The Trace Metal Composition of Marine Phytoplankton

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Abstract

Trace metals are required for numerous processes in phytoplankton and can influence the growth and structure of natural phytoplankton communities. The metal contents of phytoplankton reflect biochemical demands as well as environmental availability and influence the distribution of metals in the ocean. Metal quotas of natural populations can be assessed from analyses of individual cells or bulk particle assemblages or inferred from ratios of dissolved metals and macronutrients in the water column. Here, we review the available data from these approaches for temperate, equatorial, and Antarctic waters in the Pacific and Atlantic Oceans. The data show a generalized metal abundance ranking of $Fe \approx Zn > Mn \approx Ni \approx Cu \gg Co \approx Cd$; however, there are notable differences between taxa and regions that inform our understanding of ocean metal biogeochemistry. Differences in the quotas estimated by the various techniques also provide information on metal behavior. Therefore, valuable information is lost when a single metal stoichiometry is assumed for all phytoplankton.

INTRODUCTION

Trace metals are required for numerous processes in phytoplankton, and the past 30 years have witnessed a rapidly expanding body of research into the ways that metal availability shapes biogeochemical processes. This research has shown that metal availability can limit the growth of phytoplankton in certain environments. In addition, because groups of phytoplankton differ in their trace metal requirements, metal availability shapes the phytoplankton community in ways that influence the biogeochemistry of nonmetals such as C and N. Moreover, the metal requirements of phytoplankton are themselves a function of shifting light, macronutrient, and temperature regimes. Although much has been learned about the metal composition of phytoplankton in the ocean and its influence on patterns in marine chemistry, there has not been a systematic comparison of different lines of evidence concerning the trace element contents of phytoplankton in the ocean. Here, we review the literature on the metal content of phytoplankton determined using a variety of techniques. In so doing, we suggest limits to establishing generalized trace metal stoichiometries, note oceanographically important regional differences in trace metal content that are consistent among approaches, and discuss the implications of differences among methods.

PHYSIOLOGICAL ROLES OF METALS IN PHYTOPLANKTON

The metal quotas of phytoplankton are driven largely by the biochemical demands of the cells. Fe is required for many processes, including C and N fixation, nitrate and nitrite reduction, chlorophyll synthesis, and the electron transport chains of respiration and photosynthesis (**Table 1**). The electron flow associated with photosystems I and II alone requires nearly 22 atoms of Fe per complex (Raven et al. 1999). Fe is also incorporated into several enzymes used by cells to deal with reactive oxygen species, and the enzyme that catalyzes N fixation, nitrogenase, requires 38 Fe atoms per holoenzyme complex (Whittaker et al. 2011); these uses impart a significant Fe requirement to diazotrophs. Zn is also involved in a plethora of cell functions, often owing to its ability to function as a Lewis acid (Frausto da Silva & Williams 2001.)

The roles of other metals in cellular biochemistry are somewhat more specific and limited. Mn is involved most prominently in the O_2-evolving complex of photosystem II, and the Mn form of superoxide dismutase is also common in diatoms (Wolfe-Simon et al. 2005). Cu is used in both photosynthetic and respiratory electron transport chains as well as in the Cu-Zn form of superoxide dismutase, and it is thought to play a role in the transmembrane uptake of Fe (La Fontaine et al. 2002, Maldonado et al. 2006). Ni is associated primarily with urease and the Ni form of superoxide dismutase, whereas Co is incorporated into vitamin B_{12}. To date, the only confirmed use of Cd in phytoplankton is as a substitute for Zn in carbonic anhydrase in diatoms (Lane et al. 2005). There are also likely to be many other metalloproteins involved in phytoplankton biology that have yet to be discovered (Cvetkovic et al. 2010).

In some instances, metals may substitute for one another in proteins in response to changing environmental availability, altering cellular quotas. For example, Co and Cd may substitute for Zn in carbonic anhydrase in diatoms (Price & Morel 1990, Lane & Morel 2000). In other cases, entirely different proteins may substitute for one another if a metal is limiting. Oceanic diatoms that have evolved in low-Fe waters appear to have substituted the Cu-containing enzyme plastocyanin for the Fe-containing enzyme cytochrome c_6 in photosystem II (Peers & Price 2006). Other phytoplankton can substitute the metal-free protein flavodoxin for the Fe-S protein ferredoxin in photosystem I (LaRoche et al. 1996, McKay et al. 1999) or reduce the abundance of Fe-rich photosystem I relative to photosystem II (Strzepek & Harrison 2004). Limiting concentrations of one metal may also affect the requirements for another metal. Fe limitation increases oxidative

Table 1 Common metalloproteins present within marine phytoplankton

Metal	Protein(s)	Function(s)
Fe	Cytochromes	Electron transport in photosynthesis and respiration
	Ferredoxin	Electron transport in photosynthesis and N fixation
	Other Fe-S proteins	Electron transport in photosynthesis and respiration
	Nitrate and nitrite reductase	Conversion of nitrate to ammonia
	Chelatase	Porphyrin and phycobiliprotein synthesis
	Nitrogenase	N fixation
	Catalase	Conversion of hydrogen peroxide to water
	Peroxidase	Reduction of reactive oxygen species
	Superoxide dismutase	Disproportionation of superoxide to hydrogen peroxide and O_2
Zn	Carbonic anhydrase	Hydration and dehydration of carbon dioxide
	Alkaline phosphatase	Hydrolysis of phosphate esters
	RNA polymerase	Nucleic acid replication and transcription
	tRNA synthetase	Synthesis of tRNA
	Reverse transcriptase	Synthesis of single-stranded DNA from RNA
	Carboxypeptidase	Hydrolysis of peptide bonds
	Superoxide dismutase	Disproportionation of superoxide to hydrogen peroxide and O_2
Mn	O_2-evolving enzyme	Oxidation of water during photosynthesis
	Superoxide dismutase	Disproportionation of superoxide to hydrogen peroxide and O_2
	Arginase	Hydrolysis of arginine to ornithine and urea
	Phosphotransferases	Phosphorylation reactions
Ni	Urease	Hydrolysis of urea
	Superoxide dismutase	Disproportionation of superoxide to hydrogen peroxide and O_2
Cu	Plastocyanin	Photosynthesis electron transport
	Cytochrome oxidase	Mitochondrial electron transport
	Ascorbate oxidase	Ascorbic acid oxidation and reduction
	Superoxide dismutase	Disproportionation of superoxide to hydrogen peroxide and O_2
	Multicopper ferroxidase	High-affinity transmembrane Fe transport
Co	Vitamin B_{12}[a]	C and H transfer reactions
Cd	Carbonic anhydrase[b]	Hydration and dehydration of carbon dioxide
Mo	Nitrate reductase	Conversion of nitrate to ammonia
	Nitrogenase	N fixation

Adapted from Sunda (1988/1989), with additional information from Raven et al. (1999), Frausto da Silva & Williams (2001), and Wolfe-Simon et al. (2005).

[a] Cofactor in a number of enzymes.

[b] Has been found only in diatoms (Price & Morel 1990, Lane & Morel 2000).

stress in diatoms, and as a consequence more Mn is required for superoxide dismutase (Peers & Price 2004). A Cu-containing enzyme is likely involved in the high-affinity Fe uptake system of oceanic diatoms, and therefore low-Fe conditions increase the Cu requirements of these species (Maldonado et al. 2006, Annett et al. 2008).

Metals are also involved in accessing organic macronutrients, and thus metal requirements associated with these functions vary according to macronutrient availability. For example, Fe is required for nitrate reductase, and therefore the Fe requirements of phytoplankton grown on nitrate are higher than those of phytoplankton grown on ammonia (Maldonado & Price 1996). Zn is typically required for alkaline phosphatase (although Co may substitute; Wojciechowski et al. 2002, Gong et al. 2005, Jakuba et al. 2008), and the Zn, Co, and Cd contents of cells appear to increase under low inorganic P availability (Ji & Sherrell 2008, Twining et al. 2010). Ni requirements also likely become more significant as cells adjust to growth on organic N substrates (Price & Morel 1991; Dupont et al. 2008, 2010a; Twining et al. 2010). Furthermore, phytoplankton requirements for metals used in carbonic anhydrase appear to change according to the availability of inorganic C (Cullen et al. 1999, Sunda & Huntsman 2005, King et al. 2011). Perhaps the most well-studied link is between Fe and N fixation by diazotrophs: Owing to the high Fe content of nitrogenase, the Fe quotas of diazotrophs increase substantially when they fix N instead of growing on reduced N sources (Kustka et al. 2003). The Mo content of these cells also increases as a result of this activity owing to the Mo requirements of nitrogenase (Tuit et al. 2004).

ACCUMULATION OF METALS IN CULTURED PHYTOPLANKTON

Work with laboratory cultures has provided the range of metal quotas that may be observed and demonstrated a general ranking of metal abundances in cells. Phytoplankton metal quotas are typically considered following normalization to a proxy of cell biomass such as C or P. Here, we use metal:C or metal:P ratios interchangeably to represent cellular quotas, although these constituents can vary with respect to each other as well. We focus on P-normalized data, as P is often measured simultaneously with metals, reducing error.

The concentration of ambient dissolved metal (in particular that of the inorganic species or the free metal ion) is the primary factor controlling cellular metal contents in the environment. **Figure 1** shows the influence of the free metal ion concentration on metal quotas for the major bioactive trace metals, combining data collected for the model centric diatoms *Thalassiosira pseudonana*, *T. weissflogii*, and *T. oceanica* grown in ethylenediaminetetraacetic acid (EDTA)–buffered media. For some metals (e.g., Zn, Cd), cellular concentrations increase in direct proportion to free metal ion concentrations, whereas for others (e.g., Fe, Mn, Cu), the cells appear to regulate cellular concentrations even at low levels, likely through downregulation of cross-membrane transporters. Quotas often approach an asymptote at free metal ion concentrations above those needed to sustain maximal growth (e.g., Zn, Mn, Co). However, in some cases internal metal quotas increase with ambient free ion concentrations even above those needed to sustain maximal growth, albeit at a slower rate than is observed at lower concentrations. This pattern of luxury uptake is most obviously displayed by diatoms accumulating Fe (Sunda & Huntsman 1995b; Marchetti et al. 2006, 2009). The cellular contents of one metal can also be strongly influenced by the availability of another via competition for transport through transmembrane channels between ions with similar hydrated cross sections, coordination chemistry, or charge, allowing the concentration of one ion to influence the uptake of another. Such an interaction is observed among Zn, Co, and Cd (Sunda & Huntsman 1998a,b).

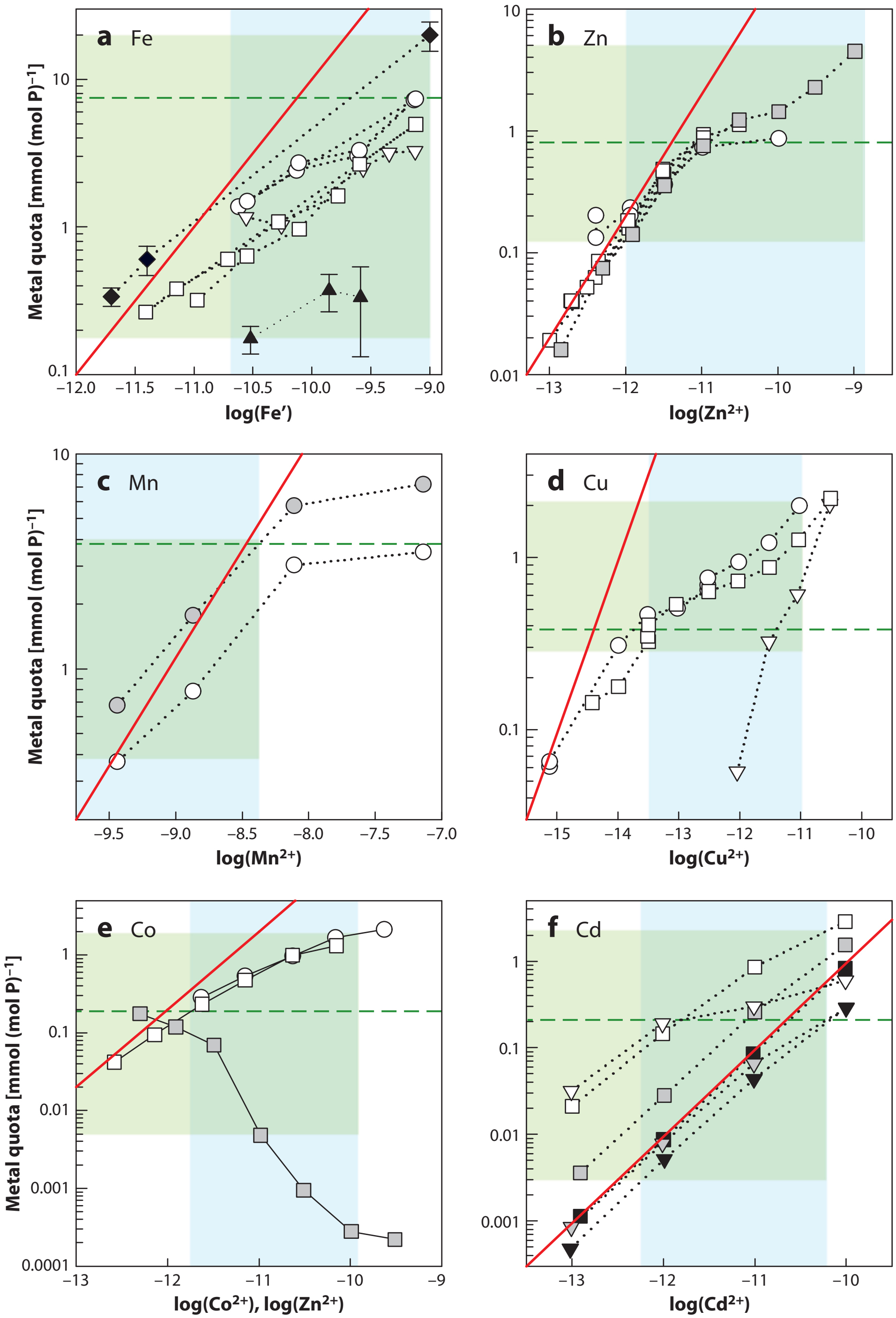
Cellular metal contents are often negatively correlated with light (Sunda & Huntsman 1998c, Finkel et al. 2006) (**Figure 1**). Although this pattern could indicate the upregulation of metal-rich photosynthetic apparatus under low light (Raven 1988, 1990), net Fe uptake rates per unit of surface area appear to remain constant, suggesting that the observed pattern reflects light-driven changes in C uptake and, therefore, the ratio of metal to C assimilation (Sunda & Huntsman 1997, 1998c). The importance of such a dilution effect is suggested by the tendency for metals to exhibit lower quotas under elevated light levels regardless of whether those metals are employed in photosynthesis, although there are exceptions to this rule (Finkel et al. 2006). Interestingly, at light levels below 50 μmol m^{-2} s^{-1} there is some indication of a net increase in uptake of many metals relative to P, many of which are not related to photosynthesis (Finkel et al. 2006). One possible explanation for such a general increase in metal uptake is a facultative increase in the storage of cellular trace metals under low growth via nonspecific mechanisms. Theoretically, dilution of cellular metal could then occur in response to the release of cells from nutrient limitation if metal uptake remains unchanged as cell growth increases.

Differences in the metal contents of distinct taxonomic groups have been observed in cultured phytoplankton, and these differences can be as large as or larger than variations caused by environmental factors. One major divide is thought to be between prokaryotes and eukaryotes. Prokaryotes such as *Synechococcus* are thought to have higher Fe contents than eukaryotes (Brand 1991, Wilhelm et al. 1996), potentially owing to the absence of C-rich structural moieties. Prokaryotes also likely have a lower Zn quota on average, given the much-reduced abundance of Zn finger proteins relative to eukaryotes (Sunda & Huntsman 1995a, Saito et al. 2002, Dupont et al. 2010b). These taxonomic trends would match those predicted by the environmental conditions present during these groups' early evolution (Frausto da Silva & Williams 2001, Saito et al. 2003).

Variation in metal content is also observed among phylogenetically divergent eukaryotic algal species, and this has been related to the evolutionary origin of the plastids within the cells, which can be separated into red and green lines based on pigment composition (Quigg et al. 2003). When grown under Fe-replete conditions, algae with red-line plastids, such as diatoms and cryptomonads, tend to have lower Fe:P and Mn:P and higher Cd:P, Co:P, and Mo:P compared with those with green-line plastids, such as chlorophytes and prasinophytes. Dinoflagellates containing red- or green-line plastids differ accordingly. However, there is a substantial amount of variability within groups that remains unexplained (Ho et al. 2003).

The potential for variation in metal quotas among species is most apparent for Fe because cellular contents have been measured for this metal in a wide number of species under a wide range of inorganic ion availabilities. Even within diatoms, Fe quotas for cells grown at a particular Fe concentration can vary by over an order of magnitude (**Figure 1**). Much of this variation reflects the area from which taxa were isolated, suggesting genotypic adaptation to local conditions. For example, *T. oceanica*, isolated from the oligotrophic North Atlantic, has significantly lower Fe quotas than the coastal strains *T. pseudonana* and *T. weissflogii* at low ambient Fe (Sunda et al. 1991), and diatoms isolated from low-Fe Southern Ocean waters (both centric and pennate forms) appear to have uniquely low Fe quotas (**Figure 1**). At the other end of the environmental gradient, some pennate diatoms have an enhanced ability to store excess Fe as ferritin for later use (Marchetti et al. 2009); the elevated Fe quotas of these pennates are also evident in **Figure 1**. Although centric diatoms can also store luxury Fe—potentially in vacuoles (Kustka et al. 2007, Nuester et al. 2012b)—this ability falls short of that in pennates.

Work with laboratory cultures has thus shown that ocean phytoplankton are likely characterized by a range of metal quotas. Although Fe appears to be the most abundant, followed by Zn, then Mn and Cu, and then Co and Cd, quotas of each of these metals may span one to two orders of magnitude. It is evident from the shaded regions in **Figure 1** that Zn, Mn, and Cu vary in *Thalassiosira* spp. diatoms by approximately an order of magnitude across the range of ambient


a Fe
Metal quota [mmol (mol P)−1]
10
1
0.1
−12.0
−11.5
−11.0
−10.5
−10.0
−9.5
−9.0
log(Fe′)
b Zn
10
1
0.1
0.01
−13
−12
−11
−10
−9
log(Zn2+)
c Mn
Metal quota [mmol (mol P)−1]
10
1
−9.5
−9.0
−8.5
−8.0
−7.5
−7.0
log(Mn2+)
d Cu
1
0.1
−15
−14
−13
−12
−11
−10
log(Cu2+)
e Co
Metal quota [mmol (mol P)−1]
1
0.1
0.01
0.001
0.0001
−13
−12
−11
−10
−9
log(Co2+), log(Zn2+)
f Cd
1
0.1
0.01
0.001
−13
−12
−11
−10
log(Cd2+)

concentrations, whereas Fe ratios vary by two orders of magnitude across a smaller concentration range. Co and Cd quotas each potentially vary by more than a factor of 100, and quotas of both metals are strongly negatively related to Zn. The ranges of possible quotas shown in **Figure 1** envelop the averaged metal stoichiometry proposed by Ho et al. (2003) for marine phytoplankton.

METAL QUOTAS OF PHYTOPLANKTON IN NATURAL COMMUNITIES

Until recently, it was impossible to determine whether the trace metal contents of living cells in situ resemble the values inferred from cultured species or bulk particulate analyses, and whether substantial variation exists among taxa or functional types. The lack of information about elemental quotas of field-collected cells meant that extrapolations from culture studies remained unverified. Although culture studies are extremely useful for probing mechanisms affecting trace element quotas, the extrapolation of culture-based quotas to field conditions is subject to several potential biases. First, the species that can be cultured are certainly not a random selection of those that constitute natural communities. Second, species can undergo significant evolution in culture over time, a problem that is particularly pertinent to several model species that have been isolated from the wild for decades. Third, culture conditions cannot replicate the wide range of physical, chemical, and biological processes affecting metal availability and acquisition in situ. And finally, monospecific cultures can only reveal changes in quotas of single species in response to ambient conditions; they do not address ecological mechanisms that may cause species with distinct quotas to replace one another over environmental gradients. The lack of information on metal quotas also confounds interpretations of patterns in particulate chemistry. To what degree are such patterns

←

Figure 1

P-normalized Fe, Zn, Mn, Cu, Co, and Cd quotas of cultured diatoms as a function of free (Me^{n+}) or inorganic (Fe') dissolved metal concentrations. Circles, inverted triangles, and squares indicate quotas for *Thalassiosira pseudonana*, *T. weissflogii*, and *T. oceanica*, respectively; in panel *a*, black diamonds and black triangles indicate the mean quotas of oceanic *Pseudo-nitzschia* spp. diatoms and Southern Ocean diatoms, respectively. Red lines indicate a 1:1 relationship between metal concentration and quota. Blue regions indicate the approximate range of open ocean metal concentrations, and green regions indicate the corresponding range of metal quotas as indicated by the plotted data. Dashed green lines indicate the average metal stoichiometries for cultured phytoplankton reported by Ho et al. (2003). (*a*) Data from Sunda & Huntsman (1995b), Marchetti et al. (2006), and Strzepek et al. (2011). Fe' concentrations are taken from Bruland & Lohan (2003), assuming that dissolved Fe in the ocean approximates Fe' in ethylenediaminetetraacetic acid (EDTA)–buffered media (following Bruland et al. 2001). (*b*) Data from Sunda & Huntsman (1995a). White and gray symbols denote Co^{2+} concentrations in the media of $10^{-13.6}$ and 10^{-12} M, respectively. The range of Zn^{2+} concentrations is taken from calculations in Sunda & Huntsman (2000). (*c*) Data from Sunda & Huntsman (1998c). White and gray symbols denote *T. pseudonana* grown at 500 and 70 μmol quanta m^{-2} s^{-1}, respectively. (*d*) Data from Sunda & Huntsman (1995c). The lower end of the Cu^{2+} range is taken from Coale & Bruland (1988), and the upper end is calculated assuming the same fraction of free Cu (0.2%) applied to the upper dissolved Cu concentration of Bruland & Lohan (2003). (*e*) Data from Sunda & Huntsman (1995a). White symbols indicate the response of Co quotas to increasing concentrations of Co^{2+} in the presence of 10^{-13} M Zn^{2+}; gray symbols indicate the response of *T. oceanica* grown at 10^{-12} M Co^{2+} to increasing concentrations of Zn^{2+}. The range of Co^{2+} was calculated using the dissolved Co concentrations from Bruland & Lohan (2003), with the assumption that 40% of dissolved Co is labile Co^{2+} (Saito et al. 2005). (*f*) Data from Sunda & Huntsman (2000). White, gray, and black symbols denote Zn^{2+} concentrations in the media of $10^{-11.4}$, $10^{-10.4}$, and $10^{-9.4}$ M, respectively. The range of oceanic Cd^{2+} concentrations is taken from Bruland & Lohan (2003), with the assumption that 30% of dissolved Cd is bound to organic ligands (Sunda & Huntsman 2000). In all instances, P-normalized quotas were calculated from published C-normalized quotas using the Redfield ratio of 106C:1P.

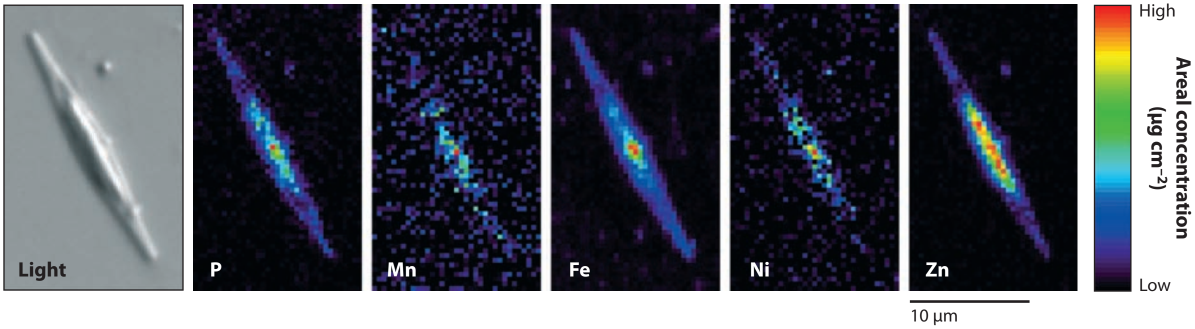


Figure 2

Light micrograph and synchrotron X-ray fluorescence (SXRF) false-color element maps of a pennate diatom collected from the equatorial Pacific. The colors show the areal concentrations of each element; the absolute color scales vary between elements.

determined by shifts in the abundance of living versus nonliving material, or indicative of shifts in the relative importance of different functional groups or species within functional groups?

To specifically address these questions, several microanalytical techniques have been used to determine the elemental and biochemical composition of single cells in situ over the past two decades. Most of these methods use a focused beam of X-rays, electrons, or protons to probe a sample (**Figure 2**). Elemental composition is determined via spectroscopic analysis of fluorescent X-rays that are produced as atoms that have been excited by the incident beam return to their ground state. Of the methods for generating X-ray fluorescence, hard X-ray beams (>6 keV) are the most sensitive for transition metals (Jones 2002, Twining et al. 2008). Owing to their transparency to organic matter, they also provide more synoptic measurements of cellular metal content and impart less radiation damage to cells than ion probes do. The development of Fresnel zone plate optics capable of submicron spatial resolution and the advent of third-generation synchrotron X-ray sources provided the necessary sensitivity to analyze nano- and picoplankton (Twining et al. 2003, 2010). Although C and N cannot be detected via synchrotron X-ray fluorescence (SXRF), elemental quotas can be normalized to P and S (as a proxy for protein), cell biovolume, or dry mass as determined by the phase shift of X-rays passing through the sample (Holzner et al. 2010). SXRF has been used to assay the metal contents of free-living cyanobacteria, diatoms, autotrophic flagellates, and heterotrophic flagellates from several putatively Fe-limited regions, including the eastern equatorial Pacific and the Pacific sector of the Southern Ocean south and north of the polar front as well as temperate waters of the Pacific off New Zealand. It has also been used to assess stoichiometric responses to Fe addition.

Metal quotas of ocean phytoplankton determined with SXRF demonstrate regional variability in some metals that match expected trends in dissolved metal availability (**Figure 3**). Fe quotas for autotrophic flagellates and cyanobacteria were found to be higher in temperate waters off New Zealand than in persistently Fe-limited regions of the equatorial Pacific and the Southern Ocean. Gradients in diatom Fe contents were also observed, with the lowest Fe quotas in the high-nutrient, low-chlorophyll (HNLC) region south of the Antarctic polar front. This supports culture data demonstrating that diatoms in the Antarctic have lower Fe requirements than diatoms in other regions (Strzepek et al. 2011). In contrast, cellular Zn:P ratios for Southern Ocean autotrophic flagellates were found to be higher in those south of the polar front than in those north of it, and Zn:P ratios in diatoms and autotrophic flagellates from temperate and equatorial Pacific waters were in turn lower than in those from the Southern Ocean. This matches dissolved Zn concentrations, which are high in the Southern Ocean south of the polar front and decline

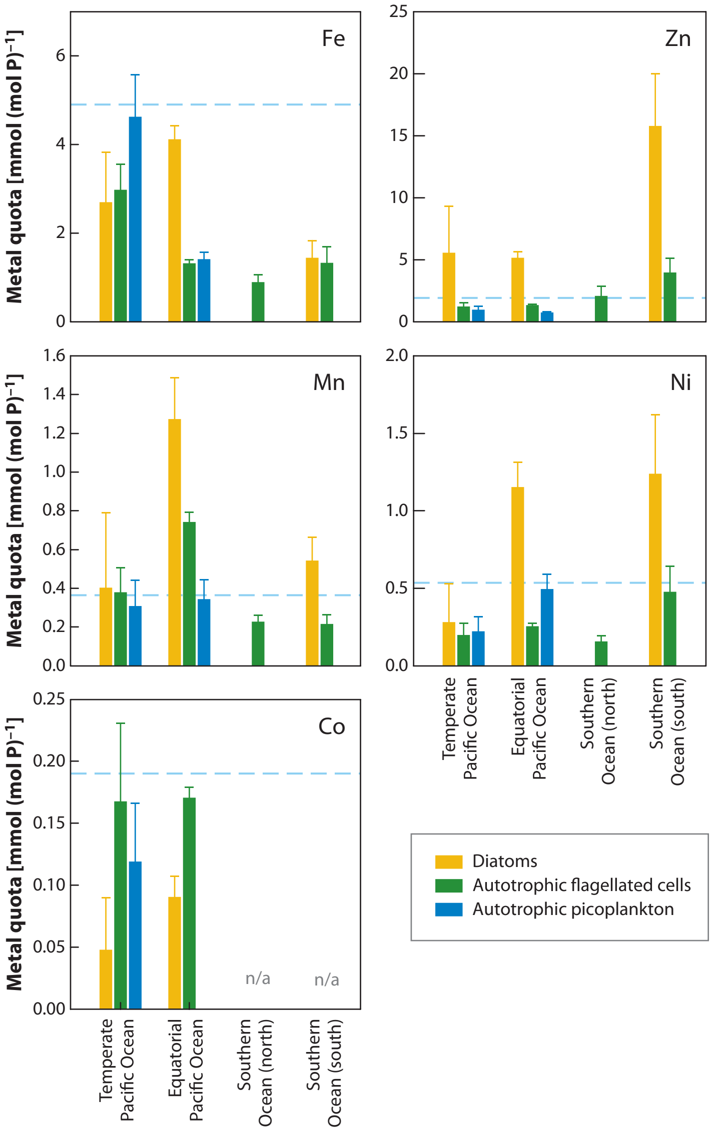


Figure 3

Taxonomic and regional variations in P-normalized metal quotas of diatoms, autotrophic flagellated cells, and autotrophic picoplankton collected from three different regions of the Pacific basin: temperate Pacific waters, equatorial Pacific waters, and the Southern Ocean. Data for temperate waters are from the FeCycle II project conducted 280 km east of the North Island of New Zealand (King et al. 2012). Metal quotas of equatorial Pacific cells are from Twining et al. (2011). Metal quotas for Southern Ocean plankton collected either north or south of the polar front are from Twining et al. (2004a,b), modified following recalculation of cellular P quotas using the same methods (Nuñez-Milland et al. 2010) as in the other studies plotted here to maximize comparability. These calculations resulted in approximately twofold increases in the P-normalized metal quotas of some cells. Bars are geometric means ± SE. Dashed blue lines show the average bulk plankton stoichiometries as summarized by Bruland et al. (1991). n/a, data not available.

northward owing to utilization and dilution with Zn-poor northern waters (Bruland 1980, Ellwood 2004, Croot et al. 2011).

A lack of information on availability limits the interpretation of regional differences in quotas of other metals. The elevated Ni contents of autotrophic flagellates south of the polar front may reflect greater availability in this upwelling region, but gradients in dissolved Ni in this region are not well documented. Likewise, the high Mn:P ratios of diatoms and autotrophic flagellates in the equatorial Pacific could reflect a greater supply of Mn to surface waters from the equatorial undercurrent. However, physiological arguments involving a greater need for O_2-evolving complexes or Mn superoxide dismutases under high-light conditions cannot be ruled out.

Besides significant interregional differences in quotas, different functional groups often exhibit systematic differences in metal quotas within a region, suggesting that changes in phytoplankton community structure can have a substantial impact on community average metal stoichiometries that is quite separate from the impacts of metal availability (**Figure 3**). Oceanic diatoms in particular frequently have higher metal quotas (normalized to P) than do other co-occurring cell types, a result that contradicts data from work with cultures (Ho et al. 2003). For example, Zn:P ratios in free-living diatoms are consistently at least four times greater than in co-occurring autotrophic flagellates and picoplankton at all sites. Zn:P ratios in picoautotrophs, in contrast, tend to be lower than those in co-occurring eukaryotic nanoplankton, much as has been reported for cultured strains (Saito et al. 2003). Lower Zn:P ratios in these cells may reflect the reduced importance of Zn finger proteins and Zn superoxide dismutase in prokaryotes compared with eukaryotes. Although Ni:P ratios in temperate diatoms are indistinguishable from those of other co-occurring cell types, they are at least five times higher than those of autotrophic flagellates in the two Fe-limited regions. The existence of high diatom Ni quotas under Fe limitation could indicate a greater dependence on reduced N sources in these regions, given the high Fe requirement for assimilatory nitrate reduction and the need for Ni as a cofactor for urease. Ni:P ratios in picoautotrophs are typically higher than those in nanoflagellates, perhaps reflecting the importance of Ni superoxide dismutase and reduced N to these prokaryotic organisms. Higher Mn:P ratios have also been observed in diatoms in the Southern Ocean and the equatorial Pacific, but not in the temperate Pacific. In contrast, autotrophic flagellates have higher Co quotas than other functional groups in both temperate and equatorial Pacific waters.

Substantial differences among species within a taxonomic group are also apparent in some cases, which may influence interregional patterns in community stoichiometries if a single species dominates the signal. The effect of species composition is particularly pronounced for Fe quotas among the diatoms. For example, in the Southern Ocean the geometric mean Fe:P in Fe-limited pennate diatoms (mostly *Fragilariopsis cylindrus*) was found to be 0.8 mmol mol^{-1} (± 1 SE $=$ 0.6–1.0 mmol mol^{-1}), whereas the value for co-occurring Fe-limited centrics was 3.1 mmol mol^{-1} (± 1 SE $=$ 2.4–4.1 mmol mol^{-1}). This fourfold difference among groups was larger (and statistically more significant) than the two- to threefold increases in Fe:P within each group following mesoscale Fe addition (Twining et al. 2004b). The rather high Fe contents exhibited by the diatom community in the equatorial Pacific may reflect the dominance of *Pseudo-nitzschia*, a genus known to store Fe intracellularly in association with ferritin (Marchetti et al. 2009). Indeed, additions of Fe to deck-board incubations resulted in 20-fold increases in Fe:P ratios for these cells, whereas a mesoscale Fe addition to the Southern Ocean resulted in a more modest 5-fold increase in SXRF samples dominated by *F. cylindrus* (Baines et al. 2011). In both the equatorial Pacific and the Southern Ocean, then, there is evidence for a wide variation in Fe:P ratios within diatoms that seems to reflect distinct strategies for dealing with Fe-limited conditions. This interspecific variation implies a substantial and generally poorly understood role for community structure in determining the average Fe:P of suspended and sinking particles.

ESTIMATES OF PHYTOPLANKTON METAL QUOTAS FROM BULK GEOCHEMICAL DATA

Phytoplankton metal concentrations have also been studied through the analysis of bulk particulate matter in settings where lithogenic and detrital contributions are expected to be minimal. Given the trace quantities of biogenic metals in phytoplankton (typically $<$1–100 ppm) and the low concentrations of phytoplankton in most open ocean systems (typically $<$10 μM particulate organic C), it is often necessary to concentrate plankton from significant volumes of seawater in order to obtain adequate quantities for these analyses. Approaches have ranged from towing nets through the water column to collecting particles in situ with submerged pumps to performing continuous in-line centrifugation of particles. Recent studies have been able to quantify most trace metals from smaller volumes of water collected with standard GO-Flo bottles (Cullen & Sherrell 1999, Twining et al. 2011, Planquette & Sherrell 2012). Early studies collected larger particles (e.g., $>$40 μm) to minimize mineral contaminants (Martin & Knauer 1973, Collier & Edmond 1984), and tracers of lithogenic materials such as Al and Ti are also used to identify and discard or correct samples with significant lithogenic fractions.

Bruland et al. (1991) summarized the results of several early particulate studies, limiting themselves to samples with particulate Al concentrations of $<$100 μg g^{-1} dry wt. Here, we add data from more recent studies focusing on metals in phytoplankton (**Table 2**). Plankton communities appear to be characterized by a generalizable metal abundance ranking of $\mathrm{Fe} \approx \mathrm{Zn} > \mathrm{Mn} \approx \mathrm{Ni} \approx \mathrm{Cu} \gg \mathrm{Co} \approx \mathrm{Cd}$. Fe is the most abundant metal in nearly all particulate matter, followed closely by Zn. Mn, Ni, and Cu are present at concentrations two- to fivefold lower, and Co and Cd appear to be present at concentrations approximately an order of magnitude lower. The similarity of Fe:P ratios reported in these studies is surprising given the diversity of settings and samples collected as well as the variations observed in different conditions and among species in culture and in situ using SXRF. Collier & Edmond (1984) collected primarily zooplankton in the low-Fe equatorial Pacific, whereas Kuss & Kremling's (1999) samples consisted primarily of small flagellated phytoplankton from the Fe-replete North Atlantic, and their averaged Fe:P ratios agreed within 10%. Martin & Knauer (1973) collected primarily large diatoms during a bloom in coastal Monterey Bay, and their overall Fe:P ratio was also within 10% of the others.

These sites were characterized by different phytoplankton communities, but the constancy across sites raises the possibility that there may be a biologically ideal Fe:P, which is maintained as species that can easily achieve this ratio under the given conditions replace others that cannot. Alternatively, these similarities may simply be fortuitous. Daily measurements of bulk size-fractioned samples collected during a spring bloom in temperate Pacific waters found that the Fe:P ratios in the nanoplankton fraction (2–20 μm) varied by more than an order of magnitude, with overall average ratios ranging from 7 to nearly 50 mmol mol^{-1} (King et al. 2012). Bulk Fe:P ratios can go even higher as detrital and lithogenic fractions increase, but they rarely drop below 3–5 mmol mol^{-1} even in Antarctic waters (e.g., Lin et al. 2011).

As seen in the single-cell analyses, there are significant interbasin differences in bulk particulate metal stoichiometries, which may indicate unique biogeochemical processes in these regions. With the exception of Fe, the P-normalized stoichiometries of the metals vary by factors of 3 to nearly 20. Zn and Cd vary by the largest degree, with both metals significantly enriched in Fe-limited Southern Ocean plankton compared with rapidly growing (and presumably Fe-replete; Hutchins et al. 1998) large coastal diatoms. As noted above, high Zn:P ratios in Southern Ocean phytoplankton may be driven by elevated dissolved Zn concentrations. However, Cullen et al. (2003) also postulated that Fe limitation of phytoplankton growth rates in this area slows the

Table 2 Plankton metal stoichiometries determined in bulk particle studies from coastal and open ocean sites in three ocean basins

Site	Metal:P ratio (mmol mol^{-1}) Fe	Zn	Mn	Ni	Cu	Co	Cd	Reference(s)
North Atlantic Ocean (mostly flagellated cells)[a]	4.6	1.9	1.6	1.4	0.37	0.19	0.51	Kuss & Kremling 1999
Monterey Bay (diatom bloom)[b]	5.2	0.84	0.33	0.21	0.18	—	0.07	Martin & Knauer 1973
Equatorial Pacific Ocean (mostly zooplankton)[c]	4.9	3.2	0.35	0.97	0.48	—	0.56	Collier & Edmond 1984
Equatorial Pacific Ocean (phytoplankton)	4.7	—	0.53	1.1	—	0.07	—	Twining et al. 2011
Southern Ocean (large diatoms)[d]	—	13.3	–	0.68	2.0	—	0.07	Collier & Edmond 1984
Southern Ocean (large diatoms)[e]	—	11.1	1.7	—	1.44	0.15	1.29	Cullen et al. 2003
North Atlantic Ocean (*Trichodesmium*)[f]	5–31	0.2–13	1–5	1–8	0.4–2.1	0.01–0.15	0.02–0.13	Tovar-Sanchez et al. 2006, Nuester et al. 2012a

[a] Data for biogenic samples, as reported by Kuss & Kremling (1999, table 4).

[b] Data for samples with Al $< 100\ \mu\mathrm{g\ g^{-1}}$ dry wt as reported by Bruland et al. (1991) because P was not reported by Martin & Knauer (1973).

[c] Data for equatorial Pacific samples with Al $< 100\ \mu\mathrm{g\ g^{-1}}$ dry wt ($n = 3$).

[d] Sample collected from Antarctic upwelling waters (station M) with high dissolved metal concentrations but low lithogenic inputs; the Fe data are not shown owing to significant Al concentrations in the particulate matter, which suggest the inclusion of significant lithogenic material.

[e] Data from Cullen et al. (2003, table 3).

[f] Data are the 25th and 75th quartiles for all *Trichodesmium* data reported by Tovar-Sanchez et al. (2006, appendix) combined with data from Nuester et al. (2012a, table 3).

effect of growth dilution and allows metals to accumulate relative to organic biomass. Indeed, when Fe was added to an ambient community, the particulate Zn:P, Mn:P, Cu:P, Co:P, and Cd:P ratios all decreased in response to phytoplankton growth, falling in line with the stoichiometries measured in Monterey Bay and the equatorial Pacific (Cullen et al. 2003). It is worth noting here that mesoscale Fe addition in the Southern Ocean resulted in *increasing* Zn, Mn, and Ni quotas of resident phytoplankton during a separate study, indicating that a variety of cellular responses are possible if cell metabolism is stimulated by Fe (Twining et al. 2004a, Marchetti et al. 2012).

Estimates of cellular metal stoichiometries in ocean communities can also be obtained from regressions of dissolved metal concentrations against dissolved macronutrients in vertical profiles, as the accumulation and subsequent remineralization of metals in deeper waters impart a geochemical signature of phytoplankton element stoichiometries on the upper water column. Such remineralization ratios should reflect the composition of the phytoplankton community under situations of minimal lateral and vertical advective and diffusive inputs. The imprint on dissolved metal profiles is easiest to detect for the metals that are associated primarily with organic fractions of cells [Zn, Ni, Cu, Cd (Collier & Edmond 1984)] rather than those that have corresponding redox/scavenging processes acting on them [Fe (Johnson et al. 1997), Mn (Landing & Bruland 1980)].

Table 3 P-normalized metal stoichiometries for the North Atlantic, North Pacific, and Southern Oceans, as calculated from regressions of dissolved concentrations in the upper water column (typically <800 m)

Site	Metal:P ratio (mmol mol^{-1})[a]						References
	Fe[b]	Zn[c]	Ni[d]	Cu[e]	Co[f]	Cd[g]	
North Atlantic Ocean	1.1 ± 0.4	2.6 ± 1.0	1.6 ± 0.1	0.30	0.061	0.24 ± 0.12	Bruland & Franks 1983, Yeats & Campbell 1983, Martin et al. 1993, Sunda 1997, Lane et al. 2009
North Pacific Ocean	0.5 ± 0.3	3.9 ± 1.2	1.0 ± 0.1	0.41 ± 0.08	0.038 ± 0.002	0.40 ± 0.11	Sclater et al. 1976, Bruland 1980, Martin et al. 1989, Sunda 1997, Lane et al. 2009
Southern Ocean	0.2 ± 0.04	6.0 ± 2.6	1.8 ± 0.1	0.53 ± 0.13	0.041 ± 0.005	0.65 ± 0.30	Sunda 1997, Löscher 1999, Ellwood 2008, Lane et al. 2009, Saito et al. 2010, Bown et al. 2011, Croot et al. 2011

[a] Mean ± SD for multiple profiles, when available.

[b] Data from Sunda (1997), calculated from profiles of Fe and apparent oxygen utilization.

[c] Data from Bruland & Franks (1983), Martin et al. (1993), and Yeats & Campbell (1983) for the North Atlantic; Bruland (1980) and Martin et al. (1989) for the North Pacific; and Löscher (1999) and Croot et al. (2011) for the Southern Ocean.

[d] Data from Yeats & Campbell (1983) and Martin et al. (1993) for the North Atlantic, Sclater et al. (1976) and Bruland (1980) for the North Pacific, and Ellwood (2008) for the Southern Ocean.

[e] Data from Martin et al. (1993) for the North Atlantic, Bruland (1980) and Martin et al. (1989) for the North Pacific, and Martin et al. (1990), Löscher (1999), and Ellwood (2008) for the Southern Ocean.

[f] Data from Martin et al. (1993) for the North Atlantic, Martin et al. (1989) for the North Pacific, and Saito et al. (2010) and Bown et al. (2011) for the Southern Ocean.

[g] Data from the compilation of Lane et al. (2009, appendix).

Some of the geographic trends noted for particulate matter and single-cell data are present in the vertical profile data as well (**Table 3**). Fe stoichiometries are highest in the North Atlantic, lower in the North Pacific, and lowest in the Southern Ocean, matching expected trends in Fe limitation. Zn and Cd show the opposite trend, with the stoichiometries being highest in the Southern Ocean and decreasing to the North Atlantic. Zn is particularly enriched in Southern Ocean plankton, with water column Zn:P ratios that are up to 8 times higher than those in the North Atlantic and North Pacific. In contrast, Cu seems to be relatively consistent across the three ocean basins, suggesting that the growth dilution effect may not be responsible for all of the differences, as presumably cellular Cu would be affected to a similar extent as the other metals. Co appears to be uniformly low in cells; however, Saito & Moffett (2002) reported high Co:P remineralization ratios in the water column near equatorial settings. The broad consistency in the regional patterns of trace metal stoichiometry observed by different methods suggests that these patterns are robust and not an artifact of methodological procedures.

Departures of plankton metal quotas from a generalized stoichiometry are also seen in studies that isolate diazotrophs from the ocean. As noted above, N fixers have elevated Fe requirements owing to the nitrogenase enzyme. Significant effort has been focused on studying the distribution and N fixation capabilities of the colonial cyanobacterial diazotroph *Trichodesmium* (Capone et al. 1997), which can be collected from the surface ocean, isolated from co-occurring phytoplankton biomass with hand-deployed nets, and analyzed with standard elemental analysis techniques (e.g., inductively coupled plasma mass spectrometry). Fe:P ratios in *Trichodesmium* collected from the tropical and subtropical North Atlantic show significant interstation variability but are generally enriched up to five- to sixfold over the ratios in the overall phytoplankton population (**Table 2**), matching expectations based on Fe use efficiencies for diazotrophic growth (Sañudo-Wilhelmy et al. 2001). These organisms appear to have altered ratios of other bioactive trace metals as well, with field-collected colonies presenting generally lower Cd but elevated Mo, Ni, and V stoichiometries compared with bulk plankton (Tovar-Sanchez & Sañudo-Wilhelmy 2011, Nuester et al. 2012a). The unique metallome of *Trichodesmium* results from the biochemical pathways of N fixation and its cyanobacterial lineage, and this metallome is likely shared by other diazotrophs, including unicellular forms (Tuit et al. 2004). Indeed, although *Trichodesmium* has received much of the research focus, unicellular N fixers are increasingly acknowledged as significant contributors to ocean N fixation (Zehr et al. 2001, Needoba et al. 2007, Moisander et al. 2010). These organisms have the potential to influence community metal stoichiometries with their unique metal signatures.

TOWARD A UNIFIED DESCRIPTION OF OCEANIC PHYTOPLANKTON METAL QUOTAS

Data from these independent methods for determining the metal contents of phytoplankton in the ocean can be combined to assess the applicability of a common "extended Redfield ratio" for metals in plankton. Similarities in the distributions of metals and macronutrients have been noted since the adoption of trace metal clean techniques in the 1970s, and Morel & Hudson (1985) connected this behavior to the composition of algae and suggested that a common generalized stoichiometry of metals in cells may be present in the ocean. This concept was advanced by Bruland et al. (1991) through a compilation of three studies of marine biogenic particulate matter, and more recently has been linked to the metal stoichiometries of various phytoplankton in laboratory cultures (Ho et al. 2003). These studies emphasize the similarities between metal stoichiometries in ocean plankton and their environment, and indeed there is great utility for a unified ratio to convert between biogenic metals and C, N, and P (e.g., Gordon et al. 1997) or to estimate the composition of a particle assemblage according to its elemental stoichiometry (e.g., Ho et al. 2007). However, valuable information is lost when biogenic metal data are aggregated into a single stoichiometry, and there is much to be learned about metal biogeochemistry from examining variations in phytoplankton metal composition across environmental, taxonomic, and spatial gradients.

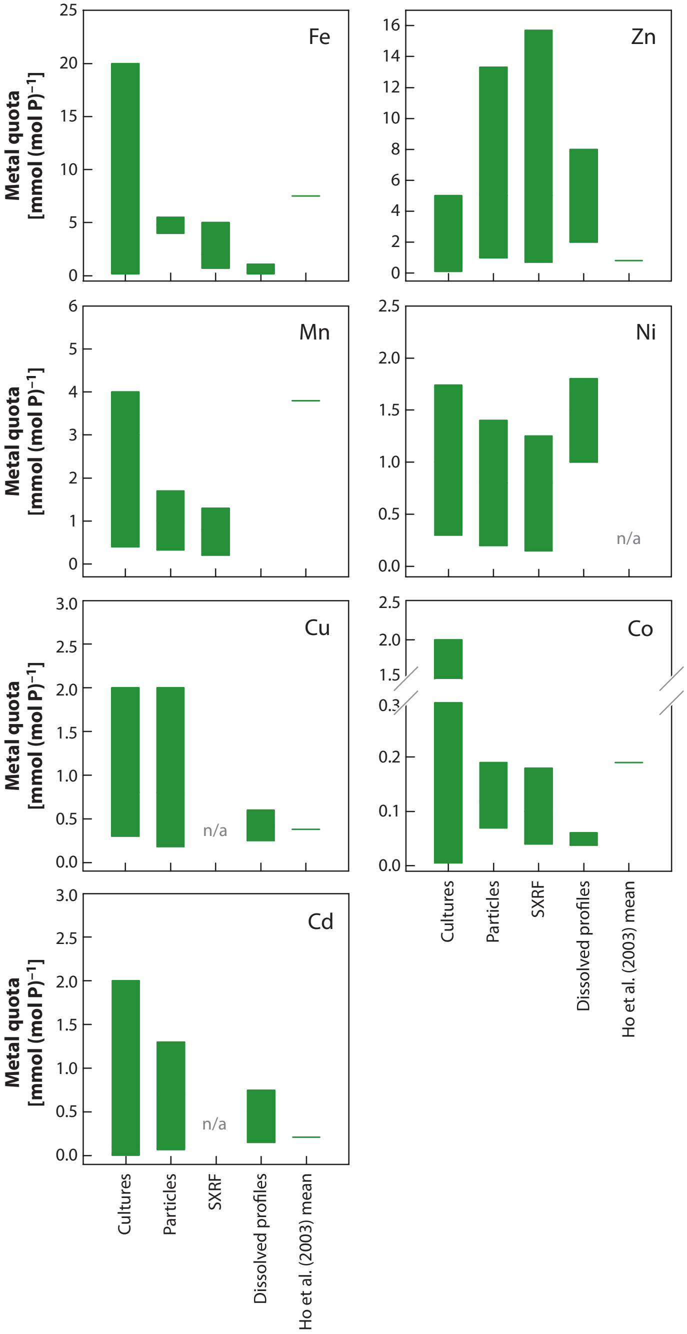
Comparisons of metal quotas determined through the various techniques suggest lessons about the interactions of bioactive trace metals and phytoplankton. Fe stoichiometries in open ocean environments are generally lower than in Fe-replete cultures, whereas Zn stoichiometries are at the upper range of culture quotas. Fe stoichiometries show a decreasing trend from bulk particles to single cells to dissolved profiles (**Figure 4**). Consistent differences between bulk particles and

⟶

Figure 4

Comparison of metal quotas calculated for ocean phytoplankton using a variety of techniques. Culture data represent metal stoichiometries measured for cultured diatoms based on estimates of the range of metal concentrations in the ocean shown in **Figure 1**; the ranges plotted here correspond to the green shaded region in each panel of **Figure 1**. Particle data are taken from the studies summarized in **Table 2**. Synchrotron X-ray fluorescence (SXRF) data are taken from the results plotted in **Figure 3**. Dissolved profile data are taken from the studies summarized in **Table 3**. Ho et al. (2003) mean data are the metal stoichiometries for 15 cultured species as reported in that study. n/a, data not available.

single cells have even been observed at the same stations (King et al. 2012). This discrepancy suggests that detrital material with Fe:P above that of phytoplankton is a significant contributor to bulk particle Fe stoichiometries in many situations. These differences are not likely caused by lithogenic (mineral) inclusions, because the bulk particulate data have been culled or corrected for high-Al samples. Rather, particulate Fe:P may be elevated—and dissolved Fe:P lowered—by



the preferential remineralization of P relative to Fe in phytoplankton cells that are consumed by planktonic grazers. Additionally, detritus is often rich in bacterial biomass, which itself may be relatively elevated in Fe (Tortell et al. 1996). Therefore, particulate Fe:P ratios are likely to be affected by the presence of biogenic detrital material.

The offset between particles and dissolved profiles is also seen for Co, which, like Fe, is prone to scavenging (Moffett & Ho 1996). These metals may be scavenged onto detritus, elevating particulate concentrations at the expense of dissolved profile stoichiometries. In contrast, generally good agreement is found for Zn, Cu, and Cd stoichiometries estimated from bulk particles and dissolved profiles (**Figure 4**). In the case of Ni, dissolved profile stoichiometries are at the high end of the range seen in particles and single cells, and most closely approach the Ni quotas measured in diatoms. As Ni is associated with both organic material and the frustules of diatoms, dissolved stoichiometries could indicate an elevated importance for diatoms in Ni export (Twining et al. 2012). Similarly, the range of Zn:P stoichiometries seen in profiles (2.6–6 mmol mol^{-1}) (**Table 3**) most resembles those measured in diatoms, again supporting a unique role for this group in Zn biogeochemistry.

The field data compiled here highlight the range of metal quotas present in the ocean and indicate that the extended Redfield ratio calculated for cultures by Ho et al. (2003) is not an accurate representation of oceanic phytoplankton for some metals. The Mn stoichiometries reported by Ho et al. (2003) are nearly 10-fold higher than most of the field data derived from bulk particles, single cells, or dissolved profiles, whereas the Zn stoichiometry is correspondingly lower than almost all of the field data and the Cd stoichiometry falls at the lower end of particle and dissolved profile data (**Figure 4**). The discrepancies observed with open ocean data likely result from relatively elevated (compared with the open ocean) Fe and Mn concentrations in the culture media, with the Mn negatively affecting cellular Zn and Cd through competition for uptake transporters (Sunda & Huntsman 1998b). In the case of Cu, the culture and oceanic profiles agree quite well. The Fe stoichiometry reported by Ho et al. (2003) is likely more representative of coastal than oceanic phytoplankton, whereas the Zn, Mn, and Cd stoichiometries do not match field data.

IMPORTANCE OF EXTRACELLULAR SCAVENGING TO PHYTOPLANKTON QUOTAS

Discrepancies between particulate Fe quotas and those derived from dissolved profiles or Fe-limited cultures suggest that extracellular scavenged Fe oxides (and other elements associated with them) may contribute to the metal quotas of cells in the field. It has long been known that the geochemical cycles of certain metals are influenced by passive adsorption or scavenging onto particle surfaces in the ocean (Goldberg 1954, Turekian 1977). This process has been most thoroughly described for natural radionuclides such as Th that have no known biological role and are thus not actively incorporated by phytoplankton (Bacon & Anderson 1982, Coale & Bruland 1985). However, although Fe has similar hydrolysis chemistry to Th and is prone to sorption onto surfaces (Waite 2001), it is also actively accumulated and regulated by cells to support biochemical demands.

Most of the evidence for the presence of scavenged metals on phytoplankton surfaces comes from the use of chemical rinses containing EDTA and either Ti(III) or oxalate (Hudson & Morel 1989, Tovar-Sanchez et al. 2003). Fe contents of >0.2-μm particulate samples from subantarctic waters decreased by 16%–86% (mean approximately 50%) following treatment with an oxalate rinse (Tovar-Sanchez et al. 2003, Frew et al. 2006), whereas experiments with ^{55}Fe added to natural Southern Ocean plankton communities indicated that scavenged (i.e., oxalate-labile) Fe accounts for approximately 80% of the Fe captured by 0.45-μm filters (Hassler & Schoemann 2009). Oxalate treatment of open ocean colonies of *Trichodesmium* suggests that approximately 60% of

Fe associated with these organisms may not be contained within cells (Sañudo-Wilhelmy et al. 2004). These data support the conclusion that bulk particulate estimates of phytoplankton Fe:P are impacted by the inclusion of noncellular material.

Although initial studies with the Ti(III)-EDTA rinse interpreted the noncellular Fe fraction as being associated with precipitated Fe oxides trapped on filters alongside cells (Anderson & Morel 1982, Hudson & Morel 1989), over time this fraction has come to be more commonly interpreted as Fe scavenged onto the outside of phytoplankton cells (e.g., Hassler & Schoemann 2009, Tovar-Sanchez & Sañudo-Wilhelmy 2011). However, data from SXRF mapping indicate that extracellular Fe does not account for a significant fraction of cellular Fe in natural settings. The distribution of Fe in most cells analyzed with SXRF shows the highest concentrations either associated with plastids or in the thickest parts of the cell, indicating control by cell volume and not surface area (Twining 2003; Twining et al. 2008; B.S. Twining, unpublished data) (**Figure 2**). Additionally, dinoflagellates collected from the Southern Ocean prior to and following repeated additions of Fe during the Southern Ocean Iron Experiment (SOFeX) showed Fe to be localized in the chloroplasts of all cells (Twining et al. 2004b). Furthermore, SXRF analyses of *Trichodesmium* collected from the western North Atlantic showed only occasional adsorbed Fe particles and no statistical difference in the Fe concentrations of trichomes rinsed with either an isotonic salt rinse or the oxalate solution (Nuester et al. 2012a). In fact, of the five bioactive elements measured in the trichomes, only P was significantly lower in oxalate-treated trichomes, similar to earlier studies that showed that P is more readily removed from *Trichodesmium* by oxalate than Fe is (Sañudo-Wilhelmy et al. 2004, Tovar-Sanchez & Sañudo-Wilhelmy 2011).

The chemical speciation of Fe in the surface ocean supports the observation that most Fe that is physically associated with cells is located intracellularly. Dissolved (<0.45-μm) Fe is typically bound to strong organic ligands present in excess (Gledhill & Buck 2012) that limit the reactivity of Fe to functional groups on cell surfaces. The presence of ligands limits scavenging of Fe in the deep ocean (Johnson et al. 1997), and it is likely to do so even more effectively in surface waters, where ligands are typically most abundant (e.g., Boye et al. 2001). Furthermore, oceanic plankton actively regulate the accumulation of metals from organic complexes (Maldonado et al. 2005, 2006; Hassler et al. 2011). It thus seems illogical that much of the Fe associated with phytoplankton cells—including in low-Fe HNLC regions, where algal competition for Fe determines ecological success—would be bound to the outside of cells in a biologically inaccessible fashion.

IRON QUOTAS IN THE OCEAN

Accurate measures of metal quotas in ocean phytoplankton are needed to parameterize and evaluate biogeochemical models. In particular, many models calculate Fe quotas (e.g., Moore et al. 2004, Tagliabue et al. 2009), and Fe quotas serve as an indicator of Fe limitation as well as a direct connection to the biogeochemical cycling of C, N, and P. However, uncertainty about the Fe quotas of ocean phytoplankton remains. In the Fe-limited Southern Ocean, dissolved profiles indicate an Fe:P of 0.2 mmol mol^{-1} (**Table 3**), whereas the minimum quotas for phytoplankton isolated from these waters can be fourfold lower and measurements of individual cells fourfold higher (Strzepek et al. 2011) (**Figure 3**). In the equatorial Pacific, the measured quotas are also above the minimum Fe quotas of diatoms isolated from this region (Maldonado & Price 1996) (**Figure 3**); however, the measured quotas match the expectations from cultures based on observed growth rates and measured dissolved Fe concentrations (Twining et al. 2011).

It may be that the taxa abundant enough to be captured for SXRF analyses are those that have been able to obtain adequate Fe for growth. However, taxa with higher dissolved Fe requirements may still respond to additions. Despite Fe:P quotas of 4.1 mmol mol^{-1} in small pennate diatoms in the equatorial Pacific, in deck-board incubation experiments the rest of the diatom community showed a strong response to added Fe through a shift toward larger pennates, indicating that these larger species had not been able to access enough dissolved Fe to bloom (Baines et al. 2011, Brzezinski et al. 2011, Twining et al. 2011).

CONCLUSIONS

The existence of regional and interspecific differences in the trace metal stoichiometries of phytoplankton suggests that two challenges prevent the development of a clearer understanding of trace element quotas. First, uncertainty about what phytoplankton perceive as available metal over the short term limits the interpretation of all but the most obvious comparisons among regions. Standardized bioassay approaches that measure organismal response to the environment may help to address this. Particularly promising are bioreporter genes whose level of transcription in well-studied indicator species can be calibrated against metal availability (Webb et al. 2001, Mioni et al. 2005, Chappell & Webb 2010).

The second challenge is to determine whether interspecific differences in metal stoichiometries reflect distinct biochemical and/or ecological solutions to problems posed by the environment in different regions. If this link can be made, then it may be possible to predict which stoichiometries may prevail on average in a given environment, given constraints imposed by evolutionary heritage, morphology, and trace element availability. This approach is already well established for predicting C:N stoichiometries in marine plankton, which exhibit far less variation (Geider et al. 1998, Armstrong 1999, Klausmeier et al. 2004). At the cellular level, metal allocation among biochemical pools related to different cellular processes, such as photosynthesis, nutrient acquisition, and antioxidant defense, must be better understood. Proteomics holds much promise on this front (Saito et al. 2011). At the same time, we will need to develop ecophysiological models that link trace element composition to performance as well as ecological models that determine effective strategies given specific environmental contexts. Finally, the continued development of field-ready molecular and cell-specific tools will be needed to test and revise the predictions of such models in the field.

SUMMARY POINTS

1. Data on phytoplankton metal quotas in the ocean from multiple independent approaches indicate consistent regional differences in the metal quotas of ocean phytoplankton. Fe quotas are highest in non-HNLC waters and lowest in the Southern Ocean. Zn and Cd are highest in the Southern Ocean south of the polar front. In contrast, Ni, Cu, and Co show negligible regional differences.
2. Taxonomic differences may drive some of the regional differences. Diatoms have elevated Zn in all regions and elevated Ni in HNLC regions. Autotrophic flagellates have elevated Co. Changes in community composition (e.g., south of the polar front) could thus affect community ratios for some metals.
3. Although Fe is usually regarded as the most abundant metal in phytoplankton, Zn is often as abundant if not more abundant in cells in the ocean, perhaps owing to different availabilities. Additionally, discrepancies between the metal stoichiometries of individual cells, particle assemblages, and dissolved profiles suggest the existence of a detrital Fe pool characterized by elevated Fe:P ratios potentially due to differential remineralization of these elements by grazing and/or Fe scavenging.

4. Substantial interspecific differences in the Fe quotas of cells collected under the same conditions may indicate different strategies under trace nutrient limitation (e.g., storage, economy, opportunism).

FUTURE ISSUES

1. This review focuses largely on osmotrophic phytoplankton such as diatoms and cyanobacteria; however, many ocean plankton utilize heterotrophy or mixotrophy to compete. Much less is known about the prevalence of these strategies and their implications for metal quotas and metal biogeochemistry, although they are recognized as important for macronutrient cycling. Additionally, cells may utilize different strategies to compete for nutrients, and these likely have implications for metal quotas. Future efforts should attempt to relate metal quotas to ecological strategies.
2. Reliable cellular metal budgets that partition metals among distinct biochemical fractions or among enzymes that perform different roles within the cell are needed to understand variations in metal quotas. Such budgets will require substantial effort to identify and quantify different metalloenzymes using molecular and mass spectrometric methods. These data will also be needed to inform new models that allocate elemental supplies not necessarily by taxonomy but by cell physiology (Follows & Dutkiewicz 2011). Application of broad spectrum genomic, transcriptomic, and proteomic methods should highlight the trade-offs associated with a shifting allocation of metals between different metalloprotein pools.
3. We still lack a detailed understanding of the impact of metal physicochemical speciation on bioavailability to phytoplankton in ocean waters. Taxon-specific measurements of quotas in natural communities provide a proxy for metal availability and may be used as indicators of ambient metal availability.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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Errata

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