

Biological factors regulating the chemical speciation of Cu, Zn, and Mn under different nutrient regimes in a marine mesocosm experiment

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Abstract

Previous mesocosm experiments and field studies have indicated a central role of major nutrients (e.g., N, P, and Si) in regulating phytoplankton abundance and species composition and influencing the rate of uptake of bioactive metals by phytoplankton. We monitored the changes in bioactive metals (Cu, Zn, and Mn) concentration and speciation that occurred over the course of a bloom of the coccolithophore *Emiliana huxleyi* under three different nutrient treatments. In each treatment, the electrochemically labile fraction of Cu decreased from 1.1 to 0.3 nmol L⁻¹ during the formation of the *E. huxleyi* bloom. This decrease was likely due to the active release of organic ligands by phytoplankton, which shut off as soon as the level of labile Cu reached 0.3 nmol L⁻¹ ([Cu²⁺] = 0.02 nmol L⁻¹). Organic Zn-binding ligands showed elevated concentrations in bag 1 (the N- and P-replete bag) only. The release of these ligands coincided with a 15-fold increase in the concentration of dead *E. huxleyi* cells in bag 1 but only a threefold increase in the P- or N-depleted bags. This suggests that the Zn-binding ligands may have originated from dead or decaying *E. huxleyi* cells. In both the P- and N-limited bags, labile Mn concentrations showed a succession of two peaks and troughs over the 2-week period; these were synchronized—but inversely correlated—with bacterial abundance. Labile Mn showed weaker fluctuations and no clear synchronization with bacterial abundance in the N- and P-replete bag. We hypothesize that the passive release of organic Zn-binding ligands under the nutrient-replete conditions may have helped restrict the detrimental effect of Zn²⁺ on Mn uptake by phytoplankton cells, thus diverting part of the dissolved Mn away from sequestration or oxidation by bacteria.

It is well established that the growth rate of phytoplankton in the sea is mainly limited by the rate of supply of limiting nutrients—typically N, P, and Si (Cloern 1996). In addition, phytoplankton species composition is strongly influenced by the relative concentrations of these nutrient elements. For example, it is possible to give rise to the selective development of different phytoplankton groups during mesocosm experiments by simply manipulating the nutrient concentrations (Egge and Aksnes 1992; Sterner and Hessen 1994). In turn, the balance struck among different phytoplankton

groups can strongly affect the export of particulate carbon and nutrients (including trace metals) from surface to bottom waters (Wassman 1998), as well as the quantity and quality of “dissolved” metal-binding organic substances, or ligands, exuded in the surface layer (Bruland et al. 1991; Croot et al. 2000). Depending on their physicochemical properties, these ligands can influence metal availability not only to the algal species that produce them but also to their competitors (Whitfield 2001). At any time during the cycle of ligand production and degradation, different microorganisms will seek to optimize their use of essential metals according to their cellular quotas (Morel et al. 1991), uptake rates (Hudson and Morel 1990, 1993), and concentration mechanisms (Hudson and Morel 1993; Sunda and Huntsman 1998), leading to changes in community structure with time. Recent work by Vasconcelos et al. (2002) has further suggested that the nature of the exudates released by a given phytoplankton species can act directly on the growth of another phytoplankton species, again potentially affecting community structure.

As indicated by previous experimental mesocosm studies (Wassmann et al. 1997), the coupling between macronutrient and carbon cycling—and hence between macronutrient and metal cycling—depends not only on the type of phytoplankton present but also on trophic processes involving the whole food web, such as grazing by zooplankton, phytoplankton-bacteria competition for limiting nutrients, degradation of organic carbon sources by bacteria, and viral lysis of bacteria

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and/or phytoplankton cells. In the coastal environment, these processes generally cannot be measured, because physical forcing due to wind stress, tidal currents, and horizontal density gradients contribute to the observed variability by influencing the rate of vertical mixing, horizontal transport, primary production, and grazing. What mesocosms ensure, compared with field studies, is to sample the same water mass with time. Our approach here was to study the complex interplay between trace metal chemistry and biology in the enclosed, yet natural, setting provided by the large (11 m³) floating mesocosms of the Bergen large-scale facility. During the first week of the experiment, the same phytoplankton community was produced via nutrient enrichment in each of the bags. During the second week, nutrients were supplied in different N:P proportions to the different bags, and the responses were examined at the food web and chemical speciation levels.

Our first objective was to examine the short-term consequences of P or N limitation on metal speciation. Our second objective was to identify the extent to which the observed changes in metal speciation are produced by specific components of the ecosystem, such as phytoplankton, bacteria, or viruses.

Materials and methods

Study site and experimental setup—Mesocosm experiments were conducted at the large-scale facility moored in Raunefjord, ~200 m from the shore, near the Marine Biological Station of the University of Bergen, on Norway's west coast. Nine enclosures, which allowed three replicates of three different treatments, were secured along the upwind, south-facing side of a large raft. The experiments took place in the first three enclosures (bags 1–3), as part of a larger experiment that included all nine enclosures. Each enclosure consisted of a heavy-duty polyethylene bag (90% transmission of photosynthetic active radiation) that was fastened to a floating frame ~0.7 m high. The bags had a diameter of 2 m, were 4 m deep, and contained a volume of 11 m³. They were open to the atmosphere, and the water inside was kept homogeneous by means of an airlift system (Williams and Egge 1998). They were filled on 6 June by pumping water from 2 m depth in the area of the fjord adjacent to the raft. More than 50 m³ of seawater was used to flush the pumping system prior to pumping seawater into the bags. The bags were pre-equilibrated with seawater for 2 d prior to being filled, to reduce contamination or loss of trace chemical constituents. They were supplied daily (between 0900 and 1000 h) with nitrate (NaNO₃; Merck Suprapur) and phosphate (K₂HPO₄; Merck Suprapur). The metal contents of the added nutrient solutions were measured and found to be an entirely negligible source of Cu, Zn, or Mn. Indeed, total concentrations of metals inadvertently added to the bags from the stock solutions of nutrients were calculated to be 0.03–0.04 nmol L⁻¹ for Cu, 0.15–0.21 nmol L⁻¹ for Zn, and 0.06–0.08 nmol L⁻¹ for Mn. Between 6 and 11 June, N and P were added in a molar N:P ratio of 15:1 (1.5 μmol L⁻¹ NO₃ and 0.1 μmol L⁻¹ PO₄). From 12 June onward, bag 1 (N and P-replete) was maintained at this ratio, whereas the P-de-

pleted bag (bag 2) was supplemented with N and P added at a molar ratio of 75:1 (1.5 μmol L⁻¹ NO₃ and 0.02 μmol L⁻¹ PO₄), and the N-depleted bag (bag 3) was supplemented with N and P at a molar ratio of 5:1 (0.5 μmol L⁻¹ NO₃ and 0.1 μmol L⁻¹ PO₄). By the end of the experiment, the N:P ratio that represented the cumulative amount of N and P added to each bag was 15, 32, and 8.3 for bags 1, 2 and 3, respectively.

Monitoring of water quality—Temperature, salinity, and the dissolved oxygen concentration were measured daily using a multiparameter environmental probe YSI Model 85 (Ocean Scientific International). No significant differences in these parameters were observed among the bags, and their overall variations were small. Therefore, these parameters were assumed to be irrelevant to explain the differences observed among bags. Nutrients were determined by standard flow injection analysis procedures adapted to a Skalar SAN⁺⁺ automated segmented flow analyzer. Triplicate samples for chlorophyll *a* analysis were filtered on 0.45-μm Sartorius filters that were then wrapped in aluminum foil and snap frozen in liquid nitrogen. Samples were stored at 20°C in darkness prior to analysis. Filters were then extracted in cold 90% acetone in darkness for 24 h prior to analysis on a Turner Design Model 10 AU fluorometer. Measurements of pH, using a Metrohm combined pH glass electrode, Metrohm Model 713 pH meter, and Metrohm standard reference buffers, were made within 2 h of sampling. These raw pH_{NBS} values were later converted to the seawater scale (pH_{SWS}) on the basis of total hydrogen ion concentrations. The conversion factors were obtained after a series of acid titrations in artificial seawater samples (Almgren et al. 1983). Total alkalinity (Alk) was determined after treating 100-ml aliquots of the samples with a 25-ml addition of 0.01 mol L⁻¹ HCl and measuring the final pH (Almgren et al. 1983).

Enumeration of phytoplankton, bacteria, and viruses—The different populations of picoplanktonic organisms were discriminated on the basis of their light scattering and fluorescence signals using a flow cytometer equipped with a 488-nm argon laser (FACSCalibur; Becton Dickinson). Flow cytometric analyses of autotrophs, heterotrophic bacteria, and viruses were done as described in Marie et al. (1999) and Brussaard et al. (2000). Autotrophic populations were discriminated on the basis of their forward and right-angle light scatter and chlorophyll and phycoerythrin fluorescence. Heterotrophic bacteria and viruses were stained using the nucleic acid-specific dye SYBR Green I (Marie et al. 1999) and discriminated on the basis of their forward and right-angle scatter versus their green-DNA fluorescence. All cellular parameters were normalized to the values measured for 1-μm beads (Molecular Probes). Data were collected in list mode files and then analyzed using CYTOWIN (available at <http://www.sb-roscoff.fr/Phyto/cyto.html>) and WinMDI version 2.7 (<http://www.bio.umass.edu/mcbfac/flowcat.html#windi>).

The different species of phytoplankton were also identified and counted using a phase-contrast microscope (Jacquet et al. 2002). Cell numbers were determined in fresh f/2 medium (Guillard and Lorenzen 1972) using a standard count-

ing chamber. A clear correlation ($r^2 = 0.83$, $P = 0.01$, $n = 69$) was found between phase-contrast microscopy counts of *Emiliana huxleyi* (live + dead) and the population referred to as high-scatter (HS) *E. huxleyi* (Jacquet et al. 2002), confirming that this population was indeed *E. huxleyi*.

Sampling and handling procedures for trace metals—Samples for pH, alkalinity, and trace metal analyses were taken in 4-liter low-density polyethylene (LDPE) bottles tied to a Kevlar line and rapidly lowered from the surface to 1.5 m with the use of a plastic-coated weight. They were brought to our laboratory within 45 min of collection. Subsamples for “dissolved” metals were immediately filtered through 0.4- μm polycarbonate membranes (Osmonics) at a vacuum of 50 mm Hg (absolute) into 500-ml LDPE bottles. Sample collection, filtration, and analysis were done using clean techniques. The latter included rigorous, multistage cleaning procedures for LDPE bottles, filtration equipment, and Teflon vials (Achterberg et al. 2001); keeping sample transfers to a minimum; handling samples while wearing plastic gloves and a nylon coat; and carrying out filtration, the dispensing of ultrapure water, and analyses under the laminar flow of HEPA-filtered air.

Total metal determinations—One 500-ml aliquot of each subsample was acidified to pH 2.2 with 0.5 ml of high-purity HCl (Aldrich double distilled) and allowed to sit at room temperature for 4 weeks. Seawater treated in this manner was then spiked with Merck Suprapur H_2O_2 (100 μl per 10 ml of sample) and ultraviolet (UV)-irradiated at 90°C for 90 min using a Metrohm 705 UV Digester to eliminate interfering organic matter. Prior to the electrochemical determination of total Cu and Zn ($[\text{Cu}_T]$ and $[\text{Zn}_T]$), the pH was raised to 5.0 by the addition of 1 ml of a 4 mol L^{-1} $\text{NH}_3/\text{NH}_4\text{OAc}$ solution (Aldrich double distilled, ppb/Teflon grade NH_3 and HOAc). The UV-irradiated portion for total Mn ($[\text{Mn}_T]$) analysis was brought to pH 7.5 with 1 mol L^{-1} NH_4OH . Total metal analyses were done by the method of standard additions. Differential pulse anodic stripping voltammetry (DPASV) was used to measure the Cu deposited at -0.70 V versus Ag/AgCl reference in an ethylenediamine medium (Scarano et al. 1992). Total Zn was measured by DPASV using a 300-s deposition step at -1.20 V (Muller and Kester 1991). Total Mn was measured by differential pulse cathodic stripping voltammetry (DPCSV) using the method developed by Roitz and Bruland (1997). The voltametric measurement system consisted of a Metrohm 693 VA Processor and a Metrohm 694 VA Stand equipped with a rotating mercury-coated glassy carbon electrode, a platinum wire counter electrode, and an Ag/AgCl reference electrode. A sample volume of 30 ml was used throughout. The relative standard deviation of sample measurements was typically 6% for $[\text{Cu}_T]$, 7% for $[\text{Zn}_T]$, and 5% for $[\text{Mn}_T]$. Detection limits were 0.3 nmol L^{-1} for Cu, 0.2 nmol L^{-1} for Zn, and 2.0 nmol L^{-1} for Mn. The accuracy of the procedures was validated using near-shore seawater reference material for trace metals (CASS-3; National Research Council of Canada). Three replicate measurements of $[\text{Cu}_T]$, $[\text{Zn}_T]$, and $[\text{Mn}_T]$ were made and the 95% confidence intervals calculated. The measured values ($[\text{Cu}_T] = 8.5 \pm 1.5$ nmol L^{-1} ;

$[\text{Zn}_T] = 16.3 \pm 2.6$ nmol L^{-1} ; and $[\text{Mn}_T] = 37.0 \pm 9.3$ nmol L^{-1}) were in good agreement with the certified ones ($[\text{Cu}_T] = 8.1 \pm 1.0$ nmol L^{-1} ; $[\text{Zn}_T] = 19.0 \pm 3.8$ nmol L^{-1} ; and $[\text{Mn}_T] = 45.7 \pm 6.5$ nmol L^{-1}).

Chemical speciation measurements—Details of the titration procedures adopted for the study of Cu and Zn complexation—except for the application of matrix exchange to DPASV measurement of Zn—are reported in detail elsewhere (Muller et al. 2001). In brief, four different titrations were done on each sample. The first was the titration of Cu present in the sample with ethylenediamine (EN) and DPASV measurement of the labile CuEN and Cu(EN)_2 complexes formed during the titration (Scarano et al. 1992). It allowed us to determine the concentration (C_{L1}) and the related conditional stability constant (K'_1) of those natural organic ligands that formed very strong Cu complexes. The second procedure was a titration with Cu(II) ions. It enabled us to probe the binding sites, characterized by C_{L2} and K'_2 , of all remaining ligands that influenced Cu speciation to a significant extent. Both procedures included a matrix exchange step whereby the sample was exchanged for a solution that contained 1.0×10^{-2} mol L^{-1} EN and 0.5×10^{-2} mol L^{-1} HCl (pH 8.4). The third procedure was a titration with Zn(II) ions, followed by measurement of the electrochemically active fraction of Zn (DPASV—labile Zn). It was thought that the presence of surface-active substances resulting from biological activity in the bags might affect the kinetics of stripping and lead to a change in peak height or shape. With this in mind, we decided to perform matrix exchange for Zn as well: after the deposition step at -1.20 V, the sample was exchanged for a solution that contained 0.4 mol L^{-1} NH_4OAc (pH 4.7). This modification ensured that the voltametric signal depended only on the parameters of the deposition step. The fourth procedure was based on a titration with Mn(II) ions and measurement of DPCSV-labile Mn, according to the method of Roitz and Bruland (1997).

In the above procedures, the “labile” species of the metal were those that contributed to the voltametric signal to the same extent as the free metal ion. The deposition potentials chosen were designed to ensure that strong organic complexes (except for those formed between Cu and EN) would be kinetically inert (nonlabile). For all metals, there was generally good agreement ($r^2 \geq 0.80$, $n = 25$) between the values of labile metal concentrations directly measured in the untreated sample and those calculated from the complexation parameters (next section) and an independent measurement of total metal concentration (previous section). As a consequence, only the calculated labile metal values are given in Tables 3–5 below.

Speciation calculations—Metal titration data were fitted to a discrete ligand model according to the linearization procedure proposed by Ruzic (1982) and van den Berg (1982). This procedure required additional input data about the extent of inorganic complexation, as expressed by the side reaction coefficient α_{inorg} . To this end, estimates of $[\text{Cl}^-]$, $[\text{OH}^-]$, $[\text{HCO}_3^-]$, $[\text{CO}_3^{2-}]$, and $[\text{SO}_4^{2-}]$ were obtained from independent measurements of S, pH, and Alk and from the calculated effects of interactions among the major seawater

Table 1. Comparison of linear and nonlinear fits to the titration results of five representative samples. The 90% confidence intervals of the estimated parameters are shown in parentheses.

Sample	Linearization method		Nonlinear regression	
	C_L (nmol L ⁻¹)	log K'	C_L (nmol L ⁻¹)	log K'
Copper				
Bag 1, 10 June	8.1 (6.6–9.6)	14.92 (14.76–15.03)	7.6 (6.6–8.6)	14.99 (14.86–15.08)
	28.0 (23.8–32.2)	9.60 (9.46–9.70)	24.5 (20.6–28.4)	9.70 (9.50–9.84)
	49.5 (43.6–55.4)	8.50 (8.47–8.53)	50.0 (43.4–56.6)	8.58 (8.48–8.66)
Bag 1, 14 June	6.2 (5.4–7.0)	14.89 (14.72–15.01)	5.8 (5.2–6.4)	14.95 (14.80–15.06)
	20.0 (17.0–23.0)	9.72 (9.62–9.80)	22.2 (18.2–26.2)	9.56 (9.36–9.70)
	23.2 (19.8–26.6)	8.34 (8.21–8.44)	20.3 (17.1–23.5)	8.42 (8.20–8.57)
Bag 1, 18 June	5.1 (4.5–5.7)	14.74 (14.64–14.82)	5.8 (5.3–6.3)	14.69 (14.61–14.75)
	55.6 (49.0–62.2)	9.96 (9.85–10.05)	60.1 (56.0–64.2)	9.86 (9.72–9.97)
Bag 1, 22 June	3.7 (3.1–4.3)	15.17 (14.97–15.31)	3.7 (3.3–4.1)	15.14 (15.01–15.24)
	55.1 (48.2–62.0)	10.41 (10.29–10.50)	61.0 (56.0–66.0)	10.35 (10.22–10.45)
Raunefjord	9.8 (8.4–11.2)	13.79 (13.65–13.89)	8.7 (7.8–9.6)	13.86 (13.76–13.94)
	75.2 (68.6–81.8)	8.53 (8.39–8.63)	77.7 (73.4–82.0)	8.40 (8.21–8.54)
Zinc				
Bag 1, 10 June	22.0 (18.7–25.3)	8.57 (8.44–8.67)	20.8 (18.0–23.6)	8.51 (8.41–8.59)
Bag 1, 14 June	22.9 (19.5–26.3)	8.36 (8.30–8.41)	21.0 (18.6–23.4)	8.39 (8.30–8.47)
Bag 1, 18 June	68.2 (61.4–75.0)	8.45 (8.38–8.51)	70.9 (65.6–76.2)	8.43 (8.37–8.48)
Bag 1, 22 June	47.1 (41.9–52.3)	8.89 (8.79–8.97)	42.6 (37.9–47.3)	8.96 (8.84–9.05)
Raunefjord	11.9 (10.0–13.8)	8.69 (8.56–8.79)	12.4 (10.6–14.2)	8.76 (8.56–9.17)
Manganese				
Bag 1, 10 June	24.0 (22.4–25.6)	8.88 (8.76–8.97)	24.3 (23.2–25.4)	8.81 (8.72–8.89)
Bag 1, 14 June	23.4 (21.6–25.2)	8.64 (8.51–8.74)	25.5 (23.4–27.6)	8.54 (8.42–8.63)
Bag 1, 18 June	28.1 (26.6–29.6)	8.54 (8.37–8.66)	27.0 (25.7–28.3)	8.58 (8.42–8.70)
Bag 1, 22 June	32.6 (30.6–34.6)	8.70 (8.60–8.78)	33.4 (31.8–35.0)	8.75 (8.66–8.82)
Raunefjord	22.7 (21.2–24.2)	9.01 (8.89–9.10)	22.5 (21.3–23.7)	9.04 (8.94–9.17)

ions (Kester 1986). A plot of $[M_{\text{labile}}]/[ML]$ versus $[M_{\text{labile}}]$ was then produced and examined for linearity. A linear plot indicated that only one ligand was required to fit the titration data. If the transformed plot was nonlinear, the linear and nonlinear portions were used to determine the two ligands involved. It is also worth pointing out that the concentration of Cu already complexed by the highest affinity ligand (L_1) at the start of the Cu titration ($[CuL_1]$) had to be subtracted from the denominator in the $[Cu_{\text{labile}}]/[CuL]$ expression used to evaluate the Cu titration data. This can be understood by noting that, in the case of Cu, the (K'_1 , C_{L1}) binding parameters have already been determined from the EN titration data, so the purpose of the Cu titration is then to determine further sets of (K'_i , C_{Li}) binding parameters. The terminology used here is that C_{Li} is the total concentration of class 1 ligands and the conditional stability constant for that ligand class is $K'_i = [ML_i]/[M^{2+}][L_i]$, where $[L_i]$ is the concentration of class 1 ligands not already bound to the metal.

Two ($i = 1, 2$) or three ($i = 1, 2, 3$) model ligands for Cu and one ($i = 1$) model ligand for Zn or Mn were required to fit the titration data. By combining the corresponding (K'_i , C_{Li}) values with the measurement of total metal concentration ($[M_T]$), it was possible to calculate the portions of each metal complexed with the model ligand(s) L_i and with the various inorganic ligands. The chemical equilibrium modeling software MINEQ (version 4.0; Schecher and McAvoy 1998) was used to perform these calculations.

Analytical error estimation—The 90% confidence intervals for the slope and intercept of all linear (standard additions for total metal determination) or linearized (Ruzic/van den Berg linearization) plots were constructed under the assumption of normally distributed errors (e.g., Walpole and Myers 1985). On the basis of error-propagation equations for sums and products, the 90% confidence intervals for the total metal, labile metal, and ligand concentrations were derived. On average, 90% confidence limits were within $\pm 10\%$, $\pm 12\%$, and $\pm 8\%$ of the measured $[Cu_T]$, $[Zn_T]$, and $[Mn_T]$ values and within $\pm 30\%$, $\pm 25\%$, and $\pm 14\%$ of the calculated $[Cu_{\text{labile}}]$, $[Zn_{\text{labile}}]$, and $[Mn_{\text{labile}}]$ values.

The influence on ligand characterization of the actual method used for the evaluation of the complexation parameters was also examined. For example, Gerringa et al. (1995) argued that, in estuarine waters with a high organic matter content, it is preferable to fit the unlinearized titration data directly, using a nonlinear curve-fitting routine. Table 1 provides a comparison of the results obtained with the linearization method and the nonlinear regression method in five samples representing the range of situations encountered for our titrations. Using the Wilcoxon signed-rank test at the 0.1 level of significance, it was not possible to establish any significant differences between the values of either C_L or log K' obtained with the two methods. The 90% confidence intervals for both C_L and log K' were generally slightly narrower when obtained with the nonlinear method (Table 1),

although the reverse was true when two ligands—and therefore two additional parameters—had to be determined from the same titration (copper: 10 and 14 June). Only results obtained with the Ruzic/van den Berg linearization method are presented below.

Results

Until 14 June (2 days after the N:P treatment was changed), the levels of all chemical parameters were statistically the same in the three bags. Choosing this date (instead of 12 June) as the natural break between the two phases of the experiment therefore makes the presentation and discussion of the results easier to follow.

Phase 1 (8–14 June)—No significant differences in the behavior of the major chemical components (Table 2 and Fig. 1) or in the development of the phytoplankton community (Jacquet et al. 2002) were recorded among the bags. A bloom of picoeukaryotes formed at the beginning of the enrichment period and then crashed over 4 d, probably because of intense grazing activity. This event produced the small initial peak in chlorophyll that is seen in Fig. 1. Until 9 June, diatoms were the most important species in terms of their carbon content (22%–62% of carbon derived from phytoplankton), whereas coccolithophores were the second most important species (17%–30%). On 14 June, the concentration of *E. huxleyi* was slightly above 10^3 cells ml⁻¹ and was similar in the three bags, which suggests that there was no specific nutrient limitation in any of the bags at the start of phase 2.

Metal concentrations, which were not manipulated in this experiment, showed somewhat greater variations among the bags than the major chemical constituents (Fig. 2). Despite precautions taken to avoid the most common forms of contamination (e.g., pumping system, conductivity-temperature-depth probe, and reagents), elevated Cu and Zn levels were observed in bag 1 at the start of the experiment. By 14 June, however, no differences in the values of [Cu_T], [Zn_T], or [Mn_T] were observed among the bags (Fig. 2). Leaving out bag 1, [Cu_T] and [Zn_T] did not show any net variation over time, whereas [Mn_T] decreased steadily over the entire period (Fig. 2).

Labile Zn concentrations were remarkably constant and similar in the three bags. [Mn_{labile}] and [Cu_{labile}] levels appeared to be less uniform, although variations were not much larger than measurement errors (Fig. 3). This was in contrast with the second phase of the experiment, which was characterized by highly significant changes in labile metal concentrations as well as significant differences among bags. Although the Cu-binding ligand (*L*₃) did not affect Cu speciation significantly (only 0%–7% of Cu was present as Cu*L*₃), it is interesting to note that its concentration, *C*_{L3}, decreased exponentially during the first phase of the experiment, to the point of becoming undetectable after 14 June (Fig. 4). In fjord waters adjacent to the bags, *C*_{L3} levels were still as high as 75 nmol L⁻¹ on 16 June—close to the initial values recorded in the bags (78, 87, and 79 nmol L⁻¹). This suggests that processes that generated *L*₃ within the fjord

environment were interrupted after fjord water was pumped into the bags.

Phase 2 (14–22 June)—After the picoeukaryote bloom's demise, the main event began with a bloom of the coccolithophore *E. huxleyi* in each of the bags, rising to a maximum concentration of 1.1×10^5 cells ml⁻¹, with chlorophyll concentrations rising to a maximum of 18.6 μg L⁻¹ in bag 1 (Fig. 1). Corresponding peak values were lower in bags 2 and 3. This was to be anticipated because of the P or N depletion induced in these bags: that average phosphate concentration in bag 2 over the period was 0.07 μmol L⁻¹ (3–4 times lower than in bags 1 and 3), whereas the average nitrate concentration in bag 3 was 0.2 μmol L⁻¹ (10–30 times lower than in bags 1 and 2). At any time during phase 2, coccolithophores were the most important species in terms of their organic carbon contribution (30%–96%), whereas diatoms were among the least important species (0.00%–0.03%). The bloom of coccolithophores crashed over 4 days (18–22 June), ~1 week after it had started. The termination of the bloom occurred 1–2 d earlier in the P-depleted bag than in the other two bags (Fig. 1). In each case, however, this was <24 h after a sudden increase in the population of viruses identified as Eh virus (i.e., a virus that specifically infects *E. huxleyi*; Jacquet et al. 2002). Heterotrophic bacterial abundance declined rapidly during the logarithmic growth phase of the *E. huxleyi* bloom, but they recovered to reach their highest values as the bloom crashed (Fig. 1).

Generally speaking, total metal concentrations were rather uniform and showed little differences among bags throughout the second phase of the experiment; the only exception was a slight depletion in [Mn_T] in the nutrient-replete bag that coincided with the highest recorded concentrations of Chl *a* and *E. huxleyi* cells between 16 and 18 June (Fig. 2). Therefore, the contrasting variation patterns in labile metal concentrations seen in different bags (see next paragraph) did not result from differences in total metal concentrations.

The different trends in labile metal concentrations can be seen in Fig. 3. For [Cu_{labile}], the general trend in each of the bags was a rapid decrease from 0.7–1.3 to 0.3 nmol L⁻¹ between 14 and 16 June (Fig. 3) that was due to the production of *L*₂ ligands (Fig. 4). The simultaneous production of Zn-binding ligands was responsible for a similar trend in [Zn_{labile}] in the nutrient-replete bag, whereas the continuous removal of Zn-binding ligands produced a steady increase in [Zn_{labile}] in the N-depleted bag (Figs. 3, 4). The behavior pattern of [Mn_{labile}] was different still, consisting of little change with time in the nutrient-replete bag but a rapid, two-fold increase followed by a more gradual decrease in the P- and N-depleted bags (Fig. 3). Contrary to levels of [Cu_{labile}] and [Zn_{labile}], variations in the level of [Mn_{labile}] could not be accounted for simply by changes in Mn-binding ligand concentrations. This may be linked to the fact that Mn tends to be incorporated into colloidal matter rather than complexed by well-defined “dissolved” organic ligands (Sulzberger 1997).

Discussion

General trends—After the initial conditioning period (phase 1), variations in the total “dissolved” metal concen-

Table 2. Auxiliary variables used to interpret the changes in metal speciation observed over the course of the mesocosm experiments. Salinity, temperature, nutrients, chlorophyll, bacteria, and HS *Emiliania huxleyi* concentrations were measured every day, but only measurements backed up by trace metal data (Tables 3–5) are shown here.

Date (June)	S	T (°C)	pH _{sws}	Alk (meq L ⁻¹)	NO ₃ ⁻			PO ₄ ³⁻ (μmol L ⁻¹)	SiO ₄ ⁻	Chl <i>a</i> (μg L ⁻¹)	H-bact (× 10 ⁶)	HS-Ehux (× 10 ⁴)
					NO ₃ ⁻	NO ₂ ⁻	NO ₃ ⁻					
Bag 1, depth=1.5 m												
8	29.8	12.3	7.89	2.170	0.6		0.12	0.2	3.53	1.50	0.12	
10	29.8	13.2	7.99	2.190	0.7		0.26	0.2	3.91	1.40	0.24	
12	29.8	12.2	8.05	2.189	1.4		0.27	0.2	3.26	2.80	0.33	
14	29.7	12.0	8.04	2.188	3.1		0.26	0.2	4.02	4.00	1.34	
16	29.7	11.6	8.04	2.167	1.8		0.24	0.2	12.06	0.46	5.20	
18	29.7	11.9	8.06	2.065	1.5		0.32	0.1	18.30	0.32	10.40	
20	29.7	12.1	8.01	2.069	1.6		0.16	0.1	12.26	3.0	3.16	
22	29.6	13.4	8.04	2.066	4.0		0.10	0.1	7.24	4.90	0.24	
Bag 2, depth=1.5 m												
8	29.8	12.2	7.97	2.170	0.5		0.15	0.2	3.59	1.40	0.18	
10	29.9	13.1	8.02	2.185	0.7		0.23	0.2	3.67	1.50	0.28	
12	29.8	12.1	8.04	2.179	1.4		0.29	0.2	2.93	2.90	0.37	
14	29.7	11.9	8.04	2.181	3.1		0.13	0.2	4.56	3.50	1.24	
16	29.7	11.6	8.02	2.115	2.8		0.17	0.3	11.62	0.21	4.46	
18	29.7	12.0	8.06	2.103	3.1		0.06	0.2	11.58	0.29	3.60	
20	29.6	12.1	8.01	2.105	6.6		0.05	0.2	6.34	1.30	0.96	
22	29.6	13.2	8.02	2.097	10.2		0.02	0.1	4.68	1.40	0.35	
Bag 3, depth=1.5 m												
8	29.9	12.1	7.73	2.182	0.5		0.14	0.1	3.26	1.00	0.16	
10	29.9	13.0	7.90	2.183	0.6		0.22	0.2	3.57	1.20	0.24	
12	29.8	12.1	7.97	2.181	1.2		0.23	0.2	3.31	2.40	0.40	
14	29.8	11.9	8.05	2.182	0.6		0.36	0.2	4.42	2.10	1.08	
16	29.7	11.6	8.03	2.106	0.2		0.36	0.2	8.94	0.31	5.12	
18	29.7	12.0	8.02	2.060	0.1		0.19	0.1	11.98	0.49	7.30	
20	29.7	12.1	7.96	2.067	0.1		0.28	0.2	8.40	2.20	5.47	
22	29.7	13.1	7.98	2.067	0.3		0.37	0.1	5.54	3.90	0.82	
Raunefjord, depth=6 m												
16	29.9	11.6	7.73	2.220	0.2		0.04	0.5	1.09	1.50	ND	

ND: not done.

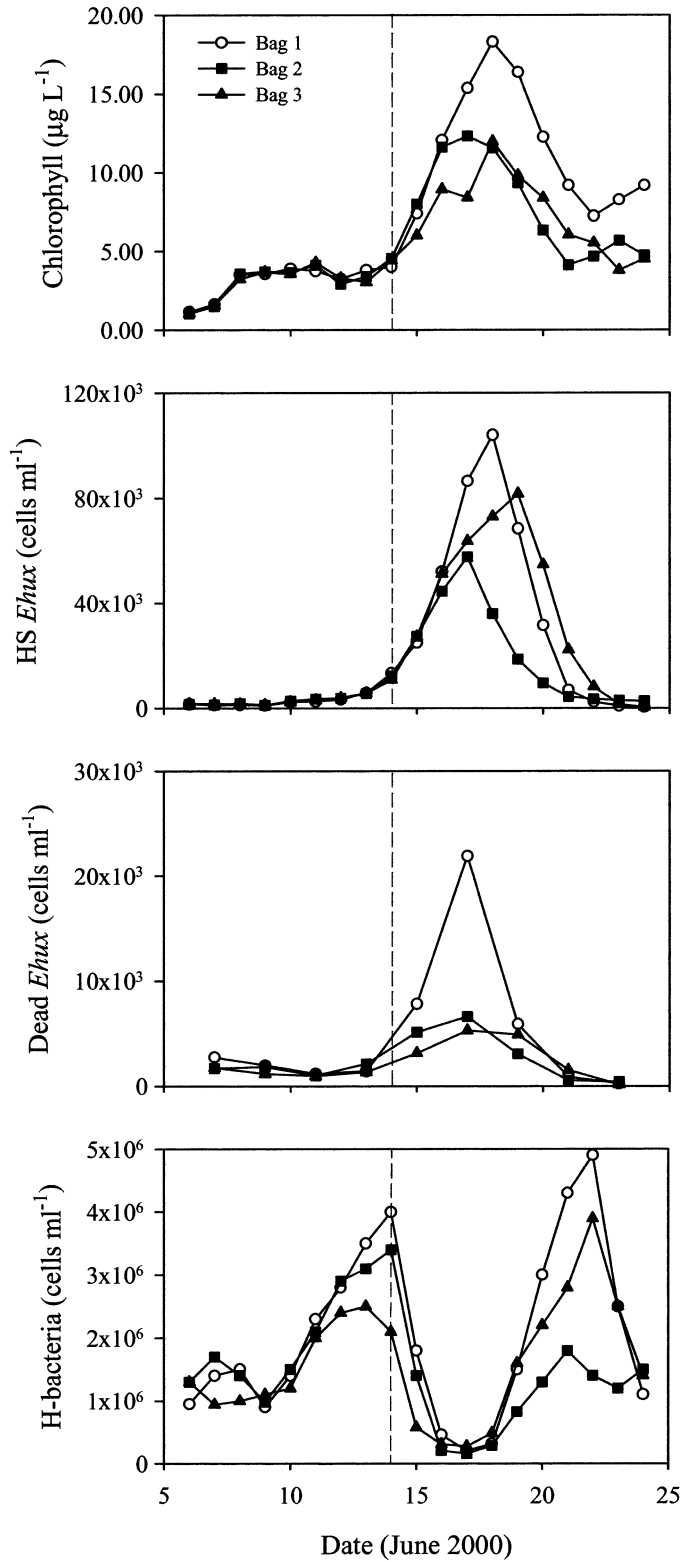


Fig. 1. Time-dependent changes in concentrations of Chl *a*, high-scatter (HS) *E. huxleyi* cells, dead *E. huxleyi* cells, and bacteria in each of the bags. The vertical line denotes the separation between the two phases of the experiment.

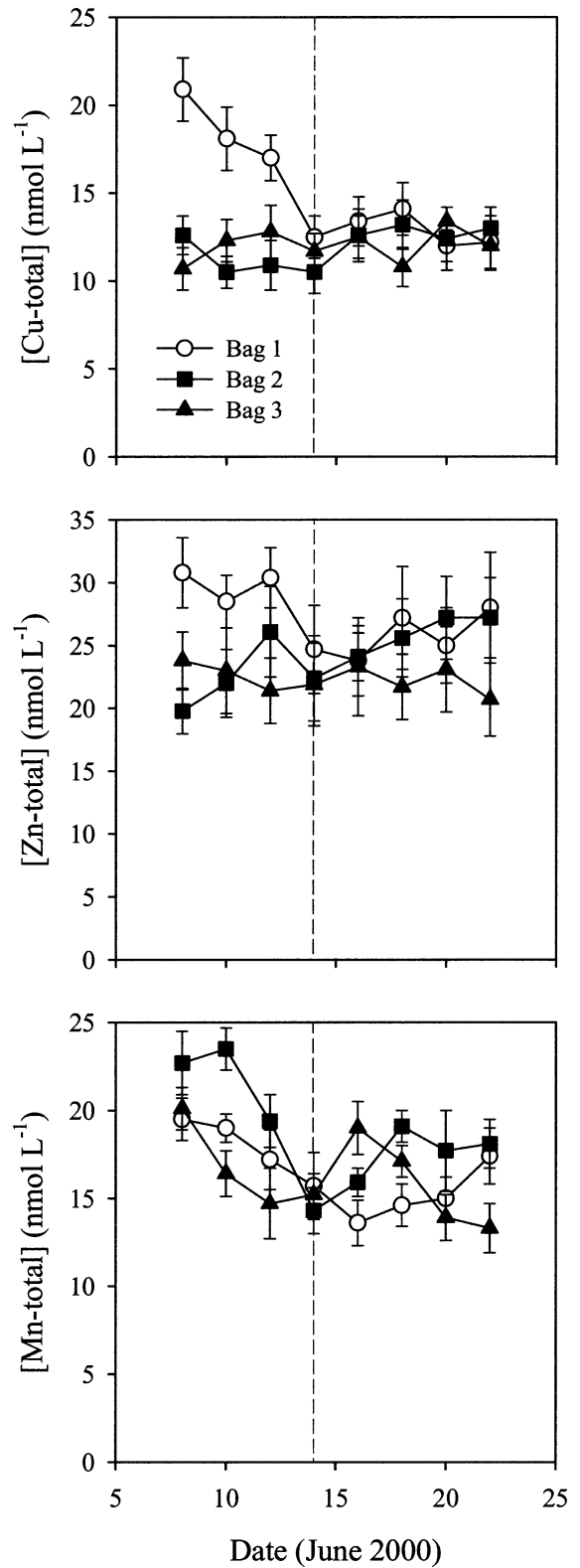


Fig. 2. Time-dependent changes in total Cu, Zn, and Mn concentrations in each of the bags. Bag 1: N- and P-replete, bag 2: P-depleted, and bag 3: N-depleted. Error bars show the 90% confidence limits for each sample.

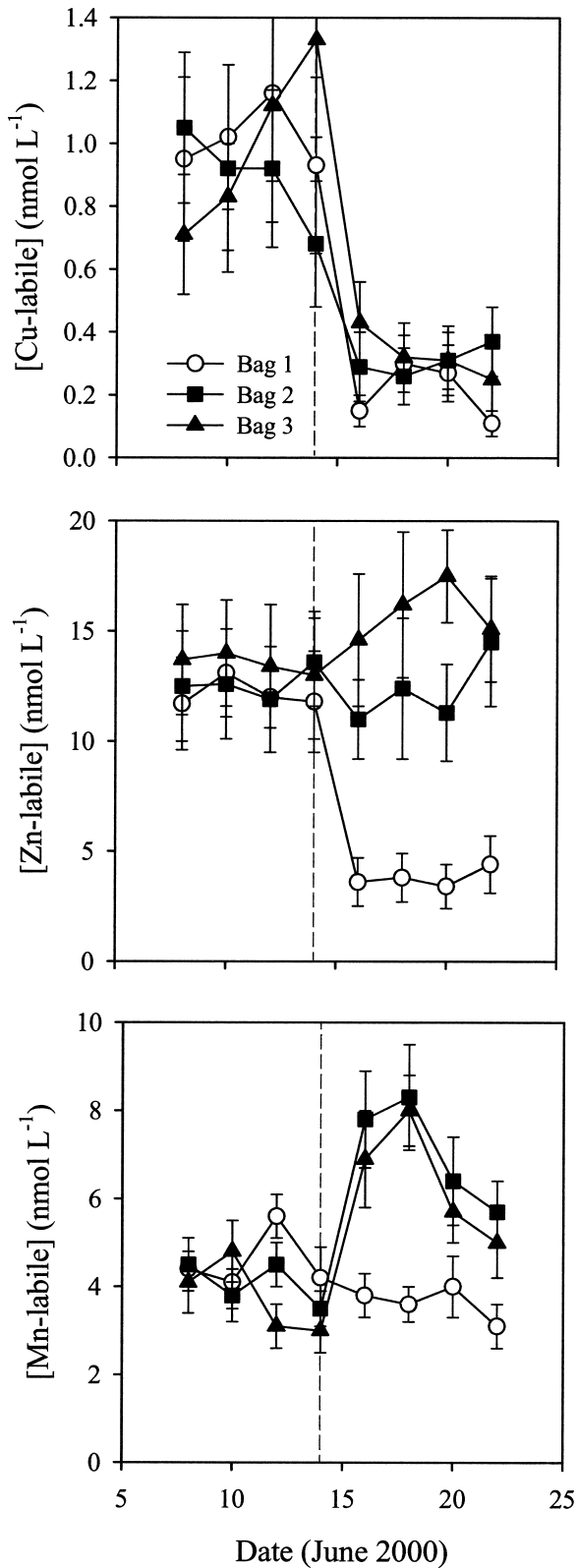


Fig. 3. Time-dependent changes in labile Cu, Zn, and Mn concentrations in each of the bags. Bag 1: N- and P-replete, bag 2: P-depleted, and bag 3: N-depleted. Error bars show the 90% confidence limits for each sample.

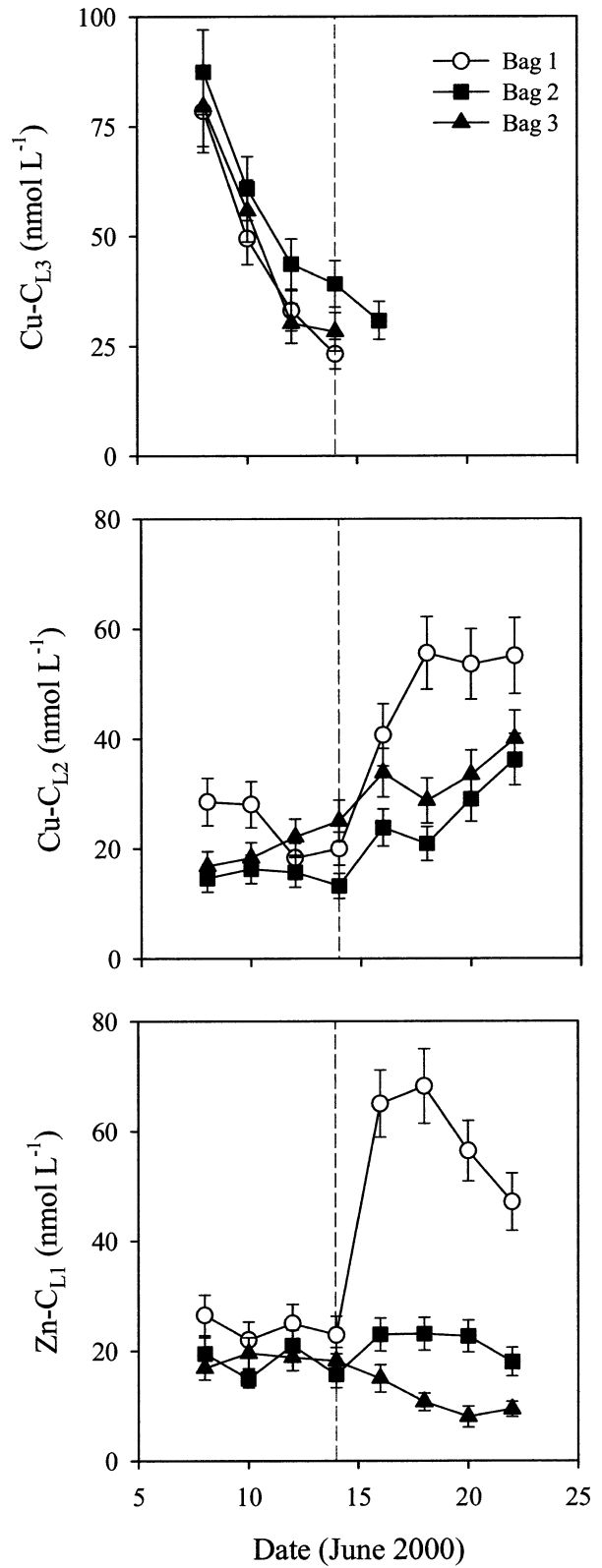


Fig. 4. Time-dependent changes in the concentrations of the weaker Cu-binding ligands (L_1 and L_2) and the concentration of the Zn-binding ligand (L_1) in each of the bags. Bag 1: N- and P-replete, bag 2: P-depleted, and bag 3: N-depleted. Error bars show the 90% confidence limits for each sample.

trations were small (Fig. 2) and were uncorrelated with those in labile metal concentrations (Fig. 3). The implication is that the transfers between the total dissolved fraction and the particulate fraction were either not significant or that they were compensating for each other during phase 2 of the experiment. Either way, there was no significant loss of metals due to sedimentation.

Fluctuations in labile Mn concentration were inversely synchronized with heterotrophic bacterial abundance in the P- and N-depleted bags (Fig. 5); the magnitude of the $[Mn_{labile}]$ fluctuations suggest that up to 40% of the dissolved Mn pool was sequestered and/or oxidized by bacteria and also that this process was readily reversible. The photoreduction of MnO_2 to Mn^{2+} (Sulzberger 1997; Sunda and Huntsman 1998) or digestion of bacteria at low pH by protozoan grazers (Hutchins and Bruland 1994) could explain the concomitance of the sharp increase in $[Mn_{labile}]$ with the decline in bacterial abundance. This synchronization was much looser and the $[Mn_{labile}]$ fluctuations were much weaker in the N- and P-replete bag, which suggests that bacterial activity and grazing were no longer dominant processes. Fluctuations in $[Cu_{labile}]$ levels were likely due to phytoplankton activity in all three bags. In particular, an increase in the organic Cu-binding ligand concentration was observed during the developing coccolithophore bloom in each of the bags. In contrast, neither the variations in coccolithophore (or other algal species) cell numbers nor in bacterial numbers could explain the marked difference in the variations of $[Zn_{labile}]$ observed between bag 1 and the other two bags. In short, although *E. huxleyi* cells were a likely source of Cu-binding ligands, there is no suggestion that they intervened in the production of the organic ligands controlling Zn speciation.

Manganese—Previous studies of Mn speciation in estuarine and marine waters (Luther et al. 1994; Roitz and Bruland 1997) have provided no evidence for significant organic complexation, except in zones characterized by high organic matter decomposition and low O_2 concentrations (Luther et al. 1994). Therefore, although a single ligand complexation model was able to reproduce the titration curves of Mn(II) quite well, one should not interpret this result as proof of complexation of Mn(II) by dissolved organic ligands. Instead, the variations in labile Mn concentrations (Fig. 3) could have resulted from transformations between the (+II) oxidation state, in which the element is primarily present as Mn^{2+} and weak inorganic complexes and is electrochemically active, and higher oxidation states (+III, +IV), in which it is strongly particle reactive and electrochemically inert. If so, one possible explanation for the relatively high conditional stability constants (Table 3) characterizing the inert form of Mn is that it is dictated by the size and shape of organic ligands that become adsorbed at the surface of the colloids produced by the oxidation of Mn(II) (Mackey and Zirino 1994). Alternatively, these small inorganic colloids may become trapped inside organic matrices (Filella and Buffle 1993; Muller 1999), such as those that may be formed by the cross-linking of large and complex polysaccharides exuded by phytoplankton. For example, *Phaeocystis* colonies, which are well known to bloom in the Raune-

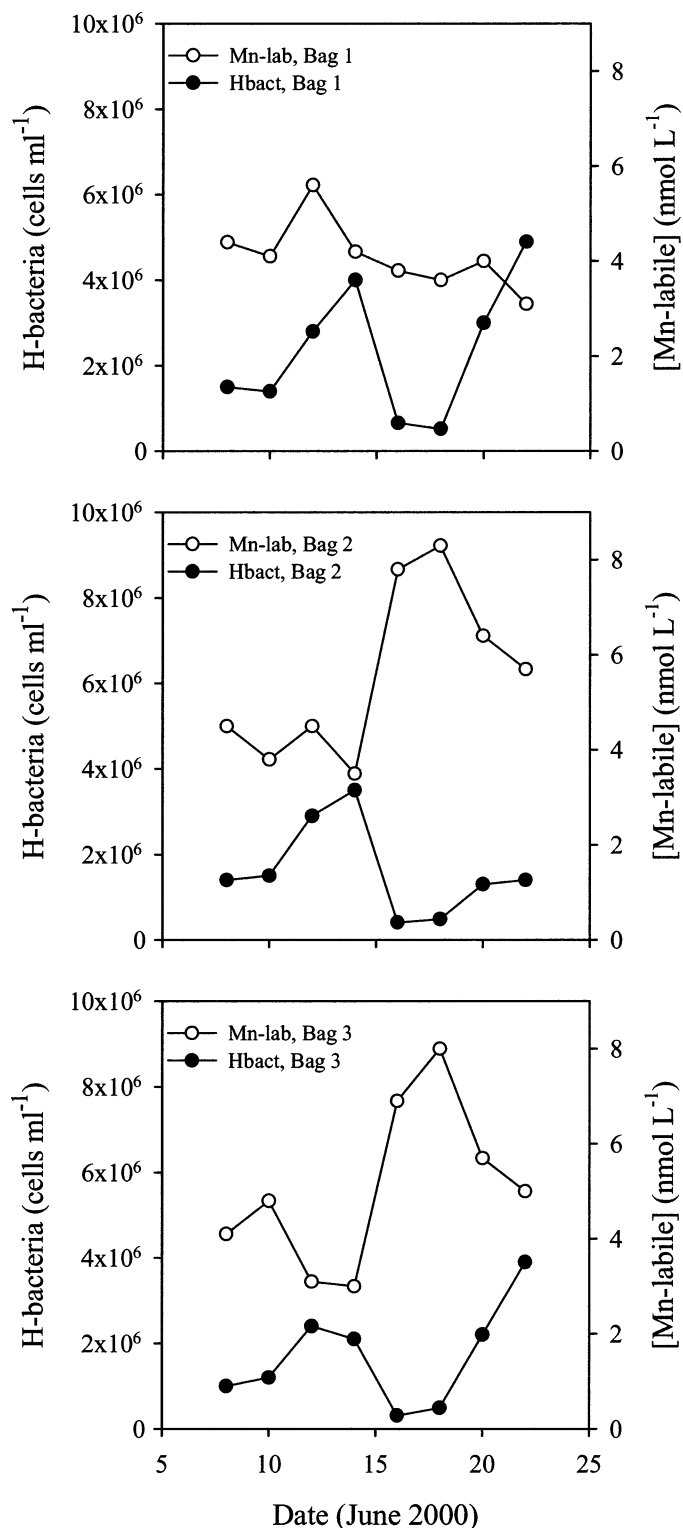


Fig. 5. Comparison of the time-dependent changes in bacterial numbers and labile Mn concentrations in each of the bags. Bag 1: N- and P-replete, bag 2: P-depleted, and bag 3: N-depleted. Error bars show the 90% confidence limits for each sample.

Table 3. Total, labile, and free Mn concentrations, Mn-binding ligand concentrations, and apparent conditional stability constants measured over the course of the mesocosm experiments.

Date (June)	[Mn _T] (nmol L ⁻¹)	α-inorg	[Mn _{labile}]	[Mn ²⁺] (nmol L ⁻¹)	C _{Li}	log K ^p
Bag 1, depth=1.5 m						
8	19.5	1.9	4.4	2.3	29.5	8.65
10	19.0	1.9	4.1	2.2	24.0	8.88
12	17.2	1.9	5.6	3.0	21.5	8.59
14	15.7	1.9	4.2	2.2	23.4	8.64
16	13.6	1.9	3.8	2.0	22.3	8.60
18	14.6	1.9	3.6	1.9	28.1	8.54
20	15.0	1.9	4.0	2.1	30.6	8.43
22	17.6	1.9	3.1	1.7	32.6	8.70
Bag 2, depth=1.5 m						
8	22.7	1.8	4.5	2.5	36.5	8.61
10	23.5	1.9	3.8	2.0	31.1	8.94
12	19.4	1.9	4.5	2.4	23.6	8.87
14	14.3	1.9	3.5	1.8	20.0	8.80
16	15.9	1.9	7.8	4.1	14.2	8.51
18	19.1	1.9	8.3	4.4	16.6	8.63
20	17.7	1.8	6.4	3.3	18.8	8.65
22	18.1	1.8	5.7	3.0	20.4	8.72
Bag 3, depth=1.5 m						
8	20.1	1.9	4.1	2.2	30.0	8.72
10	16.4	1.9	4.8	2.5	19.0	8.79
12	14.7	1.9	3.1	1.6	21.3	8.88
14	15.2	1.9	3.0	1.6	23.5	8.84
16	19.0	1.9	6.9	3.6	23.9	8.47
18	17.1	1.9	8.0	4.2	17.6	8.40
20	13.9	1.9	5.7	3.0	17.8	8.45
22	13.3	1.9	5.0	2.6	16.1	8.60
Raunefjord, depth=6 m						
16	15.9	1.8	2.5	1.4	22.7	9.01

fjord during early spring (Egge and Heimdal 1994), can accumulate Mn and Fe in their mucus (Schoemann et al. 2001). The incorporated Mn and Fe colloids could nevertheless be found in the <0.4 μm fraction if these organic/inorganic associations broke up because of filtration pressure. Whatever the explanation for the apparent selectivity of the Mn-binding sites, the formation of these sites probably involves the prior oxidation of Mn²⁺ by microbial, as opposed to purely inorganic, processes. The first line of evidence for this is that there was a remarkable inverse correspondence, at least in bags 2 and 3, between the variations in bacterial abundance and in labile Mn concentration (Fig. 5). Thus, [Mn_{labile}] was lowest between 12 and 14 June, coinciding with the first peak in bacterial cell abundance, and was highest between 16 and 18 June, coinciding with the lowest counts of bacteria recorded in the experiment. Second, inorganic Mn(II) oxidation rates reported in seawater at low (~20 nmol L⁻¹) Mn(II) concentrations are much too slow to explain the decrease in [Mn_{labile}] from ~8 to ~6 nmol L⁻¹ observed in bags 2 and 3 between 18 and 20 June. Using the Morgan (1967) homogeneous rate equation, together with the rate constant determined at 5°C or 25°C by von Langen et al. (1997), we obtained a final [Mn_{labile}] value of ~8 nmol L⁻¹, indicating that the homogeneous inorganic

mechanism was probably not important in our experiment. Microbially catalyzed oxidation, however, could explain the rapid cycling of Mn between labile and inert forms. Using the highest (6.2% h⁻¹) and lowest (0.36% h⁻¹) turnover rates of dissolved Mn (with respect to particle formation) determined by Sunda and Huntsman (1987) in a North Carolina estuary yielded a final [Mn_{labile}] value of 0.4–6.7 nmol L⁻¹, which suggests that microbial catalysis could easily have accounted for the rate of disappearance of labile Mn between 18 and 20 June. Third, the magnitude of the [Mn_{labile}] decrease (~2 nmol L⁻¹) could be explained by the presence of a relatively abundant group of bacteria that sequester large amounts of Mn and Fe on extracellular appendages in these waters (Heldal et al. 1996). On the basis of total counts and metal contents of Mn-Fe bacterial cells recorded in Raunefjord in June 1993 (Heldal et al. 1996), we estimate that this group of bacteria could potentially have trapped 2.7–7.5 nmol L⁻¹ of Mn. The processes by which the inert forms of Mn were solubilized are not clear. Not only the photoreduction of colloidal MnO₂ but also grazing on the Mn-sequestering bacteria could explain the increases in [Mn_{labile}]. If the former process dominated, then the regeneration of labile Mn would have been more intense during daylight hours. Thus, a higher measurement frequency would have been

Table 4. Total, labile, and free Cu concentrations, Cu-binding ligand concentrations, and related conditional stability constants measured over the course of the mesocosm experiments. Values of C_{L3} (or C_{L2}) are missing where titration data could be modeled with only two ligands.

Date (June)	[Cu _T] (nmol L ⁻¹)	[Cu-labile] (nmol L ⁻¹)	α-inorg	[Cu ²⁺] (nmol L ⁻¹)	C _{L1}	log K ₁	C _{L2} (nmol L ⁻¹)	log K ₂	C _{L3} (nmol L ⁻¹)	log K ₃
Bag 1, depth=1.5 m										
8	20.9	0.95	11.5	0.08	7.2	14.96	28.5	9.91	78.5	8.33
10	18.1	1.02	13.5	0.09	8.1	14.92	28.0	9.60	49.5	8.50
12	17.0	1.14	14.7	0.08	6.3	15.15	18.3	10.05	33.1	8.41
14	12.5	0.93	14.5	0.06	6.2	14.89	20.0	9.72	23.2	8.34
16	13.4	0.15	14.5	0.01	8.0	15.25	40.7	10.15		
18	14.1	0.30	14.9	0.02	5.1	14.74	55.6	9.96		
20	12.0	0.27	13.9	0.02	3.3	14.88	53.6	10.00		
22	12.2	0.11	14.5	0.01	3.7	15.17	55.1	10.41		
Bag 2, depth=1.5 m										
8	12.6	1.05	15.3	0.07	3.0	14.94	14.6	10.20	87.4	8.20
10	10.5	0.92	14.5	0.06	3.3	15.15	16.3	9.92	60.9	8.29
12	10.9	0.92	14.5	0.06	4.2	13.46	15.7	9.88	43.7	8.40
14	10.5	0.68	14.3	0.05	6.9	13.44	13.2	9.69	39.2	8.38
16	12.6	0.29	14.5	0.02	5.1	14.02	23.8	10.31	30.8	8.25
18	13.2	0.26	14.6	0.02	5.3	13.88	20.9	10.49		
20	12.4	0.31	13.9	0.02	3.8	14.90	29.0	10.25		
22	13.0	0.37	14.0	0.03	6.0	14.71	36.2	9.93		
Bag 3, depth=1.5 m										
8	10.7	0.71	8.2	0.08	2.1	14.95	16.4	9.87	79.6	8.35
10	12.3	0.83	11.7	0.07	2.5	15.04	17.9	10.06	55.8	8.44
12	12.8	1.14	13.1	0.09	4.4	14.60	22.1	9.69	30.2	8.37
14	11.7	1.33	14.7	0.09	4.8	14.91	25.0	9.82	28.3	8.47
16	12.5	0.43	14.3	0.03	3.5	14.47	33.8	10.05		
18	10.8	0.32	14.1	0.02	3.9	14.43	28.7	10.11		
20	13.4	0.31	12.9	0.02	3.3	15.18	33.4	10.25		
22	12.0	0.25	13.3	0.02	6.5	14.72	40.0	9.90		
Raunefjord, depth=6 m										
16	13.3	0.74	6.9	0.09	9.8	13.79			75.2	8.53

needed to resolve the relative extent to which these two processes were operating.

On the basis of the findings from estuarine environments (e.g., Sholkovitz et al. 1978), we might have expected aggregation and sinking to be the immediate fate of these Mn-Fe encrustations. Not only did we find no clear evidence of Mn removal from the “dissolved” phase (Fig. 2), we also observed fluctuations in labile Mn concentrations (Fig. 3), which suggests that the oxidation process was readily reversible. We conclude that the different populations of bacteria played an important role in controlling Mn speciation (and hence its availability to phytoplankton) but did not appear to facilitate the export of Mn from surface waters over the timescale of the bloom studied.

Copper—The chemical speciation of Cu could well play an important role in promoting or inhibiting phytoplankton growth within the Raunefjord ecosystem. The calculated free Cu²⁺ concentrations in the waters inside or outside the bags were close to or exceeded the toxicity thresholds for cyanobacteria (Sunda and Gillespie 1979; Brand et al. 1986; Moffett and Brand 1996) and eukaryotic phytoplankton (Brand et al. 1986). Between 14 and 16 June, these concen-

trations were reduced fourfold by the formation of strong organic complexes to reach levels ~0.02 nmol L⁻¹ in each bag (Fig. 3). Numerous studies and observations in the field have shown that the Cu-complexing ligands have a biological origin. Croot et al. (2000) proposed that prokaryotes such as cyanobacteria were the source of the bulk of the class 1 ligands—that is, those ligands with log K' values within the range of 12–14. There is also evidence from culture experiments that *E. huxleyi* can release class 2 ligands similar to thiols (log K' = 11–12) in response to Cu additions (Leal et al. 1999). Leal et al. (1999) found that toxic effects to *E. huxleyi* occurred at [Cu²⁺] levels >0.025 nmol L⁻¹, essentially the same value as observed throughout phase 2 of our mesocosm study (Table 4). The ligand that was produced in our study, however, had a different Cu-complexing strength (log K₂' = 9.9–10.5) to that of the class 2 ligand observed in single culture experiments (Leal et al. 1999; Vasconcelos et al. 2002). This difference might be explained by differences in the physiological status of the coccolithophore cells between lab cultures and mesocosms or by a different biological source (e.g., prokaryotes or grazers) in the mesocosm study. It is interesting to note in this respect that the concentration of class 2 ligands kept increas-

Table 5. Total, labile, and free Zn concentrations, Zn-binding ligand concentrations, and related conditional stability constants measured over the course of the mesocosm experiments.

Date (June)	[Zn _T] (nmol L ⁻¹)	α-inorg	[Zn-labile]	[Zn ²⁺] (nmol L ⁻¹)	C _{Li}	log K _i
Bag 1, depth=1.5 m						
8	30.8	2.0	11.7	5.5	26.5	8.67
10	28.5	2.1	13.1	6.3	22.0	8.57
12	30.4	2.1	12.0	5.7	25.0	8.68
14	24.7	2.1	11.8	5.6	22.9	8.36
16	23.8	2.1	3.6	1.7	65.0	8.44
18	27.2	2.1	3.8	1.8	68.2	8.45
20	25.0	2.1	3.4	1.6	56.4	8.58
22	28.0	2.1	4.4	2.1	47.1	8.89
Bag 2, depth=1.5 m						
8	19.8	2.1	12.5	6.0	19.5	8.00
10	22.0	2.1	12.6	6.0	14.9	8.45
12	26.1	2.1	11.9	5.7	21.0	8.57
14	22.4	2.1	13.5	6.4	15.7	8.31
16	24.1	2.1	11.0	5.2	23.0	8.40
18	25.6	2.1	12.4	5.9	23.1	8.35
20	27.2	2.1	11.3	5.4	22.7	8.64
22	27.2	2.1	14.8	7.1	18.0	8.49
Bag 3, depth=1.5 m						
8	23.8	2.0	13.7	6.5	16.9	8.36
10	23.0	2.0	14.0	6.6	19.6	8.11
12	21.4	2.1	13.4	6.4	18.8	8.06
14	21.9	2.1	13.1	6.2	18.2	8.18
16	23.3	2.1	14.6	7.0	15.0	8.30
18	21.7	2.1	16.2	7.7	10.7	8.13
20	23.1	2.1	17.5	8.3	8.0	8.43
22	20.7	2.1	14.9	7.1	9.4	8.37
Ramefjord, depth=6 m						
16	18.2	1.9	9.7	5.1	11.9	8.69

ing after 18 June in bags 2 and 3 (Fig. 4), whereas the number of HS *E. huxleyi* cells decreased (Fig. 1). This suggests either that *E. huxleyi* increased its production rate of class 2 ligands—possibly in response to decreasing log K_i values in bags 2 and 3 (Table 4)—or that it was not the only source of ligands. Whatever the reasons for the difference in Cu-binding strengths, it seems likely that the release of this class 2 ligand reflected a detoxification mechanism beneficial to phytoplankton to lower the free Cu²⁺ concentration. The fact that [Cu²⁺] was buffered at the same threshold concentration of 0.02 nmol L⁻¹ in the three treatments further suggests that reducing [Cu²⁺] to nontoxic levels was an absolute requirement for *E. huxleyi*, irrespective of the N:P regime.

Zinc—Zn is an essential element, particularly for eukaryotes, so there certainly appears to be no immediate advantage to the plankton community in reducing [Zn²⁺] through complexation by organic exudates. Therefore, although it is perhaps not surprising to find that only 25%–58% of dissolved Zn, as opposed to 90%–98% of dissolved Cu, was organically complexed in bags 2 and 3, the reason for the enhanced organic Zn complexation (92%–94%) in bag 1 after 14 June (Table 5) is not so easy to understand. Nevertheless, these Zn complexation values are comparable to those measured

in the high-salinity region of Delaware Bay (98%; Lewis et al. 1995), Chesapeake Bay (95%–99%; Henry and Donat 1996), and Narragansett Bay (97%; Kozelka and Bruland 1998) under bloom conditions. The ligands responsible for zinc complexation under the nutrient-replete conditions did seem to be produced by phytoplankton, given that their concentrations were positively correlated with chlorophyll levels ($r^2 = 0.88$, $n = 8$). Of all the biological parameters measured, however, only the concentration of dead *E. huxleyi* cells showed a clearly different behavior in the nutrient-replete bag (Fig. 1), as did the concentration of Zn-binding ligands (Fig. 4). One possibility, therefore, is that these ligands were mostly derived from the breakup or bacterial decomposition of algal cells and that the correlation with chlorophyll was no evidence of active release by phytoplankton. The resulting increase in Zn-binding ligand concentration would have helped to alleviate the antagonistic effect of Zn on Mn uptake by phytoplankton (Sunda and Huntsman 1996, 1998). The results of previous experiments that used natural seawater that contained ethylene diamine-tetraacetic acid to control [Mn²⁺] support the view that the comparatively high [Cu²⁺] and [Zn²⁺] values (0.1 and 6 nmol L⁻¹, respectively) observed throughout phase 1 of our experiment would likely induce Mn limitation in some phy-

toplankton species, especially in view of the low ambient $[\text{Mn}^{2+}]$ conditions. Whether a detrimental effect of Zn on the regulation of Mn transport actually occurred in our experiments cannot be proved. However, it is clear that $[\text{Mn}_{\text{labile}}]$ behaved completely differently in the P- and N-depleted bags than in the N- and P-replete bag from 14 June onward: a decrease from 4 to 3 nmol L^{-1} was observed in the N- and P-replete bag between 14 and 22 June, whereas an overall increase from 4 to 6 nmol L^{-1} took place in the P- and N-depleted bags over the same period (Fig. 5). This observation, coupled with the lack of correlation between $[\text{Mn}_{\text{labile}}]$ and bacterial numbers in bag 1 (Fig. 5), suggests that phytoplankton cells were perhaps better able to acquire Mn in the N- and P-replete environment in which $[\text{Zn}^{2+}]$ had been depressed by algal exudates. Conversely, the synchronized variations in $[\text{Mn}_{\text{labile}}]$ and bacterial numbers in bags 2 and 3 (Fig. 5) suggest that a significant portion of $[\text{Mn}_{\text{labile}}]$ was directed away from photosynthesis in the P- and N-depleted bags. Therefore, it can be speculated that the accumulation of $\text{Mn}_{\text{labile}}$ observed in bags 2 and 3 on 16 and 18 June was an indirect result of the absence of production of Zn-binding ligands. If so, exploring the effects of macronutrient regime on the release of Zn-binding ligands from decaying phytoplankton cells might provide predictive insight to when Mn will reach potentially limiting levels in this coastal fjordic environment.

Macronutrient influences on metal speciation—Our study was conducted on water bodies as close to the natural conditions as possible but was not subjected to horizontal exchange and dispersion. The initial conditions in the adjacent fjord were such that the study was conducted under Cu- and Zn-replete conditions. Under these conditions, it appears that the N:P regime indirectly affects the quality and quantity of Zn-complexing ligands derived from phytoplankton cells. By contrast, $[\text{Cu}^{2+}]$ levels appear to be regulated by active exudation processes independently of the N:P regime. On the basis of laboratory culture studies (Sunda and Huntsman 1996, 1998), it seems plausible that the draw-down of $[\text{Cu}^{2+}]$ and $[\text{Zn}^{2+}]$ that took place in the N- and P-replete bag had a beneficial effect on the phytoplankton cells' capability to acquire Mn. In the future, measurements of the cellular Mn pool would be useful to support or negate this hypothesis.

References

- ACHTERBERG, E. P., T. H. HOLLAND, A. R. BOWIE, F. R. C. MANTOURA, AND P. J. WORSFOLD. 2001. Determination of iron in seawater. *Anal. Chim. Acta* **442**: 1–14.
- ALMGREN, T., D. DYRSSEN, AND S. FONSELIUS. 1983. Determination of alkalinity and total carbonate, p. 99–119. *In* K. Grasshof, M. Ehrardt, and K. Kremling [eds.], *Methods of seawater analysis*. Verlag Chemie.
- BRAND, L. E., W. G. SUNDA, AND R. R. L. GUILLARD. 1986. Reduction of marine phytoplankton reproduction rates by copper and cadmium. *J. Exp. Mar. Biol. Ecol.* **96**: 225–250.
- BRULAND, K. W., J. R. DONAT, AND D. A. HUTCHINS. 1991. Interactive influences of bioactive trace metals on biological production in oceanic waters. *Limnol. Oceanogr.* **36**: 1555–1577.
- BRUSSAARD, C. P. D., D. MARIE, AND G. BRATBAK. 2000. Flow cytometric detection of viruses. *J. Virol. Methods* **85**: 175–182.
- CLOERN, J. E. 1996. Phytoplankton bloom dynamics in coastal ecosystems: A review with some general lessons from sustained investigations of San Francisco Bay, California. *Rev. Geophys.* **34**: 127–168.
- CROOT, P. L., J. W. MOFFETT, AND L. E. BRAND. 2000. Production of extracellular Cu complexing ligands by eucaryotic phytoplankton in response to Cu stress. *Limnol. Oceanogr.* **45**: 619–627.
- EGGE, J. K., AND D. L. AKSNES. 1992. Silicate as regulating nutrient in phytoplankton competition. *Mar. Ecol. Prog. Ser.* **83**: 281–289.
- , AND B. R. HEIMDAL. 1994. Blooms of phytoplankton including *Emiliania huxleyi*—effects of nutrient supply in different N/P ratios. *Sarsia* **79**: 333–348.
- FILELLA, M., AND J. BUFFLE. 1993. Factors controlling the stability of submicron colloids in natural waters. *Colloids Surf.* **73**: 255–273.
- GERRINGA, L. J. A., P. M. J. HERMAN, AND T. C. W. POORTVLIET. 1995. Comparison of the linear van den Berg/Ruzic transformation and a non-linear fit of the Langmuir isotherm applied to Cu speciation data in the estuarine environment. *Mar. Chem.* **48**: 131–142.
- GUILLARD, R. R. L., AND C. J. LORENZEN. 1972. Yellow-green algae with chlorophyllide *c*. *J. Phycol.* **8**: 10–14.
- HELDAL, M., K. M. FAGERBAKKE, P. TUOMI, AND G. BRATBAK. 1996. Abundant populations of iron and manganese sequestering bacteria in coastal water. *Aquat. Microb. Ecol.* **11**: 127–133.
- HENRY, C. W., AND J. R. DONAT. 1996. Zinc complexation and speciation in the Chesapeake Bay. *EOS Trans. Am. Geophys. Union* **77**: OS72.
- HUDSON, R. J. M., AND F. M. M. MOREL. 1990. Iron transport in marine phytoplankton: Kinetics of cellular and medium coordination reactions. *Limnol. Oceanogr.* **35**: 1002–1020.
- , AND ———. 1993. Trace metal transport by marine organisms: Implications of metal coordination kinetics. *Deep-Sea Res.* **40**: 129–150.
- HUTCHINS, D. A., AND K. W. BRULAND. 1994. Grazer-mediated regeneration and assimilation of Fe, Zn and Mn from planktonic prey. *Mar. Ecol. Prog. Ser.* **110**: 259–269.
- JACQUET, S., M. HELDAL, D. IGLESIAS-RODRIGEZ, A. LARSEN, W. H. WILSON, AND G. BRATBAK. 2002. Flow cytometric analysis of an *Emiliania huxleyi* bloom terminated by viral infection. *Aquat. Microb. Ecol.* **27**: 111–124.
- KESTER, D. R. 1986. Equilibrium models in seawater: Applications and limitations, p. 337–363. *In* M. Bernhard, F. E. Brinckman, and P. J. Sadler [eds.], *The importance of chemical speciation in environmental processes*. Dahlem Konferenzen 1986. Springer.
- KOZELKA, P. B., AND K. W. BRULAND. 1998. Chemical speciation of dissolved Cu, Zn, Cd, Pb in Narragansett Bay, Rhode Island. *Mar. Chem.* **60**: 267–282.
- LEAL, M. F. C., M.T.S.D. VASCONCELOS, AND C. M. G. VAN DEN BERG. 1999. Copper-induced release of complexing ligands similar to thiols by *Emiliania huxleyi* in seawater cultures. *Limnol. Oceanogr.* **44**: 1750–1762.
- LEWIS, B. L., G. W. LUTHER, AND T. M. CHURCH. 1995. Determination of metal-organic complexation in natural waters by SWASV with pseudopolarograms. *Electroanalysis* **7**: 166–177.
- LUTHER, G. W., D. B. NUZZIO, AND J. WU. 1994. Speciation of manganese in Chesapeake Bay waters by voltammetric methods. *Anal. Chim. Acta* **284**: 473–480.
- MARIE, D., F. PARTENSKY, D. VAULOT, AND C. BRUSSAARD. 1999. Enumeration of phytoplankton, bacteria and viruses in marine samples. *Curr. Protocols Cytom.* **11.11**: 1–15.
- MOREL, F. M. M., R. J. M. HUDSON, AND N. M. PRICE. 1991. Lim-

- itation of productivity by trace metals in the sea. *Limnol. Oceanogr.* **36**: 1742–1755.
- MOFFETT, J. W., AND L. E. BRAND. 1996. The production of strong, extracellular Cu chelators by marine cyanobacteria in response to Cu stress. *Limnol. Oceanogr.* **41**: 288–293.
- MORGAN, J. J. 1967. Chemical equilibria and kinetic properties of manganese in natural waters, p. 561–623. *In* S. D. Faust and J. V. Hunter [eds.], *Principles and applications of water chemistry*. Wiley.
- MULLER, F. L. L. 1999. Evaluation of the effects of natural dissolved and colloidal organic ligands on the electrochemical lability of Cu, Pb and Cd in the Arran Deep, Scotland. *Mar. Chem.* **67**: 43–60.
- , S. B. GULIN, AND Å. KALVØY. 2001. Chemical speciation of copper and zinc in surface waters of the western Black Sea. *Mar. Chem.* **76**: 233–251.
- , AND D. R. KESTER. 1991. Voltammetric determination of the complexation parameters of zinc in marine and estuarine waters. *Mar. Chem.* **33**: 71–90.
- ROITZ, J. S., AND K. W. BRULAND. 1997. Determination of dissolved manganese(II) in coastal and estuarine waters by differential pulse cathodic stripping voltammetry. *Anal. Chim. Acta* **344**: 175–180.
- 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.
- SCARANO, G., E. BRAMANTI, AND A. ZIRINO. 1992. Determination of copper complexation in seawater by a ligand competition technique with voltammetric measurement of the labile metal fraction. *Anal. Chim. Acta* **264**: 153–162.
- SCHECHER, W. D., AND D. C. MCAVOY. 1998. MINEQ+: Chemical equilibrium modeling system, version 4.0 for Windows. Environmental Research Software.
- SCHOEMANN, V., R. WOLLAST, L. CHOU, AND C. LANCELOT. 2001. Effects of photosynthesis on the accumulation of Mn and Fe by *Phaeocystis* colonies. *Limnol. Oceanogr.* **46**: 1065–1076.
- STERNER, R. W., AND D. O. HESSEN. 1994. Algal nutrient limitation and the nutrition of aquatic herbivores. *Annu. Rev. Ecol. Syst.* **25**: 1–29.
- SULZBERGER, B. 1997. Effects of light on the biological availability of trace metals, p. 353–380. *In* A. Gianguzza, E. Lelizzetti, and S. Sammartano [eds.], *Marine chemistry. Water science and technology library*, v. 25. Kluwer.
- SUNDA, W. G., AND P. A. GILLESPIE. 1979. The response of a marine bacterium to cupric ion and its use to estimate cupric ion activity in seawater. *J. Mar. Res.* **37**: 761–777.
- , AND S. A. HUNTSMAN. 1987. Microbial oxidation of manganese in a North Carolina estuary. *Limnol. Oceanogr.* **32**: 552–564.
- , AND ———. 1988. Effect of sunlight on redox cycles of manganese in the southwestern Sargasso Sea. *Deep-Sea Res.* **35**: 1297–1317.
- , AND ———. 1996. Antagonism between cadmium and zinc toxicity and manganese limitation in a coastal diatom. *Limnol. Oceanogr.* **41**: 373–387.
- , AND ———. 1998. Interactions among Cu²⁺, Zn²⁺, and Mn²⁺ in controlling cellular Mn, Zn, and growth rate in the coastal alga *Chlamydomonas*. *Limnol. Oceanogr.* **43**: 1055–1064.
- VAN DEN BERG, C. M. G. 1982. Determination of copper complexation with natural organic ligands in seawater by equilibration with MnO₂ - 1. *Theor. Mar. Chem.* **11**: 307–322.
- VASCONCELOS, M.T.S.D., M. F. C. LEAL, AND C. M. G. VAN DEN BERG. 2002. Influence of the nature of the exudates released by different marine algae on the growth, trace metal uptake and exudation of *Emiliania huxleyi* in natural seawater. *Mar. Chem.* **77**: 187–210.
- VON LANGEN, P. J., K. S. JOHNSON, K. H. COALE, AND V. A. ELROD. 1997. Oxidation kinetics of manganese (II) in seawater at nanomolar concentrations. *Geochim. Cosmochim. Acta* **61**: 4945–4954.
- WALPOLE, R. E., AND R. H. MYERS. 1985. *Probability and statistics for engineers and scientists*, 3rd ed. Collier Macmillan.
- WASSMANN, P. 1998. Retention versus export food chains: Processes controlling sinking loss from marine pelagic systems. *Hydrobiology* **363**: 29–57.
- , J. K. EGGE, M. REIGSTAD, AND D. L. AKSNES. 1997. Influence of dissolved silicate on vertical flux of particulate biogenic matter. *Mar. Pollut. Bull.* **33**: 10–21.
- WHITFIELD, M. 2001. Interactions between phytoplankton and trace metals in the ocean. *Adv. Mar. Biol.* **41**: 1–128.
- WILLIAMS, P. J. LE B., AND J. K. EGGE. 1998. The management and behaviour of the mesocosms. *Estuar. Coast. Shelf Sci.* **46(A)**: 3–14.

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