total metal-binding ligand concentrations decreased in the order Cu > Zn > Pb > Cd while dissolved metal concentrations decreased in the order Zn > Cu > Cd > Pb. The distribution of the specific metal-binding ligand classes differed markedly, with Cu and Zn-binding ligand concentrations decreasing from the Upper to Lower Narragansett Bay stations (Tables 1 and 3) while Cd-binding ligand concentrations remained essentially constant (Table 4). In contrast, concentrations of total Pb-binding ligands increased down-bay (Table 2).

Linearization of these dissolved (< 0.2 μm) titration data using Langmuir and Scatchard techniques

(e.g., see Kozelka and Bruland, 1998) indicated that Cu and Pb were influenced by multiple classes of metal binding ligands (i.e., having different conditional stability constants), while only one class of metal-binding ligands existed for Zn and Cd. Metal-spiked titration samples were re-analyzed at several time intervals to confirm that the added metals had equilibrated fully among the natural soluble and colloidal organic ligands within 15–40 min (data not shown). Based on total dissolved concentrations of metals and their respective ligands, the calculated free metal ion concentrations at the three sites in Narragansett Bay were: [Cu²⁺], ~ 1 pM; [Cd²⁺], 2–8 pM; [Pb²⁺], 0.4–1.2 pM; and [Zn²⁺], 0.3–13.3 nM (Kozelka and Bruland, 1998).

The colloidal ligand concentrations at the three stations, calculated by subtracting permeate ligand

Table 4

Cadmium concentrations and linearization results for conventional and permeate samples collected from three sites in Narragansett Bay in June 1994

| Site and salinity | Filtered-fraction | [Cd _T] (nM) | [Cd'] (nM) | [L _T] (nM) | log $K_{CdL_T, Cd'}^{cond}$ | Organic Cd |
|-----------------------|---------------------|-------------------------|-----------------|------------------------|-----------------------------|------------|
| Upper bay $S = 24.9$ | < 0.2 μm | 0.80 ± 0.03 | 0.22 ± 0.02 | 3.7 ± 0.4 | 8.9 ± 0.2 | 73% |
| | < 8 kDa | 0.74 | — | — | — | — |
| | < 1 kDa | 0.78 | 0.2 | 3.4 | 9.2 | 74% |
| Middle bay $S = 28.6$ | < 0.2 μm | 0.29 | 0.05 | 3.8 | 9.0 | 83% |
| | < 8 kDa | 0.26 | — | — | — | — |
| | < 1 kDa | 0.26 | 0.05 | 4.4 | 8.8 | 81% |
| Lower bay $S = 30.3$ | < 0.2 μm | 0.30 | 0.07 | 3.6 | 9.1 | 77% |
| | < 8 kDa | 0.28 | — | — | — | — |
| | < 1 kDa | 0.29 | 0.05 | 2.7 | 9.2 | 83% |

Titration results suggest only one class of Cd complexing ligands (L_T) was present.

Values below detection (< 1 pM) are shown as n.d. while (—) denotes not measured.

concentrations from dissolved ligand concentrations, are presented in Figs. 2–5. The strongest class of Cu complexing ligands (L_1) appeared to be largely soluble (< 1 kDa), except at the Upper Bay site near Providence Harbor where $\sim 60\%$ of these ligands were colloidal in nature (Fig. 2a). In contrast, the colloidal phase comprised 24–70% of total dissolved concentrations of the weaker Cu-binding ligand classes (L_2 , L_3), with these levels remaining relatively constant from Upper to Lower Narragansett Bay (Fig. 2b). The conditional stability constant of copper L_1 class ligands exceeded the analytical window of the DPASV method ($K_{\text{Cu}L_1, \text{Cu}'}^{\text{cond}} > 10^{11} \text{ M}^{-1}$) in both the dissolved and permeate samples. For copper L_2 class ligands, $K_{\text{Cu}L_2, \text{Cu}'}^{\text{cond}}$ values determined from permeate samples were somewhat lower than that measured in dissolved samples, though these differences were small (Table 1).

In contrast to Cu, the stronger Pb-binding ligand class (L_1) resided in the colloidal phase while the weaker L_2 class of ligands occurred predominantly in the soluble phase (Fig. 3). The exception was the Middle Bay station where $\sim 30\%$ of the weaker

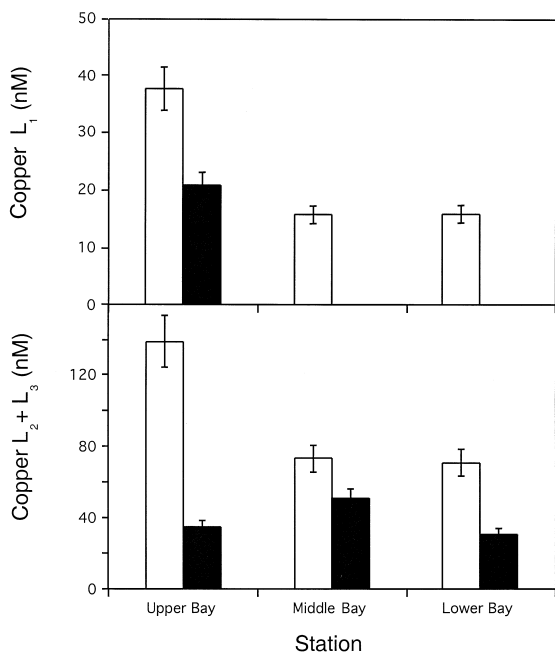


Fig. 2. The dissolved ($< 0.2 \mu\text{m}$; \square) and colloidal (1 kDa–0.2 μm ; \blacksquare) concentrations of L_1 and $L_2 + L_3$ classes of Cu complexing ligands in Narragansett Bay. Error bars are $\pm 10\%$.

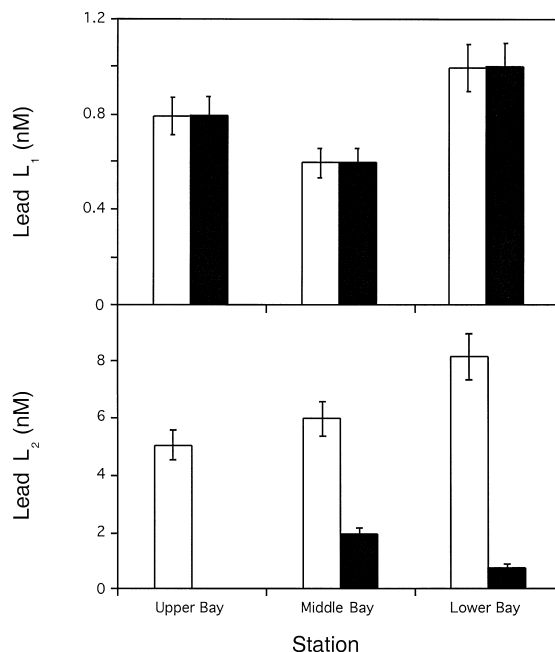


Fig. 3. Concentrations of dissolved ($< 0.2 \mu\text{m}$; \square) and colloidal (1 kDa–0.2 μm ; \blacksquare) L_1 and L_2 classes of Pb complexing organic ligands in Narragansett Bay. Error bars are $\pm 10\%$.

ligand class was colloidal. The overall strength of L_2 in the dissolved and permeate fractions was indistinguishable, with a mean $K_{\text{Pb}L_2, \text{Pb}'}^{\text{cond}}$ of $10^{8.6} \text{ M}^{-1}$.

Zn-binding ligands resided predominantly in the soluble phase, the colloidal fraction accounting for ~ 10 –15% of total ligand concentrations at the Upper Bay and Middle Bay stations while no colloidal

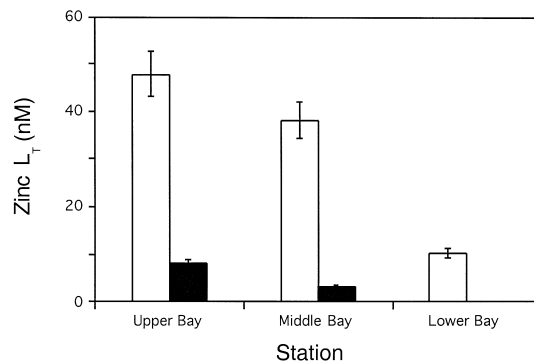


Fig. 4. Concentrations of dissolved ($< 0.2 \mu\text{m}$; \square) and colloidal (1 kDa–0.2 μm ; \blacksquare) Zn complexing organic ligands (L_T) in Narragansett Bay. Error bars are $\pm 10\%$.

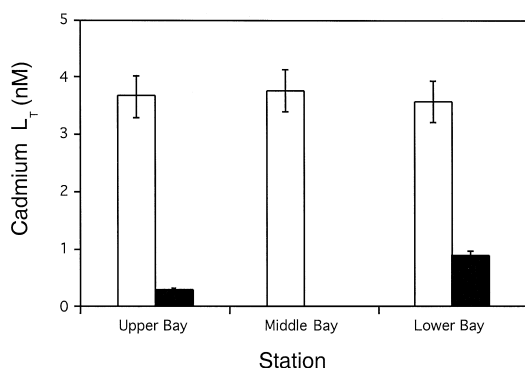


Fig. 5. The dissolved ($<0.2 \mu\text{m}$; \square) and colloidal (1 kDa–0.2 μm ; \blacksquare) Cd complexing organic ligands (L_T) in Narragansett Bay. Error bars are $\pm 10\%$.

Zn-binding ligands were seen at the Lower Bay station (Fig. 4). The $K_{\text{ZnL},\text{Zn}}^{\text{cond}}$ values were unchanged by CFF with a mean value of $10^{9.3} \text{ M}^{-1}$.

Cd-binding ligands also resided predominantly ($\sim 90\%$) in the soluble phase at the Upper and Middle Bay sites. At the Lower Bay station, $\sim 25\%$ of these ligands were colloidal; i.e., opposite to the distribution pattern seen for colloidal Zn-binding ligands (Fig. 5). As with Zn, there was no measurable change in $K_{\text{CdL},\text{Cd}}^{\text{cond}}$ among the three stations or upon CFF processing, with a mean value of $10^{8.9} \text{ M}^{-1}$.

4. Discussion

Chemical speciation studies strongly suggest that single and multiple ligand classes exist in seawaters and that these ligands are largely metal specific (Bruland et al., 1991). However, there has been little success in identifying the structures, sources, and fates of these natural substances. As a step towards better characterization of these ligands, we set out to determine whether the large range of measured conditional stability constants was matched by an equally distinct range of ligand sizes.

CFF membranes have non-uniform pore sizes and thus can only provide 'nominal' molecular weight separations. Moreover, the inherent accuracy of these nominal separations for natural substances often is poor (Buessler et al., 1996; Gustafsson et al., 1996). Manufacturer ratings of molecular weight cutoffs are

determined with model materials under processing conditions which can depart drastically from those used for natural water samples. For example, the 1 kDa Filtron membranes employed in this study have an effective cutoff closer to ~ 2 kDa in seawater using standard calibration molecules (Buessler et al., 1996), and it is reasonable to expect that the 8 kDa membranes behave similarly. Synthetic calibration molecules also cannot effectively account for the varied composition and molecular conformations expected for natural marine organic matter. As a consequence, we are restricted to obtaining information on only the relative size of the ligand compounds in seawater. Nevertheless, this information still provides a better sense of the character of these substances, and can provide insight to the processes controlling their input to surface waters.

Our findings clearly demonstrate that the natural organic ligands which complex Cu, Pb, Cd, or Zn in Narragansett Bay have broadly different architectures. Pb and Cu speciation in Narragansett Bay was controlled by both soluble and colloidal-sized ligands. For Pb, the excess strong (L_1) ligand class was present exclusively in the colloidal fraction while the weaker L_2 class ligands occurred predominantly in the soluble phase. Muller (1996) found a similar dependence of Pb speciation on colloidal phases. The < 8 kDa permeate unfortunately was not analyzed, however, given the excess of strong Pb binding ligands and the lower Pb concentrations in these permeates (Table 2) it appears that the PbL_1 complexes occurred mainly in the larger (> 8 kDa–0.2 μm) colloid fraction. Kozelka et al. (1997) found that Pb in South San Francisco Bay was bound by a class of similarly strong organic ligands ($K_{\text{PbL}_1,\text{Pb}}^{\text{cond}} = 10^{10.5} \text{ M}^{-1}$) but the PbL_1 complexes and excess unbound L_1 were primarily < 10 kDa in size. Although it is tempting to suggest that Pb-binding ligands are 8–10 kDa in size, the different ecologies of these embayments and differences in CFF processing may be important factors.

In contrast to Pb, the class of strong Cu-binding ligands (L_1) appeared to be present in the soluble phase at the Middle and Lower Bay stations, with the weaker L_2 and L_3 classes split between soluble and colloidal phases. However, our ability to accurately quantify copper L_1 concentrations by DPASV was limited (due to tuning of the analytical 'window'

towards measuring the multiple weaker ligand classes). In fact, the progressive decrease in (100% complexed) Cu concentrations in the 8 and 1 kDa permeates suggests that up to ~12% of the L_1 class ligands were > 8 kDa and ~45% were > 1 kDa; i.e., about half of the strong Cu-ligand complexes in fact were colloidal in nature. Although indirect, this evidence is striking because 100% of dissolved Cu was organically bound and there was minimal loss of Cu to the CFF system (mass balances = $95 \pm 5\%$). The presence of a colloidal L_1 class of Cu ligands differs from the findings of Gordon et al. (1996) who found that the stronger Cu binding ligand class was mainly soluble in Chesapeake Bay. However, the apparent different ligand sizes between Narragansett and Chesapeake Bays may be related more to the different methods used for molecular weight separations (centrifugation UF vs. CFF) than inherent differences in the nature of the metal-complexing organics.

Muller (1996) found a similar colloidal component (~30–65%) for 'dissolved' Cu concentrations in estuarine and coastal waters of Southern England during summer, although it is uncertain whether mass balance for Cu was achieved for these 3 kDa separations. His data also indicated the L_1 class of Cu-binding ligands were predominantly soluble in these waters. However, L_1 concentrations in his CFF permeates were 2–8 \times greater than 'dissolved' values (i.e., prior to CFF processing) at three of the four stations. This apparent 'production' of soluble L_1 ligands also was seen at other times of the year. One interpretation of this anomaly is that colloidal organic matter may have been physically disrupted in the small CFF cassettes used, releasing colloiddally associated ligand molecules to the soluble phase. It is not clear whether similar transformations may have occurred in our study—we could not determine L_1 concentrations precisely enough under the electrochemical conditions used—but if so, then our data would underestimate the component of L_1 class Cu-binding ligands residing in the colloidal phase.

Organic molecules responsible for binding Cd and Zn in Narragansett Bay surface waters resided predominantly in the soluble phase, although the colloidal fraction accounted for up to ~20% of these metal binding ligand pools (Tables 3 and 4). In the case of Cd, these findings are consistent with chemi-

cal speciation studies of size fractionated samples from the South San Francisco Bay, where most Cd species passed through a 10 kDa membrane (Kozelka, unpublished). However, the limited colloidal Cd component measured in Narragansett Bay is at least 2 times less than that reported for estuarine and coastal waters of southern England (Muller, 1996). This difference is all the more striking because the Muller study employed a larger (3 kDa) molecular weight cutoff filter, and it implies that concentrations of colloidal-sized Cd ligands may vary substantially among different coastal waters. At present there are no comparable data on the size distributions of Zn-binding ligands.

The conditional stability constants measured here are analytically identical to those measured in other seawaters (Bruland, 1989; Capodaglio et al., 1990; Bruland, 1992; Kozelka et al., 1997). Small, environmentally relevant, additions of competing metals do not significantly displace metal from these apparently specific metal ligand complexes (Bruland, 1989; Capodaglio et al., 1990; Bruland, 1992). In line with the specificity of Cd and Zn-binding ligands for their respective metals, the colloidal components of Cd and Zn ligand classes were not closely related. The small pool of colloidal Zn-binding ligands was present at the Upper and Middle Bay stations (Fig. 4) while the small fraction of Cd-binding colloidal ligands was present at the Upper and Lower Bay sites (Fig. 5). The implication from these limited data is that these ligands may have different sources or might cycle independently from one another in coastal waters.

A fundamental question surrounding the presence of dissolved chelators in seawater is whether they are exuded by growing microorganisms, or result accidentally from the predatory 'discharge' of intracellular metabolic components via zooplankton sloppy feeding and incomplete digestion, viral-induced cell lysis, or direct microbial degradation of senescent cells. The size distribution of strong metal chelators provides some basis for speculating which of these biological mechanisms are most important for introducing these substances to seawater.

Active transport of molecules across cell membranes is constrained in part by molecular size. This constraint reflects the balance between achieving specific transport of a substance (in either direction)

across the cell membrane while maintaining an effective barrier for the cell. In heterotrophic bacteria, the upper limits for assimilation of molecules (food) are on the order of ~ 700 Da, though hydrodynamic volume is the more relevant parameter (Payne, 1980). Similar constraints presumably apply to the cross-membrane transport of organic chelators by either phytoplankton or heterotrophic bacteria. It is notable, therefore, that Zn, Cd and a major fraction of Cu binding ligands were small enough (< 1 kDa) that active transport could explain their presence in seawater.

For example, marine cyanobacterium *Synechococcus* sp. are widely distributed in the oceans, including coastal embayments along the N. eastern U.S. coast (Moffett et al., 1996), but they are extremely sensitive to Cu toxicity. Moffett et al. (1996) have shown in cultures that these organisms diminish free cupric ion activities in response to Cu stress (Moffett and Brand, 1996) by releasing specific chelators with conditional stability constants very similar to the L_1 class ligands found in Narragansett Bay and elsewhere in the oceans (van den Berg et al., 1986; Coale and Bruland, 1988; Moffett et al., 1990; Donat, 1995). A marine chlorophyte, *Dunaliella tertiolecta* also has been shown to release extracellular ligands that bind Cu, Cd and Pb (Gonzalez-Davila et al., 1995; Santana-Casiano et al., 1995). However, the conditional stability constants of these chlorophyte exudates determined by DPASV are 2–3 orders of magnitude lower than those measured in Narragansett Bay. Nonetheless, the small size of strong, extracellular ligands in Narragansett Bay waters is consistent with suggestions that microorganisms actively ‘condition’ seawater to facilitate optimal metal conditions for growth (Huntsman and Sunda, 1980; Bruland et al., 1991).

It also is conceivable that some soluble metal ligand complexes in coastal seawaters are a byproduct of intracellular metal detoxification mechanisms. Cd export from the marine diatom *Thalassiosira weissflogii* is facilitated by active transport of the metal binding polypeptide phytochelatin (~ 900 Da) (Lee et al., 1996). However, this transport occurred at roughly $1000 \times$ higher Cd concentrations than measured in Narragansett Bay (Kozelka and Bruland, 1998) and the released Cd–phytochelatin complex was not very stable in seawater (Lee et al., 1996). It

is reasonable to expect that active transport of Zn–phytochelatin complexes also occurs across phytoplankton membranes, but it is not known whether the conditional stability constant in seawater is within the range for the extracellular ligands measured here ($K_{ZnL,Zn'}^{cond} \cong 10^9 M^{-1}$). Both eukaryotic and prokaryotic microorganisms also can produce small, cysteine-rich metallothionein-like proteins to complex a variety of toxic metals within the cell (Olafson et al., 1988; Hughes and Poole, 1989). Although these ligands are not highly specific for a single metals, active transport or predatory discharge of these ligands may contribute to the presence of strong metal chelators in the soluble phase (Morel and Hering, 1993).

The situation for colloidal-sized metal-binding ligands in seawater is different. Direct exudation of peptides from gram-negative bacteria (the major fraction of marine bacteria) is restricted to a few hundred Daltons in size due to molecular-sieving by the cell wall (Payne, 1980)—the specialized production of extracellular proteinases (20–40 kDa) appears to rely upon translation and extrusion of newly-formed polypeptides at membrane sites rather than cytoplasmic synthesis and transport (Law, 1980). Phytoplankton would face similar constraints for transporting large, hydrophilic molecules through the lipophilic cell membrane, making it unlikely that colloidal ligands arise via direct exudation from living cells. Another option is terrestrial humic substances, which by their size ($\gg 1$ kDa) are colloidal by definition. However, the conditional stability constants for metal complexation by humic substances are much lower than measured here (Mantoura et al., 1978). In addition, the flux of terrestrial organic matter into Narragansett Bay during summer accounts for $< 20\%$ of that derived from marine primary production (Nixon et al., 1995), suggesting that these colloidal ligands are of primarily marine origin. It seems reasonable to speculate then that the primary source of colloidal metal-complexing ligands in surface waters is predatory discharge of high-molecular weight intracellular components; probably those requiring specific metals for their metabolic function.

While there are good reasons to expect that soluble and colloidal organic ligands may have different input mechanisms, predatory discharge could explain

the presence of both. In fact, the similarity of conditional stability constants between soluble and colloidal ligands might indicate these ligands are the same low molecular weight metal complexing molecules that in some cases have become or remain associated with larger cytoplasmic or structural degradation products. But it is also possible that similarity in log scale conditional stability constants for these colloidal and soluble ligand classes simply reflects the biological application of optimally-evolved metal-binding molecular architectures to different segments of cell metabolism (e.g., extracellular 'conditioning' products as well as intracellular components).

The above discussion is founded upon interpreting electrochemical data as evidence of highly specific, highly stable metal complexing organic ligands occurring in seawater. Mackey and Zirino (1994) argue that the apparently high selectivity and high stability constants measured for organic metal-complexing ligands in seawater result from slow reaction kinetics with the electrode surface, and thus are artifacts of the analytical methods. They propose instead that these strong 'ligands' form when low and high molecular weight organic substances having comparatively weak metal-binding functional groups coalesce to embed the weakly bound metals within a colloidal organic matrix (the 'onion layer' model). The recent description of marine gel formation by spontaneous aggregation of dissolved organic matter is perhaps a realistic example of such a process (Chin et al., 1998). Because methods for determining metal speciation (including voltammetry) assume thermodynamic equilibrium exists between free and bound metal species, a physically retarded exchange between metals within the organic colloid and the electrode surface would appear as a strong complex.

These arguments are based in part on the general puzzlement over how strong chelators can be so specific for a given metal. For example, adding Cu to seawater samples does not displace Zn from the organic complexes, even though Cu theoretically should form much more stable organic complexes than Zn based on the Irving–Williams scale of reactivity. However, biological systems have evolved molecules that are remarkably tuned to form highly stable, specific complexes with individual metals

(e.g., Crumbliss, 1991; Silva and Williams, 1991). It is reasonable to expect that these molecular attributes would be partly or completely retained if these intracellular chelators were released to seawater.

If measurements of metal speciation indeed are biased by kinetic factors resulting from physical arrangements, then we would expect the highest apparent conditional stability constants to be found in the colloidal phase while soluble molecules would be less stable. However, this situation is not the case in Narragansett Bay where the weaker Cu ligand classes (L_2 and L_3) were found in the colloidal phase while the stronger ligand class was predominantly in the soluble phase (Fig. 2). Similarly, the strong Zn and Cd complexing ligands were predominantly low molecular weight substances (< 1 kDa), and the conditional stability constants of colloidal sized complexes did not differ substantially from that in the soluble phase. Our Cu, Zn and Cd data therefore are not consistent with a kinetically controlled complexation scenario as hypothesized by Mackey and Zirino (1994).

The situation for Pb is markedly different. Microorganisms have no known metabolic requirement for Pb (Silva and Williams, 1991) and so it seems unlikely that strong, highly specific Pb chelators would have evolved. It therefore seems surprising that such organic ligands occur in surface waters of Narragansett Bay (Table 2), in south San Francisco Bay (Kozelka et al., 1997) and in the eastern North Pacific (Capodaglio et al., 1990). In Narragansett Bay, the strong Pb-binding ligand class (L_1) was colloidal while the weaker ligands (L_2) were predominantly in the soluble (< 1 kDa) phase, as would be predicted by the Mackey and Zirino aggregate hypothesis (Mackey and Zirino, 1994).

It may be then that the 'strong' complexation of metals in seawater results from both highly specific, strong ligands as well as weak but kinetically restricted ligand–colloidal associations. The relative importance of these two situations might vary with the metal, with microorganisms producing specific strong ligands for metabolically required metals while non-nutrient metal speciation is affected by binding with comparatively weak non-specific ligands that can coalesce into colloidal organic phases.

The findings presented here along with those of Muller (1996) strongly imply that bioactive metal–

colloid associations are regulated differently than the non-specific surface sorption reactions thought to control Th–colloid interactions. As a consequence, determining bioactive metal–colloid partition coefficients from measurements with particulate phases, where non-specific surface sorption reactions will be most important, will significantly underestimate actual values (Wells et al., submitted). Moreover, the assumption that Th sorption to colloid surfaces is largely irreversible (e.g., Honeyman and Santschi, 1989) will not apply to bioactive metals, which have a significant concentration of strong soluble chelators competing with colloidal binding sites. It is unlikely then that Th will be an effective probe for tracing the physico-chemical cycling of soluble and colloidal bioactive metals in seawater.

The distribution of metal complexing ligands between soluble and colloidal size fractions in Narragansett Bay and the southern coastal waters of England (Muller, 1996) challenges current perceptions that organic ligands primarily serve to buffer dissolved bioactive metal concentrations, maintaining optimal conditions for organism growth (Huntsman and Sunda, 1980). Given the evidence that marine colloidal organic matter can aggregate into large, sinking particulates (Chin et al., 1998; Baskaran et al., 1992; Moran and Buesseler, 1992; Wells and Goldberg, 1993) ‘dissolved’ ligands may also be a vector for metal removal from ocean waters. Ascertaining whether metal-complexing ligands serve primarily as a buffer or sink for bioactive metals in surface waters remains a fundamental challenge for understanding the marine biogeochemistry of bioactive metals.

Acknowledgements

We are extremely grateful to Geoff Smith for logistical and sampling assistance during this study. We also extend sincere thanks to our colleagues at the University of Rhode Island–Narragansett Bay campus for accommodating and hosting our portables, in particular Dr. Al Hanson who helped coordinate our visit. We greatly appreciate comments from the two anonymous reviewers which helped us improve the manuscript. This research was supported

by ONR grants N00014-93-1-0884 and N00014-93-1-0039.

References

- Baskaran, M., Santschi, P.H., Benoit, G., Honeyman, B.D., 1992. Scavenging of Th isotopes by colloids in seawater of the Gulf of Mexico. *Geochim. Cosmochim. Acta* 56, 3375–3388.
- Benner, R., Pakulski, J.D., McCarthy, M., Hedges, J.I., Hatcher, P.G., 1992. Bulk chemical characteristics of dissolved organic matter in the ocean. *Science* 255, 1561–1564.
- Benoit, G., Oktay-Marshall, S.D., Cantu, A., Hood, E.M., Coleman, C.H., Corapcioglu, M.O., Santschi, P.H., 1994. Partitioning of Cu, Pb, Ag, Zn, Fe, Al, and Mn between filter-retained particles, colloids and solution in six Texas estuaries. *Mar. Chem.* 45, 307–336.
- Bruland, K.W., 1989. Organic zinc speciation: complexation of Zn by natural organic ligands in the central North Pacific. *Limnol. Oceanogr.* 34, 269–285.
- Bruland, K.W., 1992. Complexation of cadmium by natural organic ligands in the central North Pacific. *Limnol. Oceanogr.* 37, 1008–1017.
- Bruland, K.W., Franks, R.P., Knauer, G.A., Martin, J.M., 1979. Sampling and analytical methods for the determination of copper, cadmium, zinc, and nickel at the nanogram per liter level in seawater. *Anal. Chim. Acta* 105, 223–245.
- Bruland, K.W., Coale, K.H., Mart, L., 1985. Analysis of seawater for dissolved cadmium, copper and lead: an intercomparison of voltammetric and atomic absorption methods. *Mar. Chem.* 17, 285–300.
- Bruland, K.W., Donat, J.R., Hutchins, D.A., 1991. Interactive influences of bioactive trace metals on biological production in oceanic waters. *Limnol. Oceanogr.* 36, 1555–1577.
- Buesseler, K.O. et al., 1996. An intercomparison of cross-flow filtration techniques used for sampling marine colloids: overview and organic carbon results. *Mar. Chem.* 51, 1–31.
- Buffle, J., 1988. Complexation Reactions in Aquatic Systems: An Analytical Approach. Ellis Horwood, Chichester, 692 pp.
- Capodaglio, G., Coale, K.H., Bruland, K.W., 1990. Lead speciation in surface waters of the Eastern North Pacific. *Mar. Chem.* 29, 221–233.
- Chin, W.-C., Orellana, M.V., Verdugo, P., 1998. Spontaneous assembly of marine dissolved organic matter into polymer gels. *Nature* 391, 568–572.
- Coale, K.H., Bruland, K.W., 1988. Copper complexation in the Northeast Pacific. *Limnol. Oceanogr.* 33, 1084–1101.
- Crumbliss, A.L., 1991. Aqueous solution equilibrium and kinetic studies of iron siderophore and model siderophore complexes. In: Winkelmann, G., (Ed.), *CRC Handbook of Microbial Iron Chelates*, Chapter 7. CRC Press, NY.
- Dai, M., Martin, J.M., Cauwet, G., 1995. The significant role of colloids in the transport and transformation of organic carbon and associated trace metals (Cd, Cu and Ni) in the Rhone delta (France). *Mar. Chem.* 51, 159–175.
- Donat, J.R., 1995. The speciation of dissolved copper and cad-

- mium in the Chesapeake Bay. 208th American Chemical Society, Abstract 107.
- Donat, J.R., Bruland, K.W., 1995. Trace Elements in the Oceans. In: Salbu, B., Steinnes, E. (Eds.), Trace Elements in Natural Waters. CRC Press, Boca Raton, pp. 247–292.
- Donat, J.R., Lao, K., Bruland, K.W., 1994. Speciation of copper and nickel in South San Francisco Bay: a multi-method approach. *Anal. Chim. Acta* 284, 547–572.
- Gonzalez-Davila, M., Santana-Casiano, J.M., Perez-Peña, J., Millero, F.J., 1995. Binding of Cu(II) to the surface and exudates of the alga *Dunaliella tertiolecta* in seawater. *Environ. Sci. Technol.* 29 (2), 289–301.
- Gordon, A.S., Dyer, B.J., Kango, R.A., Donat, J.R., 1996. Copper ligands isolated from estuarine water by immobilized metal affinity chromatography: temporal variability and partial characterization. *Mar. Chem.* 53, 163–172.
- Greenamoyer, J.M., Moran, S.B., 1997. Investigation of Cd, Cu, Ni and ^{234}Th in the colloidal size range in the Gulf of Maine. *Mar. Chem.* 57, 217–226.
- Guo, L., Coleman, C.H., Santschi, P.H., 1994. The distribution of colloidal and dissolved organic carbon in the Gulf of Mexico. *Mar. Chem.* 45, 105–119.
- Gustafsson, O., Buesseler, K.O., Gschwend, P.M., 1996. On the integrity of cross-flow filtration for collecting marine organic colloids. *Mar. Chem.* 55, 93–111.
- Honeyman, B.D., Santschi, P.H., 1989. A Brownian-pumping model for trace metal scavenging: evidence from Th isotopes. *J. Mar. Res.* 47, 950–995.
- Hughes, M.N., Poole, R.K., 1989. Metals and Microorganisms. Chapman & Hall, London, 412 pp.
- Huntsman, S.A., Sunda, W.G., 1980. The role of trace metals in regulating phytoplankton growth. In: Morris, I. (Ed.), The Physiological Ecology of Phytoplankton. University of California Press, Berkeley, pp. 285–328.
- Johnson, K.S., Gordon, R.M., Coale, K.H., 1997. What controls dissolved iron concentrations in the world ocean?. *Mar. Chem.* 57, 137–161.
- Kozelka, P.B., Bruland, K.W., 1998. Chemical speciation of dissolved Cu, Zn, Cd, and Pb in Narragansett Bay, Rhode Island. *Mar. Chem.*, in press.
- Kozelka, P.B., Sanudo-Wilhelmy, S., Flegal, A.R., Bruland, K.W., 1997. Physico-chemical speciation of lead in South San Francisco Bay. *Estuar. Coast. Shelf Sci.* 44 (6), 649–658.
- Law, B.A., 1980. Transport and utilization of proteins by bacteria. In: Payne, J.W. (Ed.), Microorganisms and Nitrogen Sources. Wiley, Chichester, pp. 381–409.
- Lee, J.G., Ahner, B.A., Morel, F.M.M., 1996. Export of cadmium and phytochelatin by the marine diatom *Thalassiosira weissflogii*. *Environ. Sci. Technol.* 30 (6), 1814–1821.
- Mackey, D.G., Zirino, A., 1994. Comments on trace metal speciation in seawater or do 'onions' grow in the sea. *Anal. Chim. Acta* 284, 635–647.
- Mantoura, R.F.C., Dickson, A.G., Riley, J.P., 1978. The complexation of metals with humic materials in natural waters. *Estuar. Coast. Shelf Sci.* 6, 387–408.
- Martin, J.M., Dai, M.H., Cauwet, G., 1995. Significance of colloids in the biogeochemical cycling of organic carbon and trace metals in the Venice Lagoon (Italy). *Limnol. Oceanogr.* 40 (1), 119–131.
- Moffett, J.W., Brand, L.E., 1996. Production of strong, extracellular Cu chelators by marine cyanobacteria in response to Cu stress. *Limnol. Oceanogr.* 41 (3), 388–395.
- Moffett, J.W., Brand, L.E., Zika, R.G., 1990. Distribution and potential sources and sinks of copper chelators in the Sargasso sea. *Deep-Sea Res.* 37, 27–36.
- Moffett, J.W., Brand, L.E., Croot, P.L., Barbeau, K.A., 1996. Cu speciation and cyanobacterial distribution in harbors subject to anthropogenic Cu inputs. *Limnol. Oceanogr.* 42, 789–799.
- Moore, R.M., Hunter, K.A., 1995. Thorium adsorption in the ocean: reversibility and distribution amongst particle sizes. *Geochim. Cosmochim. Acta* 49, 2253–2257.
- Moran, S.B., Buesseler, K.O., 1992. Short residence times of colloids in the upper ocean estimated from ^{238}U – ^{234}Th disequilibrium. *Nature* 359, 221–223.
- Moran, S.B., Buesseler, K.O., 1993. Size-fractionated ^{234}Th in continental shelf waters off New England: implications for the role of colloids in oceanic trace metal scavenging. *J. Mar. Res.* 51, 893–922.
- Moran, S.B., Moore, R.M., 1989. The distribution of colloidal aluminum and organic carbon in coastal and open ocean waters off Nova Scotia. *Geochim. Cosmochim. Acta* 53, 2519–2527.
- Moran, S.B., Yeats, P.A., Balls, P.W., 1996. On the role of colloids in trace metal solid-solution partitioning in continental shelf waters: a comparison of model results and field data. *Cont. Shelf Res.* 16, 397–408.
- Morel, F.M.M., Hering, J.G., 1993. Principles and Applications of Aquatic Chemistry. Wiley, New York, 570 pp.
- Muller, F.L.L., 1996. Interactions of copper, lead and cadmium with the dissolved, colloidal and particulate components of estuarine and coastal waters. *Mar. Chem.* 52, 245–268.
- Niven, S.E.H., Kepkay, P.E., Boraie, A., 1995. Colloidal organic carbon and colloidal ^{234}Th dynamics during a coastal phytoplankton bloom. *Deep-Sea Res.* 42 (1), 257–273.
- Nixon, S.W., Granger, S.L., Nowicki, B.L., 1995. An assessment of the annual mass balance of carbon, nitrogen, and phosphorus in Narragansett Bay. *Biogeochemistry* 31, 15–61.
- Nurnberg, H.W., 1977. Potentialities and applications of advanced polarographic and voltammetric methods in environmental research and surveillance of toxic metals. *Electrochim. Acta* 22, 935–949.
- Olafson, R.W., McCubbin, W.D., Kay, C.M., 1988. Primary- and secondary-structural analysis of a unique prokaryotic metallothionein from a *Synechococcus* sp. cyanobacterium. *Biochem. J.* 251, 691–699.
- Payne, J.W., 1980. Transport and utilization of peptides by bacteria. In: Payne, J.W. (Ed.), Microorganisms and Nitrogen Sources. Wiley, Chichester, pp. 212–256.
- Pilson, M.E.Q., Hunt, C.D., 1989. Water quality survey of Narragansett Bay: A summary of results from the SINBADD 1985–1986. Graduate School of Oceanography, University of Rhode Island, #NBP-89-22.

- Plavsic, M., Krznicaric, D., Branica, M., 1982. Determination of the apparent copper complexing capacity of seawater by anodic stripping voltammetry. *Mar. Chem.* 11, 17–31.
- Powell, R.T., Landing, W., Iron Cycling in the Sargasso Sea. *Limnol. Oceanogr.*, submitted.
- Powell, R.T., Landing, W.M., Bauer, J.E., 1996. Colloidal trace metals, organic carbon and nitrogen in a Southeastern US estuary. *Mar. Chem.* 55, 165–176.
- Ruzic, I., 1982. Theoretical aspects of the direct titration of natural waters and its information yield for trace metal speciation. *Anal. Chim. Acta* 140, 99–113.
- Santana-Casiano, J.M., Gonzalez-Davila, M., Perez-Pena, J., Millero, F.J., 1995. Pb^{2+} interactions with the marine phytoplankton *Dunaliella tertiolecta*. *Mar. Chem.* 48, 115–129.
- Sanudo-Wilhelmy, S.A., Rivera-Duarte, I., Flegal, A.R., 1997. The role of colloids in the transport of trace metals in the San Francisco Bay estuary. *Geochim. Cosmochim. Acta*, accepted.
- Scarano, G., Bramanti, E., 1993. Voltammetric behavior of marine hydrophobic copper complexes: effect of adsorption processes at mercury electrode. *Anal. Chim. Acta* 277, 137–144.
- Sholkovitz, E.R., 1976. Flocculation of dissolved organic and inorganic matter during the mixing of river water and seawater. *Geochim. Cosmochim. Acta* 39, 831–845.
- Sholkovitz, E.R., Copland, D., 1981. The coagulation, solubility, and adsorption properties of Fe, Mn, Cu, Ni, Cd, Co, and humic acids in a river water. *Geochim. Cosmochim. Acta* 45, 181–189.
- Sholkovitz, E.R., Boyle, E.A., Price, N.B., 1978. The removal of dissolved humic acids and iron during estuarine mixing. *Earth Planet. Sci. Lett.* 40, 130–136.
- Sigleo, A.C., Means, J.C., 1990. Organic and inorganic components in estuarine colloids: implications for sorption and transport of pollutants. *Rev. Environ. Contam. Toxicol.* 122, 123–147.
- Silva, J.J.R.F.d., Williams, R.J.P., 1991. *The Biological Chemistry of the Elements*. Clarendon Press, Oxford, 561 pp.
- Stockner, J.G., Klut, M.E., Cochlan, W.P., 1989. Leaky filters: a warning to aquatic ecologists. *Can. J. Fish. Aquat. Sci.* 47, 16–23.
- van den Berg, C.M.G., 1982. Determination of copper complexation with natural organic ligands in seawater by equilibration with MnO_2 : I. Theory. *Mar. Chem.* 11, 307–322.
- van den Berg, C.M.G., Kramer, J.R., 1979. Determination of complexation capacities and conditional stability constants for copper in natural waters using MnO_2 . *Anal. Chim. Acta* 106, 113–120.
- van den Berg, C.M.G., Buckley, P.J.M., Huang, Z.Q., Nimmo, M., 1986. An electrochemical study of the speciation of copper, zinc and iron in two estuaries in England. *Estuar. Coast. Shelf Sci.* 22, 479–486.
- Wells, M.L. in preparation. Soluble Fe in IronEx: II. Implications for natural blooms in the Equatorial Pacific.
- Wells, M.L., Bruland, K.W., in press. An improved method for rapid preconcentration and determination of bioactive trace metals in seawater using solid phase extraction and high resolution inductively coupled mass spectrometry. *Mar. Chem.*
- Wells, M.L., Goldberg, E.D., 1991. Occurrence of small colloids in sea water. *Nature* 353, 342–344.
- Wells, M.L., Goldberg, E.D., 1993. Colloid aggregation in seawater. *Mar. Chem.* 41, 353–358.
- Wells, M.L., Smith, G.J., Bruland K.W., submitted. The distribution of colloidal and particulate bioactive metals in Narragansett Bay, RI. *Mar. Chem.*, in review.
- Whitehouse, B.G., MacDonald, R.W., Iseki, K., Yunker, M.B., 1989. Organic carbon and colloids in the Mackenzie River and Beaufort Sea. *Mar. Chem.* 26, 371–378.