

Trace Metal Substitution in Marine Phytoplankton

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Abstract

The sinking of organic matter to the deep ocean leaves extremely low concentrations of major and trace nutrients for photosynthetic organisms at the sunlit surface. As a result, marine phytoplankton make use of alternative sources of essential elements and have evolved to substitute some elements by others in various biochemical processes. A particularly intriguing example is that of Zn, which is used in many biochemical functions but is often depleted down to picomolar concentrations in surface seawater. Laboratory data show that many phytoplankton species are able to achieve high growth rates by replacing Zn with Cd or Co in cultures. One documented biochemical replacement occurs in some carbonic anhydrases that are used in the acquisition of inorganic carbon for photosynthesis. Field data show the existence of such enzymes in surface seawater and indicate a replacement of Zn by Cd and Co in the surface waters of the eastern tropical South Pacific. Those results point at interesting opportunities for future research.

- The dearth of essential elements in surface seawater has caused marine phytoplankton to substitute some trace metals by others in various biochemical processes.
- Many species can substitute Cd and/or Co for Zn as a metal center in carbonic anhydrase enzymes that are used in the acquisition of inorganic carbon for photosynthesis.

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- Field data show the presence of such enzymes in the sea and indicate a replacement of Zn by Cd and Co in the surface upwelling waters of the eastern tropical South Pacific.
- New analytical and molecular tools provide opportunities to elucidate the unusual biochemistry of marine phytoplankton.

INTRODUCTION: UTILIZATION AND VERTICAL EXPORT OF MAJOR AND TRACE NUTRIENTS

The single-cell phytoplankton that live at the surface of the oceans are responsible for nearly half of the photosynthetic fixation of CO₂ on our planet. The eventual settling of the live and dead organic matter that is produced effectively exports CO₂ out of surface seawater and the atmosphere, sequestering it in the ocean interior. This biological pump also exports all major and trace plant nutrients out of the sunlit surface ocean. Marine photosynthesis in the oceans thus depends on the slow return of these nutrients to the surface by advection and diffusion from the deep where organic matter is remineralized. As noted nearly a century ago by Harvey (1926) and Redfield (1934), nitrate and phosphate are supplied from deep water in a proportion (~16 N to 1 P) that closely matches their utilization by phytoplankton, resulting in their simultaneous elimination from sunlit surface water (**Figure 1a**). Because of their large size and dense silica frustule, diatoms play a major role in the vertical flux of biogenic material, and Si is also exported out of the surface ocean.

Along with major nutrients, biologically important trace metals are taken up by the biota in surface seawater, exported with sinking organic material, and remineralized in the water column. This is illustrated in **Figure 1a**, which shows concentration profiles of Zn, Cd, and Co in the eastern tropical South Pacific. (Those field data are the focus of the later part of this article.) The dissolved concentrations of these metals are low near the surface, and they increase with depth, as is typical of all algal nutrients. The concentrations of many trace and major nutrients are thus highly correlated, as exemplified for Zn versus Si(OH)₄ and for Cd versus PO₄ in **Figure 1b**. But, as seen in the figure, such correlations between the concentrations of nutrients are not universal: the decrease in the dissolved concentrations of Cd (dCd) and Co (dCo) appears to occur when most of the dissolved Zn (dZn) is already depleted.

The data of **Figure 1b** also seem to show that dZn is depleted when there is still residual Si(OH)₄. Similar graphs showing the elimination of Zn at the surface before that of Si or P have been reported for other areas of the oceans such as the subarctic Pacific, the northeast Pacific, and the Southern Ocean (Bruland 1980; Martin et al. 1989, 1990; as compiled by Sunda & Huntsman 1995, 2000). A depletion of Zn relative to Si was also observed in the Costa Rica upwelling dome (Chappell et al. 2016). Remarkably, the sum of the Cd and Co concentrations that disappear from the surface is a close match for the missing Zn as shown in **Figure 1b**. These observations suggest the hypothesis that diatoms and other algae may cope with depleted dZn by replacing Zn in cellular functions by Cd and Co.

The following section briefly reviews field data showing how phytoplankton obtain N and P from alternative sources in nitrate- and phosphate-poor surface water using a variety of metalloenzymes (Morel et al. 2003). Laboratory results demonstrating how phytoplankton substitute scarce metals in essential enzymatic processes are then discussed with a particular focus on Zn, Cd, and Co. The laboratory results are used to examine whether the available field data are consistent with the biological substitution of Cd and Co for Zn at the surface of the oceans, concluding with an analysis of the data from GP16 Eastern Pacific Zonal Transect of the GEOTRACES program.

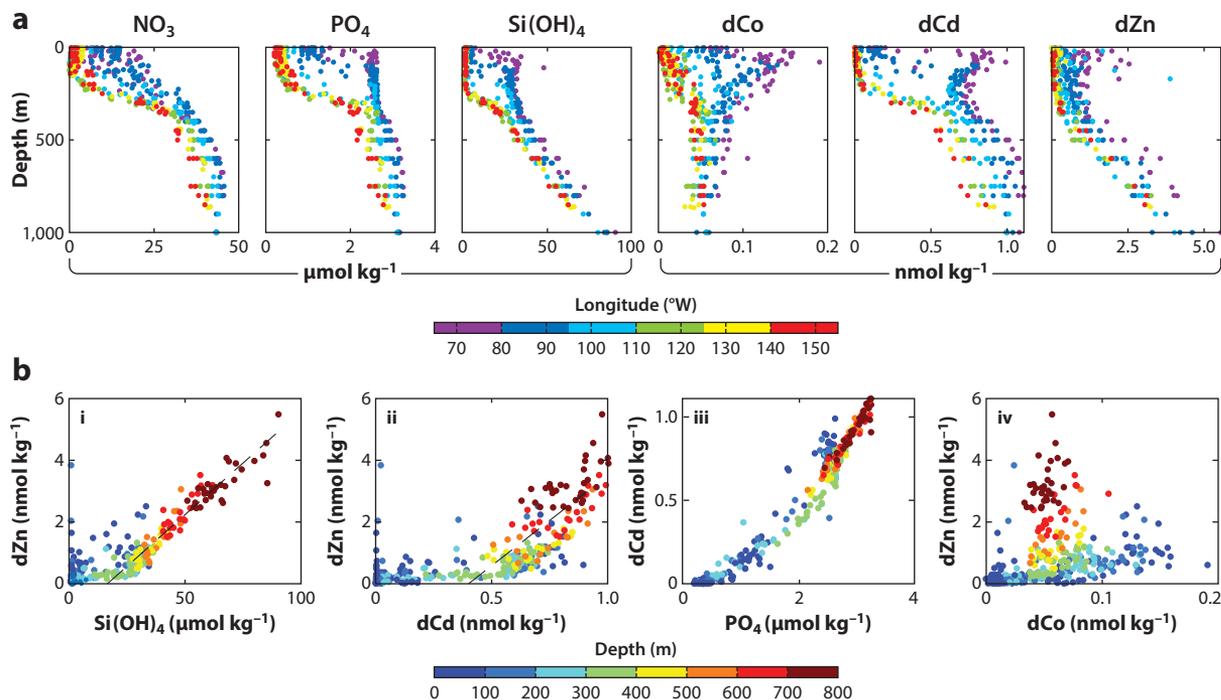


Figure 1

Profiles of nutrient concentrations in the upper 1,000 m of the tropical South Pacific. (a) Concentration profiles for macro- (left three plots) and micro- (right three plots) nutrients from the GP16 GEOTRACES cruise in the eastern tropical South Pacific along $\sim 12^\circ\text{S}$, with profiles colored by longitude. Note that all nutrients show depletion at the surface and regeneration below, with generally shallower regeneration in the coastal upwelling (purple) and transition (dark blue) zones and deeper regeneration in the more oligotrophic waters (other colors). (b) Scatterplots showing correlations between (i,iii) micro- and macronutrients and (ii,iv) dissolved Cd (dCd) or Co (dCo) and dissolved Zn (dZn), with a color bar indicating depth. Dashed lines in subpanels i and ii are the linear regressions for deep (400–1,000 m) samples between dZn and Si(OH)_4 [i: $\text{dZn} = 0.067 \cdot \text{Si(OH)}_4 - 1.1$] and dZn and dCd (ii: $\text{dZn} = 5.3 \cdot \text{dCd} - 2.3$). Note that Zn appears to be depleted before Si(OH)_4 , Cd, or Co and that the dZn versus Si(OH)_4 correlation has a negative intercept of about 1 nM, roughly equal to the $\text{dCd} + \text{dCo}$ that disappears from the solution at very low dZn.

METALS AND N AND P ACQUISITION

To palliate the scarcity of nitrate and phosphate in surface seawater, phytoplankton have evolved a variety of mechanisms to utilize alternative sources of N and P, including N_2 and recycled organic compounds. Many of the enzymes involved in the acquisition and utilization of N and P require catalytic metals, including Fe and Mo for N_2 fixation and NO_3^- reduction, Ni for urea hydrolysis, Cu for reductive deamination of amines and amino acids, and Zn or Co in alkaline phosphatase (Figure 2). The result is a complex set of linkages between the biological use of N, P, and trace metals, all of which are exported to deep seawater with the biomass (Figure 1a).

METALS AND PHOTOSYNTHESIS: THE LIGHT REACTION

Carbon fixation by photosynthesis also requires trace metals. The light reaction requires Mn in the oxygen-evolving center and large quantities of Fe to transfer electrons and energy through photosystems I (PSI) and II (PSII) (Figure 2). Fe limitation of primary production in large regions of the oceans is a well-known consequence of the high biochemical requirement for a metal

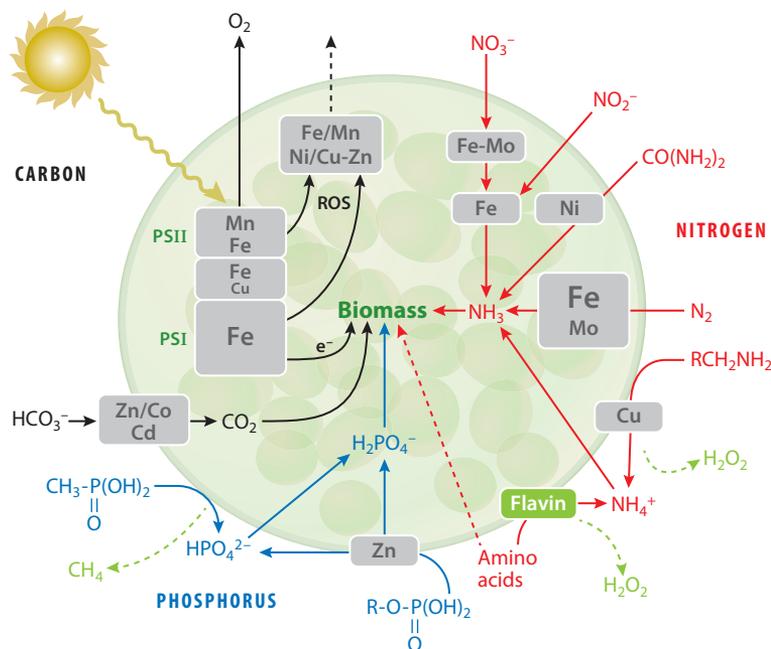


Figure 2

The use of trace metals in the uptake of light, C, N, and P by marine phytoplankton. The light and dark reactions of photosynthesis are illustrated in black, including the use of Mn in the oxygen-evolving complex of photosystem II (PSII) and of Fe in the transfer of electrons within and between PSII and photosystem I (PSI). Also shown is the use of Cu in plastocyanin in replacement of Fe in cytochrome c_6 and the use of metals to detoxify reactive oxygen species (ROSs), principally the superoxide radical O_2^- in Fe, Mn, Ni, and Cu-Zn superoxide dismutases. As discussed in the text, Zn, Cu, and Cd are used as alternative metal centers in carbonic anhydrase to catalyze the dehydration of bicarbonate, HCO_3^- , to CO_2 in the carbon concentrating mechanism for the dark reaction of photosynthesis, which is illustrated in **Figure 3**.

Mechanisms for the acquisition of N are illustrated in red: reduction of nitrate, NO_3^- , to nitrite, NO_2^- , by nitrate reductase (Fe-Mo) and of NO_2^- to ammonia, NH_3 , by nitrite reductase (Fe); hydrolysis of urea, $CO(NH_2)_2$, by urease (Ni) (McCarthy 1972, Price & Harrison 1988); uptake of amino acids (Wheeler et al. 1974); and extracellular deamination of amino-acids and primary amines by flavin- and Cu oxidases (with formation of hydrogen peroxide, H_2O_2) (Palenik & Morel 1990, 1991). Mechanisms for the acquisition of P are illustrated in blue: extracellular hydrolysis of organophosphates, $ROPO(OH)_2$, by Zn- or Co-alkaline phosphatase (Dyhrman et al. 2006, Kuenzler & Perras 1965, Saito et al. 2017, Xu et al. 2006); degradation of phosphonates, $CH_3PO(OH)_2$ (with formation of methane, CH_4) (Dyhrman et al. 2006, Karl et al. 2008); and uptake of orthophosphate, HPO_4^{2-} . Figure adapted from Morel & Price (2003).

that is both in short supply and highly insoluble in seawater. Another consequence is the adaptation of the biochemical machinery of phytoplankton, which has evolved to minimize Fe requirements. For example, marine microalgae often use Cu-containing plastocyanin (Bender et al. 2018, Erdner et al. 1999, Peers & Price 2006) instead of the Fe-containing cytochrome c_6 for transferring electrons from PSII to PSI and metal-free flavodoxin instead of Fe-containing ferredoxin in the last step of the electron transfer chain of PSI (Erdner & Anderson 1999, LaRoche et al. 1996) (**Figure 2**).

Dismutases with a variety of metal centers are used to eliminate the toxic superoxide radical, O_2^- , one of the reactive oxygen species (ROSs) formed as a by-product of light harvesting (**Figure 2**). Dealing with ROS is one of the difficulties of using sunlight as an energy source in

an oxygenated world. There are three classes of superoxide dismutases (SODs) in marine microbes, the Cu/Zn, Fe/Mn, and Ni forms. The Ni-SOD appears to be the sole SOD in the marine cyanobacterium *Prochlorococcus*, while the other two classes are distributed across different phytoplankton taxa, including other cyanobacteria. The presence of multiple classes of SODs is thought to be due to convergent evolution for a key cellular function and a response to metal variability. The Ni-SOD and Fe/Mn-SOD forms are likely vestigial from an early ocean where these metals were more abundant (Dupont et al. 2008, Kirschvink et al. 2000, Saito et al. 2003).

METALS AND PHOTOSYNTHESIS: THE DARK REACTION

CO₂ fixation into organic matter in the dark reaction of photosynthesis and the settling of this organic matter into the deep ocean maintain a low CO₂ concentration in surface waters. As a result, microalgae operate a carbon concentrating mechanism (CCM) to provide a CO₂ concentration much higher than in the ambient seawater at the site of carbon fixation. A very effective CCM is made necessary by the poor affinity of the ancient carboxylating enzyme Rubisco, which evolved when atmospheric CO₂ was many times higher and the competing substrate O₂ much lower than they are today (e.g., in diatoms $K_m^{CO_2} = 20\text{--}70\ \mu\text{M}$ and $K_m^{O_2} \sim 0.4\text{--}1\ \text{mM}$; Young et al. 2016). The CCM uses bicarbonate pumps and multiple forms of the carbonic anhydrase (CA) enzyme (**Figure 3**) in addition to a complex geometric arrangement of the components of both the light and dark reactions in the chloroplast (Mackinder et al. 2017). CA is typically a Zn enzyme that catalyzes the interconversion between bicarbonate, HCO₃⁻, and CO₂, a reaction that is slow compared to diffusion times in cells. The net result is a high Zn requirement for inorganic carbon acquisition by phytoplankton and the near complete elimination of dZn at the ocean surface where the light flux is large and photosynthesis most efficient (**Figure 1a**). Because of their high volume to surface area ratio, large phytoplankton species such as diatoms need particularly high cellular CA and Zn concentrations to power their CCM.

Zn REPLACEMENT BY Cd AND Co IN PHYTOPLANKTON CULTURES

Laboratory work starting in the early 1990s demonstrated that additions of Cd or Co increase growth rates in Zn-limited cultures of the model diatom *Thalassiosira weissflogii* (Lane & Morel 2000a; Lee & Morel 1995; Lee et al. 1995; Morel & Price 2003; Morel et al. 1994; Price & Morel 1990; Sunda & Huntsman 1995, 1998a; Yee & Morel 1996) (**Figure 4a**). A similar result was obtained later in cultures of the coccolithophore *Emiliana huxleyi* (**Figure 4a**) and then extended to other phytoplankton species (Lane & Morel 2000b; McGinn & Morel 2008a,b; Saito & Goepfert 2008; Sunda & Huntsman 2000, 2005; Xu & Morel 2013; Xu et al. 2007). In eukaryotic species, limitation of growth rates occurs at ambient unchelated Zn concentrations in the range 0.5–10 pM, with larger coastal species limited at higher concentrations than smaller open-ocean species. The beneficial effect of added Cd or Co is seen for unchelated concentrations in the same range (>0.5 pM). As discussed below, these unchelated metal concentrations are in the range measured by electrochemistry in the surface ocean. The corresponding cellular metal quotas below which the cells become limited vary from ~0.05 mmol/mol P for oceanic species to ~0.5 mmol/mol P for coastal ones.

Cd can substitute for only a fraction of the cellular Zn, about 50% depending on the species, and Zn-limited cultures often cannot reach their maximum growth rates with Cd addition. At high cellular concentrations (>1 mmol Cd/mol P), Cd becomes toxic to phytoplankton and is exported from the cells (Lee et al. 1996, Sunda & Huntsman 1996). With Co added instead of Zn in the culture medium, many species achieve full growth rate, and some species such as *E. huxleyi* even

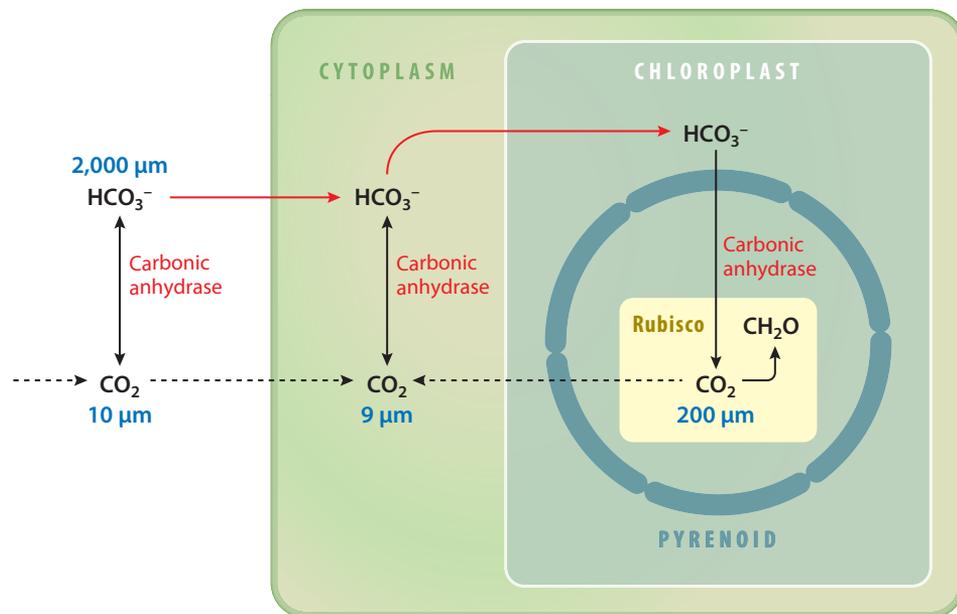
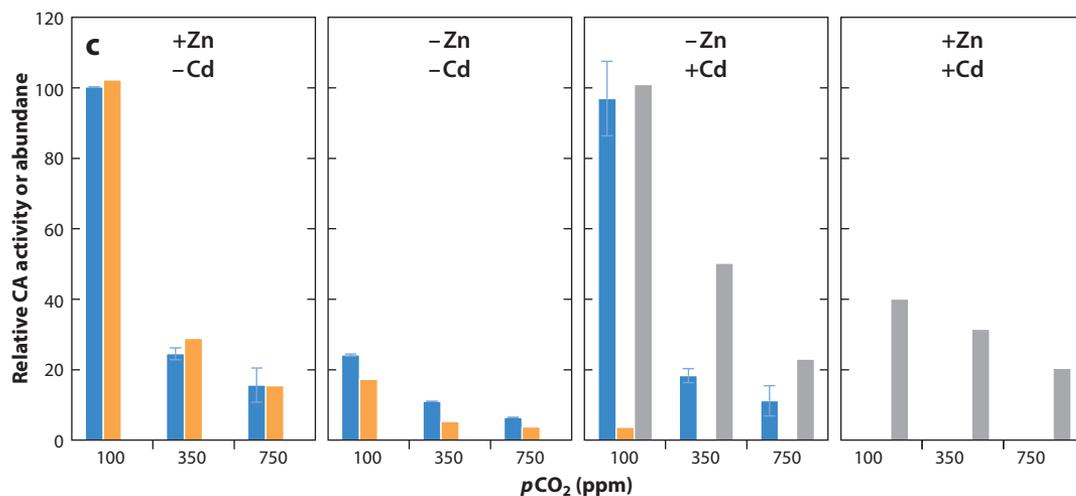
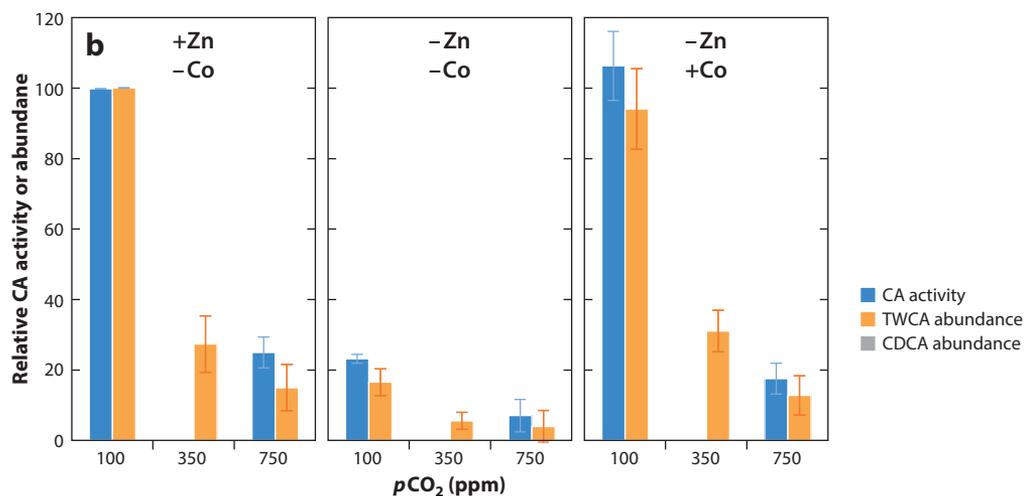
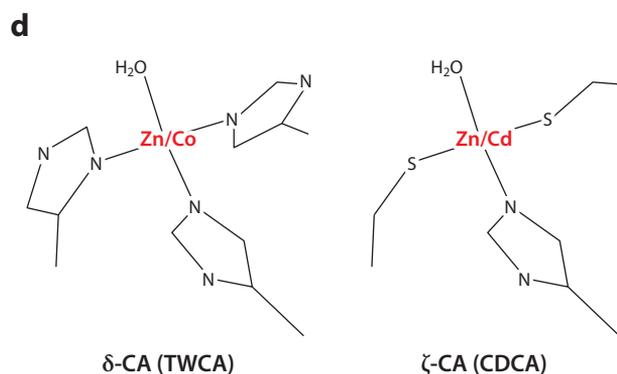
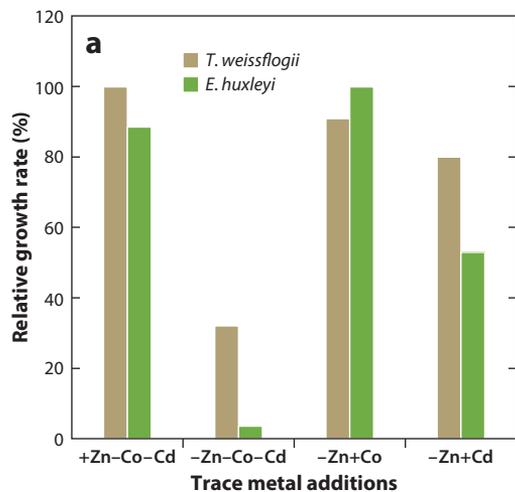


Figure 3

The carbon concentrating mechanism (CCM) of diatoms. Diatoms, which are responsible for a large fraction of CO_2 export to deep seawater, operate a CCM to elevate the concentration of CO_2 at the site of fixation and nearly saturate Rubisco, the enzyme responsible for fixation of CO_2 into organic carbon. The membranes of diatoms are highly permeable to CO_2 , resulting in a large diffusive exchange of CO_2 between the cell and external milieu. An active transport of carbon from the cytoplasm into the chloroplast is the main driver of the diatom CCM. Only one-third of this carbon flux is fixed photosynthetically, and the rest is lost by CO_2 diffusion back to the cytoplasm. Both the passive influx of CO_2 from the external medium and the recycling of the CO_2 leaking out of the chloroplast are achieved by the activity of carbonic anhydrase enzymes and the maintenance of a low bicarbonate, HCO_3^- , concentration in the cytoplasm. To achieve the CO_2 concentration necessary to saturate Rubisco, the CO_2 is concentrated in the pyrenoid, an organelle within the chloroplast where Rubisco is located. Figure adapted from Hopkinson et al. (2011).

grow faster. Some exceptions include the diatom *Chaetoceros* sp., which is incapable of substituting Co for Zn (Timmermans et al. 2001), and *Phaeocystis antarctica*, which achieves higher biomass yield with Zn (Saito & Goepfert 2008), consistent with the abundance of Zn in the Southern Ocean.

A very different situation is observed in prokaryotic phytoplankton. For example, the major marine cyanobacterium *Prochlorococcus* requires Co to grow, and under Co limitation, its growth rate is only slightly increased by Zn addition and not at all by Cd addition (Sunda & Huntsman 1995). *Prochlorococcus* (particularly its Pacific strains) is sensitive to Zn toxicity, while *Synechococcus* is sensitive to Cd toxicity (Hawco & Saito 2018, Saito et al. 2003). However, the Zn and Co requirements of these marine picocyanobacteria may increase in low P environments to serve as metal centers in alkaline phosphatase (Cox & Saito 2013, Saito et al. 2017) (Figure 2). The distinct response of cyanobacteria to Co, Cd, and Zn is likely related to their small size and their use of Co in vitamin B_{12} (also known as cobalamin), which acts as a cofactor in the biosynthesis of methionine and RNA. The relatively large surface area to volume ratio of picoplankton and the modest requirement for B_{12} , which is a major part of Co use in most organisms, enable sufficient acquisition of Co despite the scarcity of dCo in seawater. There have been reports of Co stimulation of phytoplankton growth, but the underlying biochemical mechanism remains unknown.



(Caption appears on following page)

Figure 4 (Figure appears on preceding page)

Substitution of Zn by Cd and Co in marine phytoplankton. (a) Growth rates of the diatom *Thalassiosira weissflogii* and the coccolithophorid *Emiliania huxleyi* for various combinations of Zn, Co, and Cd concentrations in the growth medium. *T. weissflogii* (clone Actin): +Zn-Co-Cd = 15 pM Zn' + no Co, Cd added; -Zn-Co-Cd = 3 pM Zn' + no Co, Cd added; -Zn+Co = 21 pM Co' + no Zn, Cd added; -Zn+Cd = 3 pM Zn' + 45 pM Cd', no Co added (Lane & Morel 2000a,b). *E. huxleyi* (CCMP 374 naked cells): +Zn-Co-Cd = 15 pM Zn' + no Co, Cd added; -Zn-Co-Cd = 0.7 pM Zn' + no Co, Cd added; -Zn+Co = 15 pM Co' + no Zn, Cd added; -Zn+Cd = 0.7 pM Zn' + 20 pM Cd', no Co added (Xu et al. 2007). (b) Carbonic anhydrase (CA) activity and δ -CA (TWCA) abundance with Co substitution for Zn as a function of $p\text{CO}_2$ in *T. weissflogii* under the same metal conditions as in panel a (Lane & Morel 2000a,b). (c) CA activity, δ -CA (TWCA), and ζ -CA (CDCA) abundance with Cd substitution for Zn as a function of $p\text{CO}_2$ in *T. weissflogii* under the same metal conditions as above (Lane & Morel 2000a,b). (d) Metal coordination in the active centers of δ -CA (TWCA) and ζ -CA (CDCA). Figure adapted with permission from Cox et al. (2000), copyright 2000 American Chemical Society.

METAL UPTAKE BY PHYTOPLANKTON: COMPETITIVE EFFECTS

The uptake of a trace metal by a microorganism requires a chemical reaction between the metal ion and transport ligands at the cell surface, and the formation of highly specific complexes usually comes at the cost of slow dissociation of the metal from the binding ligand (Morel et al. 1991). As a result, microorganisms have evolved a variety of cellular mechanisms to foster rapid and specific acquisition of trace metals, including the binding and export of unwanted or excess metal (Waldron & Robinson 2009). Emerging capabilities in developing genetic mutants of eukaryotic phytoplankton will allow the identification and characterization of metal transporters in the coming years. The problem of specificity of metal uptake is particularly true of microorganisms that live in the metal-poor surface ocean. Uptake experiments in phytoplankton demonstrate competitive kinetics between biologically important divalent metals such as Zn^{2+} , Cd^{2+} , Co^{2+} , Mn^{2+} , and Fe^{2+} (Sunda & Huntsman 1998b, 2000), where a deficiency in a given metal can lead to enhanced uptake of others. An important consequence is the increase in Cd and Co uptake at low Zn in model phytoplankton species (Sunda & Huntsman 1998a), which is beneficial for those that can replace one metal for another in a biochemical function (unless the cellular concentrations reach a toxic level). At the high Zn concentrations typical of coastal water, the uptake of Cd by phytoplankton occurs through the Mn uptake system and is affected by the ambient Mn concentration (Sunda & Huntsman 1998a). For picocyanobacteria with absolute Co requirements, even picomolar concentrations of Zn can cause toxicity by inhibiting Co uptake (Hawco & Saito 2018). Another consequence of the lack of specificity of metal transport systems is the effect of the very low Fe concentrations that are typical of the vast Fe-limited regions of the oceans on the uptake of other trace metals. In laboratory experiments with model species, the cellular concentrations of Zn and Cd increase at limiting Fe concentrations, a result attributed to the incidental uptake of Zn and Cd through the Fe(II) transport system. This effect provides an explanation for the change in the slope (the so-called kink) in the correlation between Cd and P in field data (Lane et al. 2008, 2009; Sunda & Huntsman 2000).

BIOCHEMICAL BASIS OF Cd AND Co REPLACEMENT OF Zn

The biochemical basis for the restoration of rapid growth upon Cd or Co addition in Zn-limited diatoms is due to the replacement of Zn by Co or Cd in CA. (See the section titled Postscript for the recent discovery of an Mn-CA; Jensen et al. 2019.) Zn limitation in cultures of *T. weissflogii* (Morel et al. 1994) and *T. pseudonana* (Sunda & Huntsman 2005) is easy to achieve at low $p\text{CO}_2$, leading to the hypothesis that it is caused by insufficient CA activity to power the CCM (Figure 3). Indeed, whole cell CA activity in *T. weissflogii* decreases at low Zn and is restored by either Cd or Co addition (Figure 4b,c). Under all combinations of metal concentrations, a decrease in $p\text{CO}_2$ elicits a large increase in CA activity (Figure 4b,c).

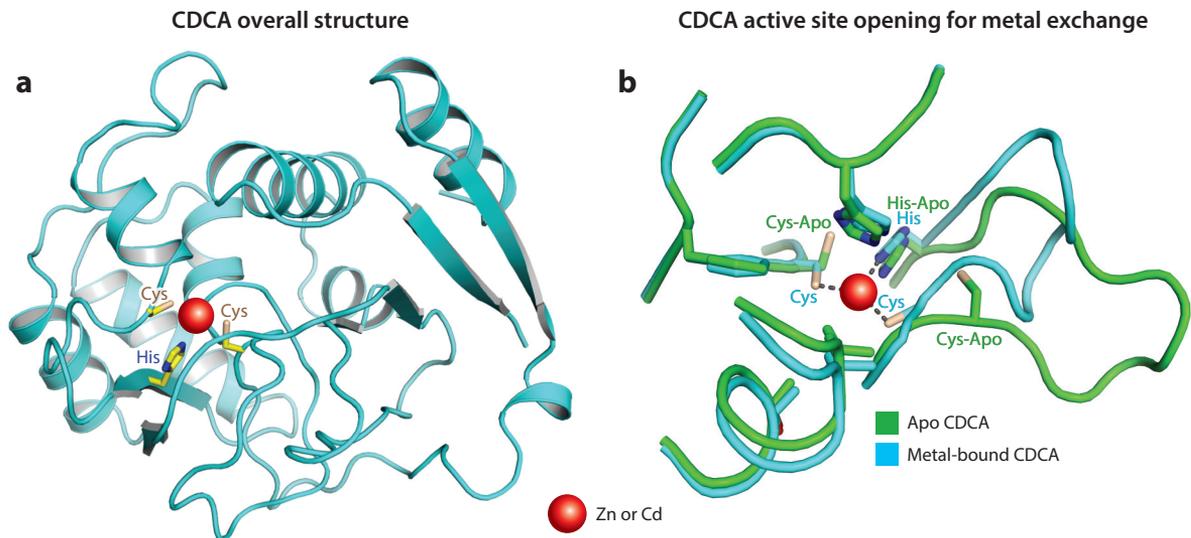


Figure 5

The ζ -CA (CDCA) of *Thalassiosira weissflogii*. (a) Overall structure of the ζ -CA of *T. weissflogii*. The metal center (Zn or Cd) is shown in red, and the metal binding residues (two cysteines and one histidine) are shown in yellow and blue. The coordination of the metal center is completed by water molecules that are themselves coordinated to conserved residues. The overall organization of the active center in a monomeric ζ -CA is similar to that in a functional dimer of a β -CA in which the metal and substrate binding residues are located on separate monomers. (b) Facile metal exchange in the active site of ζ -CA. In the absence of metal, the active site pocket of ζ -CA opens up, with one cysteine (left) flipped away from its metal binding position and the linker sequence between the other cysteine and histidine (right) rotated away from its metal-coordinating position. This rotation is made possible by the flexibility of two glycines (not shown) coordinated to the metal binding residues. Figure redrawn from Xu et al. (2008), courtesy of L. Feng and Y. Xu.

Two different enzymes that have been discovered in *T. weissflogii* are responsible for the increased CA activity upon Co or Cd additions in Zn-limited cultures of diatoms: δ -CA and ζ -CA, which are distinct from the α -CA and β -CA of higher plants. The δ -CA, TWCA, exchanges Co for Zn in its active site (Figure 4d), while the ζ -CA, CDCA, exchanges Cd for Zn (Figure 4d). In the active site of TWCA, the metal is coordinated to three histidines as occurs in α -CAs, while in CDCA, the metal binding is achieved with one histidine and two cysteines as in β -CAs. Crystal structures of CDCA (Figure 5a) have been obtained under various conditions, providing molecular insight into the catalytic and metal exchange mechanisms. The arrangement of the key residues of the active center is a close mimic of the active center of the functional dimer of β -CAs. Of particular interest is the opening of the active site pocket, which is made possible by two glycines that flank the linker sequence between the metal-coordinating His and Cys (Figure 5b). This opening allows a facile exchange between Zn and Cd in the active site and makes CDCA a true cambialistic enzyme.

The exchange between Zn and Cd in CDCA occurs rapidly in the presence of an excess of the incoming metal bound to the phytochelatin dimer (γ -glu-cys)₂-gly (PC2) (Figure 6a), a mechanism likely relevant to the functioning of CDCA in marine phytoplankton in the field. Both the mechanism of phytochelatin synthesis (Gupton-Campolongo et al. 2013) and its utilization in diatoms (Ahner & Morel 1995, Ahner et al. 1994, 1995) are different from those in higher plants and seem adapted to the low metal concentrations in seawater. In metal-polluted coastal waters, phytoplankton produce high phytochelatin concentrations (Figure 6b) and export the PC complex of various trace metals including Cd to avoid toxicity (Ahner et al. 1997). But no PC export

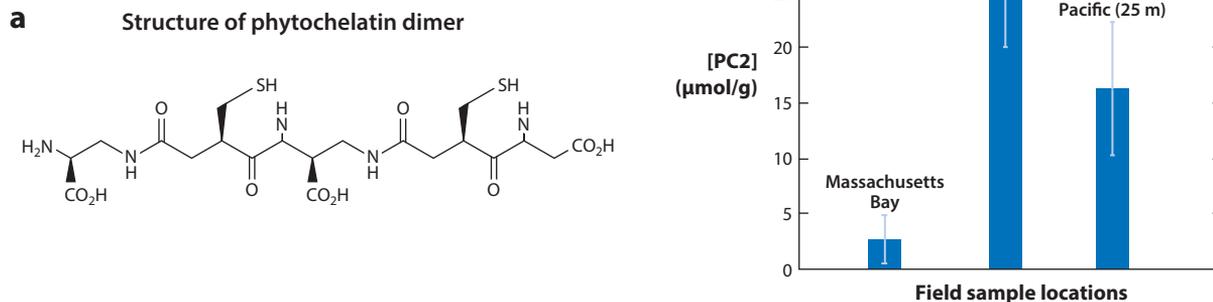


Figure 6

Phytochelatin structure and abundance in seawater. (a) Structure of the phytochelatin dimer (γ -glu-cys) $_2$ -gly (PC2). (b) Concentrations of PC2 in field samples: Massachusetts Bay (18 samples >20 km from Boston Harbor; data from Ahner et al. 1997); Eel Pond in Woods Hole, Massachusetts (10 samples; data from Ahner et al. 1994); and equatorial Pacific (9 samples; data from Ahner et al. 1998).

occurs at low metal concentrations ($< \sim 0.5$ mmol Cd/mol P; Lee et al. 1996); instead, an increase in cellular phytochelatin is seen under Zn limitation in the laboratory and at low Zn concentration in the field (Ahner et al. 1998) (**Figure 6b**). It appears that, in phytoplankton, phytochelatins are used for the homeostasis of divalent metals, both controlling their cellular concentrations through export of the PC-bound metal and maintaining them in nontoxic complexes that allow exchange with metalloproteins.

The work that has been done to date on CAs in microalgae has focused exclusively on the role of the enzyme in inorganic carbon acquisition. It is however very likely that CAs play other roles in these organisms as they do in others from bacteria to humans because the equilibrium between the ubiquitous solutes HCO_3^- and CO_2 must be achieved on a timescale shorter than that of diffusion in individual cells. The diatom genomes contain a number of CAs besides TWCA and CDCA (Armbrust et al. 2004). The prymnesiophyte *E. huxleyi* also contains as many as 15 CAs that likely contribute to a wide range of cellular processes (Read et al. 2013). It is thus possible, perhaps likely, that substitution of Cd or Co for Zn in CA plays a role in physiological functions other than inorganic carbon acquisition in phytoplankton. One may speculate, for example, that in coccolithophores, CAs play a role in the formation of the calcite liths as they do in the calcification of corals (Chen et al. 2018) and the formation and resorption of bones (Sly & Hu 1995). This could be the reason for the beneficial substitution of Cd for Zn in Zn-limited *E. huxleyi*, which does not appear to involve inorganic carbon acquisition.

PREVALENCE AND PHYLOGENY OF CARBONIC ANHYDRASES IN THE OCEANS

The recent transcriptome sequencing of marine eukaryotic microbes (Keeling et al. 2014) allows an updated examination of the distribution of the diatom CAs.

The distribution of δ -CAs (TWCA) is particularly broad throughout marine eukaryotic microbes. These enzymes are found in prymnesiophytes (haptophytes), heterokonts, and the chlorophytic prasinophytes (**Figure 7a**). All extant δ -CA genes appear to share a common ancestor, implying that the appearance of this particular class of CA must be very ancient, dating from the

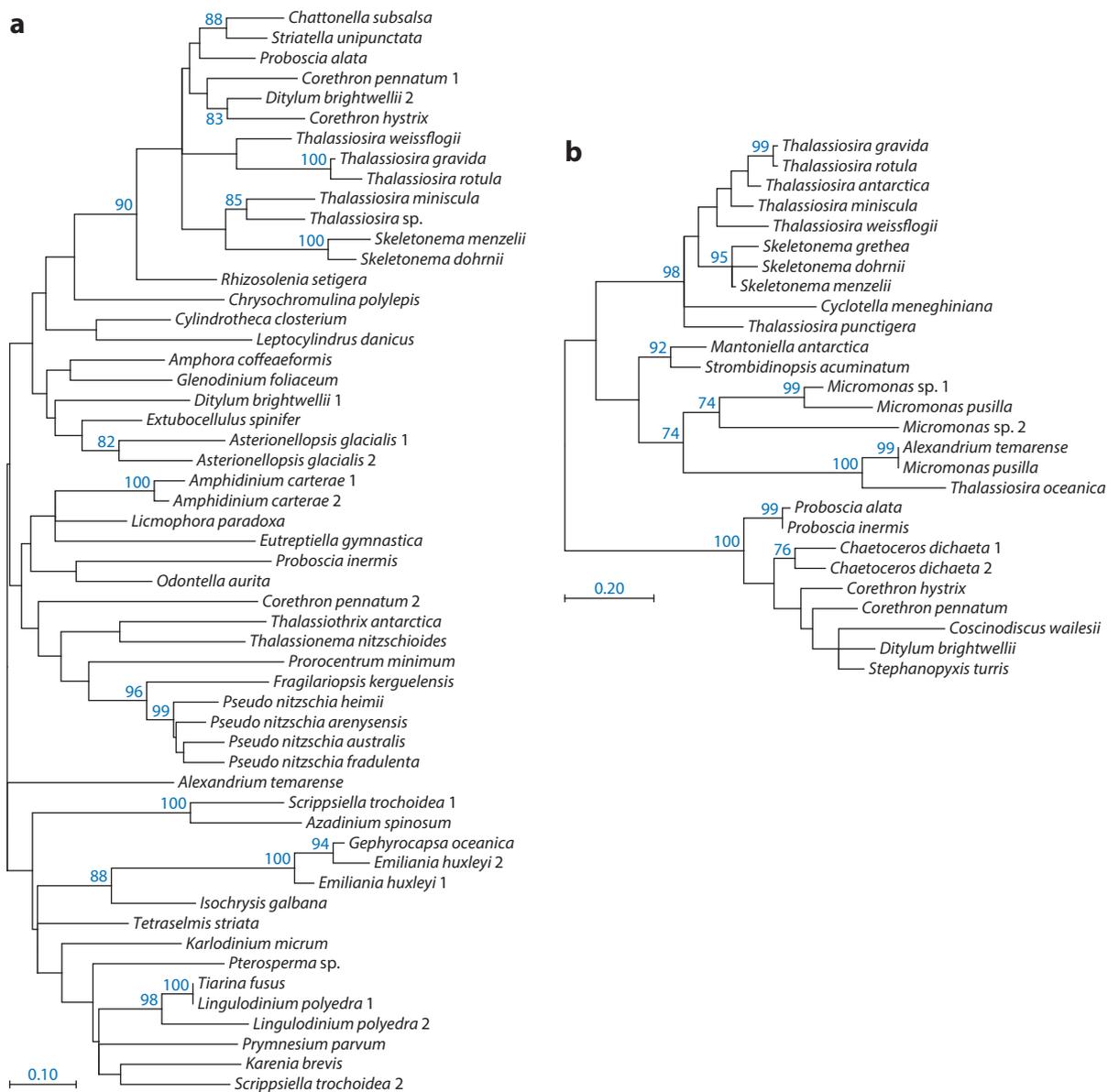


Figure 7

Phylogenetic trees for (a) δ -CA (TWCA) and (b) ζ -CA (CDCA). The taxonomic distribution of the δ -CA (TWCA) and ζ -CA (CDCA) enzymes in marine eukaryotes was investigated using the Marine Microbial Eukaryote Transcriptome Sequencing Project data set (Keeling et al. 2014). BLAST+ searches (Camacho et al. 2009) were conducted using query amino acid sequences from *Thalassiosira weissflogii*, the diatom in which both carbonic anhydrase enzymes were discovered. Phylogenetic analysis was conducted on amino acid sequences with e-values less than $1e^{-5}$ from representative taxonomic groups. Using MEGA-X software (Kumar et al. 2018), sequences were aligned using MUSCLE and Maximum Likelihood trees created using the JTT model, allowing partial deletion of residues, and 100 bootstrap replicates (values greater than 70% shown). Sequences representing multiple gene copies within the transcriptome, multiple repeats within genes, and shorter sequences were removed. Identifiers followed by 1 or 2 represent different strains and/or gene copies within a strain.

Triassic or Early Jurassic about 200 million years ago, before the divergence of the green and red phytoplankton lineages (Falkowski et al. 2004). The broad phylogenetic distribution of δ -CAs implies that these enzymes may be more appropriately named marine carbonic anhydrases rather than a diatom CA or a *Thalassiosira* CA (TWCA), as previously done in the literature. In contrast, ζ -CAs (CdCA), which are present in a number of diatom species, are found in only a few other marine eukaryotes, such as the green algae *Micromonas* and *Mantoniella*, the dinoflagellate *Alexandrium*, and some others (**Figure 7b**). It is interesting to consider if these differences in the distributions of δ -CAs and ζ -CAs might be related to the ecological niches of the organisms and if those organisms with CdCA may be found in regions where Zn and Co are more often depleted. Both the δ -CAs and ζ -CAs have been identified in DNA and RNA isolated from natural environments, demonstrating the use in natural populations of these two CAs that were originally identified from diatom cultures (McGinn & Morel 2008b, Park et al. 2008).

Zn, Cd, AND Co CONCENTRATIONS, CHELATION, AND BIOAVAILABILITY IN SEAWATER

dZn and dCd accumulate in the ocean interior as a result of increasing water mass age, with concentrations of 2 nM and 0.4 nM, respectively, in the North Atlantic and increasing to more than 10 nM and 1 nM, respectively, in the North Pacific. In contrast, dCo concentrations decrease with water mass age due to removal by scavenging processes, with values around 80 and 40 pM in the North Atlantic and North Pacific basins, respectively (Hawco et al. 2016, 2018). The resulting concentration ratios Zn:Co are quite different in the interiors of the Atlantic and Pacific Oceans: ca. 25:5:1 versus 250:25:1, respectively. Notably, Zn has a deeper remineralization length scale than the other two metals, as observed in its correlation with Si, and its concentration gradient in the water column is not as steep as those of Cd and Co (**Figure 1a**). As these metals are transported from the deep to the euphotic zone by upwelling and diffusion, Zn can become particularly depleted through biological uptake, resulting in Cd:Zn and Co:Zn concentration ratios that approach or exceed unity in the upper euphotic zone (see the section titled Case Study: Zn, Cd, and Co in the Eastern Tropical South Pacific). These similar concentrations at the ocean surface likely resulted in the selection pressure for the substitution of Cd and Co as alternative metals at the catalytic sites of Zn enzymes.

In laboratory cultures, Zn limits growth at unchelated concentrations of a few picomolar and can be substituted by Cd or Co at similar concentrations. One way to decide whether the field data are consistent with the biological use of Cd and Co is to compare bioavailable concentrations in the field and in laboratory cultures. (See the next paragraph for the relation between unchelated and bioavailable.) There have been few measurements of Zn, Cd, and Co complexation in the oceans, so that information must be boldly extrapolated from sparse data. Electrochemical titrations performed by anodic stripping voltammetry (ASV) in the North Pacific 30 years ago (Bruland 1989, 1992; Donat & Bruland 1990) indicated unchelated concentrations of about 2 pM for Zn and of 0.7 pM for Cd ($Zn_T = 0.15\text{--}0.3$ nM; $Cd_T = 2\text{--}4$ pM) at the surface. More recent measurements by ASV of unchelated Zn and Cd in the metal-poor Subantarctic waters east of New Zealand (Ellwood 2004) yielded somewhat lower unchelated concentrations near the surface, about 0.5 pM for both metals ($Zn_T \sim Cd_T = 5\text{--}10$ pM). In the North Pacific and the Subantarctic, unchelated metal concentrations obtained by cathodic stripping voltammetry (CSV) were up to 10 times higher than those obtained by ASV (Donat & Bruland 1990), providing context for CSV data collected in other field studies in the eastern Atlantic (Ellwood & Van den Berg 2000) and in the North Pacific (Lohan et al. 2005). Measured concentrations of unchelated Zn at the surface increased from 1.5 pM near the Azores to 50 pM at 10°W and 300 pM at the entrance

of the English Channel ($Zn_T = 0.3\text{--}60\text{ nM}$) (Ellwood & Van den Berg 2000). At Station Papa in the North Pacific, Zn addition caused a rapid transient increase in Zn-binding ligands, with a return to an unchelated Zn concentration of $\sim 2\text{ pM}$ within a week, implying a dynamic control of Zn speciation by the extant biota (Lohan et al. 2005). Except for those obtained near the coast, these sparse data show unchelated Zn concentrations in the range that limits phytoplankton in the laboratory and unchelated Cd concentrations that partially relieve that limitation.

One may wonder whether the electrochemical measurements of unchelated Zn and Cd actually provide a good quantitative estimate of the bioavailability of these metals to phytoplankton and whether they can usefully be compared to the results of laboratory experiments in which the metals are buffered by complexation with an artificial chelating agent such as ethylenediaminetetraacetate (EDTA). One study compared the unchelated concentration of a metal measured by electrochemistry with its bioavailability to phytoplankton in culture (Kim et al. 2015). This study is directly relevant here since it dealt with Zn availability to *T. weissflogii* in EDTA-buffered medium. The results show good agreement between the electrochemical measurements of reducible Zn and the rates of Zn uptake by phytoplankton. Interestingly, the quantitative correlation between biological uptake rates and reducible metal concentrations remains in the presence of relatively weak Zn complexing agents such as cysteine and histidine (Aristilde et al. 2012, Xu et al. 2012). For example, the 1:1 Zn-histidine complex is both available to phytoplankton and reduced at the plating potential of -1.2 V (versus Ag/AgCl) used for ASV measurements (Aristilde et al. 2012, Kim et al. 2015, Xu et al. 2012). The unchelated concentrations measured in the field by electrochemistry should thus include Zn complexes with relatively weak organic ligands and provide a good measure of bioavailable Zn. This may be less true of ASV measurements of unchelated Cd, which are sometimes made at less negative plating potentials.

In contrast, organic complexes of Co in the modern ocean tend to be exceptionally strong to the point of being chemically inert to dissociation, reflecting the fact that they stabilize the Co(III) oxidation state of the metal (Saito et al. 2005). These complexes likely contain corrin ring structures including vitamin B₁₂ and its degradation products. But the B₁₂ molecule itself is generally present at a concentration of about 1 pM or less and hence is only a small fraction of the organically complexed Co reservoir (Menzel & Spaeth 1962, Sañudo-Wilhelmy et al. 2012).

EVIDENCE FOR Zn AND Co (AND Fe) LIMITATION IN FIELD EXPERIMENTS

While most of the fieldwork on trace metal limitation in marine phytoplankton has focused on Fe, a growing number of studies have examined the role of other metals. The very low unchelated Zn concentrations measured in the field and laboratory experiments showing that Zn limitation is easily (sometimes inadvertently) achieved in phytoplankton cultures have inspired a number of attempts at testing Zn limitation in field samples—a difficult task in view of the prevalence of Zn contamination. Co is less prone to contamination but is also found at extremely low concentrations in the euphotic zone and, as mentioned above, plays a key biochemical role in vitamin B₁₂, aside from its potential substitution for Zn in other metalloenzymes.

The few successful observations of Zn or Co stimulatory effects on phytoplankton growth have been made in Fe-limited regions. Such effects usually pale in comparison to the dramatic response to Fe addition and evince a role for these metals in colimitation or secondary limitation. Stimulation of phytoplankton growth was observed in the highly productive Costa Rica dome in response to Zn (Franck et al. 2003) and Co (Saito et al. 2005) additions. In subsequent studies in the same region, Fe or Zn amendments did not increase phytoplankton growth, but addition of either together with Si did (Chappell et al. 2016). Stimulatory effects have also been observed

for Zn additions in the North Pacific Ocean (Jakuba et al. 2012, Martin et al. 1989) and for Co additions in the Benguela upwelling region (Browning et al. 2017) and the Ross Sea (Co added as B₁₂) (Bertrand et al. 2007). A number of experiments have shown no response to Zn addition (Coale et al. 2003, Ellwood 2004) or a modest increase in chlorophyll *a* and/or in nutrient uptake (Coale 1991, Crawford et al. 2003).

B₁₂ limitation is a curious and increasingly observed phenomenon in nature, particularly in polar coastal environments where B₁₂ bacterial production may be stunted by scarce dissolved organic carbon (Bertrand et al. 2007). B₁₂ limitation in diatoms can result in the use of alternative methionine biosynthesis, MetE, which is much less efficient than B₁₂, MetH. Both enzymes require Zn, but the use of the inefficient MetE under B₁₂ scarcity results in an increased Zn demand (Bertrand et al. 2013). Observations of Co stimulation of phytoplankton populations may be due to either its substitution for Zn or allowing the biosynthesis of B₁₂ (Browning et al. 2017, Saito et al. 2005).

The extraordinary scarcity of Zn, Cd, and Co at the ocean surface and the observations of Zn and Co stimulation in some experiments are consistent with the physiological and biochemical laboratory results described above. The very low surface concentrations of Zn, Cd, and Co likely result from a combination of uptake via high affinity Zn transport systems (John et al. 2007, Sunda & Huntsman 1992) and upregulated divalent metal transporters that are not very specific. Upregulated Fe(II) transporters can result in incidental uptake and low ambient concentrations of Zn, Co, and Cd (Lane et al. 2008, 2009). This provides an explanation for the negative results of several Zn amendments experiments in Fe-limited ocean water with very low ambient Zn concentrations (e.g., Ellwood 2004). But it is also conceivable that the accumulation of Zn, Cd, and Co by phytoplankton, which causes the depletion of these metals in low Fe surface water, may be particularly useful to the organisms under conditions of Fe limitation. For example, Fe uptake may require extracellular pH buffering and CA activity. Such speculation would fit with the high cellular Si content of diatoms observed in the field at low Fe (Hutchins & Bruland 1998, Takeda 1998), with SiO₂ serving as the buffer for extracellular CA activity (Milligan & Morel 2002).

Indirect evidence supporting the notion of the physiological replacement of Zn by Cd and Co in the field comes from measurements of phytochelatin concentrations. In the equatorial Pacific where metal concentrations are very low, cellular phytochelatin concentrations are surprisingly higher than in polluted coastal areas (**Figure 6b**). This is consistent with what is observed in laboratory cultures where high cellular phytochelatin concentrations are produced under Zn-limiting conditions in the presence of picomolar concentrations of Cd or Co. The phytochelatin complexes of Cd and Co may serve as an intracellular buffer for these metals, making them available for use in biochemical functions (Ahner et al. 1998).

CASE STUDY: Zn, Cd, AND Co IN THE EASTERN TROPICAL SOUTH PACIFIC

The field data of **Figures 1, 8, and 9** from a GEOTRACES zonal transect in the South Pacific (**Figure 8j**, roughly along 12°S; Moffett & German 2018) and similar data from other regions demonstrate the near disappearance of dCd and dCo from the surface ocean when Zn concentrations are very low. This is *prima facie* evidence of biological uptake of Cd and Co under conditions of low Zn concentrations at the sea surface, a process that is further examined here using available chemical and biological data from the upper 200 m of this transect. The cruise track of **Figure 8j** is particularly advantageous for our purpose as it covers a wide range of primary production but no region where Fe availability limits the growth of phytoplankton (Bruland et al. 2005) and may promote the incidental uptake of Cd and Co (*vide supra*). Furthermore, inadvertent Cd uptake

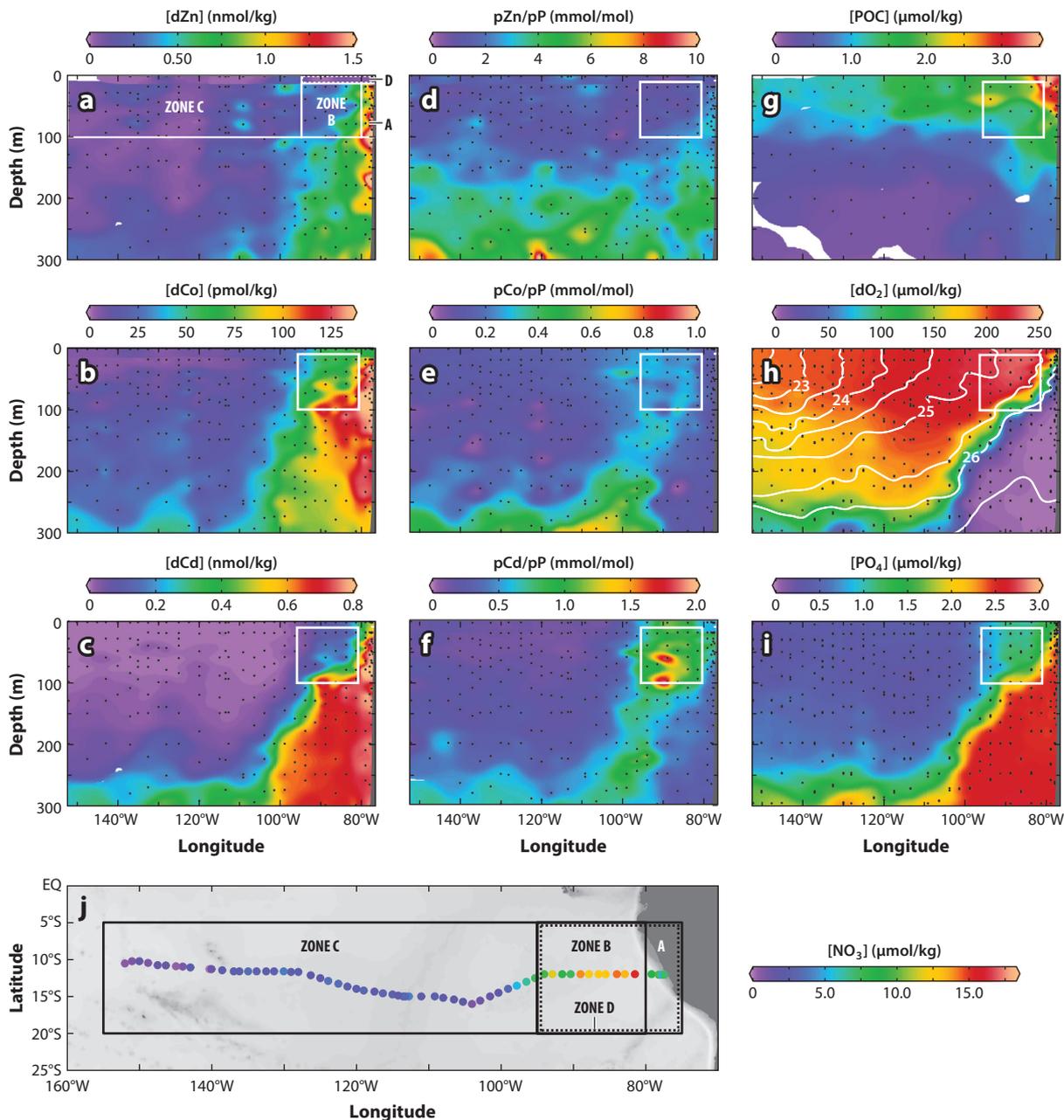


Figure 8

Contour plots of metals, organic C, and phosphate concentrations in the top 300 m of GP16. Shown are depth sections of (*a–c*) dissolved Zn (dZn), Co (dCo), and Cd (dCd), and (*d–f*) particulate Zn (pZn), Co (pCo), and Cd (pCd) and (*g*) particulate organic carbon (POC), (*h*) oxygen, and (*i*) phosphate in the upper 300 m of the (*j*) GP16 transect in the eastern tropical South Pacific. Particulate trace metal concentrations are normalized to particulate P (pP), a biomass tracer. The coastal upwelling zone, transition zone, and oligotrophic gyre (zones A, B, and C, respectively) are marked as solid white boxes in the dissolved Zn section (panel *a*) and as black boxes on the map of the cruise track showing surface nitrate concentrations (panel *j*). Surface zone D (top 20 m above A and B) is marked as a dotted white box in panel *a* and a dotted black box in panel *j*. White contour lines in panel *h* are potential density anomalies relative to the surface ($\sigma_{\theta,0}$) in kg/m^3 . The continental margin is indicated as the dark wedge along the right axis of each panel.

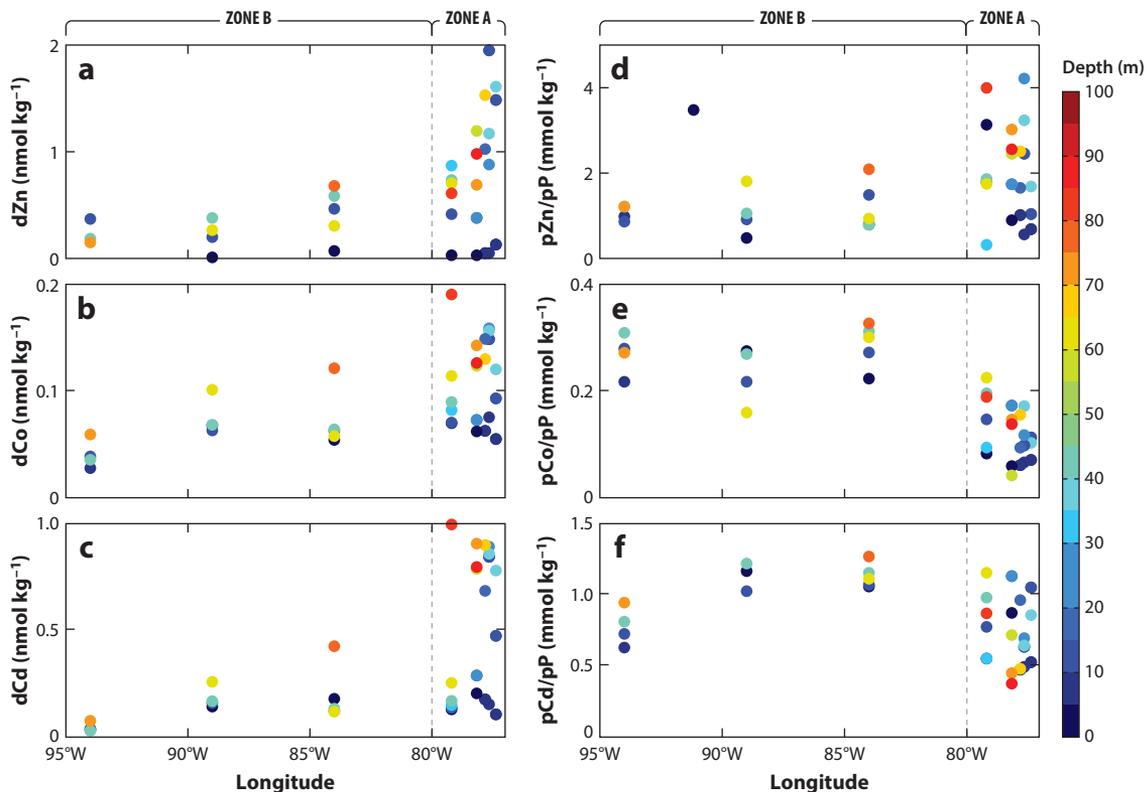


Figure 9

Dissolved and particulate metal concentrations near the surface in GP16. Dissolved Zn (dZn), Co (dCo), and Cd (dCd) and particulate Zn/P (pZn/pP), Co/P (pCo/pP), and Cd/P (pCd/pP) in the upper 100 m of the upwelling (zone A, east of 80°W) and transition zones (zone B, 80–95°W). All dissolved trace metals decrease westward, as does pZn/pP. In contrast, pCo/pP and pCd/pP increase westward, reaching a maximum in the transition zone. The color bar shows the sample depth.

through the Mn transporter (Sunda & Huntsman 1996) is unlikely since dissolved Mn in surface waters is high throughout this transect (Resing et al. 2015). Finally, the large dissolved and particulate metal data sets from the trace metal clean rosette and surface fish sampler are complemented by major particulate phase (particulate organic carbon, biogenic silica, CaCO₃) and particulate trace metal concentrations of size-fractionated particles collected by in situ filtration, as well as pigment concentrations (John et al. 2018; Lam et al. 2018; Lee et al. 2018; Ohnemus et al. 2017; Roshan et al. 2017, 2018; Schlitzer et al. 2018). We make the simplifying assumption that total particulate Zn, Cd, and Co concentrations in the upper 100 m normalized to P reflect chiefly the cellular concentration of these metals (cf. Twining & Baines 2013).

Before we examine in detail the field data to see if they are consistent with substitutions of trace metals by the biota at the ocean surface, it is useful to recall that the biochemical use of trace metals by phytoplankton is only one of several processes influencing the overall distribution of dissolved and particulate trace metal concentrations in the ocean. Other processes that may obscure clear evidence of biochemical substitution include differential sources and (nonbiochemical) sinks of various metals. For example, the well-documented correlation between Zn, which has many known biochemical functions, and silica rather than with phosphate, which is the classic nutrient tracer, has puzzled oceanographers for decades. It is only with the recent explosion in data coverage

from the GEOTRACES program that it has become clear that this correlation is largely set by intense uptake of both Zn and silica by Fe-limited diatoms in the Southern Ocean (Roshan et al. 2018, Vance et al. 2017, Weber et al. 2018), particularly in the Subantarctic mode water formation region. This water mass fills the intermediate depths (~500–1,000 m) of a large portion of the world's oceans (Sarmiento et al. 2004) with waters that are particularly depleted in Zn and silica, resulting in a deeper regeneration of Zn and silica compared to other nutrients and, hence, an ocean-wide Zn-Si correlation (independent of any biochemical process that may connect the two in the Southern Ocean). In contrast, the dominant supply of Co to the surface ocean is from low-oxygen continental margin environments, including the vast eastern tropical South Pacific oxygen minimum zone that underlies the eastern half of the GP16 transect (Hawco et al. 2016, Tagliabue et al. 2018).

Figure 8 illustrates the broad features of the upper water column data obtained in the South Pacific GEOTRACES transect, from the highly productive Peru coastal upwelling to the east (zone A, east of 80°W) through the transition region (zone B, 80–95°W) to the more oligotrophic waters further west in the gyre (zone C, west of 95°W), with the dissolved concentrations of major nutrients and trace metals decreasing westward. Like dissolved trace metal concentrations, particulate Zn/P ratios decrease westward through the transition zone (**Figure 9**), yet both particulate Cd/P and Co/P increase westward into the transition region. This inverse relationship between particulate Zn/P and particulate Cd/P and Co/P is what is expected for the replacement of Zn by Co and Cd in phytoplankton and makes this GEOTRACES transect a good case study for biological substitution of trace metals in the field.

To understand how the uptake of Co and Cd by phytoplankton is related to ambient dZn concentrations, we must first look at the very surface (~4 m, above the shallowest 20-m rosette samples and obtained in part from fish samples) of zones A and B (east of 95°W). In this very shallow region (zone D), the dissolved concentration of Zn is extremely low (<0.2 nM) and those of Co and Cd are much lower than in the upwelling water below (**Figures 8–10**). Remarkably, the particulate Cd/P concentrations in these surface waters are high, similar to those in the water column below (zone A), and much higher than those of zone C, which have similarly low dZn concentrations (**Figure 10**). The high particulate Cd/P in zone D, at the water surface where the dZn concentrations are very low, provides compelling evidence of biological utilization of Cd. This assertion is supported by the biological data, which evince a diatom bloom with chlorophyll *a* and fucoxanthin concentrations reaching above 3,000 ng/L and 800 ng/L, respectively, at the surface near the coast (**Figure 10e**) and decreasing as the newly upwelled surface water is advected toward the west. Coccolithophores are also abundant in this near-surface zone, with 19'-hexanoyloxyfucoxanthin concentrations above 300 ng/L at some stations (**Figure 10f**). Interestingly, the particulate Co/P is relatively low in these surface waters (**Figure 10d**), likely reflecting a dominant use of Cd rather than Co enzymes.

Below the surface (>20 m) in the upwelling stations (zone A), the cellular Cd and Co concentrations increase as dZn decreases (**Figure 10c,d**). Large diatoms are important in zone A, as indicated by high concentrations of fucoxanthin (**Figure 10e**) and biogenic silica (**Figure 10g**) as well as the high fraction of particulate organic carbon in the large (>51 µm) size fraction (Lam et al. 2018). Although they are less dramatic than the surface data (zone D), these results are also consistent with substitution of Zn in diatoms, in this case by both Cd and Co. The inorganic carbon requirements increase proportionally with the volume and the growth rate of the cells, necessitating a very high cellular CA concentration despite the high ambient CO₂ concentration of newly upwelled surface water in zone A, up to 3 times equilibrium with the atmosphere (Bates 2018).

Cellular Cd and Co concentrations reach their maximum values of 1.2 and 0.35 mmol/mol P, respectively (**Figure 10c,d**), in the transition region (zone B) at intermediate dZn concentrations

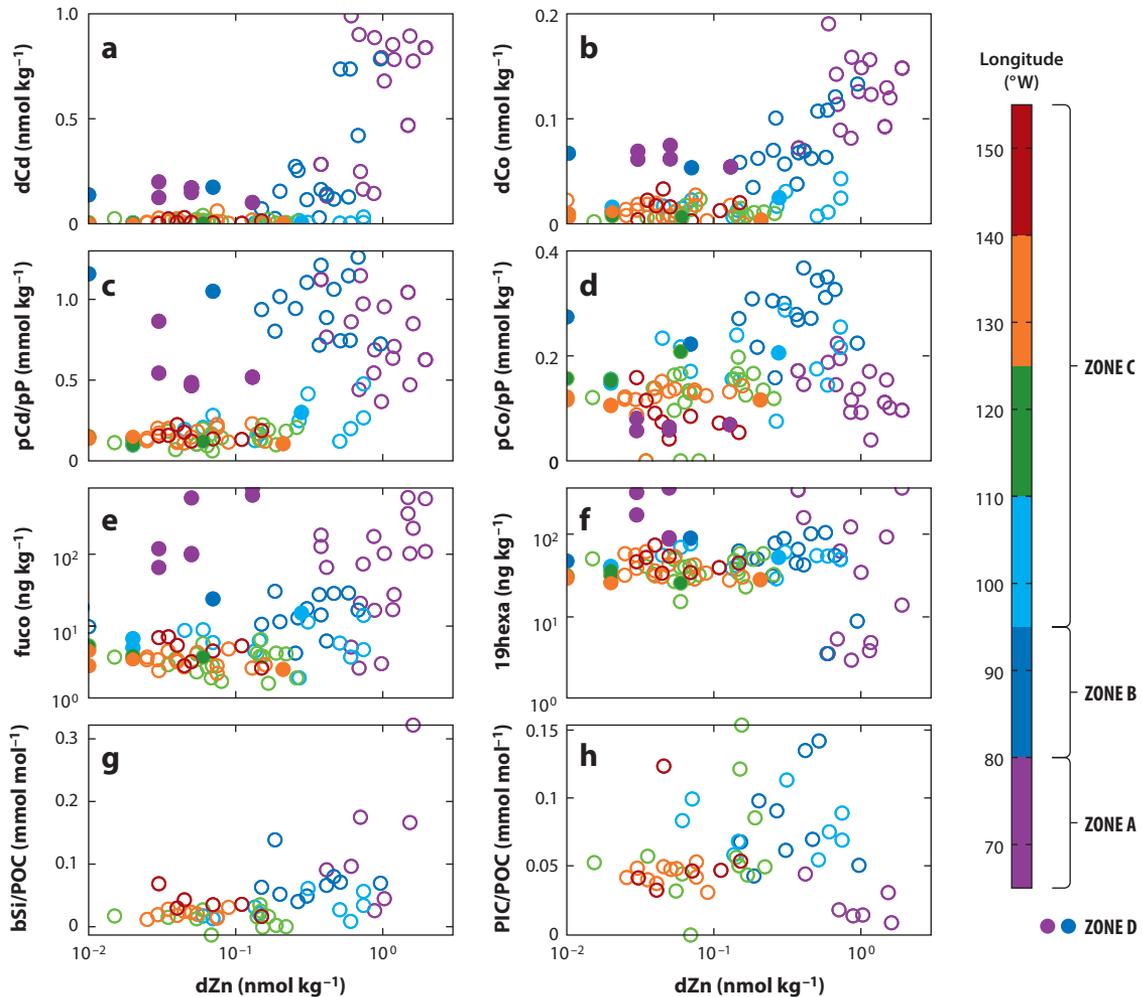


Figure 10

Cd, Co, algal pigments, and hard parts as a function of dissolved Zn (dZn) in the upper 1,000 m of GP16. Shown are variations as a function of dZn of (*a,b*) dissolved Cd (dCd) and Co (dCo); (*c,d*) particulate Cd (pCd) and Co (pCo) normalized to particulate P (pP); (*e,f*) the pigments fucoxanthin (fuco) and 19'-hexanoyloxyfucoxanthin (19hexa), indicators of diatoms and coccolithophores, respectively; and (*g,h*) biogenic silica (bSi) and particulate inorganic carbon (PIC) normalized to particulate organic carbon (POC). All data are from the upper 100 m. The color bar indicates the longitude of samples, with zone A in purple, zone B in dark blue, and zone C in other colors. Surface (~4 m) samples are filled circles, with Zone D surface samples indicated as filled purple and dark blue circles. No bSi, PIC, or POC measurements were made on zone D samples.

(~0.5 nmol/kg). This zone has a lower concentration of diatoms than in the east (lower fucoxanthin and biogenic silica/POC ratio; **Figure 10e,g**) and higher coccolithophore concentrations, with an increase in the 19'-hexanoyloxyfucoxanthin pigment and high particulate inorganic carbon (PIC)/POC ratios (**Figure 10f,h**). The high cellular Cd and Co concentrations may reflect the use of the metals in CDCA and TWCA and possibly in other yet-to-be-discovered functions in coccolithophores.

Further offshore (zone C), all the metal concentrations decrease westward, with dCo and dCd down to a few picomolars and Zn to a few tens of picomolars toward the end of the transect

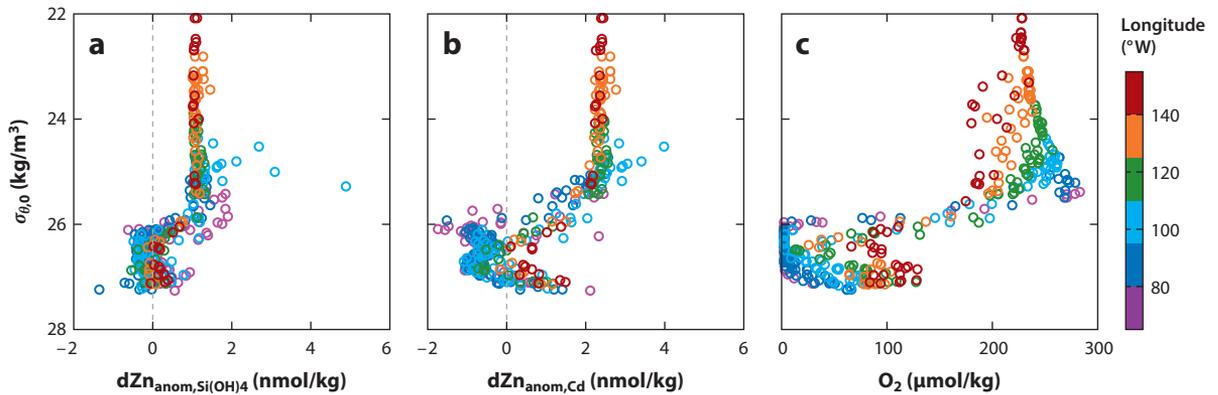


Figure 11

Zn anomalies ($dZn_{anom,X}$) and oxygen concentrations on density surfaces ($\sigma_{\theta,0}$). Zn anomalies are defined as $dZn_{anom,X} = Zn_{obs} - (Zn/X)_{deep} * X_{obs}$, where X is Cd (a), PO_4 (b), and $Si(OH)_4$ (c), and $(Zn/X)_{deep}$ is the slope of the linear relationship between Zn and X between 400 and 1,000 m (see **Figure 1b**). Zn anomalies for all stations (indicated by the color of their longitudes) collapse when plotted against the potential density anomalies relative to the surface instead of depth. Refer to **Figure 8b** for the relationship between depth and $\sigma_{\theta,0}$ in the upper 300 m. The minimum Zn anomalies (greatest deficiencies in Zn) occur between $\sigma_{\theta,0} = 26\text{--}27 \text{ kg/m}^3$, where oxygen concentrations are at a minimum. Abbreviation: dZn, dissolved Zn.

(**Figures 8 and 10**). The cellular concentrations of Co and Cd also decrease along with those of Zn (**Figure 10c,d**), providing no obvious clue regarding their use by the ambient flora. The draw-down of dissolved silicate, Cd, and Co at low dZn we noted in the Introduction is confined largely to the upper mesopelagic (200–400 m) zone of the oligotrophic waters of zone C (**Figure 1a,b**). This feature is unlikely to be tied to the large Zn requirements of fast-growing photosynthetic organisms since it is found below the euphotic zone. Instead, its placement in the upper mesopelagic suggests a water mass and/or remineralization feature that leads to a regeneration in the supply of silicate, Co, and Cd but not Zn. Indeed, culture and field studies show that Zn is rapidly scavenged back onto organic matter during remineralization, a process not observed for Cd or phosphate (John & Conway 2014). It is possible that a nonphotosynthetic biological demand for Zn in this depth zone, such as for Zn-centered proteases, could also explain this feature (Fukuda et al. 2000).

While oligotrophic stations exhibit the lowest absolute dZn concentrations, it is the upwelling and transition zone stations that exhibit the lowest Zn concentrations relative to other macro- and micronutrients. This can be quantified by calculating the Zn anomalies (see the definitions in the caption of **Figure 11**) in the upper water column relative to the deepwater relationships between dZn and phosphate, silicate, or dCd (cf. **Figure 1b**). Negative Zn anomalies, which indicate a relative Zn deficiency, occur between the $26\text{--}27 \text{ kg/m}^3$ potential density surfaces for all stations (**Figure 11**), corresponding to water depths of 250–500 m offshore. Further east, at the upwelling and transition zone stations, these negative anomalies occur at shallower depths (cf. **Figure 8b**) and reach within 15 m of the surface near the coast. Throughout the transect, the region of negative Zn anomalies corresponds to the oxygen minimum zone (**Figure 11**). The cause of these anomalies could be excess Zn uptake by phytoplankton at the base of the euphotic zone, particularly in the upwelling regions of very high productivity (Saito et al. 2010) and/or abiotic scavenging related to low oxygen (Chappell et al. 2016, Conway & John 2014, John & Conway 2014).

In sum, the regions with the most compelling evidence for the biological substitution of Co and Cd for Zn are in the waters of the coastal upwelling and transition zones of this eastern tropical South Pacific transect. With the exception of the surface-most layer, these are not the regions with the lowest Zn concentrations, which are found in the offshore waters of zone C. Rather, these are

the regions in which there is a high enough supply of major nutrients to promote a growth rate that outstrips the diffusive supply of Zn to phytoplankton, as well as an adequate supply of Co and Cd compared to Zn to allow substitution into otherwise Zn-centered enzymes.

Upwelling zones satisfy these requirements by providing a plentiful supply of major nutrients, as well as a ready supply of Co and Cd, which have shallower remineralization depths than Zn. In contrast, oligotrophic gyres, which generally have the lowest dZn concentrations, also have such low supplies of major nutrients that diffusive supply of CO_2 may be able to keep up with the carbon fixation rates of tiny picoplankton. The expected resulting lower demand for CA, as well as low supply of Co and Cd, makes this an unlikely locale to detect the replacement of Zn by Cd or Co in CA, certainly by the crude methods used here.

Similarly, despite the function of Co and Cd as substitutes for Zn in CA, the regions in which this substitution occurs are not necessarily those with the lowest pCO_2 . As noted above, the coastal and upwelling zones that provide compelling evidence of Co or Cd substitution for Zn sometimes have pCO_2 values several times above atmospheric equilibrium. The presumed high demand for CA even in high pCO_2 waters is to keep up with a carbon fixation rate by large diatoms that outstrips the diffusive supply of CO_2 to Rubisco. Other environments that could be visited in future studies of Co and Cd replacement for Zn are those where phytoplankton blooms induce significant depletion of CO_2 . While coastal waters are generally thought to be replete in micronutrients, many observations of metals substitution and sparing mechanisms have been observed in coastal phytoplankton, implying they do experience conditions of metal and, perhaps, carbon scarcity (Mackey et al. 2015, Morel et al. 1994).

CONCLUSION

Laboratory experiments have shown that some phytoplankton species have the ability to utilize Cd and/or Co as partial replacements for Zn in cultures with low bioavailable Zn concentrations. A demonstrated mechanism for this replacement is metal substitution at the active site of CAs: Co for Zn in the δ -CA of many eukaryotic phytoplankton species from different phyla and Cd for Zn in the ζ -CA of diatoms and some other eukaryotic phytoplankton groups. The metal replacement occurs over free metal concentrations that are relevant to field conditions, and DNA and RNA sequences that match those coding for δ -CA and ζ -CA are found in many species and in the few field samples that have been examined. Field data from the GP16 transect of the GEOTRACES program in the South Pacific strongly support the use of Cd by diatoms in the highly productive surface waters off Peru and are consistent with the use of Cd and Co as replacements for Zn further west where both diatoms and coccolithophores are abundant.

The high affinity metal uptake systems of marine phytoplankton appear to have poor selectivity for divalent metals such as Zn^{2+} , Cd^{2+} , and Co^{2+} . It is thus perhaps not surprising that some phytoplankton species growing in the metal-poor waters of the surface ocean have evolved the means to replace one metal by another in some enzymes. The widespread distribution of δ -CA and ζ -CA among eukaryotic phytoplankton phyla indicates that Co and Cd substitution for Zn may be ancient, perhaps reflecting a change in metal availability in the ocean over geological times. In view of the many uses of CAs in organisms, it is possible that substitution of Zn by Cd and Co in phytoplankton may be involved in cellular processes other than inorganic carbon acquisition.

Substitution of Cd and Co for Zn in key biochemical functions provides at least a partial explanation for the paucity of field experiments demonstrating Zn limitation of phytoplankton in regions of the ocean where ambient Zn concentrations are extremely low. (Another explanation is that several of these experiments were conducted in regions that are severely Fe limited and where Zn amendments can have only a minor effect on the growth of the extant phytoplankton

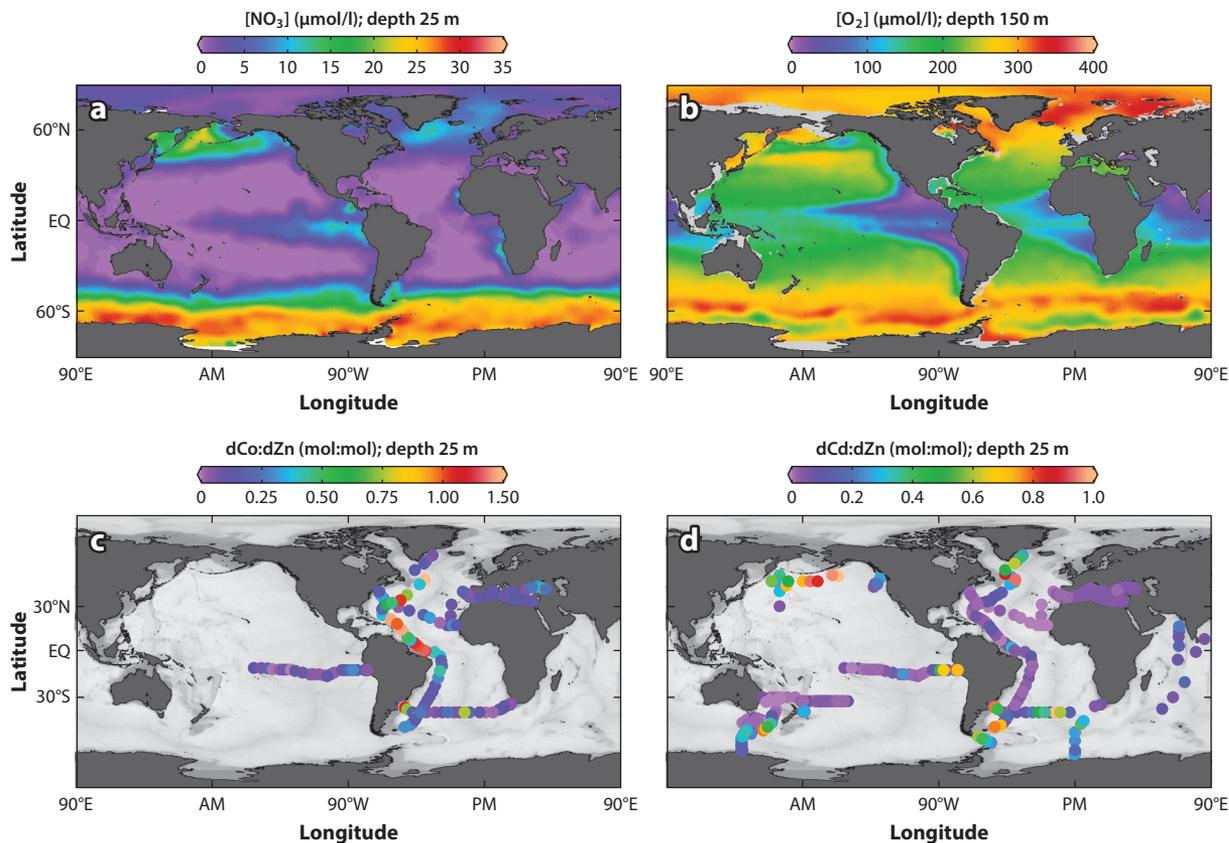


Figure 12

Identifying oceanic regions where Co and Cd may substitute for Zn in biochemical functions. Shown are isosurface maps of (a) nitrate at 25 m and (b) oxygen at 150 m from the *World Ocean Atlas 2009* (Garcia et al. 2010a,b) and the ratios of (c) dissolved Co:dissolved Zn ($d\text{Co}:d\text{Zn}$) and (d) dissolved Cd:dissolved Zn ($d\text{Cd}:d\text{Zn}$) (Schlitzer et al. 2018). Upwelling regions showing residual nitrate whose source waters are low in oxygen may be particularly prone to Zn deficiency. Indeed, these regions often have elevated $d\text{Co}:d\text{Zn}$ and/or $d\text{Cd}:d\text{Zn}$ and may be good places to look for evidence of biochemical substitution of Co and Cd for Zn.

population.) The ability to substitute Cd or Co provides a clear advantage in surface waters with very low Zn concentrations such that Zn limitation should occur only as a transient phenomenon (the ambient flora not being able to substitute other metals for Zn) or as a colimitation by Zn, Co, and Cd when the bioavailable concentrations of all three metals are very low. Future efforts to conduct very demanding Zn (and Cd and Co) amendment experiments in highly productive coastal systems with low $p\text{CO}_2$ could reveal the occurrence of such metal substitutions in phytoplankton.

Based on the observations from the GP16 cruise, one can make some predictions for where else in the ocean Co and Cd may substitute for Zn in CA. **Figure 12** shows the available surface $d\text{Cd}/d\text{Zn}$ and $d\text{Co}/d\text{Zn}$ ratios from the GEOTRACES Intermediate Data Product 2017 (Schlitzer et al. 2018) plotted next to surface nitrate concentrations and oxygen concentrations at 150 m. Upwelling regions with residual surface nitrate whose source waters are low in oxygen may be particularly prone to Zn deficiency. Although the available data are still relatively limited, the western subarctic Pacific and Peru upwelling zone fulfill these criteria and have elevated $d\text{Cd}:d\text{Zn}$ ratios. The subpolar North Atlantic and the Southern Ocean, which do have residual surface nitrate but are not underlain by low oxygen, also have elevated surface $d\text{Cd}:d\text{Zn}$. These

are regions in which high biological productivity and high Zn demand may favor the biochemical substitution of Zn by Cd in CA and possibly other enzymes. While the available Co data are much more sparse, there are regions of elevated dCo/dZn, such as the Mauritanian upwelling region of the eastern tropical Atlantic and the western tropical Atlantic, that do not have elevated dCd/dZn. These are regions in which Co substitution for Zn in CA, and possibly in other enzymes, may be occurring.

The biochemistry of the marine biota, particularly that of phytoplankton, is adapted to the extraordinary impoverishment of biologically essential elements in the surface ocean caused by the sinking of organic matter. Over the past 30 years much of the attention of oceanographers has focused on Fe, an element that accounts for about a third of Earth mass and less than 0.1% of the dry mass of phytoplankton yet limits the productivity of large regions of the oceans. As discussed in this article, the biochemistry of oceanic phytoplankton must also reflect the paucity of other essential elements and involve biomolecules and pathways that are distinct from those in terrestrial microorganisms. The greatly expanded availability of metal concentration data (provided in part by the GEOTRACES program), the novel ability to perform omic analyses of the biota in ocean samples, and the burgeoning availability of tools to transform marine phytoplankton should provide opportunities to address this question.

POSTSCRIPT

Recently, an interesting paper by Jensen et al. (2019) reported a novel Mn-CA in the marine diatom *T. pseudonana*. This enzyme, which has very low identity with other known CAs and cannot use Zn, Cd, or Co as a metal center, plays a role in the CCM. It is widely distributed among phytoplankton taxa and occurs also in marine bacteria and archaea. The high concentration of Mn compared to that of other transition metals in marine surface waters presents an interesting conundrum regarding the simultaneous use of CAs with different metal centers. Localization of the Mn-CA at the periphery of the chloroplast in *T. pseudonana* and preliminary data showing a relatively low specific activity indicate a particular yet unknown role for this enzyme in the CCM (for example, perhaps converting the CO₂ leaking out of the chloroplast into HCO₃⁻). These are exciting times for CA research.

DISCLOSURE STATEMENT

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