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The effect of pH on the uptake of zinc and cadmium in marine phytoplankton: Possible role of weak complexes

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Abstract

In natural samples from the New Jersey coast and the Gulf of Alaska, zinc (Zn) and cadmium (Cd) uptake rates by phytoplankton decreased on average about 30% as pH was decreased from 8.5 to 7.9 or 7.7, and the partial pressure of carbon dioxide (PCO_2) increased accordingly. The underlying mechanism was explored with the model species, *Thalassiosira weissflogii* and *Emiliania huxleyi*, using ethylenediaminetetraacetic acid (EDTA), desferrioxamine B, phytochelatin, and cysteine as complexing agents. Experiments with single complexing agents did not reproduce the effect of pH seen in field samples, ruling out two possible mechanisms: a direct effect on the uptake machinery or down-regulation of uptake at high PCO_2. Zn and Cd bioavailability must thus somehow decrease at low pH in natural seawater, which is counterintuitive since the protonation of complexing agents at low pH should increase the total free concentration of metals. However, in the presence of both a strong and a weak complexing agent, metal uptake rate may decrease at low pH if formation of the weak complex decreases and the metal in the weak complex is more "available" than in the strong complex. We obtained proof of concept for such a two-ligand mechanism for Zn uptake in the presence of EDTA + phytochelatin and EDTA + cysteine. Weak ligands that bind a small fraction of essential metals in surface seawater may thus be important in metal uptake by phytoplankton, and the dual effects of strong and weak complexing agents may control not just the magnitude but also the sign of the effect of pH–PCO_2 on metal uptake rates.

The ongoing acidification of seawater through dissolution of anthropogenic carbon dioxide (CO_2) in the surface ocean is likely to have complex effects on marine phytoplankton. Some effects will result from direct physiological responses to the decrease in pH and the increase in the partial pressure of CO_2 (PCO_2; Riebesell et al. 2000; Kranz et al. 2009; Hopkinson et al. 2010). Other effects will be mediated through complex changes in the chemistry of seawater. For example, it has been shown that acidification can result in a change in the chemistry of iron (Fe) and lead to a decrease in the rate of Fe uptake by phytoplankton (Shi et al. 2010). Here we examine the question of the effect of acidification on the uptake of zinc (Zn) and cadmium (Cd) by phytoplankton. This question is made particularly interesting because those two metals are involved in inorganic carbon acquisition in marine phytoplankton (Morel et al. 2002). The Zn and Cd requirements of the organisms may thus be affected by the increase in ambient PCO_2, while the uptake of the metals may be affected by a change in chemical speciation caused by the lower pH of the medium.

Zn, which is used as a cofactor in many enzymes, is an essential element for phytoplankton. Cd is known to replace Zn for some biological functions in some phytoplankton, including the model species *Thalassiosira weissflogii* and *Emiliania huxleyi*, fostering better growth under Zn-limited conditions (Lee and Morel 1995; Lane and Morel 2000*a*; Xu et al. 2007). In *E. huxleyi*, the biochemical function of Cd is unknown (Xu et al. 2007); while, in *T. weissflogii*, Cd can replace Zn as a cofactor in the cadmium-carbonic anhydrase (CDCA; Lane et al. 2005; Xu et al. 2008). Carbonic anhydrases (CAs) are abundant enzymes that play a key role in the carbon-concentrating mechanism (CCM) of marine phytoplankton. As a result, CA expression is very sensitive to variations in pH–PCO_2 (Lane and Morel 2000*b*; Sunda and Huntsman 2005; McGinn and Morel 2008). Previous studies have shown a substantial decrease in CA activity in the diatoms *T. weissflogii* and *Thalassiosira pseudonana* when PCO_2 increases. This should result in a lower Zn and Cd requirement at high PCO_2, as observed for Zn in *T. pseudonana* under moderate Zn limitation (Sunda and Huntsman 2005).

As demonstrated by uptake kinetics, there are at least two kinds of transport systems for Zn in eukaryotic phytoplankton, a high-affinity and a low-affinity system (Sunda and Huntsman 1992, 1998*b*, 2005). The former is inducible and is up-regulated by cells under metal limitation, and the latter is either constitutive or under only minimal regulation. Cd can be taken up via the Zn transport system at low Zn and via the manganese (Mn) transport system at high Zn (Sunda and Huntsman 1998*a*,*b*, 2000). There is no molecular characterization of metal uptake systems in eukaryotic phytoplankton, and we do not know the nature or the acid–base properties of the molecules involved in transport.

In the surface oceans, the bulk of the dissolved Zn and Cd is bound to very strong organic chelators (Bruland 1989, 1992; Ellwood 2004). We have estimates for the stability constants of the metal complexes, but we do not know the nature of the functional groups involved in metal binding or their acid–base chemistry, except that some chelators appear to be hydroxamate siderophores (Mawji

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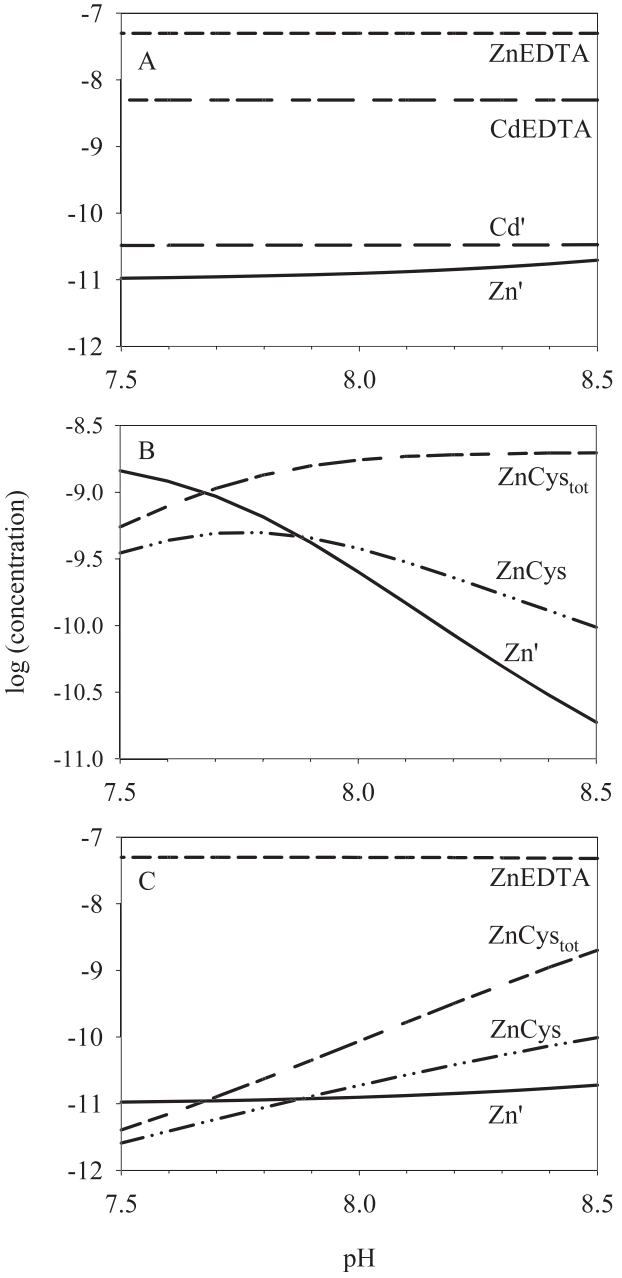

Fig. 1. Zn speciation as a function of pH in the presence of (A) EDTA, (B) Cys, and (C) both EDTA and Cys. Panel A also shows Cd speciation as a function of pH in the presence of EDTA. The metal and ligand concentrations are (A) 50 nmol L^{-1} Zn or 5 nmol L^{-1} Cd and 100 μmol L^{-1} EDTA; (B) 2 nmol L^{-1} Zn and 2 μmol L^{-1} Cys; (C) 50 nmol L^{-1} Zn, 100 μmol L^{-1} EDTA, and 2 μmol L^{-1} Cys. [$ZnCys_{tot}$] = [ZnCys] + [$Zn(Cys)_2$].

et al. 2008). Metal competition studies with natural seawater samples generally show that the Zn-binding ligands may be specific for Zn, but the results for the Cd-binding ligands are variable (Bruland 1992; Ellwood 2004). The low concentration of Zn in the open ocean suggests that this metal could limit phytoplankton growth in some areas, although this idea is supported by the results of only a few field studies (Coale 1991; Crawford et al. 2003; Franck et al. 2003). A study in California coastal waters showed that the Cd content of phytoplankton (as quantified by the particulate Cd : P) increased at low PCO_2 and at low cellular Zn. Short-term uptake experiments showed a lower Cd uptake rate at lower pH–higher PCO_2, and the effect was attributed to the change in metal requirement responding to the change in PCO_2 (Cullen et al. 1999).

The binding of Zn and Cd by strong chelators in surface seawater results in very low concentrations of the free metal ions, [Zn^{2+}] and [Cd^{2+}], and of the total free metals, Zn′ and Cd′, which we define as the sum of the concentrations of the metal complexes with the major inorganic ligands of seawater—principally, CO_3^{2-}, Cl^-, and SO_4^{2-}, in addition to OH^- and H_2O. Numerous laboratory studies, using chiefly the artificial chelating agent ethylenediaminetetraacetic acid (EDTA), have shown that the total free metal concentration controls the kinetics of metal uptake by phytoplankton. In other words, in the presence of EDTA, Zn′ and Cd′ provide a measure of the bioavailability of Zn and Cd (Anderson and Morel 1982; Sunda and Huntsman 1992; Xu et al. 2007). In an EDTA-buffered medium subjected to a change in pH, we thus expect a change in Zn and Cd uptake rate proportional to the change in Zn′ and Cd′, when the uptake system is not saturated. In seawater, Zn^{2+}, $ZnCl_x$, $Zn(SO_4)_x$, $Zn(OH)_x$, and $ZnCO_3$ all account for a significant fraction of Zn′ (ca. 48%, 21%, 4%, 4%, and 3%, respectively, at pH 8.1; Byrne et al. 1988; Millero et al. 2009); and the bulk of the EDTA is bound to calcium ion (Ca^{2+}). The net result is that a decrease in the concentration of $Zn(OH)_x$ and $ZnCO_3$ at lower pH leads to a modest decrease in Zn′ (Fig. 1A). In contrast, chloride complexes account for the bulk of Cd′ ([$CdCl_x$] = 96.4% of Cd′) in seawater, while $Cd(OH)_x$ and $CdCO_3$ are almost negligible (Byrne et al. 1988; Millero et al. 2009). As a result, in an EDTA-buffered medium, pH has almost no effect on Cd′ (Fig. 1A).

But EDTA has an unusually high affinity for Ca^{2+}. For other complexing agents with relatively lower affinity for Ca^{2+}, the major species in seawater are protonated to various extents depending on the pK_as of their acid groups. Thus, H^+ and Zn^{2+} (or H^+ and Cd^{2+}) compete with each other for binding to the complexing agent, such that a decrease in pH (increase in [H^+]) leads to a dissociation of the Zn (or Cd) complexes. The net result is that, in the presence of many complexing agents, the free concentrations Zn′ and Cd′ should increase with decreasing pH, as illustrated in Fig. 1B for the Zn complexes with cysteine (Cys). A qualitatively similar result would obtain for most complexing agents and most metals (except for metals such as Fe, which forms stable hydrolysis species throughout the pH range; Shi et al. 2010).

In addition to strong, and perhaps specific, trace metal chelators, surface seawater also contains nonspecific, weaker complexing agents (van den Berg 1985), which must bind a small fraction of any trace metal. As a result, the total dissolved Zn in seawater, Zn_T, can be classified into three pools: the strong chelate(s), ZnY, the weak complex(es), ZnL, and the total free Zn, Zn′; Zn_T = [ZnY] + [ZnL] + Zn′; and, equivalently, Cd_T = [CdY] + [CdL] + Cd′. The

concentration of the weak complexes [ZnL] is only a small fraction of the total dissolved Zn ([ZnL] $\ll$ [ZnY] $\approx$ Zn_T), but it can be larger than the total free concentration Zn′. This is illustrated in Fig. 1C for a model system containing both EDTA and Cys, where the sum of the concentrations of the Zn–Cys complexes is only 4% of the total Zn but is seven times greater than the total free Zn at pH 8.0. Figure 1C also illustrates that it is possible for the concentration of the weak complexes to decrease at lower pH, a point that will be significant in our study.

There are three possible and not mutually exclusive mechanisms by which a change in pH may affect the uptake of Zn and Cd by phytoplankton in seawater: (1) the kinetic parameters of the Zn and Cd uptake systems may be directly affected; (2) the uptake systems for Zn and Cd may be up- or down-regulated; and (3) the bioavailability of the metals may change as discussed above. Mechanisms 1 and 3, which are purely chemical effects on transport molecules or on metal speciation in the medium, should presumably operate rapidly and can be studied in short-term experiments. In contrast, the second mechanism, which is a physiological acclimation of the organisms, should require a relatively long exposure to the new pH–PCO_2.

Here we report first the results of field experiments designed chiefly to identify the sign and the approximate magnitude of the possible effect of pH on Zn and Cd uptake by phytoplankton. A series of laboratory experiments with model species and metal complexing agents with variable functionalities are then used in an attempt to identify the mechanisms responsible for the observed effects.

Methods

Field experiments—Surface seawater was collected from the New Jersey coast (N 39°26′, W 74°14′) in October 2009 and the Gulf of Alaska (N 53°66′, W 158°00′) between August and September 2007, using a trace metal clean pumping system. Experiments with New Jersey water (background Zn concentration, 2–9 nmol L^{-1}; Field et al. 1999; Wright et al. 2010) were carried out at Princeton University within 4 h of collection after transport at the collection temperature (ca. 15°C). The water was dispensed into acid-cleaned, 2.7-liter polycarbonate bottles and incubated at 20°C under continuous light ($\sim$ 50 μmol quanta m^{-2} s^{-1}) with addition of 30 μmol L^{-1} NO_3^-, 3 μmol L^{-1} PO_4^{3-}, and 30 μmol L^{-1} SiO_2. Triplicate bottles were used for each treatment. The pH (initial pH = 8.06) was manipulated by adding ultrapure hydrochloric acid (HCl) or sodium hydroxide (NaOH) and measured on the total hydrogen ion scale (pH_T) by thymol blue (Zhang and Byrne 1996). To measure chlorophyll *a* (Chl *a*), cells were filtered onto GF/F filters, pigments were extracted with 90% acetone in a freezer overnight, and then a Turner 10-AU fluorometer was used for quantification. After 4 d of incubation, a 100-mL subsample from each bottle was poured into a small bottle amended with a few microliters of stock solutions (^{65}Zn in 1 mmol L^{-1} HCl and ^{109}Cd in 5 mmol L^{-1} HCl) to obtain 0.5 nmol L^{-1} ^{65}Zn and $<$ 0.1 nmol L^{-1} ^{109}Cd. The uptake bottles were incubated under the same conditions. After 3 to 4 h, samples for Zn and Cd uptake were filtered onto a 3-μm polycarbonate membrane filter, washed with an oxalate–EDTA solution, and counted by liquid scintillation counting (Tovar-Sanchez et al. 2003; Tang and Morel 2006). For the uptake experiment without prior pH acclimation, incubation was done without pH manipulation, then subsamples were poured into small bottles and pH was adjusted to target experimental values before radioisotopes were spiked.

In the Gulf of Alaska cruise, seawater was taken from the high-nutrient, low-chlorophyll (HNLC) region with background Zn concentration from 0.04 to 0.62 nmol L^{-1} (Lohan et al. 2002). Aliquots were dispensed into acid-cleaned, 4-liter, low-density polyethylene cubitainers. The pH was manipulated as described above and measured using a pH electrode (Oakton pH 11 meter with Oakton 35811–71 probe) calibrated daily with National Institute of Standards and Technology pH standard buffers. Intercalibrations between the electrode measurements and spectrophotometric measurements using thymol blue (Zhang and Byrne 1996) were made on seawater samples to arrive at pH_T values. The two methods differed by less than 0.1 pH units. The initial measured pH was 7.88, although we expected a value near 8.0 based on dissolved inorganic carbon (DIC) and alkalinity data (National Ocean Data Center: http://www.nodc.noaa.gov/cgi-bin/OC5/SELECT/builder.pl). For metal addition treatments, 2 nmol L^{-1} Fe was added as $FeCl_3$, 2 nmol L^{-1} Zn as $ZnSO_4$, or 2 nmol L^{-1} Cd as $CdCl_2$. Chl *a* was measured as described above. Each treatment was duplicated. The cubitainers were sealed and placed in on-deck, flow-through incubators screened with neutral density screening to 20% of surface irradiance. Subsamples (100 mL) were poured into small, acid-cleaned polycarbonate bottles, spiked with a few microliters of stock solutions (^{65}Zn in 10 mmol L^{-1} HCl and ^{109}Cd in 10 mmol L^{-1} HCl) to achieve 1.13 nmol L^{-1} ^{65}Zn and $<$ 0.1 nmol L^{-1} ^{109}Cd, and incubated under the same conditions. After about 3 h, samples for Zn and Cd uptake were filtered onto 1-μm polycarbonate membrane filters, washed with seawater three times, and counted by liquid scintillation counting.

Culturing—T. weissflogii CCMP1336 was obtained from the Provasoli-Guillard National Center for Culture of Marine Phytoplankton in Maine and *E. huxleyi* strains PLY 92E and PLY B92/11 from the Plymouth Culture Collection of Marine Algae in the UK. Bottles used for all culturing and uptake experiments were acid-cleaned polycarbonate bottles. Culture media were prepared using 0.2-μm-filtered and microwave-sterilized Gulf Stream seawater (DIC = 2050 μmol L^{-1}; Shi et al. 2010) enriched with chelexed and filter-sterilized macronutrients and filter-sterilized trace metals (20 nmol L^{-1} copper (Cu), 120 nmol L^{-1} Mn, 10 nmol L^{-1} selenium (Se)) buffered with 100 μmol L^{-1} EDTA and vitamins. For *T. weissflogii*, 100 μmol L^{-1} NO_3^-, 10 μmol L^{-1} PO_4^{3-}, 100 μmol L^{-1} SiO_2, 1 μmol L^{-1} Fe, and various concentrations of Zn were added. For *E. huxleyi*, 50 μmol L^{-1} NO_3^-, 2 μmol L^{-1} PO_4^{3-}, and 87 nmol L^{-1} Fe were added. Cells for cultures and short-term uptake were grown at 20°C under continuous light (80–100 μmol quanta m^{-2} s^{-1}).

Short-term uptake of Zn and Cd—The uptake media were prepared using 0.2-μm-filtered Gulf Stream seawater (Sunda et al. 2005), and the pH was manipulated as described above and was measured by thymol blue. When EDTA was used as the metal binding ligand, 100 μmol L^{-1} EDTA was added to the seawater together with Zn or Cd, and the medium was equilibrated overnight before adding cells. ^{65}Zn and ^{106}Cd were used as tracers with specific activities adjusted in each experiment to provide precise measurements of cellular uptake. When desferrioxamine B (DFB) or phytochelatin (PC2; (γGlu-Cys)$_2$-Gly) was used as the only ligand, Zn or Cd was equilibrated with the ligand in a small volume of Milli-Q water at about pH 7 overnight before being introduced into the uptake medium. When Cys or PC2 was used as the ligand in the two-ligand system, Zn was equilibrated with it in a small volume of Milli-Q water at about pH 7 for 2–4 h, and then the complex was added into the seawater with or without EDTA to equilibrate for another 2–4 h before adding cells. Tris(2-carboxyethyl)phosphine (TCEP) was also added as the reducing agent for PC2 or Cys; control experiments comparing media with EDTA + TCEP and EDTA alone showed that TCEP has no effect on metal uptake. The concentrations for ligands and metals in each experiment are given in the figure legends. Zn-limited exponentially growing cells were filtered onto acid-cleaned polycarbonate membrane filters, rinsed five times with 0.2-μm-filtered Gulf Stream seawater, and resuspended in seawater. Aliquots were then dispensed into the uptake media. At an interval of 0.5–1 h for a total period of 2–4 h, 20-mL aliquots from each bottle were removed and filtered onto polycarbonate membrane filters. Three-micrometer filters were used for *T. weissflogii* and 1-μm filters for *E. huxleyi*. Cells were then washed with an oxalate–EDTA solution for 5 min, and ^{65}Zn or ^{109}Cd retained on the membrane was measured via liquid scintillation counting. The pH measured at the beginning and the end of the uptake experiments remained constant.

Steady-state uptake of Zn and Cd—The culture media were prepared as described above. ^{65}Zn, ^{109}Cd, and ^{14}C were added as tracers to measure the cellular concentration of Zn, Cd, and C. The pH of the media was manipulated by adding ultrapure HCl or NaOH, measured by potentiometry during the experiments, and calibrated with thymol blue at the beginning and end of the experiments. All bottles were tightly capped throughout the experiments, except for removing daily aliquots to measure cell density and pH. Cells were acclimated in the low-Zn media at experimental pH for a week before transfer into the experimental media. Cells were counted with a Multisizer II Coulter Counter, and specific growth rate was calculated from the linear regression of the natural log of cell density vs. time for the exponential phase of growth. All experiments were conducted at low cell densities (final concentrations were < 7000 cells mL^{-1} for *T. weissflogii* and $< 30{,}000$ cells mL^{-1} for *E. huxleyi*) to prevent any significant drawdown of CO_2 and to keep the pH increase throughout all experiments below 0.1 unit. The cells were allowed to grow for seven to eight cell divisions and then were harvested by filtration onto polycarbonate membrane filters and washed with an oxalate–EDTA solution. One milliliter of 1% HCl was added to the filter to remove inorganic ^{14}C before the scintillation fluid was added into the vial for counting. ^{65}Zn was measured with a gamma counter and ^{14}C and ^{109}Cd by liquid scintillation counting. Steady-state Zn and Cd uptake rates were calculated from cellular Zn : C and Cd : C ratios and specific growth rates. The metal speciation was calculated using MINEQL$^+$ (Environmental Research Software), and the stability constants were taken from Morel and Hering (1993).

Results

Zn and Cd uptake in natural samples—We tested the effect of pH on the short-term uptake of Zn and Cd by natural phytoplankton assemblages in surface seawater samples collected from the New Jersey coast and the Gulf of Alaska. The initial Chl *a* concentration in the water from the New Jersey coast was 1.1 μg L^{-1}. Microscopic examination revealed a high concentration of diatoms in the water samples. The water from the Gulf of Alaska had an initial Chl *a* concentration of 0.7 μg L^{-1}. The phytoplankton community was dominated equally by haptophytes and chlorophytes, with a substantial contribution from diatoms (Hopkinson et al. 2010).

Metal uptake experiments with the New Jersey coastal water were performed after a 4-d incubation with addition of macronutrients and with or without adjustment of pH (Fig. 2). The final Chl *a* concentrations after incubation reached about 35 μg L^{-1} and were comparable among the various pH treatments (data not shown). The Zn uptake rate normalized to Chl *a*, with and without prior pH acclimation, decreased slightly (16% and 11%, respectively) as pH decreased from 8.5 to 7.9, but the difference was not statistically significant ($p = 0.11$ and 0.16, one-way analysis of variance [ANOVA]; Fig. 2A). The Cd uptake rate normalized to Chl *a*, with and without prior pH acclimation, showed a large decrease as pH decreased (40% and 45%, respectively; $p = 0.002$ and 0.09, one-way ANOVA; Fig. 2B). The similar sign and magnitude of the pH effect on metal uptake by natural assemblages, with and without prior pH acclimation, indicates that the main effect is probably rapid and does not result from a physiological acclimation of phytoplankton.

Surface-water samples from the HNLC region of the Gulf of Alaska were incubated for 5 d with or without addition of Fe at three pH levels before the uptake experiments. Without addition of Fe, there was no obvious change in Chl *a* concentration; with Fe addition, Chl *a* concentration increased substantially more at low than at high pH as a response to the higher PCO_2 (Hopkinson et al. 2010). We observed a large effect of pH on the rate of Zn uptake normalized to Chl *a* in both −Fe and +Fe treatments: a decrease of 49% and 68%, respectively, as pH decreased from 8.5 to 7.7 ($p = 0.001$ and 0.008, respectively, one-way ANOVA; Fig. 2C). Similarly, pH had an effect on normalized Cd uptake rates in −Fe and +Fe treatments: a decrease of 22% and 45%, respectively,

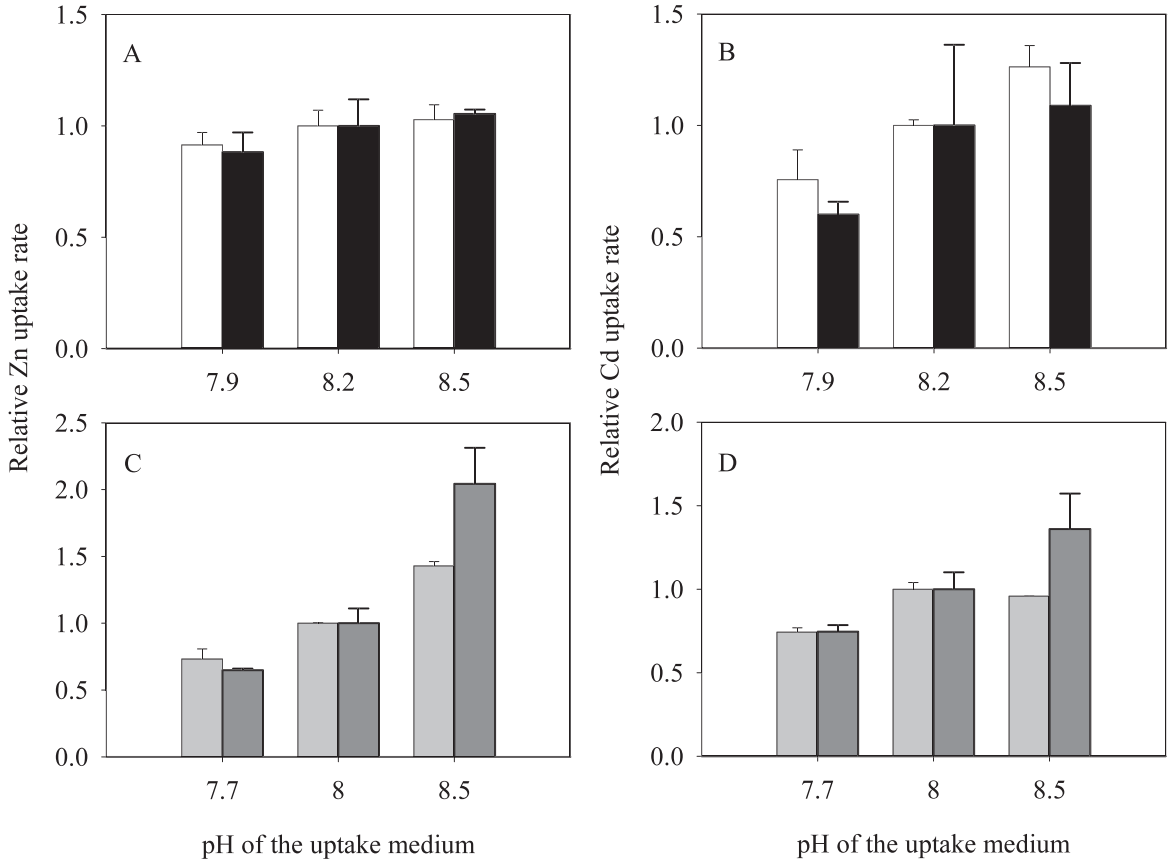

Fig. 2. (A, C) Relative short-term Zn and (B, D) Cd uptake rate at different pHs by natural assemblages from Zn and Cd bound to natural ligands. (A, B) Surface water collected from the New Jersey coast. White bars represent uptake with prior pH acclimation and black bars uptake without prior pH acclimation ($n = 3$). (C, D) Surface water collected from the Gulf of Alaska ($n = 2$). Light gray bars: incubation without Fe addition; dark gray bars: incubation with Fe addition. Uptake rate of the tracers per Chl *a* concentration are shown normalized to the rate at medium pH.

as pH decreased from 8.5 to 7.7 ($p = 0.005$ and 0.05, respectively, one-way ANOVA; Fig. 2D).

The effect of pH on Zn uptake in New Jersey samples is small compared to the results obtained for Cd in New Jersey samples and for Zn and Cd in the Gulf of Alaska samples. The reason for this difference is not known. The biological Zn uptake signal in coastal water may be partly obscured by nonspecific binding to nonliving particulate material—a phenomenon that should be minimal in oligotrophic water in which particulate detritus are rare and relatively less important for Cd that is bound to Cl^-. Except for Zn uptake in New Jersey samples, our field data show a systematic and generally significant decrease in metal uptake rates by natural phytoplankton with decreasing pH: an average decrease of 58% for Zn and 38% for Cd as pH decreases by 0.6 to 0.8 units. To sort out the underlying mechanisms, we carried out a series of laboratory experiments with model phytoplankton under a variety of conditions.

Direct effect of pH–PCO_2 on the kinetic parameters of metal transport systems: Short-term experiments in the presence of EDTA—To examine the direct effect of pH on the Zn and Cd transport system of phytoplankton, we performed short-term uptake experiments with Zn-limited cultures of the diatom *T. weissflogii* and the coccolithophore *E. huxleyi* in the presence of excess EDTA. Under these conditions, the bioavailability of the metals is determined by the total free concentrations, Zn′ and Cd′, in the medium.

The short-term Zn uptake rate in *T. weissflogii* was measured at pH 7.7, 8.1, and 8.4 in the presence of 100 μmol L^{-1} EDTA, with Zn_T ranging from 1.1×10^{-9} mol L^{-1} to 7.6×10^{-6} mol L^{-1} (Fig. 3A) and Zn′ from $10^{-11.6}$ mol L^{-1} to $10^{-8.7}$ mol L^{-1} (Fig. 3B). The uptake rate increased with Zn concentration up to $Zn_T = 1$ μmol L^{-1} (Zn′ = 0.27 nmol L^{-1} at pH 8.1), at which point it began to saturate, essentially identical to the data of Sunda and Huntsman (1992). When Zn_T was below 1 μmol L^{-1}, we observed a small but systematic decrease in the uptake rate with decreasing pH, with an average of about 30% between pH 8.4 and pH 7.7 (Fig. 3A bars). Similar experiments with *E. huxleyi* at $Zn_T = 38$ nmol L^{-1} gave a similar result: the uptake rate at pH 7.7 was about 35% lower than at pH 8.4. The observed decrease in Zn uptake rate at low pH is in good agreement with the calculated decrease in Zn′ of 36%. We conclude that the bulk of the effect of pH on Zn uptake rates by our model

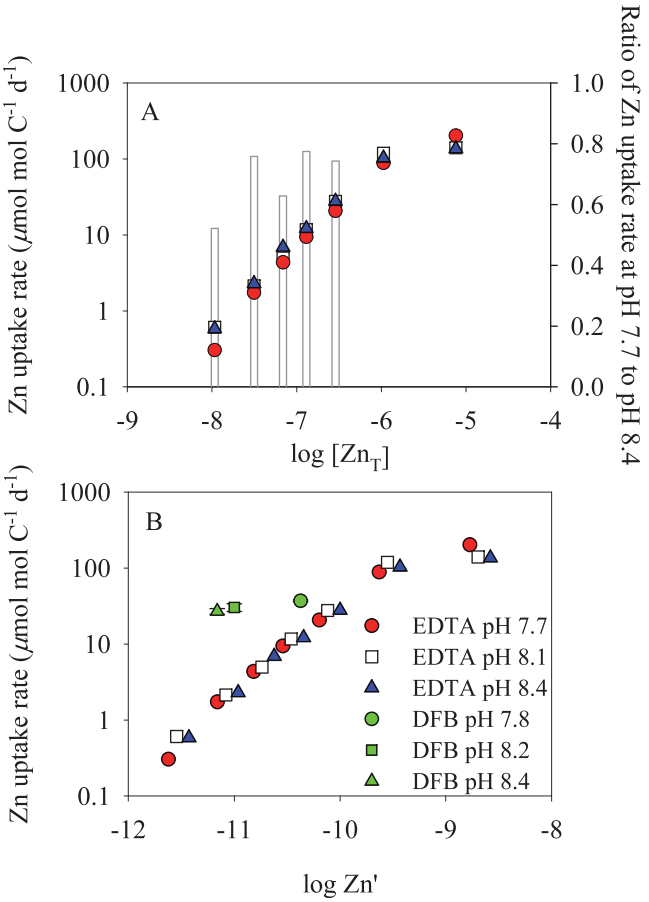

Fig. 3. (A) Symbols show the short-term Zn uptake rate as a function of the concentration of total dissolved Zn, Zn_T, by Zn-limited *Thalassiosira weissflogii* at different pHs in EDTA-buffered media. Left y-axis: red circles, pH 7.7; white squares, pH 8.1; blue triangles, pH8.4; no replicate. Bars show the ratio of Zn uptake rate at pH 7.7 to that at pH 8.4 ($n = 2$). (B) Short-term Zn uptake rate as a function of the concentration of free Zn, Zn′, by Zn-limited *T. weissflogii* at different pHs. For Zn bound to EDTA: red circles, pH 7.7; white squares, pH 8.1; blue triangles, pH 8.4; no replicate. For Zn bound to DFB: green circle, pH 7.8; green square, pH 8.2; green triangle, pH 8.4; $n = 2$. EDTA = 100 μmol L^{-1}.

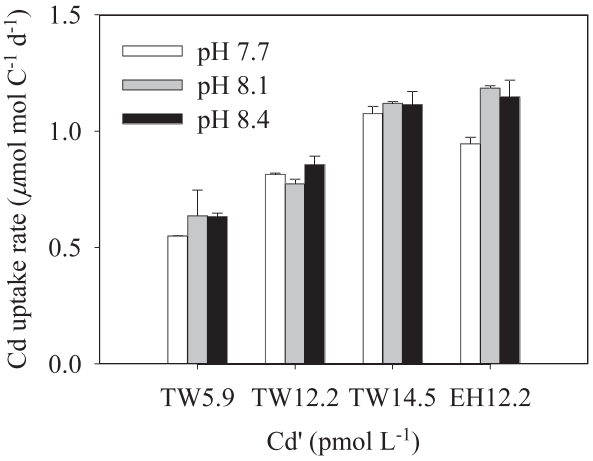

Fig. 4. Short-term Cd uptake by Zn-limited cells from Cd bound to EDTA at different pHs. Numbers after the prefix TW (*T. weissflogii*) or EH (*Emiliania huxleyi*) on the x-axis are values of Cd′. EDTA = 100 μmol L^{-1}.

organisms in the presence of EDTA is accounted for by the change in Zn′ and that any direct effect of pH on the kinetics of the Zn uptake system must be small or nil.

Similar uptake experiments with Cd showed no significant effect of pH in either *T. weissflogii* or *E. huxleyi*, except for one unexpected low value at pH 7.8 in *E. huxleyi* (Fig. 4). In the presence of EDTA, there is no change in Cd′ over the experimental range of pH (Fig. 1A); so that these data provide a direct demonstration that pH has a negligible direct effect on the Cd uptake systems of the organisms. Since Cd is probably taken up by the Zn transport system, as has been shown in some other species (Sunda and Huntsman 1998*a*), this result also corroborates the lack of a significant direct effect of pH on the Zn transport system.

Effect of pH–PCO_2 on Zn and Cd requirements and regulation of uptake: Steady-state experiments in the presence of EDTA—Changes in PCO_2, which affect the demands on the CCM of phytoplankton, are known to modulate the cellular requirement for Zn, the main cofactor in CA (Sunda and Huntsman 2005). We measured growth rates and cellular Zn concentrations in Zn-limited cultures of *T. weissflogii* acclimated at different pH–PCO_2. As expected, the growth rate increased with increasing Zn in the medium at a given pH (Fig. 5A) and was higher at higher PCO_2 at a given Zn′ (Fig. 5B). A lower requirement for Zn at higher PCO_2 can clearly be seen in a plot of growth or carbon fixation rate as a function of the cellular Zn concentration (Fig. 5C,E). The more dramatic effect seen in carbon fixation rate compared to growth rate is explained, at least in part, by the smaller size of the cells in the high pH–low PCO_2 cultures (data not shown). For a given Zn′, the higher growth rate at low pH (Fig. 5B) is compensated by a lower intracellular Zn concentration (Fig. 5D), such that the steady-state Zn uptake is independent of pH (Fig. 5F). This steady-state uptake rate is equal to the short-term uptake rate measured in Zn-limited cells at the same Zn′ (Fig. 5F, line). In these experiments, the Zn uptake system is up-regulated as much as it can be and does not change with pH–PCO_2. A higher PCO_2 allows a down-regulation of the CCM and hence a lower Zn requirement, leading to an increase in growth rate at a given Zn′.

The coccolithophore *E. huxleyi* has a relatively weak CCM and low CA activity at low PCO_2 (Elzenga et al. 2000; Schulz et al. 2007). Its Zn requirement should thus not be very sensitive to variations in PCO_2. We observed indeed that the growth rate of *E. huxleyi* and its cellular Zn concentration were essentially independent of PCO_2 (data not shown). As in Zn-limited *T. weissflogii*, the steady-state Zn uptake rate in *E. huxleyi* thus depended only on Zn′ and not on pH–PCO_2.

If, as it appears, Cd is taken up via the same transport system as Zn, the uptake rate of Cd, like that of Zn, should not be affected by pH in these steady-state experiments.

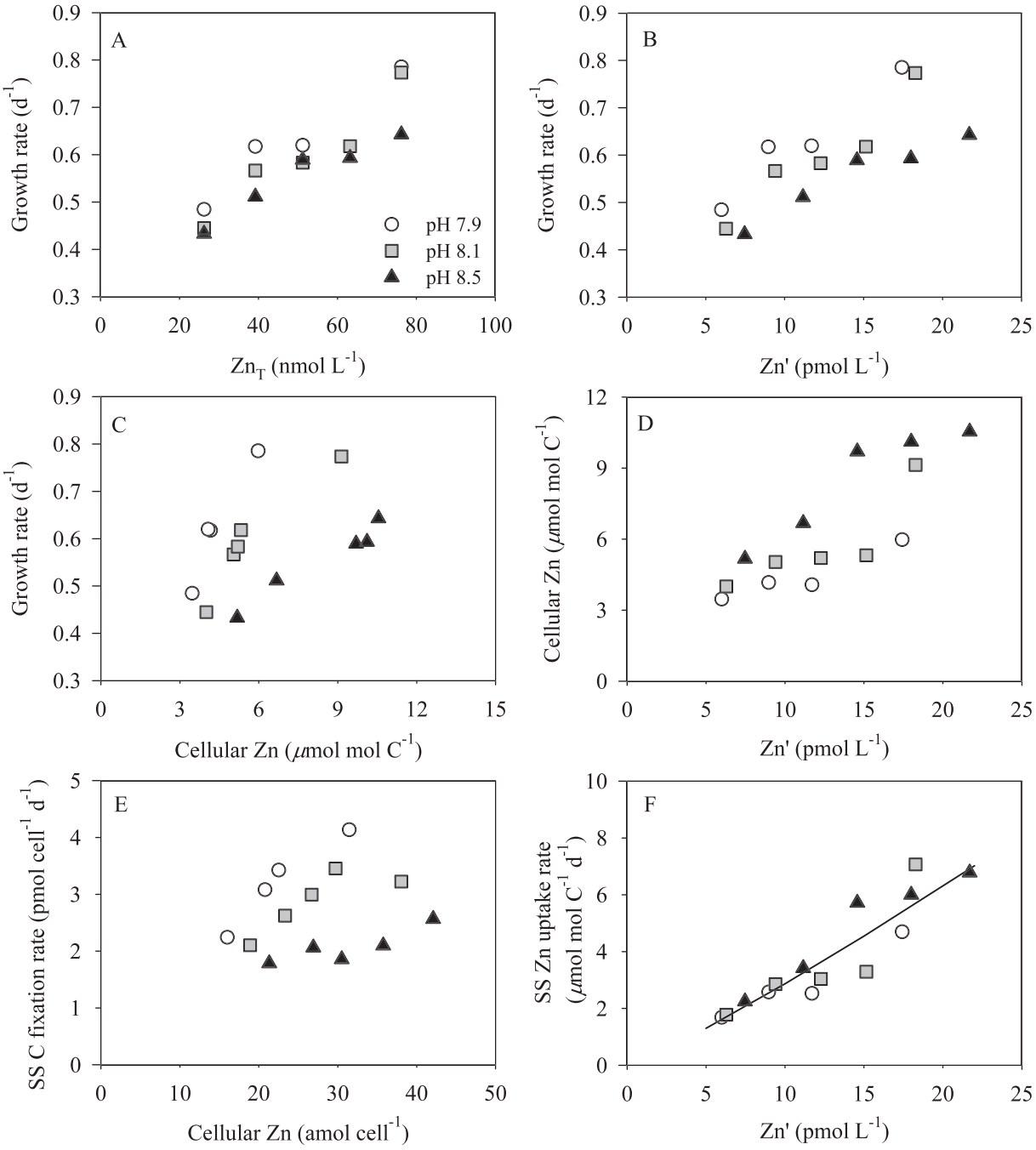

Fig. 5. *T. weissflogii*. Relations at various pHs between (A) growth rate and total dissolved Zn concentration, Zn_T, (B) growth rate and free Zn concentration, Zn′, (C) growth rate and cellular Zn concentration normalized to cellular carbon, (D) cellular Zn concentration normalized to carbon and Zn′, (E) steady-state carbon fixation rate normalized to cell number and cellular Zn concentration normalized to cell number, and (F) steady-state Zn uptake rate normalized to cellular carbon. (F) Line shows data from Fig. 2. EDTA = 100 μmol L^{-1}.

This is what we observed in Zn-limited *T. weissflogii* cultures at pH values 7.8 and 8.4 (data not shown).

Our results from steady-state experiments confirm that the Zn and Cd requirements of phytoplankton vary with the ambient PCO_2. But they also show that the uptake rates of Zn and Cd at low ambient concentrations are maintained at maximum rates, which depend only on the bioavailable concentrations of Zn and Cd in the medium and are not regulated by the ambient pH–PCO_2. In this situation, the steady-state uptake of Zn and Cd can be affected by pH only via its effect on the chemical speciation and bioavailability of the metals, as seen in short-term uptake data (Fig. 3 and below).

Effect of pH on Zn and Cd bioavailability in the presence of one complexing agent—Our results in EDTA-buffered

media show that Cd uptake is invariant with pH and that Zn uptake decreases slightly with decreasing pH, as expected because of the modest decrease in $ZnCO_3$ and $Zn(OH)_x$ concentrations. But for other complexing agents that are partly protonated in seawater, we expect the free concentrations Zn′ and Cd′ to increase with decreasing pH (Fig. 1B), presumably leading to an increase in Zn and Cd uptake rates to an extent that depends on the number of protons released by complexation of the metals. We examined the effects of various coordinating functionalities on the pH dependence of Zn and Cd uptake by phytoplankton by conducting experiments in the presence of the thiol-containing PC2 and the tri-hydroxamate DFB, in addition to the aminocarboxylate EDTA.

Phytochelatins are small peptides synthesized and released by phytoplankton (Ahner et al. 1994; Lee et al. 1996; Wei and Ahner 2005). Although we do not have thermodynamic data for the Zn and Cd complexes of PC2, we know that thiol and carboxyl groups have a high affinity for both metals and that Zn and Cd form complexes with PC2 in vivo (Gekeler et al. 1988). The uptake rate of Zn and Cd by both model organisms increased as pH decreased ($p < 0.01$, except $p = 0.045$ for Cd in *E. huxleyi*, one-way ANOVA, Fig. 6A,B), qualitatively consistent with the expected increase in Zn′ and Cd′. The magnitude of the pH effect on uptake was similar for both metals in both organisms, which is also expected since the increase in protonation of PC2 with decreasing pH should be the same for both metals and should dwarf the small effect of pH on $ZnCO_3$ and $Zn(OH)_x$ concentrations. While a quantitative analysis of the pH effect is not warranted at this point (*see* below), the sizeable effect of pH is consistent with a release of more than one proton upon complexation of Zn or Cd by PC2, as expected for the involvement of thiol and carboxyl groups, which are partially protonated at seawater pH (Morel and Hering 1993; Spain and Rabenstein 2003; Chekmeneva et al. 2008).

Although DFB is a siderophore produced by organisms to mobilize iron, it also complexes other metals and has a moderate affinity for Zn^{2+} and Cd^{2+} (Hernlem et al. 1996; Kiss and Farkas 1998). The pK_as of DFB and the formation constant for its Zn complex are similar to those of Cys. While its affinity for Ca^{2+} and magnesium ion (Mg^{2+}) is much lower than that of EDTA, it is sufficiently high that a significant fraction of the DFB should be bound to Mg^{2+} in seawater. The effect of pH on the bioavailability of Zn and Cd bound to DFB should thus be intermediate between those of EDTA and PC2. This is what we observed; the uptake rate of both metals by *T. weissflogii* and *E. huxleyi* in the presence of DFB increased by 30–50% as pH decreased from 8.4 to 7.8 ($p < 0.01$, except $p = 0.012$ for Cd in *T. weissflogii* and $p = 0.61$ for Cd in *E. huxleyi*, one-way ANOVA, Fig. 6C,D).

Besides the limiting case of EDTA, whose binding of metals in seawater does not release protons, the ligands we tested showed an increase in Zn and Cd availability to phytoplankton at low pH, qualitatively consistent with a predicted increase in Zn′ and Cd′. Yet this is contrary to our field observations.

A quantitative analysis of the data makes it doubtful, however, that the observed uptake rates measured in the presence of PC2 or DFB can be fully ascribed to uptake of the free metals. For example, even though there is uncertainty in the calculated value of Zn′ in the presence of DFB (due to uncertainty in the formation constant for the Zn–DFB complex), the rate of uptake of Zn by *T. weissflogii* in the presence of DFB is much higher than expected for the corresponding values of Zn′, particularly at high pH (Fig. 3B). There is no evidence of DFB uptake by our model organisms, whether bound to metals or not. But the Zn–DFB complex might nonetheless be "available," for example, if DFB is able to exchange Zn with uptake ligands via formation of a ternary complex, without releasing free Zn in solution. In the same way, the relatively weak complexes Zn–PC2, Cd–DFB, and Cd–PC2 might confer a degree of bioavailability to the bound metals.

Effect of pH on Zn and Cd bioavailability in the presence of both a strong and a weak complexing agent: The two-ligand mechanism—The proposition that trace metals in weak complexes are "bioavailable" in the experiments of Fig. 6 is speculative. But it suggests a possible and testable mechanism for the lower rather than higher metal availability with decreasing pH seen in field samples. This would occur if the decrease in metal uptake caused by a lower concentration of a bioavailable weak complex would overwhelm the increase in metal uptake caused by the higher total free metal concentration. This is not possible in the presence of only one complexing agent for, in this situation, the decrease in the concentration of the organic complex at low pH must necessarily be reflected in an increase in the total concentration of the free metal, which is taken up rapidly. But in the presence of both a weak and a strong complexing agent, the total free metal could be maintained at a very low concentration and a decrease in the concentration of the bioavailable weak complex at low pH could dominate the overall effect of pH on uptake. To test for the possibility of such a mechanism, we performed a Zn uptake experiment with *T. weissflogii* in the presence of PC2 with and without an excess of EDTA in the medium (Fig. 7A). The uptake of Zn in the presence of PC2 alone was higher at lower pH, consistent with our previous experiment (Fig. 4A), and the addition of excess EDTA markedly decreased the Zn uptake rate, as expected. Most importantly, in the presence of both PC2 and EDTA, the Zn uptake rate was lower at lower pH, providing proof of concept for the two-ligand mechanism.

To confirm this result, we repeated the experiment with another weak ligand, Cys, a compound that is also known to be released by some phytoplankton species and for which good thermodynamic data are available (Morel and Hering 1993; Dupont and Ahner 2005). The presence of Cys markedly increased the Zn uptake rate compared to the EDTA-only control, demonstrating a degree of bioavailability of Zn in the Cys complexes (Fig. 5B). As in previous experiments, only a very small effect of pH was seen in the presence of EDTA alone, but a marked decrease in Zn uptake rate was observed at low pH in the treatment with both EDTA and Cys. A preliminary quantitative analysis of the data shows that the Zn uptake rate (ρ) follows approximately the linear relation

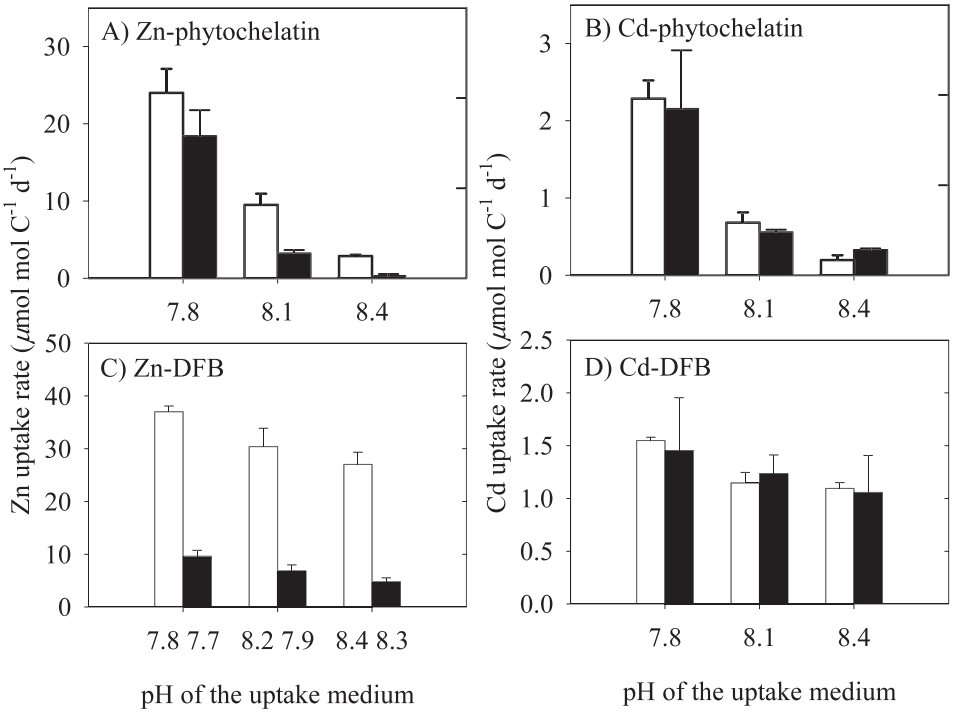

Fig. 6. (A, C) Short-term Zn and (B, D) Cd uptake at different pHs by Zn-limited cells from Zn and Cd bound to (A, B) PC2 and (C, D) DFB. White bars, *T. weissflogii*; black bars, *E. huxleyi*; $n = 2$–3. The metal, ligand, and TCEP concentrations in each panel are (A) 1.2 nmol L^{-1} Zn, 1 μmol L^{-1} TCEP, and 500 nmol L^{-1} PC2; (B) 1.03 nmol L^{-1} Cd, 1 μmol L^{-1} TCEP, and 500 nmol L^{-1} PC2; (C) 1.2 nmol L^{-1} Zn and 50 μmol L^{-1} DFB; (D) 0.05 nmol L^{-1} Cd and 50 μmol L^{-1} DFB.

$$\rho = 0.4 \times (\mathrm{Zn}' + [\mathrm{ZnCys}]) \quad (1)$$

for experiments with EDTA alone and experiments with EDTA + Cys at pH 7.9 and 8.1, considering that only the 1 : 1 complex (ZnCys but not $Zn(Cys)_2$) is available for uptake (Fig. 7C).

Discussion

Our laboratory data demonstrate that, in model phytoplankton species, modest variations in pH–PCO_2 (within 0.4 pH units of average seawater) have a negligible effect on the Zn and Cd uptake system, which is neither directly affected by pH nor regulated as PCO_2 varies. The decrease in Zn and Cd uptake rates we observed at low pH in natural samples is thus apparently due to a decrease in the bioavailability of the metals. This counterintuitive result leads us to postulate that Zn and Cd in (some) weak complexes are available for uptake by phytoplankton in surface seawater and that the concurrent effects of strong and weak complexing agents are responsible for the observed pH effect.

The absence of effect of pH on the trace metal uptake machinery of phytoplankton observed here and in Shi et al. (2010) is somewhat surprising. A decrease in Cd and Mn uptake rate at low pH in freshwater microalgae has been reported and ascribed to a conformational change in membrane proteins (François et al. 2007; Boullemant et al. 2009). But this effect is relatively modest and is seen only over a much wider variation of pH than in our experiments. A more direct effect could result from the protonation of cellular uptake ligands at low pH, which should decrease the binding of metal substrates and their uptake rate. It is, of course, possible that the metal binding moieties of the uptake ligands are not protonated in seawater. A more likely explanation is that the pH at the surface of the cells is tightly buffered by numerous acid and base groups, including organic moieties and the surface hydroxyls of the frustules and coccoliths. Variation in pH in the bulk medium should be greatly attenuated at the surface of the cells as it is at particle surfaces with acid–base functionalities (Dzombak and Morel 1990).

As exemplified in Fig. 1, a decrease in pH can cause only a modest decrease in Zn′ and none in Cd′ if the metals are bound to a non-protonated chelator; if the dominant complexing agents are partially protonated, Zn′ and Cd′ increase at low pH. Regardless of the functionalities of the strong natural Zn and Cd chelators, the negative effect of low pH on Zn and Cd uptake seen in field samples cannot be explained by a decrease in total free metal concentrations. Some degree of bioavailability of Zn and Cd in organic complexes thus seems necessary to explain the field data, but it is not sufficient. In a system containing only one complexing agent, the decrease in the concentration of a potentially available metal complex with decreasing pH is accompanied by a corresponding increase in the total free

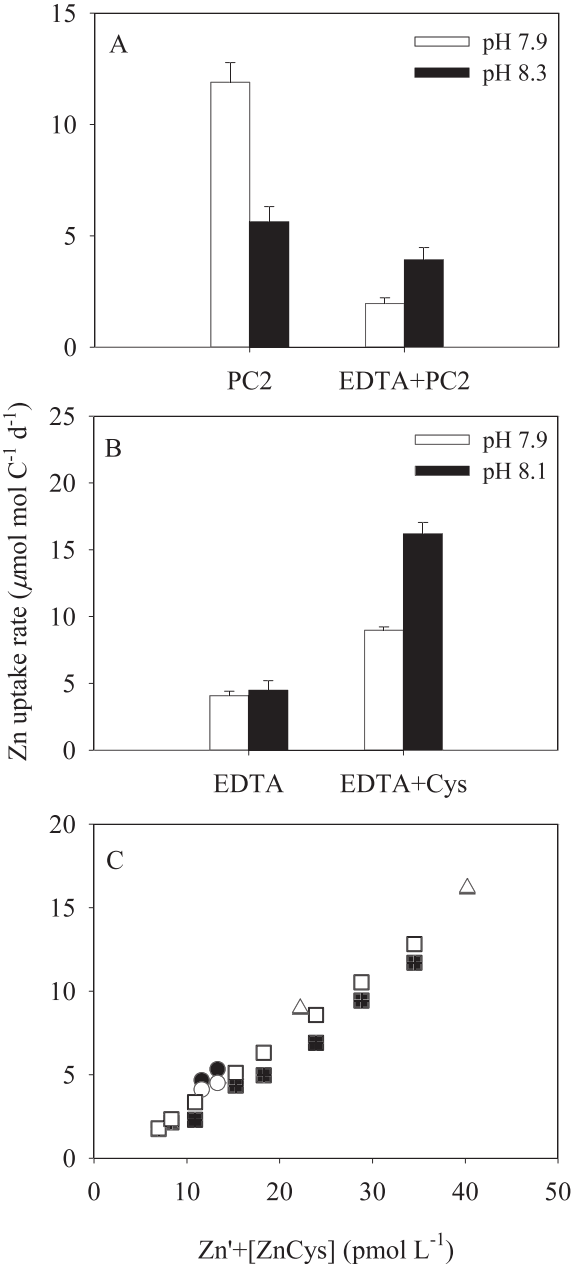

Fig. 7. Short-term Zn uptake by Zn-limited *T. weissflogii* at different pHs. The metal, ligand, and TCEP concentrations in each panel are: (A) 0.67 nmol L^{-1} Zn, 0.67 μmol L^{-1} TCEP, and 0.33 μmol L^{-1} PC2 (PC2); 33 nmol L^{-1} Zn, 0.67 μmol L^{-1} TCEP, 0.33 μmol L^{-1} PC2, and 100 μmol L^{-1} EDTA (EDTA + PC2); (B) 50 nmol L^{-1} Zn and 100 μmol L^{-1} EDTA (EDTA); 50 nmol L^{-1} Zn, 2 μmol L^{-1} Cys, 20 μmol L^{-1} TCEP, and 100 μmol L^{-1} EDTA (EDTA + Cys); $n = 3$. (C) The Zn uptake rate in the presence of EDTA alone or EDTA + Cys as a function of Zn′ + [ZnCys]. Black symbols show measured uptake rates; white symbols show calculated uptake rates (reaction 1). Circles: EDTA alone (data from panel B); triangles: EDTA + Cys (data from panel B, note: black triangles are covered by white triangles due to their similar values); squares: EDTA alone (data from Fig. 3).

metal concentration. Unless the metal complex is more available than the free metal, the net result is an increase in metal availability with decreasing pH as exemplified in Fig. 6. The negative effect of decreasing pH on Zn and Cd uptake may then best be explained by the joint effects of weak and strong complexing agents.

Most of the work on speciation of trace metal in seawater has focused on the very strong complexing agents, Y, and has shown that their complexes, e.g., ZnY, account for the bulk of the dissolved metal concentration. The presence of weaker complexing agents, L, would not be detected by many of the electrochemical methods that have been used to demonstrate metal binding to strong ligands (Bruland 1989), but there is little doubt that such weak ligands exist (van den Berg 1985; Coale and Bruland 1988) in both the dissolved and colloidal phases and that they include humic substances (Wells et al. 1998; Kogut and Voelker 2001). Using Zn as an example, the concentrations of weak complexes, ZnL, and of the free metal, Zn′, are effectively controlled by the following reactions (omitting charges and protonation of the complexes for simplicity):

$$\mathrm{ZnY} + \mathrm{H}_{\alpha}\mathrm{L} = \mathrm{ZnL} + \mathrm{H}_{\beta}\mathrm{Y} + (\alpha - \beta)\mathrm{H}^{+} \quad (2)$$

$$\mathrm{ZnY} + \beta\mathrm{H}^{+} = \mathrm{Zn}' + \mathrm{H}_{\beta}\mathrm{Y} \quad (3)$$

Where α and β are the average degree of protonation of the complexing agents L and Y in seawater, respectively. For such a system to produce a decrease in metal availability at low pH, the factor ($\alpha - \beta$) must be positive; i.e., the weak complexing agent must have a higher degree of protonation than the strong chelator, so that the formation of the weak complex is disfavored at low pH (reaction 2 goes to the left). But almost nothing is known at this point of the acid–base chemistry of either strong or weak complexing agents in seawater. In our model system with EDTA as the strong chelator, the desired result is easily obtained since EDTA is bound to Ca^{2+} and $\beta = 0$. Partial binding of strong natural chelators to the abundant alkaline earth cations Ca^{2+} and Mg^{2+} in seawater, as also occurs for DFB, can lead to a qualitatively similar result.

To obtain a decrease in metal bioavailability at low pH, it is also necessary that the weak complex be bioavailable and that the decrease in its concentration with decreasing pH (reaction 2) overcome the possible increase in total free metal concentration (reaction 3). The concentration of some weak complexes can be larger than the total free metal concentration, even if it is only a small fraction of the total dissolved metal (e.g., Fig. 1C). So only a modest degree of bioavailability of the weak complexes may be necessary. The same principle should apply to Cd as well. Because weak complexing agents do not provide a convenient buffer for the free concentrations of essential trace metals, they have rarely been utilized in laboratory studies of metal uptake and requirements in phytoplankton. Nonetheless, metal uptake by phytoplankton from weak organic complexes has been implicated in some studies (Maldonado et al. 2002; Hassler et al. 2011) and is directly demonstrated for Zn–Cys by the results of Fig. 7B. It remains to be determined how prevalent such an effect

might be and whether it is mediated through specific or nonspecific mechanisms: e.g., uptake of a particular complex via a specific transporter vs. transfer of the metal from a complexing agent to a transport molecule via a ternary complex. The role played in iron uptake by weak complexing agents such as domoic acid has received particular attention (Maldonado et al. 2002). In this respect, we note that, like the formation of hydrolysis species (Shi et al. 2010), the formation of weak organic complexes of Fe, which has been documented in seawater (Buck and Bruland 2007), could also lead to a decrease in uptake rate at low pH if these complexes are bioavailable.

In surface seawater in which total free metal concentrations are kept low by binding to strong chelators in excess, uptake from weak complexes may be an important process for the acquisition of several essential metals. The dual roles of strong and weak complexing agents in controlling trace metal speciation and bioavailability may determine the effect of ocean acidification on the uptake of trace metals by phytoplankton.

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References

Ahner, B. A., N. M. Price, and F. M. M. Morel. 1994. Phytochelatin production by marine phytoplankton at low free metal ion concentrations—laboratory studies and field data from Massachusetts Bay. Proc. Natl. Acad. Sci. USA **91:** 8433–8436, doi:10.1073/pnas.91.18.8433

Anderson, M. A., and F. M. M. Morel. 1982. The influence of aqueous iron chemistry on the uptake of iron by the coastal diatom *Thalassiosira weissflogii*. Limnol. Oceanogr. **27:** 789–813, doi:10.4319/lo.1982.27.5.0789

Boullemant, A., M. Lavoie, C. Fortin, and P. G. C. Campbell. 2009. Uptake of hydrophobic metal complexes by three freshwater algae: Unexpected influence of pH. Environ. Sci. Technol. **43:** 3308–3314, doi:10.1021/es802832u

Bruland, K. W. 1989. Complexation of zinc by natural organic ligands in the central North Pacific. Limnol. Oceanogr. **34:** 269–285, doi:10.4319/lo.1989.34.2.0269

———. 1992. Complexation of cadmium by natural organic ligands in the central North Pacific. Limnol. Oceanogr. **37:** 1008–1017, doi:10.4319/lo.1992.37.5.1008

Buck, K. N., and K. W. Bruland. 2007. The physicochemical speciation of dissolved iron in the Bering Sea, Alaska. Limnol. Oceanogr. **52:** 1800–1808, doi:10.4319/lo.2007.52.5.1800

Byrne, R. H., L. R. Kump, and K. J. Cantrell. 1988. The influence of temperature and pH on trace metal speciation in seawater. Mar. Chem. **25:** 163–181, doi:10.1016/0304-4203(88)90062-X

Chekmeneva, E., R. Prohens, J. M. Daaz-Cruz, C. Arino, and M. Esteban. 2008. Thermodynamics of Cd^{2+} and Zn^{2+} binding by the phytochelatin $(\gamma\text{-Glu-Cys})_4$-Gly and its precursor glutathione. Anal. Biochem. **375:** 82–89, doi:10.1016/j.ab.2008.01.008

Coale, K. H. 1991. Effects of iron, manganese, copper and zinc enrichments on productivity and biomass in the subarctic Pacific. Limnol. Oceanogr. **36:** 1851–1864, doi:10.4319/lo.1991.36.8.1851

———, and K. W. Bruland. 1988. Copper complexation in the northeast Pacific. Limnol. Oceanogr. **33:** 1084–1101, doi:10.4319/lo.1988.33.5.1084

Crawford, D. W., and others. 2003. Influence of zinc and iron enrichments on phytoplankton growth in the northeastern subarctic Pacific. Limnol. Oceanogr. **48:** 1583–1600, doi:10.4319/lo.2003.48.4.1583

Cullen, J. T., 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, doi:10.1038/46469

Dupont, C. L., and B. A. Ahner. 2005. Effects of copper, cadmium, and zinc on the production and exudation of thiols by *Emiliania huxleyi*. Limnol. Oceanogr. **50:** 508–515, doi:10.4319/lo.2005.50.2.0508

Dzombak, D. A., and F. M. M. Morel. 1990. Surface complexation modeling: Hydrous ferric oxide. John Wiley & Sons.

Ellwood, M. J. 2004. Zinc and cadmium speciation in subantarctic waters east of New Zealand. Mar. Chem. **87:** 37–58, doi:10.1016/j.marchem.2004.01.005

Elzenga, J. T. M., H. B. A. Prins, and J. Stefels. 2000. The role of extracellular carbonic anhydrase activity in inorganic carbon utilization of *Phaeocystis globosa* (Prymnesiophyceae): A comparison with other marine algae using the isotopic disequilibrium technique. Limnol. Oceanogr. **45:** 372–380, doi:10.4319/lo.2000.45.2.0372

Field, M. P., J. T. Cullen, and R. M. Sherrell. 1999. Direct determination of 10 trace metals in 50 μL samples of coastal seawater using desolvating micronebulization sector field ICP-MS. J. Anal. At. Spectrom. **14:** 1425–1431, doi:10.1039/a901693g

Franck, V. M., K. W. Bruland, D. A. Hutchins, and M. A. Brzezinski. 2003. Iron and zinc effects on silicic acid and nitrate uptake kinetics in three high-nutrient, low-chlorophyll (HNLC) regions. Mar. Ecol. Prog. Ser. **252:** 15–33, doi:10.3354/meps252015

François, L., C. Fortin, and P. G. C. Campbell. 2007. pH modulates transport rates of manganese and cadmium in the green alga *Chlamydomonas reinhardtii* through non-competitive interactions: Implications for an algal BLM. Aquat. Toxicol. **84:** 123–132, doi:10.1016/j.aquatox.2007.02.019

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, doi:10.1007/BF00425162

Hassler, C. S., V. Schoemann, C. M. Nichols, E. C. V. Butler, and P. W. Boyd. 2011. Saccharides enhance iron bioavailability to Southern Ocean phytoplankton. Proc. Natl. Acad. Sci. USA **108:** 1076–1081, doi:10.1073/pnas.1010963108

Hernlem, B. J., L. M. Vane, and G. D. Sayles. 1996. Stability constants for complexes of the siderophore desferrioxamine B with selected heavy metal cations. Inorg. Chim. Acta **244:** 179–184, doi:10.1016/0020-1693(95)04780-8

Hopkinson, B. M., Y. Xu, D. L. Shi, P. J. McGinn, and F. M. M. Morel. 2010. The effect of CO_2 on the photosynthetic physiology of phytoplankton in the Gulf of Alaska. Limnol. Oceanogr. **55:** 2011–2024, doi:10.4319/lo.2010.55.5.2011

Kiss, T., and E. Farkas. 1998. Metal binding ability of desferrioxamine B. J. Inclusion Phenom. Mol. Recognit. Chem. **32:** 385–403, doi:10.1023/A:1008046330815

Kogut, M. B., and B. M. Voelker. 2001. Strong copper-binding behavior of terrestrial humic substances in seawater. Environ. Sci. Technol. **35:** 1149–1156, doi:10.1021/es0014584

Kranz, S. A., D. Sultemeyer, K. U. Richter, and B. Rost. 2009. Carbon acquisition by *Trichodesmium*: The effect of pCO_2 and diurnal changes. Limnol. Oceanogr. **54:** 548–559, doi:10.4319/lo.2009.54.2.0548

Lane, T. W., and F. M. M. Morel. 2000*a*. A biological function for cadmium in marine diatoms. Proc. Natl. Acad. Sci. USA **97:** 4627–4631, doi:10.1073/pnas.090091397

———, and ———. 2000*b*. Regulation of carbonic anhydrase expression by zinc, cobalt, and carbon dioxide in the marine diatom *Thalassiosira weissflogii*. Plant Physiol. **123:** 345–352, doi:10.1104/pp.123.1.345

———, M. A. Saito, G. N. George, I. J. Pickering, R. C. Prince, and F. M. M. Morel. 2005. A cadmium enzyme from a marine diatom. Nature **435:** 42, doi:10.1038/435042a

Lee, J. G., B. A. Ahner, and F. M. M. Morel. 1996. Export of cadmium and phytochelatin by the marine diatom *Thalassiosira weissflogii*. Environ. Sci. Technol. **30:** 1814–1821, doi:10.1021/es950331p

———, and F. M. M. Morel. 1995. Replacement of zinc by cadmium in marine phytoplankton. Mar. Ecol. Prog. Ser. **127:** 305–309, doi:10.3354/meps127305

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, doi:10.1016/S0967-0645(02)00215-1

Maldonado, M. T., M. P. Hughes, E. L. Rue, and M. L. Wells. 2002. The effect of Fe and Cu on growth and domoic acid production by *Pseudonitzschia multiseries* and *Pseudonitzschia australis*. Limnol. Oceanogr. **47:** 515–526, doi:10.4319/lo.2002.47.2.0515

Mawji, E., and others. 2008. Hydroxamate siderophores: Occurrence and importance in the Atlantic Ocean. Environ. Sci. Technol. **42:** 8675–8680, doi:10.1021/es801884r

McGinn, P. J., and F. M. M. Morel. 2008. Expression and regulation of carbonic anhydrases in the marine diatom *Thalassiosira pseudonana* and in natural phytoplankton assemblages from Great Bay, New Jersey. Physiol. Plant. **133:** 78–91, doi:10.1111/j.1399-3054.2007.01039.x

Millero, F. J., R. Woosley, B. Ditrolio, and J. Waters. 2009. Effect of ocean acidification on the speciation of metals in seawater. Oceanography **22:** 72–85, doi:10.5670/oceanog.2009.98

Morel, F. M. M., and J. G. Hering. 1993. Principles and application of aquatic chemistry. John Wiley and Sons.

———, and others. 2002. Acquisition of inorganic carbon by the marine diatom *Thalassiosira weissflogii*. Funct. Plant Biol. **29:** 301–308, doi:10.1071/PP01199

Riebesell, U., I. Zondervan, B. Rost, P. D. Tortell, R. E. Zeebe, and F. M. M. Morel. 2000. Reduced calcification of marine plankton in response to increased atmospheric CO_2. Nature **407:** 364–367, doi:10.1038/35030078

Schulz, K. G., B. Rost, S. Burkhardt, U. Riebesell, S. Thoms, and D. A. Wolf-Gladrow. 2007. The effect of iron availability on the regulation of inorganic carbon acquisition in the coccolithophore *Emiliania huxleyi* and the significance of cellular compartmentation for stable carbon isotope fractionation. Geochim. Cosmochim. Acta **71:** 5301–5312, doi:10.1016/j.gca.2007.09.012

Shi, D. L., Y. Xu, B. M. Hopkinson, and F. M. M. Morel. 2010. Effect of ocean acidification on iron availability to marine phytoplankton. Science **327:** 676–679, doi:10.1126/science.1183517

Spain, S. M., and D. L. Rabenstein. 2003. Characterization of the acid/base and redox chemistry of phytochelatin analogue peptides. Anal. Chem. **75:** 3712–3719, doi:10.1021/ac0207426

Sunda, W. G., and S. A. Huntsman. 1992. Feedback interactions between zinc and phytoplankton in seawater. Limnol. Oceanogr. **37:** 25–40, doi:10.4319/lo.1992.37.1.0025

———, and ———. 1998*a*. 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, doi:10.1021/es980271y

———, and ———. 1998*b*. Processes regulating cellular metal accumulation and physiological effects: Phytoplankton as model systems. Sci. Total Environ. **219:** 165–181, doi:10.1016/S0048-9697(98)00226-5

———, and ———. 2000. Effect of Zn, Mn, and Fe on Cd accumulation in phytoplankton: Implications for oceanic Cd cycling. Limnol. Oceanogr. **45:** 1501–1516, doi:10.4319/lo.2000.45.7.1501

———, and ———. 2005. Effect of CO2 supply and demand on zinc uptake and growth limitation in a coastal diatom. Limnol. Oceanogr. **50:** 1181–1192, doi:10.4319/lo.2005.50.4.1181

———, N. M. Price, and F. M. M. Morel. 2005. Trace metal ion buffers and their use in culture studies, p. 35–63. *In* R. A. Andersen [ed.], Algal culturing techniques. Elsevier.

Tang, D. G., and F. M. M. Morel. 2006. Distinguishing between cellular and Fe-oxide-associated trace elements in phytoplankton. Mar. Chem. **98:** 18–30, doi:10.1016/j.marchem.2005.06.003

Tovar-Sanchez, A., S. A. Sanudo-Wilhelmy, M. Garcia-Vargas, R. S. Weaver, L. C. Popels, and D. A. Hutchins. 2003. A trace metal clean reagent to remove surface-bound iron from marine phytoplankton. Mar. Chem. **82:** 91–99, doi:10.1016/S0304-4203(03)00054-9

van den Berg, C. M. G. 1985. Determination of the zinc complexing capacity in seawater by cathodic stripping voltammetry of zinc APDC complex ions. Mar. Chem. **16:** 121–130, doi:10.1016/0304-4203(85)90017-9

Wei, L. P., and B. A. Ahner. 2005. Sources and sinks of dissolved phytochelatin in natural seawater. Limnol. Oceanogr. **50:** 13–22, doi:10.4319/lo.2005.50.1.0013

Wells, M. L., P. B. Kozelka, and K. W. Bruland. 1998. The complexation of "dissolved" Cu, Zn, Cd and Pb by soluble and colloidal organic matter in Narragansett Bay, RI. Mar. Chem. **62:** 203–217, doi:10.1016/S0304-4203(98)00041-3

Wright, D. D., T. K. Frazer, and J. R. Reinfelder. 2010. The influence of river plume dynamics on trace metal accumulation in calanoid copepods. Limnol. Oceanogr. **55:** 2487–2502, doi:10.4319/lo.2010.55.6.2487

Xu, Y., L. Feng, P. D. Jeffrey, Y. G. Shi, and F. M. M. Morel. 2008. Structure and metal exchange in the cadmium carbonic anhydrase of marine diatoms. Nature **452:** 56–61, doi:10.1038/nature06636

———, D. Tang, Y. Shaked, and F. M. M. Morel. 2007. Zinc, cadmium, and cobalt interreplacement and relative use efficiencies in the coccolithophore *Emiliania huxleyi*. Limnol. Oceanogr. **52:** 2294–2305, doi:10.4319/lo.2007.52.5.2294

Zhang, H. N., and R. H. Byrne. 1996. Spectrophotometric pH measurements of surface seawater at in situ conditions: Absorbance and protonation behavior of thymol blue. Mar. Chem. **52:** 17–25, doi:10.1016/0304-4203(95)00076-3

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