Effects of dissolved carbon dioxide, zinc, and manganese on the cadmium to phosphorus ratio in natural phytoplankton assemblages

Jay T. Cullen[1,2]

Institute of Marine and Coastal Sciences, Rutgers University, 71 Dudley Road, New Brunswick, New Jersey 08901

Robert M. Sherrell

Institute of Marine and Coastal Sciences and Department of Geological Sciences, Rutgers University, 71 Dudley Road, New Brunswick, New Jersey 08901

Abstract

We report the results of a field study, in productive waters off California, of the factors that control the particulate cadmium (Cd) : phosphorus (P) composition of natural assemblages of marine phytoplankton, the dominant vector of both elements to the deep ocean. Controlled shipboard incubation experiments (~2–4 d) demonstrated that while manipulation of $p\text{CO}_2$ and dissolved zinc (Zn) and manganese (Mn) concentrations had little effect on the species composition or C : nitrogen (N) : P ratios of natural, diatom-dominated phytoplankton assemblages, their Cd : P ratio was negatively correlated to each of these variables. The particulate Cd : P ratios of phytoplankton were two to five times higher for cells grown at low $p\text{CO}_2$ than for cells acclimated to growth at $p\text{CO}_2$ at or above atmospheric equilibrium values. Addition of Zn to incubations at five- to 20-fold above background concentrations decreased Cd uptake and phytoplankton Cd : P ratios across $p\text{CO}_2$ and Mn treatments and suppressed short term Cd uptake rates by a factor of approximately two to four, compared to controls. A broad pattern of Mn suppression of Cd uptake was also evident in our incubations. We propose that natural variability in surface water $p\text{CO}_2$ and dissolved Zn and Mn, related to water mass history and biological drawdown, likely govern the degree of Cd uptake and, therefore, the evolution of the dissolved Cd : PO_4 ratio in recently upwelled, high-productivity surface waters.

The vertical and horizontal distribution of cadmium (Cd) in the ocean resembles that of the inorganic nutrient phosphate (PO_4) (Boyle et al. 1976; Bruland et al. 1978). Like PO_4, vertical distributions are typified by acute surface depletions that increase rapidly to maximum concentrations in the main thermocline, remaining relatively constant with depth. This ''nutrient-type'' behavior (Donat and Bruland 1995) implies a biogeochemical cycle for the element, whereby its distribution results primarily from an association with or incorporation into organic material. Despite fundamental differences in aqueous chemistry, Cd and phosphorus (P) are both removed from ocean surface waters by organisms during growth and are released almost quantitatively to the ocean interior when organic material is remineralized at depth. While the essential biochemical and structural functions of P are well understood, a physiological requirement for Cd has yet to be established for any marine organism. The fundamental question of why these chemically dissim-ilar elements are so strongly linked biogeochemically in the ocean has provided no satisfactory answer.

Unlike the modern deep-water relationships that are remarkably constant, in the upper waters of the ocean (depth <1,000 m) there exist well-documented deviations of dissolved Cd : PO_4 ratios from the global average (Boyle 1988; Rutgers van der Loeff et al. 1997; Löscher et al. 1998). In general, surface water dissolved Cd : PO_4 ratios are lower (de Baar et al. 1994) and show more spatial variability (Elderfield and Rickaby 2000) than ratios in underlying deep waters. One must consider that in the central gyres where Cd and PO_4 are both drawn down to nearly undetectable levels, extreme dissolved Cd : PO_4 ratios carry significant errors, and the deviations in these surface waters must therefore be interpreted with caution. Indeed, our understanding of both dissolved Cd and PO_4 cycling in the upper water column of oligotrophic gyres is limited currently by a lack of temporal and spatial coverage with high-sensitivity analyses and, in particular, poor knowledge of the exchange of P between inorganic and organic pools (Karl and Tien 1997). Despite these gaps in understanding, the broad pattern of Cd depletion in surface waters indicates that either (1) Cd is removed preferentially to P relative to the dissolved Cd : P in deep waters that is transported to the surface by upwelling or (2) P remineralization occurs at shallower depths (in the mixed layer) than Cd (Boyle et al. 1981; Boyle 1988; de Baar et al. 1994). While neither mechanism can be ruled out completely given available data, one way for such a pattern to persist would be the production of particulate matter in surface waters with Cd : P ratios significantly greater than the average dissolved upwelled Cd : PO_4 ratio (Collier and Edmond 1984). Many authors have suggested that phytoplank-

[1] Corresponding author (jcullen@uvic.ca).

[2] Present address: School of Earth and Ocean Sciences, University of Victoria, P.O. Box 3055 STN CSC, Victoria, British Columbia V8W 3P6, Canada

ton may remove Cd preferentially from seawater during periods of intense productivity, leading to lower dissolved Cd: PO$_4$ ratios (Knauer and Martin 1973; Boyle et al. 1981; Löscher et al. 1998), but mechanistic explanations for these variations have been largely lacking from field investigations.

Much effort has been expended by geochemists and algal physiologists to identify processes that remove Cd from surface waters and to understand how removal rates of Cd, relative to P, both from surface waters and from the ocean as a whole, are controlled. Concurrent yet largely independent progress has been achieved through direct field measurements of dissolved and particulate Cd and from measurements of Cd uptake in laboratory cultures of marine phytoplankton under different controlled environmental conditions (Boyle et al. 1976; Bruland et al. 1978; de Baar et al. 1994).

Laboratory culture experiments have demonstrated that added Cd can accelerate the growth of some species of marine phytoplankton when zinc (Zn) concentrations are limiting (Price and Morel 1990). Subsequent work, including work wth additional species, indicated that Cd's stimulating effect likely results from its substituting for or supplementing Zn in carbonic anhydrase (CA) (Lee and Morel 1995; Cullen et al. 1999; Lane and Morel 2000*a*), a class of enzymes thought to be essential for growth at low $[CO_2]_{aq}$ (Kaplan and Reinhold 1999). CA catalyzes the dehydration reaction of HCO_3^- to produce H_2O and CO_2, supplying CO_2 to ribulose bisphosphate carboxylase-oxygenase (RuBisCO), the primary carbon (C)-fixing enzyme, allowing for maximal inorganic C fixation rates when ambient $[CO_2]_{aq}$ would otherwise lead to diffusion limitation of growth rates (Riebesell et al. 1993). These findings intimately link the biogeochemical cycling of Cd, Zn, and inorganic carbon to the production of photosynthetic biomass in the ocean.

Detailed studies of the uptake kinetics of Cd in single-species laboratory cultures of members of the bacillariophyceae, chlorophyceae, and prymnesiophyceae indicate that Cd uptake is inversely related to the concentration of dissolved free Zn and, in the case of diatoms, manganese (Mn) in the growth media (Lee and Morel 1995; Sunda and Huntsman 2000). The modeled effect of these two metals, according to the above studies, is to inhibit the uptake of Cd through cell surface transport proteins that are under negative feedback regulation by cellular Zn and Mn levels. Across gradients of free Cd, Zn, and Mn concentrations representative of those found in the ocean, laboratory-grown cultures of phytoplankton were demonstrated to have 100–1,000-fold variations in Cd content (Sunda and Huntsman 1998, 2000). The global utility of these Michaelis–Menten competitive uptake relationships to explain the variability of dissolved Cd: PO$_4$ ratios in surface waters is limited currently by our understanding of trace-metal speciation (Cd, Zn, and Mn, in this case) and by lack of direct measurements of free ion concentrations for the salient metals in the oceanic euphotic zone. However, using values for important kinetic parameters estimated or determined for some diatom species, these models of particulate phytoplankton Cd: P composition adequately account for vertical profiles of dissolved Cd, Zn, and P in selected oceanic regimes (Sunda and Huntsman 2000).

Why phytoplankton take up Cd from seawater and what

factors control how natural assemblages fractionate Cd relative to P in surface waters must be understood in order to model how dissolved Cd: PO$_4$ ratios might vary temporally and spatially, including over geological time scales. Whether emerging paleoceanographic proxies of surface-water nutrient distributions based on planktonic foraminiferal Cd records (Elderfield and Rickaby 2000) can be used quantitatively will depend on how predictably Cd is fractionated from P in an upwelled water mass by biological and abiotic removal processes. Therefore, an understanding of the factors driving the variability in phytoplankton Cd: P composition is of critical importance.

We initiated a field study of the uptake of Cd by natural assemblages of marine phytoplankton. Our approach elaborated on previous work (Cullen et al. 1999) combining direct, low-level analysis of Cd: P ratios in surface-water particulate material in relation to natural chemical gradients of the important variables pCO$_2$ (Morel et al. 1994), Zn, and Mn (Sunda and Huntsman 1996, 2000) with shipboard incubations that allowed for these variables to be manipulated independently. By investigating the control of particulate phytoplankton Cd: P ratios, we constrain potential temporal and spatial variability in surface-water dissolved Cd: PO$_4$ ratios as this variability pertains to the application of emerging paleo-nutrient proxies. The results may also have implications for future changes in metal cycling driven by increasing anthropogenic CO$_2$, a trend likely to have important implications for the ecology of phytoplankton communities and interconnected biogeochemical processes in the ocean (Feely et al. 2004).

Materials and methods

Field study sites—Sampling sites for field experiments were located in coastal and offshore waters off central California (May 1998, July 1999, and July 2000) (Fig. 1). Field work was carried out aboard the R/V *Pt. Sur* (1998, 2000) and R/V *New Horizon* (1999). The study area experiences seasonal (spring–summer) wind-driven upwelling events that bring nutrient-laden subsurface waters to the photic zone, promoting the development of large diatom ($>25 \mu m$)–dominated phytoplankton blooms (Wilkerson et al. 2000), with surface-water chlorophyll *a* (Chl *a*) concentrations of 10–35 μg L^{-1}. The advantage of this system is that a wide range of biological, physical, and chemical parameters can be sampled on relatively short spatial scales.

Incubation experiments—Trace-metal clean methodology was utilized throughout to minimize potential contamination during sample collection and subsequent handling. Incubation studies were undertaken to examine the response of phytoplankton Cd: P composition to varying dissolved CO$_2$, Zn, and Mn concentrations. Seawater was collected through Teflon™-lined polyethylene tubing mounted on a polyvinyl chloride hydrodynamic fish that was lowered from the bow of the research vessel to 15 m in depth (Vink et al. 2000). A continuous flow of seawater was sent to the laboratory by way of a polypropylene, Teflon™ diaphragm pump that could be selectively diverted to and isolated in metal clean polyethylene 20-liter carboys (four connected in parallel).
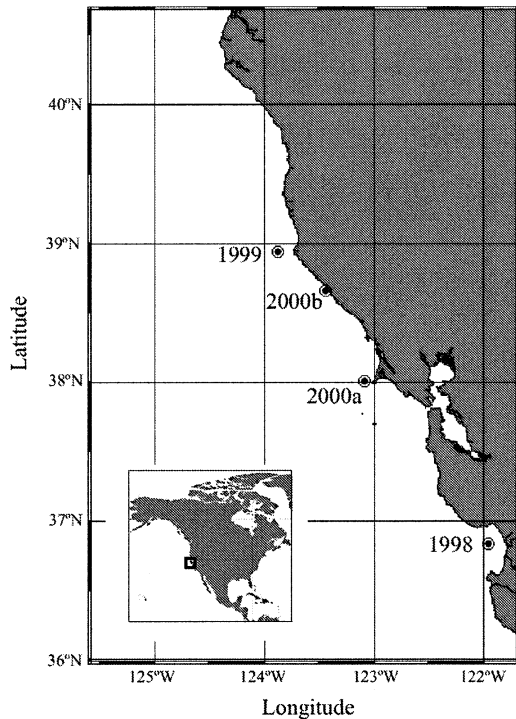
Fig. 1. Station locations for research cruises off central California R/V *Pt. Sur,* May 1998, July 2000; R/V *New Horizon,* July 1999. Stations at which water for shipboard incubations was collected are identified by the year of the experiment.

During periods of intense upwelling, this water was used unaltered, except in experiments 2000*a* and *b,* in which high-nutrient subsurface water (collected at 60 m in depth by hand-lowering the hydrodynamic fish) was mixed with nutrient-poor surface water to promote the growth of the resident phytoplankton community. After homogenizing the seawater by gentle shaking, 1.5 liters were dispensed to 2-liter acid-washed polycarbonate incubation bottles in a Class 100 laminar flow bench. For metal manipulations, high-purity metal stock solutions (Mn and Zn; 1,000 ppb; High-Purity Standards) that had been equilibrated with 0.2-μm–filtered surface seawater were added to treatment bottles. Bottles were then incubated on deck in an acrylic chamber that was continually flushed with surface seawater and covered with neutral density screening to reduce available light to 25% of surface irradiance levels. The pCO$_2$ of the culture solution was controlled by bubbling incubation bottles with 0.2-μm–filtered, commercially prepared air-CO$_2$ mixtures with 100, 200, 350, 500, and 800 ppm CO$_2$ (Scott Specialty Gas and Airgas). At the incubation temperatures and alkalinities (measured by Gran titration), pCO$_2$ treatments lead to a range of [CO$_2$]$_{aq}$ from ~4 to ~32 μmol L^{-1}, spanning those values found in surface waters of today's ocean, as calculated from reported pCO$_2$ ranges (Takahashi et al. 2002 and references therein).

Over the course of the incubations, fluorometric Chl a was analyzed on 25-mm GF/F filters to monitor the growth rate of the phytoplankton community. Growth rates were calculated from linear regression analysis of the natural logarithm of Chl a against time for replicate incubation bottles. Cells

for radiotracer uptake experiments and samples for particulate trace element analysis were collected during the late-exponential phase of growth.

^{109}Cd and ^{14}C uptake experiments—Following an acclimation period (at least 20–27 h, ~1 doubling) and in late-exponential growth phase, samples were transferred to 250-ml acid-cleaned polycarbonate bottles (Nalgene), in which Cd and C uptake were measured in separate duplicate sub-samples (250 ml) by short term (5–6 h) uptake of added H^{14}CO$_3^-$ and carrier-free ^{109}Cd (pre-equilibrated with 0.2-μm–filtered surface seawater during acclimation time). The total Cd addition to incubation bottles, as calculated from the specific activity of the primary stock, was ~10 pmol L^{-1}. Once the radioisotopes were added, the incubation bottles were returned to neutral-density screened incubators on the ship's deck. After the prescribed incubation time had elapsed, cells were harvested by gentle (<20 kPa) vacuum filtration onto 3-μm polycarbonate membrane filters. Cells were then exposed to a 4 mmol L^{-1} diethylenetriamine penta-acetic acid solution for 5 min, then washed with 0.2-μm–filtered seawater to remove extracellular surface-adsorbed ^{109}Cd (Lee et al. 1995) and assayed for ^{109}Cd and ^{14}C activity by liquid scintillation counting. The Cd:C uptake ratio was determined from total inorganic carbon calculated for each pCO$_2$ level determined from incubation temperature, salinity, and total alkalinity and from total dissolved Cd (Field et al. 1999), measured for incubation starting water or in treatment bottle.

Short-term ^{109}Cd uptake experiments—Three experiments were conducted during May 1998 to determine the effect of Zn amendments on short-term uptake rates of dissolved Cd by natural phytoplankton assemblages. Replicate 500-ml seawater samples were collected from depth (15 m) using a trace-metal clean pumping system (Vink et al. 2000). Phytoplankton were concentrated onto 3-μm polycarbonate filters and resuspended into 250 ml of 0.45-μm–filtered seawater that was in equilibrium with atmospheric pCO$_2$. Zn stocks pre-equilibrated with 0.2-μm–filtered seawater were added to amendment bottles to achieve final total dissolved concentrations of 25 nmol L^{-1}. Carrier-free ^{109}Cd was added as described for incubations above, bottles were returned to the incubators on deck, and the incorporation of the label over the 4–6 h experiment was determined by liquid scintillation counting. The pCO$_2$ in the short-term incubations was not controlled by bubbling with compressed gases, as in other experiments, but likely stayed at or near atmospheric equilibrium over the 4–6 h time span of the incubations. Chl a normalized Cd uptake was calculated from total label added, the concentration of dissolved Cd, and the length of the experiment.

Particulate Cd, Zn, and P measurements—Samples for particulate trace-metal analysis by high resolution-inductively coupled plasma-mass spectrometry (HR-ICP-MS) and carbon, hydrogen, nitrogen (CHN) analysis were collected from late–exponential phase incubation experiments (Cullen and Sherrell 1999). Cells were harvested by gentle vacuum filtration (<20 kPa) onto two 47-mm filters stacked (0.45

μm polysulfone and 5 μm polycarbonate) or onto one nominal 0.8-μm quartz microfiber filter housed in an acid-washed, polysulfone filter tower (Nalgene) inside a Class 100 laminar flow hood. Filters were removed to acid-cleaned Petri slides and stored at $-20°C$ until they could be analyzed in the laboratory.

For trace-metal analysis, filters (47-mm 0.45-μm polysulfone and 5-μm polycarbonate filters) were subsampled (50% $\pm$ 4% of area by mass), placed in 15-ml screw-cap Teflon vials (Savillex), and digested completely by refluxing in a mixture of 950 μl 16 mol L^{-1} HNO$_3$ and 50 μl 32 mol L^{-1} HF on a hotplate at 120°C for 4 h (Cullen and Sherrell 1999). Digests of samples, filter blanks, and digest blanks (acid only) were analyzed for trace elements and P by magnetic sector HR-ICP-MS (Element, Finnigan-MAT) using a combination of internal and external standardization (Cullen et al. 2001). Samples were corrected for filter blanks and for the presence of terrigenous material using crustal abundance data (Wedepohl 1995), making the assumption that particulate aluminum derived wholly from terrigenous particles.

Subsamples (1.21 cm^2) of 47-mm quartz filters were fumed over HCl for 24 h and analyzed for organic C and N on a CHN analyzer (Carlo-Erba model NA 1500 Series 2 or Perkin Elmer model 2400 CHN).

Results

Incubation experiments: Species composition—Natural phytoplankton assemblages in all incubation studies were dominated by large diatom species that were retained in the >5-μm fraction. Prevalent species were of the genera *Skeletonema, Thalassiosira,* and *Nitzschia,* as identified by microscopic observation of selected incubation bottles (Cullen unpubl. data). The rapid increase of biomass in incubation bottles was due to the proliferation of all of these large diatoms across all dissolved CO$_2$, Zn, and Mn manipulations, indicating that treatments had little effect on phytoplankton species composition on the time scale of the experiments.

Growth rates and C:N:P composition—For five incubations (1998–2000), the effect of pCO$_2$ and/or Zn and Mn manipulations on phytoplankton growth rates and gross chemical composition are summarized in Table 1. Over the 3-yr period of study, growth rates of the phytoplankton populations, calculated from log–linear transformed measurements of Chl a taken over the course of shipboard incubations, varied from 0.78 $\pm$ 0.2 d^{-1} to 1.3 $\pm$ 0.2 d^{-1} and were unaffected by varying pCO$_2$, Zn, and Mn treatments. The gross chemical composition of phytoplankton assemblages (C:N and N:P mol mol^{-1}) bracketed Redfield values varying from 3.3 $\pm$ 1 to 10 $\pm$ 0.8 for C:N and from 101 $\pm$ 9 to 111 $\pm$ 11 for C:P. Reported errors are 1-sigma uncertainties, representing subsampling and analytical reproducibility. The C:N:P ratios of the phytoplankton assemblages were constant within measurement error for each experiment, across pCO$_2$ and dissolved Zn and Mn treatments. The tight range and near-Redfield values for C:N:P ratios support our use of P, measured by HR-ICP-MS on filters, as a proxy for phytoplankton biomass.

Particulate Cd:P ratios: Modulation of particulate Cd:P by pCO$_2$—Particles retained by the 0.45-μm filter will include intact phytoplankton cells as well as biodetritus and marine bacteria. While microscopic examination of a subset of filters and the C:N and C:P ratios of the particles indicated that this material was largely dominated by intact marine diatoms, measured particulate Cd:P ratios must also reflect to some degree the presence of detrital material and marine prokaryotes. In incubations 1998, 1999a, 2000a, and 2000b, cells acclimated to grow at low dissolved CO$_2$ had Cd:P ratios that were consistently elevated relative to assemblages grown at higher pCO$_2$, regardless of dissolved Zn or Mn availability (Table 2). For example, in incubation 2000b, cells acclimated to 100 ppm CO$_2$ had Cd:P ratios of 0.25 $\pm$ 0.03 mmol mol^{-1}, values that were approximately three times higher than those of 800-ppm acclimated cells (0.09 $\pm$ 0.02 mmol mol^{-1}). Cells grown at intermediate pCO$_2$ (200, 350, and 500 ppm) had Cd:P ratios of 0.13 $\pm$ 0.06, 0.08 $\pm$ 0.03, and 0.12 mmol mol^{-1}, respectively, similar to values for high pCO$_2$ acclimated cells. Analysis of size-fractionated samples showed that significant differences existed between large (>5-μm) and small (0.45–5-μm) cells across CO$_2$ treatments. Large cells had Cd:P ratios that were ~3–10 times greater than those of the smaller phytoplankton classes also composed mostly of diatoms (Fig. 2).

Modulation of particulate Cd:P by dissolved Zn and Mn—Addition of dissolved Zn to incubation 1998 led to lower Cd:P ratios across the experimental range of pCO$_2$ (Table 2) and at the lowest pCO$_2$ in incubation 1999a (Fig. 3). Cells from incubation 1998, grown in the presence of additional dissolved Zn, showed a similar trend in Cd:P, with 100 ppm pCO$_2$ acclimated cells having significantly higher Cd:P ratios (0.17 mmol mol^{-1}) than 350-ppm (0.05 $\pm$ 0.001 mmol mol^{-1}) and 800-ppm (0.04 $\pm$ 0.01 mmol mol^{-1}) acclimated assemblages. Zn amendments to incubation 1998 (25 nmol L^{-1}) and 1999 (5 nmol L^{-1}) resulted in phytoplankton assemblages that had consistently lower Cd:P ratios than control treatments across pCO$_2$ treatments in 1998 and at the lowest pCO$_2$ in 1999 (Table 2). Multiple short-term Cd uptake experiments conducted in Monterey Bay, California, in 1998 demonstrated significant reduction of biomass-normalized Cd uptake rates in the presence of amended dissolved Zn (Fig. 4). Cd uptake was suppressed, on average, by a factor of three in Zn treatments, relative to controls.

In experiment 1999b, the effect on phytoplankton Cd:P of manipulating both dissolved Zn and Mn concentrations was investigated (Table 2). The effect of increasing Zn was similar to that of previous experiments; Cd:P ratios measured in natural phytoplankton assemblages were reduced as dissolved Zn concentrations increased and Mn was held constant at 1.7 nmol L^{-1}, ranging from 0.50 $\pm$ 0.04 mmol mol^{-1} at ambient Zn down to 0.21 $\pm$ 0.03 mmol mol^{-1} with 10 nmol L^{-1} additional dissolved Zn. The addition of 30 nmol L^{-1} Mn had no effect at low Zn concentrations, with phytoplankton Cd:P ratios of 0.5 $\pm$ 0.04 and 0.7 $\pm$ 0.2 mmol mol^{-1} at dissolved Mn levels of 1.7 nmol L^{-1} and 32 nmol L^{-1}, respectively. At higher dissolved Zn concentrations, increasing dissolved Mn concentrations led to reductions of

Table 1.   Effect of variable dissolved CO_2 and Zn on the steady-state growth rates and major element composition of natural assemblages of marine phytoplankton.

Date and study site	Latitude	Longitude	Incubation time (d)	CO_2 concentration (ppm)	Growth rate (d^{-1})	C:N (mol:mol)	C:P (mol:mol)
May 1998, Monterey Bay, California	36°49.92	121°57.36	1.5	100	0.92±0.08	4.08±0.52	106±16
				350	0.95±0.06	3.85±0.79	101±9
				800	0.97±0.09	3.43±0.46	102±7
				100	0.98±0.06	4.0±0.57	100±14
				350	0.96±0.10	4.15±0.33	107±3
				800	0.94±0.08	3.27±1.2	104±13
July 1999a, California Current	38°56.21	123°52.55	3.51	100	0.85±0.21	5.48±0.35	105±9
				350	0.85±0.05	5.33±0.15	103±11
				800	0.78±0.17	5.15±0.21	109±15
				100	0.80±0.07	5.35±0.05	107±10
				350	0.88±0.15	5.53±0.16	108±10
				800	0.87±0.11	5.31±0.12	100±10
July 1999b, California Current	38°56.21	123°52.55	4.38		0.89±0.04	5.48±0.35	116±8
			4.38		0.85±0.06	5.33±0.15	120±11
			4.38		0.86±0.12	5.15±0.21	113±9
			4.38		0.95±0.08	4.98±0.36	118±10
			4.38		0.88±0.10	5.47±0.25	116±6
			4.38		0.84±0.10	5.45±0.28	119±7
July 2000a, Pt. Reyes, California	38°00.29	123°04.9	2.94	100	0.88±0.04	4.08±0.37	101±13
				200	0.89±0.03	3.99±0.48	111±11
				350	0.92±0.09	3.86±0.21	110±12
				500	0.92±0.06	3.73±0.53	105±11
				800	0.88±0.02	4.14±0.35	107±19
July 2000b, Pt. Arena, California	38°39.53	123°26.33	2.83	100	1.24±0.10	9.66±1.8	104±8
				200	1.28±0.17	9.06±1.4	106±13
				350	1.27±0.11	10.1±0.84	100±9
				500	1.29±0.29	8.89±3.1	109±9
				800	1.30±0.21	7.31±1.5	100±11

Cd:P ratios from 0.36 ± 0.07 to 0.14 ± 0.04 mmol mol^{-1} at 5 nmol L^{-1} additional Zn and from 0.21 ± 0.03 to 0.15 ± 0.01 mmol mol^{-1} at 10 nmol L^{-1} additional Zn. While little evidence of Mn modulation of algal Cd:P was evident based on experiment 1999b, there is a general pattern of Mn suppression of phytoplankton Cd uptake across pCO$_2$ manipulation experiments over the 4-yr field study (Fig. 5). HR-ICP-MS measured Cd:P ranged from ~0.8 down to 0.05 mmol mol^{-1} as Mn concentrations increased from 1.7 to 40 nmol L^{-1}.

For experiment 1999a, both radiotracer-derived and ICP-MS–determined particulate Cd:P data were obtained (Table 2). Ratios determined by ICP-MS are significantly higher for each treatment when compared with Cd:P ratios calculated from the uptake of ^{109}Cd and ^{14}C and measured C:P ratios from the incubations. A number of plausible reasons for these differences exist. This discrepancy could reflect that the radiotracer uptake experiments, carried out on 4–6-h time scales, provide a snapshot of the instantaneous relative uptake rates of Cd and C (which is subsequently converted to P using measured C:P) across the experimental treatments, while total particulate Cd:P measured by ICP-MS reflects accumulated changes in the cellular reservoirs of Cd and P in response to the metal and pCO$_2$ treatments. On these time scales, ^{14}C is likely measuring something between net and gross C fixation (Falkowski and Raven 1997) and

would, therefore, underestimate particulate Cd:P. Another possibility is that the different filter pore sizes used in the radioisotope uptake experiments (3 μm) versus the 0.45-μm filters used for ICP-MS analysis sampled different size classes of the plankton with fundamentally different Cd:P composition. Even so, we consider the differences between the two approaches to be minor given that the radioisotope approach calculates Cd:P from Cd:C uptake rate ratios and particulate C:P measured on separate filters taken from the incubation bottles, while the ICP-MS directly detects particulate Cd and P on the same filter.

Discussion

The goal of this study was to investigate the factors that modulate the Cd:P composition of natural assemblages of marine phytoplankton and hence to understand the fractionation of Cd relative to PO$_4$ during biological uptake in ocean surface waters. Our study was motivated by previous work with model organisms cultured in chemically defined media in the laboratory that demonstrated considerable variability in the Cd:P composition of diatoms in response to changes in the availability of dissolved Cd, Zn, and Mn (Price and Morel 1990; Lee et al. 1995; Sunda and Huntsman 2000) and ambient pCO$_2$. This study is the first to combine trace elemental analyses of biogenic particulate material with con-

Table 2. Effect of variable dissolved CO_2 and Zn on the Cd:P composition of natural assemblages of marine phytoplankton.

Date and study site	Latitude	Longitude	Incubation time (d)	CO_2 concentration (ppm)	[Cd]γ (nmol L^{-1})	[Zn]γ* (nmol L^{-1})	[Mn]γ (nmol L^{-1})	Cd:Pγ (nmol:mol)†	Cd:P (nmol:mol)†	Cd:P (nmol:mol)‡
May 1998, Monterey Bay, California	36°49.92	121°57.36	1.5	100	0.7	0.7	40	0.25±0.02		—
				350	0.7	0.7	40	0.1±0.03		—
				800	0.7	0.7	40	0.13±0.04		—
				100	0.7	26.0	40	0.17±0.01		—
				350	0.7	26.0	40	0.05±0.001		—
				800	0.7	26.0	40	0.04±0.01		—
July 1999a, California Current	38°56.21	123°52.55	3.51	100	0.7	<3	1.7	0.36±0.07		0.77±0.01
				350	0.7	<3	1.7	0.06±0.01		0.71±0.03
				800	0.7	<3	1.7	0.05±0.09		0.60±0.03
				100	0.7	5§	1.7	0.19±0.06		0.90±0.1
				350	0.7	5§	1.7	0.07±0.01		0.84±0.02
				800	0.7	5§	1.7	0.07±0.02		0.64±0.09
July 1999b, California Current	38°56.21	123°52.55	4.38	ND	0.65	<3	1.7	0.50±0.04		—
			4.38	ND	0.65	5§	1.7	0.36±0.07		—
			4.38	ND	0.65	10§	1.7	0.21±0.03		—
			4.38	ND	0.65	<3	32	0.69±0.16		—
			4.38	ND	0.65	5§	32	0.14±0.04		—
			4.38	ND	0.65	10§	32	0.15±0.01		—
July 2000a, Pt. Reyes, California	38°00.29	123°04.9	2.94	100	0.73	<3	13			0.33±0.02
				200	0.73	<3	13			0.24±0.07
				350	0.73	<3	13			0.14±0.03
				500	0.73	<3	13			0.10±0.02
				800	0.73	<3	13			0.08±0.03
July 2000b, Pt. Arena, California	38°39.53	123°26.33	2.83	100	0.67	<3	16			0.16±0.03
				200	0.67	<3	16			0.09±0.04
				350	0.67	<3	16			0.06±0.03
				500	0.67	<3	16			0.04
				800	0.67	<3	16			0.05±0.01

* Values reported as <3 nmol L^{-1} were below detection limit for the analytical run in question and were conservatively estimated.
† Calculated from ^{109}Cd and ^{14}C uptake and the measured particulate C:P ratio ± propagated standard deviation based on duplicate measurements of Cd and C uptake rates.
‡ Measured by HR-ICP-MS; ratios calculated for pooled size fractions >0.45 μm and reported error is range of measurements from duplicate treatment bottles.
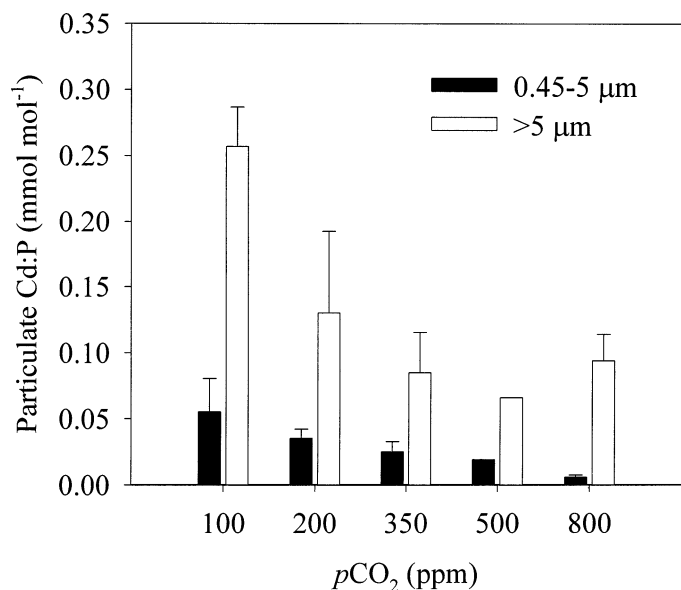§ Concentration of Zn added to incubation starting water in addition to <3 nmol L^{-1} m control treatment. ND, not determined.

Fig. 2. Size-fractionated particulate Cd:P measured by HR-ICP-MS versus incubation pCO_2 for late–exponential phase shipboard incubation 2000b. Error bars represent range of measurements from duplicate treatment bottles.

Fig. 4. Biomass-normalized Cd uptake rates with and without $+25$ nmol L^{-1} Zn additions for three short-term uptake experiments in Monterey Bay, California, May 1998. Error bars represent the standard deviation of duplicate Cd uptake measurements.

trolled incubation experiments using natural assemblages of marine phytoplankton to investigate factors controlling the cycling of Cd and PO_4 in the surface ocean. While manipulations of pCO_2, Zn, and Mn had no effect on steady-state growth rates or on the C:N:P composition of natural phytoplankton assemblages, significant variability in the Cd:P composition of the algae resulted. These variations were qualitatively similar to those observed previously in labo-
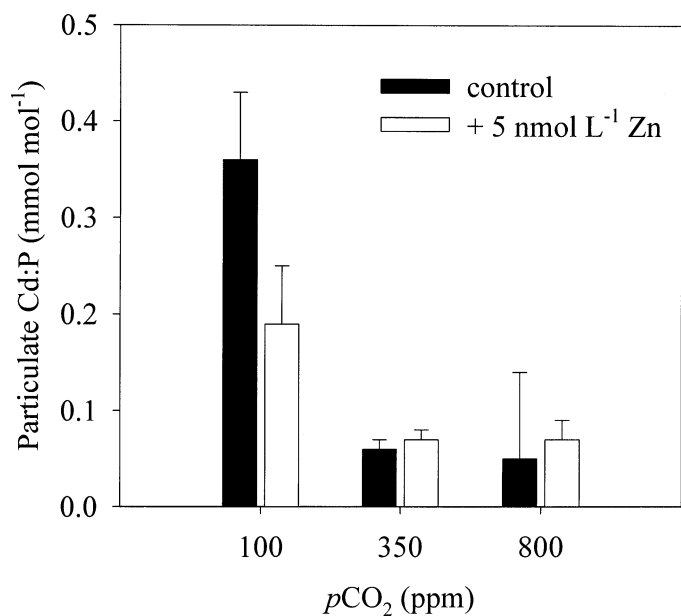
ratory experiments with diatom isolates. Taken together, the laboratory results, kinetic models, and our field studies indicate that preferential biological removal of dissolved Cd in ocean surface waters is a likely mechanism that can account for observed depletions of the dissolved Cd:PO_4 ratio. We suggest that inhibition of Cd uptake by elevated Mn concentrations is probably only significant in coastal waters but that anomalously low dissolved Cd:PO_4 ratios are most likely to occur in productive waters in which dissolved Zn

Fig. 3. Particulate Cd:P calculated from ^{109}Cd:^{14}C uptake rates and particulate C:P versus incubation pCO_2 for experiment 1999a. Error bars represent propagated standard deviation based on duplicate measurements of Cd and C uptake rates.
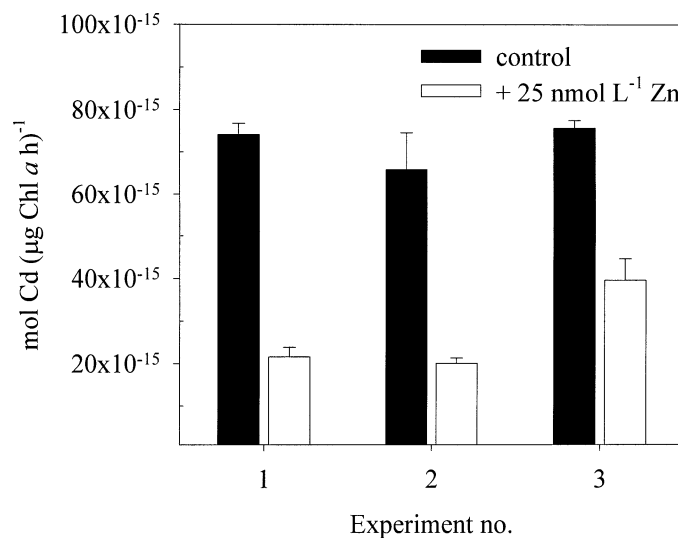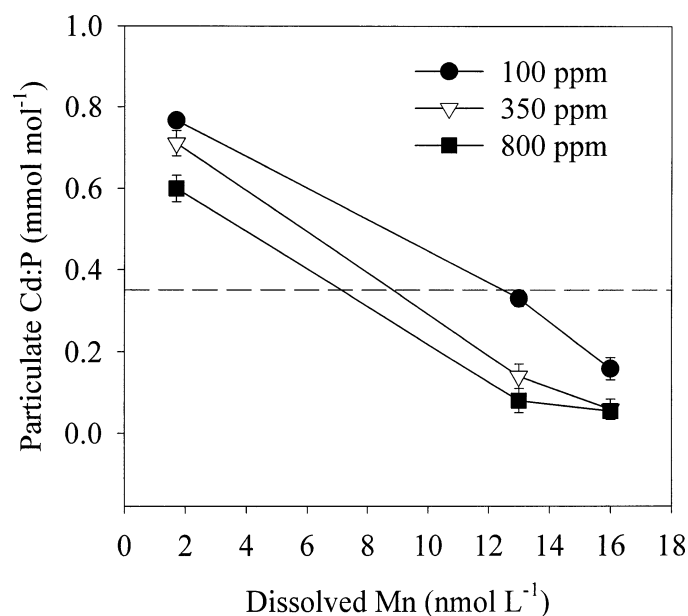

Fig. 5. Particulate Cd:P (>0.45 μm) measured by HR-ICP-MS versus incubation dissolved Mn concentration for experiments 1999, 2000a, and 2000b. Error bars represent standard deviation of measurements from duplicate treatments. Dashed line represents average deep-water dissolved Cd:P ratio ($\sim$0.35 mmol mol^{-1}).

availability and ambient $p\text{CO}_2$ are reduced. The study demonstrates that field populations of phytoplankton subjected to varying $p\text{CO}_2$ and dissolved Zn and Mn concentrations have a Cd:P composition that is remarkably plastic and that significant differences in the Cd:P ratio of different size classes of particulate matter result. Both these findings indicate that the surface-water biogeochemistry of Cd and the resulting variability in the surface water dissolved Cd:PO$_4$ ratio may be a direct result of preferential uptake of Cd by large phytoplankton that results in the export of high Cd:P particulate matter from the euphotic zone.

Dissolved concentrations of Mn, Zn, and $p\text{CO}_2$ exhibit pronounced variability in coastal ocean waters owing to their proximity to terrestrial sources of metals, inputs from shelf sediments, and the occurrence of wind-driven upwelling events that bring high-nutrient subsurface waters to the surface and fuel intense phytoplankton blooms. The range of $p\text{CO}_2$ measured in coastal surface waters can be extreme, spanning values from as low as 117 ppm during intense phytoplankton blooms up to 1,734 ppm when upwelling conditions predominate (Ianson et al. 2003). Dissolved concentrations of Mn and Zn are generally a factor of 10 to 100 higher in unpolluted coastal waters compared to shelf and open-ocean waters. Dissolved Mn in the surf zone off the North American coast reaches values of 17 nmol L^{-1} and can be as high as 40–60 nmol L^{-1} in Californian shelf waters and surface waters of the Mid–Atlantic Bight (Cullen and Sherrell unpubl. data). Dissolved Zn is $\sim$10 nmol L^{-1} in the subsurface waters in the northeast Pacific that feed coastal upwelling centers (Lohan et al. 2002) and can reach concentrations of >20 nmol L^{-1} in English coastal waters (Achterberg et al. 1999). In this study we chose our treatments to reflect the natural range of dissolved Mn and Zn, and the $p\text{CO}_2$ in coastal waters and upwelling centers, to examine the effect of these variables on particulate Cd:P ratios.

Regulation of Cd:P ratios by $p\text{CO}_2$—In all incubation experiments, the Cd content of phytoplankton was enhanced as ambient $p\text{CO}_2$ was lowered (Table 2). There is increasing evidence that Cd's nutrient-like behavior, like that of Zn, results, at least in part, from a role in carbon acquisition by marine phytoplankton (Cullen et al. 1999; Lane and Morel 2000*a*,*b*). Cd is known to increase the activity of the enzyme CA, normally a Zn-containing metalloenzyme, by supplementing or substituting for Zn in some species of phytoplankton under Zn limitation in culture (Price and Morel 1990; Lee and Morel 1995; Lee et al. 1995). The biogeochemical cycling of Cd, and therefore Cd:P ratios, are thus linked by CA to Zn and C cycling in ocean surface waters. This enzyme is thought to play an important role in phytoplankton carbon acquisition strategies at low external inorganic carbon concentrations (Tortell et al. 1997, 2000) and Zn availability (Morel et al. 1994; Lane and Morel 2000*b*). In a previous study we demonstrated for the first time the regulation of phytoplankton Cd content by $p\text{CO}_2$ and Zn (Cullen et al. 1999). This regulation is qualitatively consistent with the use of Cd in a recently discovered Cd-specific CA that is similarly regulated in cultures of the marine diatom *Thalassiosira weisfloggii* (Lane and Morel 2000*a*). Whether or not the increases in particulate Cd:P in response

to lower $p\text{CO}_2$ found in this study result from incorporation of Cd into CA or simply from nonspecific uptake driven by upregulation of Zn or Mn transporters (Sunda and Huntsman 2000) or by indirect effects of an altered carbonate system on dissolved metal availability (Wei and Sherrell unpubl. data) is not known. Below we attempt to constrain the relative contribution of Cd–CA and/or possible Cd substitution for Zn in Zn–CA to the total intracellular Cd pool measured in high Cd:P cells collected off central California.

As of yet, no quantitative measurements of total Cd associated with Cd–CA in natural assemblages or cultured phytoplankton have been published. However, estimates of total cellular content of CA (C_{CA}, mol CA cell^{-1}) calculated from the activity (a_{CA}, mol CO_2 s^{-1}) and catalytic rate (a_{MM}, mol CO_2 catalyzed mol CA^{-1} s^{-1}) of the enzyme

$$C_{CA} = \frac{a_{CA}}{a_{MM}} \tag{1}$$

for various planktonic diatoms acclimated to low CO_2 vary from 0.4–880 $\times$ 10^{-20} mol cell^{-1} (Riebesell pers. comm.). Assuming 0.6 pg Chl *a* cell^{-1} and a 1:1 ratio of Cd to CA, the range of expected Cd for low–CO_2 acclimated diatoms is 0.008 to 20 pmol Cd μg Chl a^{-1}. The range of Cd content for phytoplankton collected off central California spans values from 1–220 pmol Cd μg Chl a^{-1} (assuming Chl a:C = 0.3 mmol mol^{-1} and Chl a = 893.5 g mol^{-1}) (Cullen et al. 1999; Sunda and Huntsman 2000). Unless almost 100% of intracellular Cd is associated with the Cd–CA or has completely substituted for Zn in Zn–CA within this diatom assemblage, it is unlikely that Cd–CA can account for a majority of cellular Cd in our highest Cd:P cells.

The high levels of intracellular Cd reported here for cells growing at low $p\text{CO}_2$ are near to or exceeding levels known to be toxic to coastal marine diatoms (Payne and Price 1999). The growth and coincident Cd accumulation in our diatoms indicates that they are able to detoxify or limit Cd toxicity within the cell. Eucaryotic phytoplankton exposed to elevated concentrations of Cd or reduced concentrations of Zn at typical oceanic concentrations of Cd are known to synthesize intracellular metal binding polypeptides called phytochelatins (PC) (Ahner and Morel 1995; Ahner et al. 1995, 1998). In some species, including an oceanic diatom, these sulfur-containing ligands bind Cd in roughly a 2:1 stoichiometry, with total phytochelatin concentrations varying from 3–2,800 μmol (g Chl a)$^{-1}$ (Gekeler et al. 1988; Ahner et al. 1995; Wei et al. 2003). This corresponds to a cellular Cd content of roughly 2–1,400 pmol Cd (μg Chl a)$^{-1}$ if all phytochelatin were bound to Cd. The similarity between the range of Cd content predicted from studies of phytochelatin production and the Cd content of diatoms measured in shipboard incubations and from cells collected off central California (1–220 pmol Cd μg Chl a^{-1}) indicates that the bulk of intracellular Cd could be bound to phytochelatin in our high–Cd:P samples. As a dynamic intracellular pool of Cd that is present even when ambient Cd concentrations are extremely low (Ahner et al. 1995), PC–Cd may play an important role in maintaining metal homeostasis within the cell and may potentially feed Cd to functional metalloenzymes like Cd-CA, as needed. Quantification of

Cd–CA in relation to other intracellular Cd pools is required to determine the relative importance of Cd–CA in explaining surface-water Cd depletions in low–pCO$_2$ areas off central California.

Increased Cd incorporation by phytoplankton growing under low pCO$_2$ could also result from pH-driven changes in the relative availability of Cd versus Zn ([Cd′]/[Zn′]) in productive ocean surface waters. Similar to our incubation experiments, the pH of surface waters increases by ~1.5 units when pCO$_2$ is reduced from upwelling values of ~800 ppm to ~100 ppm by the removal of total inorganic carbon during photosynthesis. Given that the degree of organic complexation of Cd and Zn is likely pH dependent, we may expect substantial shifts in the free ion concentrations of these elements. While our understanding of organic complexation of these metals continues to grow (Bruland 1989, 1992; Ellwood 2004), the effect of variable pH on the stability constants of organic chelators for Cd and Zn and resulting Δ[Cd′]/Δ[Zn′] cannot be predicted easily. It is possible, however, that higher pH as a result of phytoplankton growth could raise [Cd′]/[Zn′], as available evidence indicates that Zn is organically complexed to a greater degree than is Cd (Bruland 1989, 1992; Ellwood 2004), favoring increased Cd incorporation by cells growing under low–pCO$_2$ conditions.

A distinct pattern of cell-size dependence for Cd : P ratios in the plankton was evident in the study, with larger (>5-μm) cells having higher ratios than cells in the 0.45–5-μm size range. This size dependence has been reported before for these waters. Cullen et al. (1999) reported that >53-μm diatoms collected from coastal California surface waters had Cd : P ratios (0.11–6.4 mmol mol^{-1}) that were up to 25-fold higher than those of the 0.45–5-μm size fraction. Larger cells having more Cd relative to their biomass is consistent with their lower surface area–to-volume ratio, which places them at greater risk of diffusion limitation for CO$_2$ uptake (Wolf-Gladrow and Riebesell 1997). Under these conditions, some large diatoms are known to facilitate carbon acquisition using a Cd-specific CA (Lane and Morel 2000a). The data presented here and that of Cullen et al. (1999) tend to support this interpretation, as the largest enrichment of particulate Cd relative to P was found in cells harvested in low–pCO$_2$ waters. However, the larger fraction in the incubations reported here had higher Cd : P ratios across the full range of pCO$_2$ treatments, suggesting that factors besides CO$_2$ diffusion limitation are behind the elevated Cd : P ratios in the large phytoplankton. Another explanation is that the larger size fractions may contain more diatoms, while the 0.45–5-μm fraction may contain organisms like heterotrophic and autotrophic bacteria, which could have substantially lower Cd : P ratios.

Regulation of Cd : P ratios by dissolved Zn and Mn—We observed inverse relationships between the Cd content of phytoplankton assemblages and the availability of dissolved Zn and Mn in shipboard incubations, similar to those found for single-species marine diatom cultures performed in the laboratory (Lee and Morel 1995; Sunda and Huntsman 1998, 2000) (Table 2; Figs. 4, 5). The suppression of Cd uptake by Zn and Mn is thought to result from competitive inhibi-

tion at cell surface transport sites that are under negative feedback regulation by cellular Zn and Mn content (Sunda and Huntsman 1998, 2000). Experiments designed to test the effect of increased Zn concentrations on short-term Cd uptake indicate highly reproducible and significant suppression of rates in diatom-dominated assemblages in Monterey Bay, California (Fig. 4). In two experiments investigating Cd : P composition in response to varying Zn and pCO$_2$, Zn additions significantly reduced phytoplankton ratios across our range of pCO$_2$, with the exception of experiment 1999a, in which the Zn effect was only significant at 100 ppm. The lack of an effect of Zn on Cd uptake and resulting Cd quota in the 1999a experiment is consistent with the study of Sunda and Huntsman (1998), in which increasing Zn only affected Cd uptake by the coastal diatom *Thalassiosira pseudonana* when Mn concentrations were high (log [Mn] mol L^{-1} = −6.5). For experiment 1999a, we observed the lowest dissolved Mn concentration in surface waters sampled as part of the study (1.7 nmol L^{-1}; Table 2). Indeed, additions of Mn in experiment 1999b suppressed Cd uptake and led to lower phytoplankton Cd : P only in incubation bottles in which Zn concentrations were supplemented with additions of 5 and 10 nmol L^{-1} (Table 2). Taken together, the incubation experiments support and generalize the conclusion that, as for Cd uptake in single-species laboratory cultures, the availability of dissolved Zn and Mn can modulate the Cd : P composition, and we observe the same qualitative Cd–Zn–Mn antagonisms in natural phytoplankton assemblages as are reported for *T. pseudonana* in laboratory culture (Sunda and Huntsman 1998).

In coastal environments, the overriding control on phytoplankton Cd : P ratios may be the high concentrations of Zn and Mn, leading to low Cd uptake by the biota, despite higher total dissolved Cd. Changes in phytoplankton Cd : P ratios due to onshore–offshore gradients in total dissolved Cd, Zn, and Mn can explain observed onshore–offshore increases in particulate Cd : P in the Mid–Atlantic Bight (Cullen et al. unpubl. data). This effect can be seen in broad patterns of dissolved-metal distributions, which demonstrate that Zn and Mn decrease rapidly with distance offshore, while Cd decreases are much more gradual with distance, as, for example, in the northwest Pacific (Bruland 1980). Larger variability in dissolved Cd : P ratios in coastal waters and enclosed basins (de Baar et al. 1994) is not surprising, as these environments experience temporally and spatially variable trace-metal and nutrient inputs (riverine and aeolian) that can cause dissolved Cd : P to deviate from the open-ocean values and that can additionally affect phytoplankton fractionation. Both effects are likely to lead to dissolved Cd : P distinct from that supplied by upwelling (Rutgers van der Loeff et al. 1997).

Caveats and questions for further study—There are other factors, in addition to those studied here, that may affect the relative removal of Cd and PO$_4$ from ocean surface waters. Recent work indicates that iron limitation, through a number of physiological effects, may be responsible for the lower dissolved Cd : PO$_4$ ratios observed in high nutrient low chlorophyll (HNLC) regions of the world ocean (Sunda and Huntsman 2000; Cullen et al. 2003). While this study fo-

cused on factors affecting the removal of Cd as the root of variations in surface-water dissolved Cd : PO_4 ratios, the observed variability could also result from changes in the relative depths and/or rates of remineralization of the elements in the main thermocline. Secular variations in the biological removal of P and the inorganic/organic P uptake ratio of algae, as well as variable P content of growing phytoplankton, may also drive some of the variability in dissolved Cd : PO_4 ratios. Changes in the availability of interacting metals (speciation) driven by pH variations or the concentration and/or binding strength of organic ligands, shifts in cell size, and species composition of the resident phytoplankton community (Ho et al. 2003) could result in different degrees of Cd removal on millennial and annual time scales. Most laboratory culture work with single species of algae is carried out in trace-metal buffer systems to control the speciation of relevant metals during experiments because, with few exceptions (Hutchins et al. 1999; Maldonado and Price 1999), phytoplankton appear to access the free-metal ion of nutrient and toxic metals (Hudson and Morel 1993). In our studies, only total metal concentrations were manipulated and monitored, and little attention was paid to organic complexation and metal speciation. Future studies should focus on the relationship between the carbonate system, metal speciation, metal–metal interactions, and the resulting Cd content of dominant phytoplankton species.

We have demonstrated that Cd : PO_4 cycling in surface waters is intimately linked to trace-metal and carbon assimilation in marine phytoplankton. The principal variables that govern the Cd : P composition of diatom-dominated natural phytoplankton assemblages in our experiments are the ambient pCO_2 and the availability of dissolved Zn and Mn to the growing algae, in agreement with previous work in single-species laboratory culture experiments. Our results imply that the preferential Cd removal from ocean surface waters is initiated in productive areas of the world ocean, where high nutrient concentrations ($\sim$10 μmol L^{-1} NO$_3^-$; Wilkerson et al. 2000) promote the growth of large diatoms, which in turn effectively lowers ambient pCO_2 and depletes dissolved Zn and Mn. A great proportion of these zones of preferential Cd uptake, like the productive areas of the Polar Front and equatorial upwelling in the Pacific, are distant from terrestrial metal inputs, indicating that suppression of Cd uptake by Mn may be of minor importance on the global scale. More work is needed to determine the factors that affect the dissolved Cd : PO_4 in the large oceanic gyres, where the concentrations of both Cd and PO_4 are greatly reduced by biological drawdown and where organic species of P may be biologically more relevant than PO_4.

This study is directly relevant for validating the assumption of constant surface-water Cd : PO_4 fractionation over the past 400–500 kyr, used by emerging paleonutrient proxies based on the amount of Cd preserved in fossil planktonic foraminifera (Rosenthal et al. 1997; Elderfield and Rickaby 2000). Elderfield and Rickaby (2000) modeled observed variability in surface-water dissolved Cd : P ratios by applying a Rayleigh fractionation model with a constant fractionation factor ($\alpha_{Cd:P} = 2.5$), thus treating Cd : PO_4 in a manner analogous to nutrient isotope systems (e.g., C, N, and silicon).

However, surface-water Cd biogeochemistry is modulated by environmental factors, particularly surface-water pCO_2, that show pronounced fluctuations in the geologic record (Petit et al. 1999; Augustin et al. 2004). This implies that the relationship between dissolved Cd and P may have varied regionally or globally over the last 420 kyr. For example, during the last glacial, maximum surface-water pCO_2 was, on average, nearly equilibrated with an $\sim$200-ppm atmosphere (Petit et al. 1999; Augustin et al. 2004), with substantially lower values in areas of intense phytoplankton productivity. Under these conditions, Cd removal by phytoplankton would be enhanced, leading to lower dissolved Cd : PO_4 ratios in ocean surface waters. Reconstructions of surface-water PO_4 concentrations based on planktonic foraminiferal Cd : calcium (Ca) and the modern dissolved Cd : PO_4 ratio (Boyle 1988; Elderfield and Rickaby 2000) would overestimate nutrient drawdown and the efficiency of the biological carbon pump in the past ocean under these conditions. This would tend to enhance the degree of discordance between the δ^{13}C, δ^{15}N of sedimentary organic matter and planktonic foraminiferal Cd : Ca–based reconstructions of nutrient utilization in the Southern Ocean during the last glacial period (Elderfield and Rickaby 2000). At the same time, changes in upwelling rates could lead to variations in dissolved Cd : Zn or absolute Zn concentration in the euphotic zone, further complicating predictions of Cd : PO_4 uptake ratios. This modulation of Cd : P uptake ratios is inconsistent with a constant $\alpha_{Cd:P}$ (Elderfield and Rickaby 2000) or regionally specific algal Cd : P composition (Saager and Baar 1993) and is incompatible with a true Rayleigh model of elemental fractionation. The control of phytoplankton Cd uptake and surface-water Cd : P by factors like pCO_2, dissolved Cd, Zn, and Mn introduces an additional level of complexity to reconstructions of past surface-water dissolved Cd : PO_4 or absolute PO_4 concentrations.

References

ACHTERBERG, E. P., C. COLOMBO, AND C. M. G. VAN DEN BERG. 1999. The distribution of dissolved Cu, Zn, Ni, Co and Cr in English coastal surface waters. Cont. Shelf Res. **19:** 537–558.

AHNER, B. A., S. KONG, AND F. M. M. MOREL. 1995. Phytochelatin production in marine algae. 1. An interspecies comparison. Limnol. Oceanogr. **40:** 649–657.

———, J. G. LEE, N. M. PRICE, AND F. M. M. MOREL. 1998. Phytochelatin concentrations in the equatorial Pacific. Deep-Sea Res. I **45:** 1779–1796.

———, AND F. M. M. MOREL. 1995. Phytochelatin production in marine algae. 2. Induction by various metals. Limnol. Oceanogr. **40:** 658–665.

AUGUSTIN, L., AND OTHERS. 2004. Eight glacial cycles from an Antarctic ice core. Nature **429:** 623-628.

BOYLE, E. A. 1988. Cadmium: Chemical tracer of deepwater paleoceanography. Paleoceanography **3:** 471–489.

———, S. S. HUESTED, AND S. P. JONES. 1981. On the distribution of copper, nickel and cadmium in the surface waters of the North Atlantic and North Pacific Ocean. J. Geophys. Res. **86:** 8048–8066.

———, F. SCLATER, AND J. M. EDMOND. 1976. On the marine geochemistry of cadmium. Nature **263:** 42–44.

BRULAND, K. W. 1980. Oceanographic distributions of cadmium,

zinc, nickel, and copper in the north Pacific. Earth Planet. Sci. Lett. **47:** 176–198.

———. 1989. Complexation of zinc by natural organic ligands in the central North Pacific. Limnol. Oceanogr. **34:** 269–285.

———. 1992. Complexation of cadmium by natural organic ligands in the central North Pacific. Limnol. Oceanogr. **37:** 1008–1017.

———, G. A. KNAUER, AND J. H. MARTIN. 1978. Cadmium in northeast Pacific waters. Limnol. Oceanogr. **23:** 618–625.

COLLIER, R., AND J. EDMOND. 1984. The trace element geochemistry of marine biogenic particulate matter. Prog. Oceanogr. **13:** 113–199.

CULLEN, J. T., Z. CHASE, K. H. COALE, S. FITZWATER, AND R. M. SHERRELL. 2003. Effect of iron limitation on the cadmium to phosphorus ratio of natural phytoplankton assemblages from the Southern Ocean. Limnol. Oceanogr. **48:** 1079–1087.

———, M. P. FIELD, AND R. M. SHERRELL. 2001. Determination of trace elements in filtered suspended marine particulate material by sector field HR-ICP-MS. J. Anal. Atmos. Spectrom. **16:** 1307–1312.

———, T. W. LANE, F. M. M. MOREL, AND R. M. SHERRELL. 1999. Modulation of cadmium uptake in phytoplankton by seawater CO_2 concentration. Nature **402:** 165–167.

———, AND R. M. SHERRELL. 1999. Techniques for determination of trace metals in small samples of size-fractionated particulate matter: Phytoplankton metals off central California. Mar. Chem. **67:** 233–247.

DE BAAR, H. J. W., P. M. SAAGER, R. F. NOTLING, AND J. VAN DER MEER. 1994. Cadmium versus phosphate in the world ocean. Mar. Chem. **46:** 261–281.

DONAT, J. R., AND K. W. BRULAND. 1995. Trace elements in the oceans, p. 247–281. *In* B. Salbu and E. Steinnes [eds.], Trace elements in natural waters. CRC Press.

ELDERFIELD, H., AND R. E. M. RICKABY. 2000. Oceanic Cd/P ratio and nutrient utilization in the glacial Southern Ocean. Nature **405:** 305–310.

ELLWOOD, M. J. 2004. Zinc and cadmium speciation in subantarctic waters east of New Zealand. Mar. Chem. **87:** 37–58.

FALKOWSKI, P. G., AND J. A. RAVEN. 1997. Aquatic photosynthesis. Blackwell Science.

FEELY, R. A., C. L. SABINE, K. LEE, W. BERELSON, J. KLEYPAS, V. J. FABRY, AND F. J. MILLERO. 2004. Impact of anthropogenic CO_2 on the $CaCO_3$ system in the oceans. Science **305:** 362–366.

FIELD, M. P., J. T. CULLEN, AND R. M. SHERRELL. 1999. Direct determination of 10 trace metals in $50 \mu L$ samples of coastal seawater using desolvating micronebulization sector field ICP-MS. J. Anal. Atmos. Spectrom. **14:** 1425–1431.

GEKELER, W., E. GRILL, E.-L. WINNACKER, AND M. H. ZENK. 1988. Algae sequester heavy metals via synthesis of phytochelatin complexes. Arch. Microbiol. **150:** 197–202.

HO, T., A. QUIGG, Z. V. FINKEL, A. J. MILLIGAN, K. WYMAN, P. G. FALKOWSKI, AND F. M. M. MOREL. 2003. The elemental composition of some marine phytoplankton. J. Phycol. **39:** 1145–1159.

HUDSON, R. J. M., AND F. M. M. MOREL. 1993. Trace metal transport by marine microorganisms: Implications of metal coordination kinetics. Deep-Sea Res. I **40:** 129–150.

HUTCHINS, D. A., A. E. WITTER, A. BUTLER, AND G. W. LUTHER III. 1999. Competition among marine phytoplankton for different chelated iron species. Nature **400:** 858–861.

IANSON, D., S. E. ALLEN, S. L. HARRIS, K. J. ORIANS, D. E. VARELA, AND C. S. WONG. 2003. The inorganic carbon system in the coastal upwelling region west of Vancouver Island, Canada. Deep-Sea Res. I **50:** 1023–1042.

KAPLAN, A., AND L. REINHOLD. 1999. CO_2 concentrating mechanisms in photosynthetic microorganisms. Annu. Rev. Plant Physiol. Plant Mol. Biol. **50:** 539–570.

KARL, D. M., AND G. TIEN. 1997. Temporal variability in dissolved phosphorus concentrations in the subtropical North Pacific Ocean. Mar. Chem. **56:** 77–96.

KNAUER, G. A., AND J. H. MARTIN. 1973. Seasonal variations of cadmium, copper, manganese, lead and zinc in water and phytoplankton in Monterey Bay, California. Limnol. Oceanogr. **18:** 597–604.

LANE, T. W., AND F. M. M. MOREL. 2000*a*. A biological function for cadmium in marine diatoms. Proc. Nat. Acad. Sci. USA **97:** 4627–4631.

———, AND ———. 2000*b*. Regulation of carbonic anhydrase by zinc, cobalt, and carbon dioxide in the marine diatom *Thalassiosira weissflogii*. Plant Physiol. **123:** 345–352.

LEE, J. G., AND F. M. M. MOREL. 1995. Replacement of zinc by cadmium in marine phytoplankton. Mar. Ecol. Prog. Ser. **127:** 305–309.

———, S. B. ROBERTS, AND F. M. M. MOREL. 1995. Cadmium: A nutrient for the marine diatom *Thalassiosira weissflogii*. Limnol. Oceanogr. **40:** 1056–1063.

LOHAN, M. C., P. J. STATHAM, AND D. W. CRAWFORD. 2002. Total dissolved zinc in the upper water column of the subarctic North East Pacific. Deep-Sea Res. II **49:** 5793–5808.

LÖSCHER, B. M., J. T. M. DE JONG, AND H. J. W. DE BAAR. 1998. The distribution and preferential biological uptake of cadmium at 6°W in the Southern Ocean. Mar. Chem. **62:** 259–286.

MALDONADO, M. T., AND N. M. PRICE. 1999. Utilization of iron bound to strong organic ligands by plankton communities in the subarctic Pacific Ocean. Deep-Sea Res. II **46:** 2447–2473.

MOREL, F. M. M., J. R. REINFELDER, S. B. ROBERTS, C. P. CHAMBERLAIN, J. G. LEE, AND D. YEE. 1994. Zinc and carbon colimitation of marine phytoplankton. Nature **369:** 740–742.

PARSONS, T. R., Y. MAITA, AND C. M. LALLI. 1984. A manual of chemical and biological methods for seawater analysis. Pergamon.

PAYNE, C. D., AND N. M. PRICE. 1999. Effects of cadmium toxicity on growth and elemental composition of marine phytoplankton. J. Phycol. **35:** 293–302.

PETIT, J. R., J. JOUZEL, D. RAYNAUD, N. I. BARKOV, J.-M. BARNOLA, AND I. BASILE. 1999. Climate and atmospheric history of the past 420,000 years from the Vostok ice core, Antarctica. Nature **399:** 429–436.

PRICE, N. M., AND F. M. M. MOREL. 1990. Cadmium and cobalt substitution for zinc in a marine diatom. Nature **344:** 658–660.

RIEBESELL, U., D. A. WOLF-GLADROW, AND V. SMETACEK. 1993. Carbon dioxide limitation of marine phytoplankton growth rates. Nature **361:** 249–251.

ROSENTHAL, Y., E. A. BOYLE, AND L. LABEYRIE. 1997. Last glacial maximum paleochemistry and deepwater circulation in the Southern Ocean: Evidence from foraminiferal cadmium. Paleoceanography **12:** 787–796.

RUTGERS VAN DER LOEFF, M., E. HELMERS, AND G. KATTNER. 1997. Continuous transects of cadmium, copper, and aluminium in surface waters of the Atlantic Ocean, 50°N to 50°S: Correspondence and contrast with nutrient-like behaviour. Geochim. Cosmochim. Acta **61:** 47–61.

SAAGER, P. M., AND H. J. W. DE BAAR. 1993. Limitations to the quantitative application of Cd as a paleoceanographic tracer, based on results of a multi-box model (MENU) and statistical considerations. Global Planet. Change **8:** 69–92.

SAÑUDO-WILHELMY, S. A., AND A. R. FLEGAL. 1996. Trace metal concentrations in the surf zone and in coastal waters off Baja California, Mexico. Environ. Sci. Technol. **30:** 1575–1580.

SUNDA, W. G., AND S. A. HUNTSMAN. 1996. Antagonisms between

cadmium and zinc toxicity and manganese limitation in a coastal diatom. Limnol. Oceanogr. **41:** 373–387.

———, AND ———. 1998. Control of Cd concentrations in a coastal diatom by interactions among free ionic Cd, Zn, and Mn in seawater. Environ. Sci. Technol. **32:** 2961–2968.

———, AND ———. 2000. Effect of Zn, Mn, and Fe on Cd accumulation in phytoplankton: Implications for oceanic Cd cycling. Limnol. Oceanogr. **45:** 1501–1516.

TAKAHASHI, T., AND OTHERS. 2002. Global sea-air CO_2 flux based on climatological surface ocean pCO^2, and seasonal biological and temperature effects. Deep-Sea Res. II **49:** 1601–1622.

TORTELL, P. D., G. H. RAU, AND F. M. M. MOREL. 2000. Inorganic carbon acquisition in coastal Pacific phytoplankton communities. Limnol. Oceanogr. **45:** 1485–1500.

———, J. R. REINFELDER, AND F. M. M. MOREL. 1997. Active uptake of bicarbonate by diatoms. Nature **390:** 243–244.

VINK, S., E. A. BOYLE, C. I. MEASURES, AND J. YUAN. 2000. Automated high resolution determination of the trace elements iron and aluminium in the surface ocean using a towed Fish

coupled to flow injection analysis. Deep-Sea Res. I **47:** 1141–1156.

WEDEPOHL, K. H. 1995. The composition of the continental crust. Geochim. Cosmochim. Acta **59:** 1217–1232.

WEI, L., J. R. DONAT, G. FONES, AND B. A. AHNER. 2003. Interactions between Cd, Cu, and Zn influence particulate phytochelatin concentrations in marine phytoplankton: Laboratory results and preliminary field data. Environ. Sci. Technol. **37:** 3609–3618.

WILKERSON, F. P., R. C. DUGDALE, R. M. KUDELA, AND F. P. CHAVEZ. 2000. Biomass and productivity in Monterey Bay, California: Contribution of the large phytoplankton. Deep-Sea Res. II **47:** 1003–1022.

WOLF-GLADROW, D., AND U. RIEBESELL. 1997. Diffusion and reactions in the vicinity of plankton: A refined model for inorganic carbon transport. Mar. Chem. **59:** 17–34.