Limnol. Oceanogr., 53(1), 2008, 276–290

Some thoughts on the concept of colimitation: Three definitions and the importance of bioavailability

Mak A. Saito[1] *and Tyler J. Goepfert*
Marine Chemistry and Geochemistry Department, Woods Hole Oceanographic Institution, 360 Woods Hole Rd., Woods Hole, Massachusetts 02543

Jason T. Ritt
McGovern Institute for Brain Research, Massachusetts Institute of Technology, 46-2166, 77 Massachusetts Ave., Cambridge, Massachusetts 02139

Abstract

We discuss the concept of colimitation of primary productivity in aquatic environments, with an emphasis on reconciling this concept with recent advances in marine bioinorganic chemistry. Colimitations are divided into three categories on the basis of their mathematical formulations and visualizations: type I, independent nutrient colimitation (e.g., N and P); type II, biochemical substitution colimitation (e.g., Co and Zn); and type III, biochemically dependent colimitation (e.g., Zn and C), where the ability to acquire one nutrient is dependent upon sufficient supply of another. The potential for colimitation occurring in the marine environment and the critical importance of understanding nutrient bioavailability are discussed.

The notion of simultaneous limitation by multiple elements, or colimitation, is an important yet often misunderstood concept. Our aim in this manuscript is to clarify and define the types of colimitation, review and discuss their mathematical descriptions, and present three-dimensional examples to promote a visual understanding. There is a particular emphasis on the role of trace metals in colimitation, as well as the potential importance of organic complexation to nutrient bioavailability and colimitation in marine waters. These discussions are necessitated in part by the recent advances in marine bioinorganic chemistry, where it is now believed that limitation by certain trace metals could reverberate through coupled biogeochemical systems by hindering the biosynthesis of key metalloenzymes (Morel et al. 2003).

The water column of the marine environment contains micronutrients and macronutrients that nourish the growth of autotrophic life. Much has been written about the nutrition of oceanic primary production (e.g., Redfield et al. 1963; Droop 1973; Moore et al. 2004). A useful recurring theme in the marine literature is Liebig's law of the minimum (de Baar 1994). This law was the 33rd of 50 principles of agricultural chemistry: "When a given piece of land contains a certain amount of all the mineral constituents in equal quantity in an available form, it becomes barren for any one kind of plant when, by a series of crops, one only of these constituents—as for example soluble silica—has been so far removed, that the remaining quantity is no longer sufficient for a crop." (Liebig 1855 as cited in de Baar 1994). While Liebig's law exerts itself on biological systems by controlling the overall yield of biomass, an alternate concept of limitation is rate limitation (also known as Blackman limitation) where growth rate is reduced rather than yield. These can be interrelated concepts; for example, both types of limitation have been clearly observed in the metal limitation experiments (e.g., Saito and Goepfert 2008), where the gradual replenishment of free metals in solution from the metal-buffered media operates as a chemical chemostat, inducing growth rate limitation. Once significant biomass is achieved in the culture relative to the amount of cobalt and zinc needed for nutrition, yield is limited and the buffer is effectively "blown" (either by kinetics of the back reaction of the metal–buffer complexes, or by actual depletion of the total metal).

Liebig's law implies that there is a single limiting nutrient. But the concept of limitation (either Liebig or Blackman) is frequently expanded to more than one nutrient, often by invoking the term "colimitation." The surface oceans are particularly prone to colimitation because of the simultaneous scarcity of many nutrients. In particular, improvements in trace metal analytical methods that occurred at the end of the last century have allowed researchers to demonstrate the potential for

[1] Corresponding author (msaito@whoi.edu).

Acknowledgments

We thank Sonya Dyhrman, Scott Doney, Seth John, and François Morel for comments on this manuscript, as well as Bill Sunda, Yeala Shaked, and Eric Webb for helpful discussions. Thanks to two anonymous reviewers for their significant reviewing efforts and for encouraging this to be a stand-alone manuscript. We are particularly indebted to our colleagues, many cited here, who have established the novel and exciting field of marine bioinorganic chemistry. We thank Michael Droop for inspiration and for our borrowing of his manuscript title (Droop 1973). We dedicate this manuscript to François Morel and Bill Sunda, who have led and continue to lead the study of many of the colimitations described here.

This work was funded by the National Science Foundation through Chemical Oceanography and the Office of Polar Programs (OPP-0440840 and OCE-0327225 and OCE-0452883), the Office of Naval Research, and through the Center for Environmental Bioinorganic Chemistry at Princeton and the Center for Microbial Oceanography Research and Education.

limitation of trace metals acting as micronutrients. While iron is now known to exert a global influence on marine primary productivity (Moore et al. 2004), many of the other trace elements are also close to their potentially limiting values (Sunda and Huntsman 1995*a*). An example of this is the cobalt–zinc colimitation of the carbonic anhydrase enzyme (Morel et al. 1994; Sunda and Huntsman 1995*a*; Saito et al. 2002). Colimitation has also been described as a potentially important process in freshwater environments, such as the Great Lakes in North America (North et al. 2007).

Methods

Calculations of potential autotrophic production per element (PAPPE)—The values used in these simple calculations have a large influence on the results, and hence were carefully chosen to reflect current knowledge. Cellular quotas can vary slightly for major nutrients and tremendously for micronutrients. For example, variability in major nutrient composition of cellular material has been observed (Sambrotto et al. 1993), and even larger variability is observed in cellular metal quotas of phytoplankton, usually spanning several orders of magnitude (Sunda and Huntsman 1995*a*). As a result, trace metal cellular quota values were selected to be representative of cultures experiencing slight metal limitation. Calculations are based on these laboratory cellular quota experiments as well as typical surface water nutrient and trace metal measurements. Values for C, N, and P stoichiometry are the Redfield ratio values (as updated in Redfield et al. 1963). Fe, Co, and Zn phytoplankton composition values (cellular quotas) were taken from Sunda and Huntsman growth rate studies (Sunda and Huntsman 1995*a,b*). Fe quotas under Fe-induced growth-limiting conditions can vary from below 3 to 13.1 μmol mol^{-1} C depending on the phytoplankton species. Moore et al. (2004) utilized values of 2.5 and 6 μmol for minimum and optimum Fe mol^{-1} C for both their diatom and small phytoplankton categories in their ecosystem models on the basis of Fe : C ratios calculated from sinking particulate matter by Sunda and Huntsman (1997). Likewise, Co quotas under Co-limiting conditions can vary significantly between phytoplankton groups, with 0.08, 0.40, and 0.95 μmol mol^{-1} C measured in *Synechococcus bacillarus*, *Thalassiosira oceanica*, and *Emiliania huxleyi* when no Zn was added (Sunda and Huntsman 1995*a*). Finally, Zn quotas varied from 0.38 to 0.83 μmol mol^{-1} C for *T. oceanica* and *E. huxleyi*, respectively. On the basis of these studies, cellular metal quotas of 2.5, 0.40, and 0.38 μmol mol^{-1} C were used for Fe, Co, and Zn, respectively. Obviously caution should be taken when interpreting these calculations, especially with regard to the significant diversity and plasticity of metal cellular quotas in phytoplankton.

Values for seawater concentrations of trace metals are divided into organically complexed and inorganic categories to reflect the dissolved organic forms of N and P, and the complexation by organic ligands of Fe, Co, and Zn. N refers to the summation of nitrate and nitrite concentrations; P refers to soluble reactive phosphate values measured using low-level techniques for the oligotrophic regions (Wu et al. 2000). Typical total dissolved Fe measurements from each region are utilized (Martin et al. 1989; Rue and Bruland 1995; Wu and Boyle 2002). Fe speciation reflects actual measured Fe speciation at station ALOHA (Rue and Bruland 1995), or approximate estimates on the basis of ~99.9% complexation (slightly less than the 99.97% measured at ALOHA), but do not include the current complexities associated with colloidal fractions (Wu et al. 2001). Total dissolved Co values are from each region as measured by electrochemical techniques (20 pmol L^{-1} annual average at BATS [Saito and Moffett 2002], 90 pmol L^{-1} in surface waters in the North Pacific high-nutrient low-chlorophyll (HNLC) region [Saito unpubl. data], and near the Hawaiian islands [Noble and Saito, unpubl. data]). Co speciation values reflect ~99% complexation in each of these regions, on the basis of labile Co measurements showing Co electrochemical signals that are indiscernible from the analytical blank and conditional stability constants in excess of $10^{16.8}$ (Saito et al. 2004, 2005). Total dissolved Zn measurements are representative of near-surface values in the HNLC of the North Pacific and Sargasso Sea (Wisniewski 2006). Zn speciation values from the HNLC of the North Pacific are utilized (Wisniewski 2006), and the other values are estimated on the basis of 98% binding on the basis of Bruland (1989).

For comparison of the PAPPE calculations described above with actual regional productivity, average particulate organic carbon (POC) concentrations were calculated from the Sargasso Sea Bermuda Atlantic Time Series (BATS) and Hawaiian Ocean Time-series project (HOT) using the mean of all BATS data available to present between 20- and 30-m depth, and the mean of HOT data from 1988 to 2004 between 22 and 28 m (Bermuda Atlantic Time-series Station http://bats.bbsr.edu/; Hawaii Ocean Time-series: http://hahana.soest.hawaii.edu/hot/hot-dogs/interface.html). A range of POC values for the iron-limited regions of the North Pacific were obtained from the Joint Global Ocean Fluxes Study-World Ocean Circulation Experiment (JGOFS/WOCE) data sets (http://ocean.tamu.edu/~pdgroup) and Station Papa and North Pacific cruises (Ichikawa 1982; Bishop et al. 1999; Harrison 2002). These time-series POC values were not used in the PAPPE calculations, but are intended to give a reality reference point to these idealized and highly simplified calculations.

Results and discussion

A simple *Gedanken* (thought) experiment of Liebig limitation in seawater is presented in Fig. 1 by calculating the amount of fixed carbon (as POC) that can be autotrophically produced from the components found in a single liter of surface seawater (adapted from Saito 2001). There are numerous geochemical and biological subtleties that must be pointed out when performing these calculations, and their descriptions are presented in the subsequent paragraphs. There are two obvious conclusions from these calculations: First, if only inorganic nutrient forms are considered, then the surface oceans are close to Liebig limitation for multiple nutrients simultaneously; for example, nitrogen, phosphorus, and iron could all be potentially

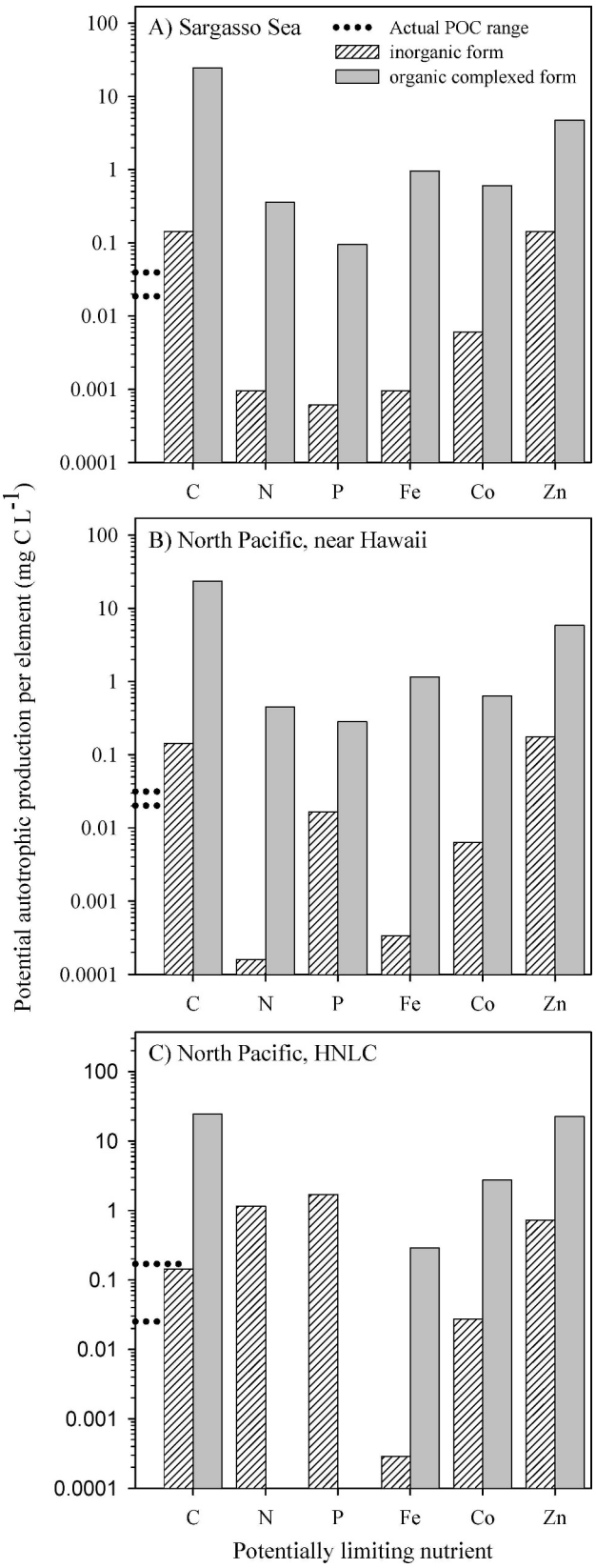

Fig. 1. A *Gedanken* (thought) experiment emphasizing the importance of bioavailability by applying Liebig's law of the minimum to (A) the Sargasso Sea, (B) the North Pacific near Hawaii, and (C) the North Pacific high-nutrient low-chlorophyll region (HNLC). Each bar refers to the amount of biomass that could stoichiometrically be produced using the quantity of each element in a liter of seawater assuming all other nutrients were replete (or potential autotrophic production element). The nutrients with the lowest bar(s) in each graph should be limiting. Both organic (or organically complexed for metals, so as to not imply the direct organometallic metal–carbon bond) and inorganic forms of the nutrients are shown because there is growing evidence for the bioavailability of organic/complexed forms of macronutrients and metals. Current understanding of metal bioavailability suggests that the organic iron (FeL) is bioavailable, but only the inorganic Co and Zn forms are bioavailable to eukaryotic phytoplankton (with the exception of organic cobalt being bioavailable to cyanobacteria). This results in cobalt being closer to limiting than iron in all three environments, yet the capability for zinc–cobalt biochemical substitution would likely alleviate much of this limitation (see Fig. 2, type II). In addition, this makes the oversimplifying assumption that all organically complexed iron is uniformly bioavailable. Carbon calculations are based on CO_2 and dissolved inorganic carbon (DIC) concentrations rather than dissolved organic carbon. These calculations are a simplification of primary productivity in oceanic ecosystems since they do not account for regeneration, recycling, advective input, aeolian input, or numerous other biogeochemical processes, as well as oversimplifying the variability in cellular metal quotas and surface water metal concentrations. See text for further caveats (adapted from Saito 2001).

limiting in the oligotrophic Sargasso Sea, according to this calculation. Second, an understanding of the bioavailability of elements associated with organic molecules (for N and P) or organic complexes (for metals) is critical to determining whether or not each element is Liebig limiting.

While this thought experiment is conceptually useful in demonstrating the importance of bioavailability, there are numerous simplifications, generalizations, and caveats that must be pointed out so that the reader understands its limitations. The true limiting potential of each macronutrient and micronutrient is governed by geochemical and biological factors, many of which are only somewhat elucidated at this point. Indeed, each nutrient has its own active research field and hence a detailed discussion is beyond our scope here, with the exception of a brief overview. By setting limitations on this single liter of seawater, we simplify by ignoring biological, physical, and chemical inputs, transformations, regenerations, and exports from the system, as well as limitation effects induced by differential uptake rates between different phytoplankton groups (e.g., differences in diffusion limitation and uptake affinities). The amount of POC that can be generated by each nutrient, when assuming all other nutrients are replete, is calculated by dividing the concentration of the nutrient solute in a liter of seawater by the cellular quotas (nutrient : C ratio) of each nutrient and micronutrient, and is then converted to units of milligrams of C per liter. This production is basically the PAPPE that can be produced from a liter of surface seawater.

It is important to recognize that these simple calculations are produced from very limited data and hence make no attempt to account for the variability of nutrient concentrations and cellular quotas that are known to occur. This

is probably especially true for the trace elements iron and cobalt, which have short residence times in the ocean and can be influenced by aeolian or physical processes (e.g., Saito and Moffett 2002, Wu and Boyle 2002). An order of magnitude scale variability in the size of these bars would not be surprising, and this should be taken into account when comparing the potential for limitation of these elements in a given environment.

The bioavailability of nutrients and micronutrients can vary significantly because of the chemical form of the nutrient. For trace elements, chemical speciation is dominated by the formation of metal–ligand complexes with strong organic ligands in seawater, often reducing the bioavailability of metals such as Fe, Zn, and Co by two to three orders of magnitude (Sunda and Guillard 1976; Anderson and Morel 1982; Saito et al. 2002). Trace metal speciation is difficult to measure and there are only limited data sets available (e.g., Rue and Bruland 1997; Saito and Moffett 2001, and references therein; Ellwood 2004). Moreover, it is currently unclear the extent to which organically complexed forms are bioavailable to phytoplankton. For Fe complexes, there is evidence demonstrating utilization of FeL complexes (Maldonado and Price 2001), presumably through a ferric reductase uptake system like that found in the diatom *Thalassiosira pseudonana* (Armbrust et al. 2004). For Co complexes, there is evidence for utilization of CoL by cyanobacteria (Saito et al. 2002), but these complexes may be so strong in seawater that they do not readily dissociate and hence may not be available to some eukaryotic phytoplankton, as empirically suggested by higher Co : P utilization ratios when cobalt is labile in the Peru upwelling system (Saito et al. 2004, 2005). There is little experimental evidence for the bioavailability of natural ZnL complexes. These Zn complexes are significantly weaker than the CoL complexes, with conditional stability constants of $\sim 10^{11}$ versus $>10^{16.8}$ for CoL (Bruland 1989; Saito et al. 2005), suggesting that ZnL may be easier to acquire than CoL, or at least are subject to exchange reactions relative to the seeming inertness of CoL. On the basis of culture experiments, the prevailing thought is that this ZnL is not readily bioavailable to phytoplankton (Sunda and Huntsman 1992, 1995*a*, 2000). Together, this suggests that the chemical species that are currently thought to be bioavailable (to eukaryotic phytoplankton) and hence that should be compared on these figures are FeL, inorganic Co, and inorganic Zn. This would suggest that Co should be limiting in the North Pacific HNLC, except that it is also known that Zn and Co substitute for a biochemical function in many eukaryotic phytoplankton (Sunda and Huntsman 1995*a*; Saito and Goepfert 2008), and inorganic Zn is just slightly "less limiting" than iron in Fig. 1, and hence all three elements may be close to colimiting.

This discussion points out the crucial importance of understanding the bioavailability of metals (and macronutrients) in seawater. The large differences in conditional stability constants between metal–ligand complexes also suggests that there could be unique kinetic issues for each metal regarding replenishment of each reservoir of inorganic metal with the much larger reservoir of metal–ligand complexes, zinc complexes being relatively weak versus the cobalt complexes that appear almost inert. It is also possible that the reservoir of complexed metals is available to some fraction of the phytoplankton community through specialized uptake systems such as iron reductases or metallophore (siderophore or cobalophore) acquisition pathways (Maldonado and Price 2001; Saito et al. 2002; Shaked et al. 2005). The use of those specialized uptake pathways likely comes at additional physiological cost and difficulty relative to metal cation transport. The speciation of major nutrients is also believed to be important, especially in oligotrophic regions where nitrate and soluble reactive phosphate are scarce (Karl and Yanagi 1997). The utilization of phosphonates, for example, is now believed to be important, in addition to the utilization of soluble reactive phosphate (Dyhrman et al. 2006).

The variation in POC that can be generated from carbon is approached differently from the other nutrients to reflect ideas on the potential for carbon limitation in seawater (Riebesell et al. 1994). Only inorganic carbon is considered here, since utilization of dissolved organic carbon would no longer qualify as autotrophy. Values for dissolved inorganic carbon (DIC) are from each region, and approximate concentrations of CO_2 are used (Winn et al. 1994; Bates et al. 1996; Wong et al. 2002). Carbon limitation of marine phytoplankton growth is often considered as being rate limiting rather than biomass limiting (Wolf-Gladrow and Riebesell 1997), since concentrations of DIC in seawater are quite high ($\sim$2,000 μmol L^{-1}) and the amount of DIC existing as the CO_2 species is only $\sim$1% of the total DIC. The major dissolved chemical species, bicarbonate (HCO_3^-), can undergo protonation and dehydration to form $CO_{2(aq)}$, and is governed by thermodynamic equilibrium. However, this dehydration step is kinetically slow without catalysis by the enzyme carbonic anhydrase. Carbon acquisition in phytoplankton typically involves the use of some form of carbon concentrating mechanism (CCM) to deal with the low CO_2 concentrations in aqueous environments, which are typically lower than the half-saturation constant of the Rubisco enzyme involved in carbon fixation. These CCMs often include bicarbonate transporters that allows access to the larger DIC reservoir (Tortell and Morel 2000). Moreover, CCMs have been shown to be affected by zinc deficiency, by reducing zinc carbonic anhydrase activity, resulting in carbon limitation (Morel et al. 1994).

These PAPPE calculations can be compared with actual POC concentrations from the Sargasso Sea and the Pacific Ocean near Hawaii and in the North Pacific (Fig. 1, $\pm$1 SD of mean of BATS and HOT data, range of North Pacific HNLC values, see Methods). The mean POC values are somewhat higher than what the inorganic N and P concentration calculations yield. This is likely due to a combination of factors including the contribution to POC from heterotrophic biomass, nitrogen fixation, physical transport processes, and the utilization of organic nutrient forms. Moreover, regeneration and recycling of nutrients that exist within the standing crop of POC is not considered by these calculations (as well as DOC utilization by heterotrophic bacteria), but are obviously fundamental components of the microbial loop (Azam et al. 1983).

Table 1. Examples of potential nutrient colimitation pairs in the marine environment.

Nutrient couple	Colimitation type (targeted enzyme)		Example refs.†
Zinc and cobalt (Cyanobacteria)	0 or I	Only one nutrient/independent	a,b
Nitrogen and phosphorus	I	Independent	c
Nitrogen and light	I	Independent	—
Nitrogen and carbon	I	Independent	d
Iron and cobalt	I	Independent	e
Iron and zinc	I	Independent	f
Iron and phosphorus	I	Independent	g
Iron and vitamin B_{12}	I	Independent	h
Zinc and cobalt (eukaryotic phytoplankton)	II	Biochemical substitution (CA)*	b,i
Zinc and cadmium (diatoms)	II	Biochemical substitution (CA)*	j
Iron and Manganese (or Ni, or Cu-Zn)	II	Biochemical substitution (SOD)*	k
Zinc and cobalt (hypothesized)	II	Biochemical substitution (AP)*	l,m
Iron on light	III	Dependent	n
Zinc on phosphorus	III	Dependent (AP)*	l
Cobalt on phosphorus	III	Dependent (AP)*	m
Zinc on carbon	III	Dependent (CA)*	j
Cobalt on carbon	III	Dependent (CA)*	j
Cadmium on carbon	III	Dependent (CA)*	k
Copper on iron	III	Dependent (FRE and MCO)*	o
Iron on nitrate	III	Dependent (NR)*	p
Iron on nitrogen (N_2 fixation)	III	Dependent (NIF)*	q
Molybdenum on nitrogen (N_2 fixation)	III	Dependent (NIF)*	r
Nickel on urea (nitrogen)	III	Dependent (urease)	s
Copper on amines	III	Dependent (amine oxidase)	t

* CA, carbonic anhydrase; SOD, superoxide dismutase; AP, alkaline phosphatase; FRE, ferric reductase; MCO, multicopper oxidase; NR, nitrate reductase; NIF, nitrogenase.

† Example references: a, Saito et al. 2002; b, Sunda and Huntsman 1995*a*; c, Benitez-Nelson 2000; d, Hein and Sand-Jensen 1997; Riebesell et al. 1994; e, Saito et al. 2005; f, Franck et al. 2003, Wisniewski 2006; g, Mills et al. 2004; h, Bertrand et al. 2007; i, Morel et al. 1994; Yee and Morel, 1996; j, Price and Morel 1990; k, Tabares et al. 2003; Wolfe-Simon et al. 2005; l, Shaked et al. 2006; m, Sunda and Huntsman 1995*a*; n, Boyd et al. 2001; Maldonado et al., 1999; Sunda and Huntsman 1997; o, Peers et al. 2005; Robinson et al. 1999; p, Raven 1988, 1990; Maldonado and Price 1996; q, Falkowski 1997; Berman-Frank et al. 2001; r, Howarth and Cole 1985; s, Price and Morel 1991; t, Palenik and Morel 1991.

This simple gedanken experiment in Fig. 1 illustrates (1) how multiple nutrients are simultaneously close to limiting concentrations in the marine environments, and (2) the importance of research on the bioavailability of different chemical species to the concept of colimitation. Indeed, some of our recent field observations agree with these calculations: cobalt–iron colimitation has been found in the North Pacific HNLC (Saito et al. unpubl. data) and the Costa Rica upwelling dome (Saito et al. 2005). One can also see the potential for an interrelated colimitation scenario, where cobalt–zinc concentrations are limiting enough to potentially influence carbon acquisition through an inability to synthesize the metalloenzyme carbonic anhydrase as part of the CCM (Morel et al. 1994). This potential for colimiting nutrients and the different types of colimitation that are possible are not often discussed in the literature. The next section aims to clarify some thoughts on this subject.

The concept of colimitation—One reason for the ambiguity associated with the term colimitation is the fact that there are really several distinct types of colimitations, as we and others have previously briefly discussed (e.g., Arrigo 2005; Saito et al. 2005). According to a strict interpretation of Liebig's law, one nutrient is the primary limiting nutrient and the next most limiting is a secondary limiting nutrient, rather than a colimiting nutrient. In contrast, true colimitation could be defined more precisely as a situation where growth rate is actually influenced by two limiting nutrients simultaneously, rather than secondarily (or sequentially) affecting growth as described above (e.g., alleviation of the first nutrient causes limitation by the second). These alternate conceptions of the influence of multiple potentially limiting nutrients are not incompatible with our classifications in the subsequent text, since we describe differences that manifest themselves on the biochemical and bioinorganic level, rather than the physiological and ecological level. In Table 1, we list many of the known nutrient limitation scenarios that can be ascribed to the notion of colimitation, divided here into three distinct types. There have also been previous studies discussing the mathematics of colimitation of phytoplankton (Legovic and Cruzado 1997; Klausmeier et al. 2004); however, they have not addressed the problem of the bioinorganic metal substitution phenomenon, as exemplified in our related manuscript (Saito and Goepfert 2008).

The first scenario, which we will describe as "Type I. Independent nutrient colimitation," concerns two elements that are generally biochemically mutually exclusive, but are also both found in such low concentrations as to be potentially limiting. Here, "independent" is operationally

defined relative to the clear biochemical interactions of the next two types of colimitation described below, since at the cellular level everything is obviously interrelated to some extent. A second scenario, described here as "Type II. Biochemical substitution colimitation," involves two elements that can substitute for the same biochemical role within the organism. There are two permutations of this scenario, first where the two elements can substitute effectively within the same enzyme, and second where there are two enzymes that carry out the same function, but each utilizing a different element. A third scenario, "Type III. Biochemically dependent colimitation," refers to the limitation of one element that manifests itself in an inability to acquire another element. Common examples of the three types of colimitations are nitrogen–phosphorus, zinc–cobalt, and zinc–carbon colimitations, respectively. It should also be pointed out that the concept of colimitation is closely aligned to that of competitive inhibition, where one substrate can interfere with the acquisition of another (e.g., Mn inhibition of Zn uptake; Sunda and Huntsman 2000, and references therein). Our focus on the biochemical basis for colimitation in this manuscript results in our ignoring these ecologically important competitive effects at this time.

Type I: Independent nutrient colimitation—Mathematical expressions for nutrient limitation of microbes and phytoplankton can be generally classified into at least two related types of equations. First, the Monod equation is an empirical relationship that relates concentration to growth rate using a hyperbolic saturation curve for growth rate or nutrient uptake that is controlled by the substrate concentration (S) (Monod 1942; de Baar 1994), using the biological constants of maximal growth rate μ_{max} and the half-saturation constant K_m (Eq. 1). Second, the effect of an intracellular pool of nutrients and micronutrients can be incorporated into the expressions of growth limitation (equations not shown). This pool of intracellular nutrients, often referred to as a cellular quota (Q), needs to be significantly depleted before growth limitation occurs (Droop 1973; Sterner and Elser 2002). Moreover, the Monod treatment is best suited to steady-state conditions, whereas the Droop cellular quota approach can take into account nonsteady-state perturbations (Morel 1987). In addition, it is generally well recognized that micronutrient quotas (e.g., Fe, Co, Zn) have a much broader range than those of macronutrients (C, N, P), where large trace metal luxury quotas can be generated and maintained by the cell. Growth rate and cellular quota equations for algal growth and nutrient uptake have been shown to be interrelated and result in equivalent growth rates when applied to cultures under steady-state culture conditions (Burmaster 1979). Because the types of culture experiments considered here are usually grown under steady-state conditions using metal-buffered media or chemostats, and because this data set is based on growth rates rather than uptake rates (although under steady-state conditions, the results should be equivalent: where $\rho_{ss} = Q\mu$, with ρ_{ss} representing the steady-state uptake rate [mol cell^{-1} d^{-1}] ; Q = intracellular concentration [mol cell^{-1}], and μ = growth rate [d^{-1}]), our descriptions will use examples built on the steady-state equation for substrate limitation of growth rates (Eq. 1).

$$\mu = \frac{\mu_{max}[S]}{K_m + [S]} \quad (1)$$

Recent applications of growth equations to multiple limitation scenarios has primarily relied on one of two simple approaches thus far (e.g., Moore et al. 2004). Both approaches add an additional term for the second substrate (or for each of the nth substrates), but in the first approach these terms are multiplied (Eq. 2) and in the second the minimum of the two terms is utilized (Eq. 3). Each of these equations can be extended to multiple limitations by extending the polynomial with nth substrates. Type I. Colimitation—multiplicative form:

$$\mu = \mu_{max} \frac{[S_1]}{K_{m1} + [S_1]} \cdot \frac{[S_2]}{K_{m2} + [S_2]} \quad (2)$$

Type I. Colimitation—minimum form (Liebig's law):

$$\mu = \min\left(\frac{\mu_{max}[S_1]}{K_{m1} + [S_1]}, \frac{\mu_{max}[S_2]}{K_{m2} + [S_2]}\right) \quad (3)$$

Droop (1973) points out that the application of Eq. 2 (and the related cellular quota multiplicative equations) to a large number of nutrients is problematic because the concentrations of nonlimiting nutrients would have to be high for their aggregate product to not significantly depress the calculated growth rate. This is because each substrate term imposes its degree of nutrient limitation as a value between 0 and 1; thus several almost-saturated nutrients together can significantly reduce the growth rate from the maximal growth rate. Equation 3 avoids this problem by imposing a strict Liebig limitation ideology, where only the most limiting nutrient is allowed to influence growth rate. This Liebig approach has been used recently in examinations of this type-I-style multiple limitations (Klausmeier et al. 2004). In addition, the multiplicative approach was used in a global marine ecosystem model for iron–light colimitation to allow significant iron and light colimitation effects to occur, while the minimization form was used for all other nutrients (Sunda and Huntsman 1997; Timmermans et al. 2001; Moore et al. 2004). An example type I colimitation plot is represented in Fig. 2, where the multiplicative Eq. 2 form is on the left and the more angular effect of the Liebig minimization Eq. 3 is on the right.

Experimental examination of the validity of each of these type I colimitation equations was conducted independently in two studies (Droop 1974 for P and B_{12}; Rhee 1978 for N and P), where both studies did not find evidence for a multiplicative effect of multiple limitations (Eq. 2), and instead found that colimitation results fit the threshold Liebig minimum expression (Eq. 3). Interestingly, Droop notes that from his cellular quota studies on phytoplankton: "One is driven to the conclusion that the biochemical details of uptake and utilization of the various nutrients have very little bearing on the appearance of the kinetic

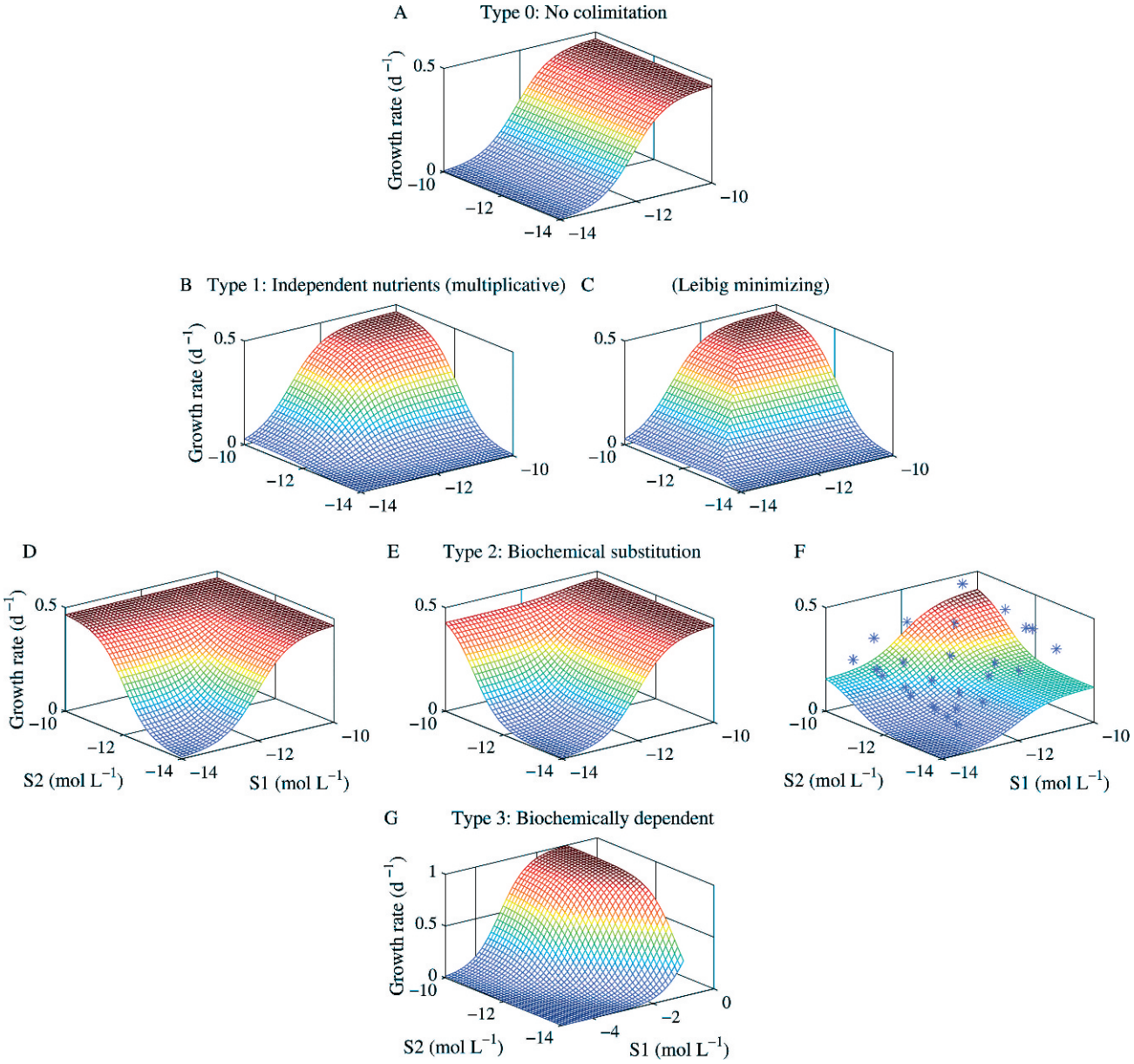

Fig. 2. Three-dimensional representations of colimitation scenarios, where S_1 and S_2 are the substrate nutrients. (A) Type 0, no colimitation, concerns two elements, where only one is a nutrient (Eq. 1). Type I, independent nutrients, concerns two nutrients that do not share a specific biochemical function, such as nitrogen and phosphorus. Two expressions of type I are plotted: (B) the multiplicative form (Eq. 2), and (C) the minimization (Liebig) form (Eq. 3). Type II, biochemical substitution, concerns two micronutrients that can substitute for the same biochemical function, usually due to a metalloenzyme that can be active with two different metals (e.g., Zn and Co). Three scenarios are presented, (D) where two nutrients substitute perfectly for each other (Eq. 4, $K_{m1} = K_{m2}$), (E) where the two nutrients have unique half-saturation constants (Eq. 4), and (F) where two nutrients only partially substitute for each other, leaving a nonsubstitutable component of each biochemical quota (Eq. 6). In this case, maximal growth occurs when both nutrients are present, representing a situation where the cambialistic metalloenzyme constitutes only a fraction of the total S_1 and S_2 quotas (Fig. 3). For simplicity, $K_{m3} = K_{m1}$ and $K_{m4} = K_{m2}$, using values from *Phaeocystis antarctica* grown under Zn-Co colimiting conditions (Saito and Goepfert 2008), and $\mu_{Nosub} = 0.27$ and $\mu_{Camb} = 0.17$ chosen to sum to a slightly lower maximal growth rate than observed in that study (0.44 d^{-1} vs. 0.47 d^{-1}) so that overlaid *P. antarctica* data remain visible above the surface. (G) Type III, biochemically dependent colimitation, concerns two nutrients where the acquisition of one (S_1) is dependent on the sufficient nutrition of the other (S_2) (e.g., C and Zn). The equation from Buitenhuis et al. (2003) is used (Eq. 9) using their values. See Table 1 for further colimitation examples.

relationship between substrate concentration and growth (Droop 1974).'' He continues that: ''The burden of this argument holds some comfort for ecologists, for it suggests that they may be spared the necessity of becoming biochemists in addition to being mathematicians (Droop 1974).'' It is interesting that the very few empirical analyses of growth rates under type I colimitation seem to follow the minimum (threshold) response of Eq. 3, whereas it seems

clear to us that the biochemical response to nutrient stress must result in the up-regulation of high-affinity transport systems even under conditions of multiple nutrient stresses. For these few studies to not observe a multiplicative effect suggests that the energetic cost of this up-regulation of the necessary biochemical machinery is too small to detect, a result we find difficult to reconcile. However, one can imagine that the biochemical cost associated with B_{12} acquisition is too small to be significant in these physiological measurements, given its extremely small cellular quota (Droop 1974). Perhaps colimitation experiments as comprehensive and detailed as Droop's studies would be better suited for a pair of nutrients known to demand significant cellular machinery. It seems then that further experimental examination, perhaps with the biochemical emphasis that Droop wished to spare us of, is needed to better assess the merits of the multiplicative and minimum parameterizations of type I colimitation.

Type II: Biochemical substitution colimitation—With progress in marine bioinorganic chemistry, it has become apparent that various metals can substitute for an active site within a metalloenzyme. Enzymes with this characteristic are termed cambialistic (Sugio et al. 2000; Tabares et al. 2003; Wolfe-Simon et al. 2005). In addition, multiple forms of enzymes with equivalent functionalities but with different metals in the active site can co-occur within an organism. Two of the best studied examples of this type of system in phytoplankton are (1) the carbonic anhydrases in the marine diatoms that can utilize zinc, cobalt, or cadmium (e.g., Morel et al. 1994; Sunda and Huntsman 1995*a*; Lane et al. 2005), and (2) a variety of superoxide dismutases (SODs) that contain Ni, Mn, Fe, or both Cu and Zn in their active sites (see Wolfe-Simon et al. 2005 and references therein). Biochemical (cambialistic) substitution has also been observed for Mn and Fe within a single SOD enzyme (Sugio et al. 2000; Tabares et al. 2003; Wolfe-Simon et al. 2005), although this substitution does not appear to be present in marine phytoplankton: the diatoms and cyanobacteria examined appear to rely on MnSOD or NiSOD, respectively (Peers and Price 2004; Wolfe-Simon et al. 2005; Dupont et al. 2006). We have previously used three-dimensional (3D) representations of growth-rate data for cobalt and zinc culture experiments as a means to intuitively display this type II biochemical substitution effect (Saito 2001; Saito et al. 2002, 2003). If cobalt could completely substitute for all of zinc's biochemical functions, and vice versa, we could utilize the colimitation equation (Eq. 4) for ammonia–nitrate colimitation (O'Neill et al. 1989). This equation allows unique K_m values for each nutrient (unique transport systems), but assumes that each nutrient alone can provide the full nutritional requirement needed to achieve μ_{max} (perfect biochemical substitution), which is not necessarily true for cobalt and zinc interreplacement. Type II. Colimitation with perfect biochemical substitution:

$$\mu = \mu_{max}\left(\frac{S_1/K_{m1} + S_2/K_{m2}}{1 + S_1/K_{m1} + S_2/K_{m2}}\right) \quad (4)$$

Rewriting this equation produces Eq. 5, a more intuitive version where two Monod terms are added to allow the substitution effect, but with an extra attenuating term in each denominator, avoiding growth rates greater than μ_{max}.

$$\mu = \mu_{max}\left(\frac{S_1}{K_{m1} + S_1 + \frac{S_2 K_{m1}}{K_{m2}}} + \frac{S_2}{K_{m2} + S_2 + \frac{S_1 K_{m2}}{K_{m1}}}\right) \quad (5)$$

The multiple limitation described by Eq. 4 produces a 3D surface similar to that observed for cobalt–zinc colimitation (Saito et al. 2002). If K_m values are set equal, a symmetrical pattern is produced that clearly shows interreplacement of the two nutrients and that is quite distinct from the type I colimitation described above (Fig. 2D, type II). Trace element uptake studies and genomic analysis of marine phytoplankton both demonstrate that there are likely multiple transport systems for each trace element (Sunda and Huntsman 1995*a*; Armbrust et al. 2004; John et al. 2007), as in the cases of zinc and cobalt, and that a single transporter can transport different elements with different affinities (Sunda and Huntsman 1995*a*, 2000). As a result, Eq. 4 is also graphically presented using unique K_m values for each metal, giving it a nonsymmetrical shape (Fig. 2E, type II).

It has been observed that although substitution of certain trace elements allows for significant recovery of growth rates, optimal growth often occurs with one of the two trace elements. For example, the marine centric diatoms appear to have a preference for zinc, which can be partially replaced by cobalt (Sunda and Huntsman 1995*a*). Conversely, the coccolithophore *E. huxleyi* appears to have a preference for cobalt, which can be partially replaced by zinc (Sunda and Huntsman 1995*a*; Saito et al. 2002). *Phaeocystis antarctica* appears to be distinct from either of these examples, displaying a maximal growth rate when both cobalt and zinc are replete (Saito and Goepfert 2008). The notion of a "preference" for a given metal is somewhat vague. There are two potential underlying biochemical processes that could explain these physiological phenomena. First, metalloenyzmes are known to have different enzyme activities when constituted with different metal active sites, as has been observed with carbonic anhydrase enzymes (Tripp et al. 2004). This could manifest itself in physiological differences in growth rate (e.g., a higher growth rate with Zn than Co or vice versa). Second, most biochemical functions involving a metal center are metal specific, and hence cannot retain activity if metal substitution occurs. If the physiology of the cell is considered as a summation of its biochemical pathways, then these two situations described above are likely to both be occurring with respect to a given metal's roles within the cell. We can treat this as an additive scenario, with the combination of two metals acting as independent nutrients for distinct components of the cellular biochemistry, while also simultaneously having a biochemical substitution effect for another component of cellular biochemistry (a cambialistic enzyme). Equation 6 is an example of this, combining the type I independent nutrient colimitation

form for a nonsubstitutable component of the growth rate (μ_{Nosub}, Eq. 2) and the type II biochemical substitution form for the cambialistic component of the growth rate (μ_{Camb}, Eq. 5), and an example plot is shown in Fig. 2F (Type II). This equation does not account for subtle differences in a cambialistic enzyme's activity that might result from substituting S_1 for S_2, as mentioned above (Tripp et al. 2004). Moreover, we have somewhat arbitrarily chosen the multiplicative form of the type I component (Eq. 2), on the basis of our comments above. However, the difference between multiplicative and minimizing forms of the type I component is likely to be small relative to the influence of the biochemical substitution component (Eq. 5). This type II equation (Eq. 6) does a reasonable job of representing our observations in *P. antarctica* of zinc–cobalt substitution with a zinc preference (Saito and Goepfert 2008), where a significant component of the Co and Zn quotas appears to be involved in nonsubstituting processes (Fig. 2F, type II, far right), in contrast to diatom and coccolithophore results that show near-complete recovery when substituting Zn for Co (Fig 2D, E, type II) (Saito et al. 2002; Sunda and Huntsman 1995*a*). Type II. Colimitation combining independent and biochemical substitution:

$$\mu = \mu_{\text{Nosub}}\left(\frac{S_1}{K_{\text{m1}} + S_1} \cdot \frac{S_2}{K_{\text{m2}} + S_2}\right) + \mu_{\text{Camb}}\left(\frac{S_1}{K_{\text{m3}} + S_1 + \frac{S_2 K_{\text{m3}}}{K_{\text{m4}}}} + \frac{S_2}{K_{\text{m4}} + S_2 + \frac{S_1 K_{\text{m4}}}{K_{\text{m3}}}}\right) \quad (6)$$

This formulation of colimitation assumes that the nutrient colimitation affects the independent and cambialistic functions proportionally. In reality, we know that there is very tight control of intracellular metal concentrations and intracellular metal transport via metal chaperone proteins (O'Halloran et al. 1999), but quantitative information on how this might affect relative reservoirs of metalloenzymes or biomolecules is not available at this time. Although it is tempting to derive a colimitation model on the basis of intracellular equilibrium for two metals and a suite of nonsubstituting and substituting enzymes, the data on tight intracellular control of metals imply kinetic (biological) rather than equilibrium control of metals within the cell.

The biochemical underpinnings for maximal growth rate occurring only when both nutrients are present are based on the elemental cellular quotas comprising numerous biochemical components, only one of which is a cambialistic enzyme such as the Co-Zn carbonic anhydrase. This is depicted by the idealized cartoon in Fig. 3 for the Zn and Co quotas, where the carbonic anhydrase is capable of biochemical interreplacement of Zn and Co, although vitamin B_{12} and zinc finger proteins are not known to substitute alternative metals in vivo. This reflects the expectation that biochemical interreplacement is involved in only a subset of the enzymes that utilize a particular metal within the cell. A specific example of this is the active

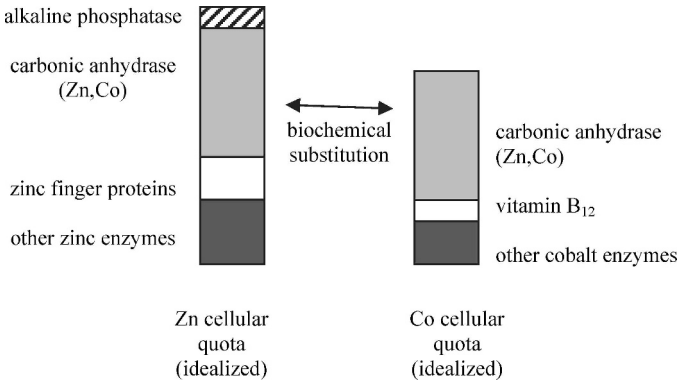

Fig. 3. An idealized schematic of zinc and cobalt cellular quotas in an eukaryotic phytoplankton cell, where the vertical axis is proportional to the number of zinc or cobalt atoms. Zinc and cobalt can substitute biochemically within a carbonic anhydrase metalloenzyme (a DCA1-type enzyme; Morel et al. 2002 and references therein), and there are no other known substitutions between the other bioinorganic functions of these two elements.

site of the DCA1 carbonic anhydrase in diatoms, which can substitute Zn and Co effectively. Ideally, we would consider the influence of limitation of each metal on the regulation of each metalloenzyme system and the subsequent effect on phytoplankton growth rates. However, in reality this summation of the numerous factors for each cellular micronutrient quota is difficult to quantify. In addition to the known functions of trace elements within the cell, novel functions may be discovered in the coming years that will require a reconsideration of the metallome of the cell. Such estimations have been performed for iron, manganese, and molybdenum (Raven 1988, 1990), but quantitative estimates for intracellular cobalt and zinc uses have not yet been determined. For zinc, there are thousands of proteins (primarily zinc finger binding proteins), with many existing at very low copy number (Berg and Shi 1996). Moreover, these cellular quotas and the relative abundance of metalloenzymes are believed to change significantly on the basis of the demands of the environmental setting. Future studies that are enabled by quantitative proteomics, both theoretical (Dupont et al. 2006) and experimental, will allow a decomposition of the cellular trace element quota.

Type III: Biochemically dependent colimitation—The third type of colimitation involves two substrates where the uptake of one substrate is dependent on the sufficient nutrition with regard to the second. There are numerous potential examples of this such as zinc–carbon colimitation via the carbonic anhydrase, nickel–urea colimitation via the nickel metalloenzyme urease, and copper–iron colimitation via the multicopper oxidase and ferric reductase coupled systems (see Table 1). Morel et al. demonstrated the colimitation effect of zinc and carbon dioxide in diatoms originally (Price and Morel 1990; Morel et al. 1994). Carbon acquisition by phytoplankton is an area of active research, and CCMs in marine diatoms have been found to be inhibited by zinc limitation due to the loss of carbonic anhydrase activity (Tortell and Morel 2000), and although the exact details of this pathway have yet to be definitively

worked out, this phenomenon of zinc–carbon colimitation is indisputably observed in physiological studies (Morel et al. 1994, 2002). Buitenhuis et al. (2003) provided an example of an equation for this type of biochemically dependent colimitation, where the K_m for bicarbonate (described here as substrate no. 1 or S_1, Eqs. 7 and 8) is negatively affected by a Michaelis–Menten saturation term for zinc. As zinc becomes limiting the half-saturation constant for bicarbonate increases (Eq. 9, Fig. 2G, type III). It should be pointed out that zinc limitation is not believed to affect the bicarbonate transporter directly, and instead this is approximating the overall effect of the loss of zinc carbonic anhydrase activity upon the CCM. This relationship can be described as a facilitating dependence of S_1 growth on S_2, where in this case S_1 is HCO_3^-, S_2 is Zn^{2+}, α_{S1} is the affinity for bicarbonate, and there are K_m half-saturation values for growth of each respective substrate. Type III. Biochemically dependent colimitation (equations from Buitenhuis et al. 2003):

$$\alpha_{S_1} = \alpha_{S1\max} \cdot \frac{[S_2]}{K_{mS2} + [S_2]} \tag{7}$$

$$K_{mS1} = \frac{\mu_{\max}}{\alpha_{S1}} = \frac{\mu_{\max}}{\alpha_{S1\max}} \left(\frac{[S_2] + K_{mS2}}{[S_2]} \right) \tag{8}$$

$$\mu = \frac{\mu_{\max}[S_1]}{([S_2] + K_{mS2})\mu_{\max}/([S_2]\alpha_{S1\max}) + [S_1]} \tag{9}$$

Type 0 colimitation—Appropriately described as "type 0," this scenario applies to a situation where only one substrate is actually a nutrient (Eq. 1), and is particularly pertinent to the micronutrients cobalt and zinc. For example, in the marine cyanobacteria cobalt is an essential micronutrient and cadmium has no known biological function (Sunda and Huntsman 1995*a*; Saito et al. 2003). This is in contrast to the marine diatoms that can substitute cobalt and cadmium for carbonic anhydrase activity (Lane et al. 2005). Hence, this type 0 descriptor then is primarily useful for comparing organisms with very different micronutrient requirements. Moreover, its graphical representation is useful for comparison with the three actual colimitation types (Fig. 2).

Cobalt and zinc colimitation in marine cyanobacteria is also effectively a type 0 colimitation scenario, on the basis of studies with *Prochlorococcus* and *Synechococcus* (Sunda and Huntsman 1995*a*; Saito et al. 2002). In those studies an absolute requirement for Co was observed, and no Zn substitution was observed for the Co requirement. A Zn limitation effect was not observed in *Synechococcus*, and only a small Zn limitation effect was observed in *Prochlorococcus*, resulting in a 3D growth curve with a type 0 shape (Saito et al. 2002). To be completely accurate, however, Co-Zn colimitation in *Prochlorococcus* may be closer to a type I scenario if a future study with a lower blank showed an absolute Zn requirement, although the Zn concentrations necessary to demonstrate this may be so low as to not be environmentally relevant and hence making Co-Zn colimitation in the marine cyanobacteria an effective type 0 scenario.

Clarification on the criticisms of the concept of colimitation—There is some debate as to whether or not the notion of colimitation is actually valid, where detractors argue for a strict interpretation of Liebig's law of the minimum limitation where only a single nutrient can limit a phytoplankton species at any moment. As described above (type I), the next limiting nutrient is then discussed in terms of being "secondarily limiting." Placing this debate within the context of our three types of limitation outlined above, it is clear that this particular debate concerns only the type I colimitation between independent nutrients, and specifically whether their diminutive effect should be multiplicative (Eq. 2) or not (the minimizing Liebig form of Eq. 3). It is important to point out that type II and type III colimitations are distinct from this debate about Liebig limitation, and should be carefully separated from the discussion. The biochemistries of types II and III colimitations are such that the two (or more) nutrients clearly can act to colimit growth rates simultaneously, either through the effect of biochemical substitution (type II) or depressing the ability for the uptake of another nutrient (type III), and both types have been clearly demonstrated to occur in laboratory cultures (e.g., Morel et al. 1994; Saito and Goepfert 2008).

Going beyond colimitation to multiple limitations—Although the discussion of multiple limitation thus far has focused on two elements being simultaneously limiting and their biochemical manifestations, it is possible that three or more elements could be limiting. Moreover, this multiple limitation scenario is possible as a type I, II, III, or II/III limitation situation as described above. For example, cobalt–zinc colimitation could also negatively affect the ability to acquire carbon, thus resulting in a four-dimensional system where cobalt, zinc, and carbon are affecting growth rate. This added complexity, unfortunately, does not allow the utilization of the 3D plots as a visual aid once we move into four-dimensional space. However, we can imagine that the primary productivity in the oceans, if driven by bottom-up controls as many believe, is likely a combination of types I, II, and III colimitations exerting their influence simultaneously and uniquely on each species of phytoplankton.

In situations where we believe there are colimitation(s) of the phytoplankton community occurring, the phytoplankton community growth dynamics would be comprised of the growth rate of each phytoplankton species in a water body being governed by pertinent colimiting nutrients from the three types described here (Fig. 2) that are applicable (where some species may not have the specific biochemistries to allow a certain type II or III colimitation). (This is of course assuming steady-state conditions and limiting our discussion to influences on growth rates, since mortality terms are not considered here.) Most of the nutrients a phytoplankter requires will likely be saturating and hence not limiting, but the combination of those that are

potentially limiting will evolve as the availability of those nutrients changes. We can imagine a situation such as spring in the Ross Sea when ice cover retreats, making a nutrient-rich environment available for exploitation by eukaryotic phytoplankton. The ecosystem is known to move from iron and nutrient replete to rapid drawdown and export of trace elements (Sedwick et al. 2000), and eventually significant pCO_2 drawdown. A possible succession of nutrient scenarios includes: nutrient and micronutrient replete, to iron-limited (type I), then adding zinc–cobalt colimitation (type II), then finally adding zinc–cobalt–carbon colimitation (type III). It will be exciting when both analytical micronutrient methods and molecular stress diagnostics are advanced and reliable enough to detect such nutrient colimitation succession patterns.

Thoughts on the potential for colimitation in the marine environment—We have briefly reviewed the literature and added to the concept of colimitation (the type II scenario), outlined three types of colimitation, and presented graphical representations of how these types of colimitation are different. In Table 1, we have presented examples of each of these types of colimitation on the basis of previous studies of specific nutrient pairings. This section provides a very brief discussion of our knowledge of the potential for selected scenarios to occur in nature. The reader is directed to the referenced sources and references therein in each nutrient's literature for a comprehensive discussion (Table 1).

One of the goals of this manuscript is to use our understanding from laboratory studies to clarify thinking and provide a common language with which we can discuss the problem of colimitation of marine primary productivity. Taking this understanding to the field to determine the extent to which colimitation occurs in nature is yet another challenge. A complicating factor is that many of these nutrients can be found in multiple types of colimitation, such as nitrogen, with its potential type I (ammonia–phosphate) and type III (urea–nickel), or zinc, which could be found in all three categories (see Table 1). Of the three distinct types of colimitation we have described, type I with independent nutrients remains the form that most envision when discussing the concept, is likely the easiest to assay for, and has been proposed to occur beyond major nutrient colimitation to also include cobalt, zinc, and vitamin B_{12} (e.g., Fe-Zn, Fe-Co, and Fe-B_{12} colimitation; Franck et al. 2003; Saito et al. 2005; Bertrand et al. 2007). There is definitive evidence for types II and III occurring in laboratory settings (Price and Morel 1991; Morel et al. 1994; Sunda and Huntsman 1995*a*).

The ability to probe for colimitation in the oceans is difficult because the traditional methods, such as bottle incubations, may mask the subtleties of a colimitation nutrient pair behind experimental error or changes in community structure (or multiple limitations for three or more nutrients). In particular, the assay methods for finding type II and type III colimitations may need to be fundamentally different from that of type I. The standard trace metal clean bottle incubation experiments used to demonstrate iron limitation of HNLC regions is often considered as the yardstick to assay for limitation. Although a major breakthrough when initially developed (Martin and Fitzwater 1988), this type of incubation is arguably the application of a tool comparable with a sledgehammer: enrichment incubations have been powerful in clearly demonstrating iron limitation, but may not be agile or subtle enough to reliably detect colimitation. In iron enrichment experiments, the addition of iron allows eukaryotic phytoplankton, usually diatoms, to race ahead of the remainder of the phytoplankton community presumably through a combination of rapid growth rates and a temporary lack of grazing pressure (Landry et al. 2000). In marked contrast, the cyanobacteria in HNLC regions were believed to not be iron limited (Wells et al. 1994) until sophisticated in situ cell-cycle growth-rate techniques were applied (Mann and Chisholm 2000). Because the grazers of these smaller cyanobacteria were able to respond almost immediately to the doubling of *Prochlorococcus* growth rates, the actual biomass of *Prochloroccoccus* did not increase, and hence the increase in growth rate, and hence the nutrient limitation, was not observed using standard bottle incubation techniques. The arrival of molecular diagnostics for nutrient stress provides us with more dexterous tools that can simultaneously target specific phytoplankton groups and nutrients. Examples of such diagnostics include the alkaline phosphatase–phosphorus stress assay (Dyhrman et al. 2002) and expression studies of iron stress genes (Webb et al. 2001).

The type II/III zinc–carbon (and cobalt–carbon, cadmium–carbon) colimitation scenario is likely another system that will be difficult to assay for in the natural environment. Although there is evidence for low pCO_2 causing increases in Cd uptake rates in bottle incubation experiments off the California coast (Cullen et al. 1999), attempts to detect actual Co-C, Zn-C, or Cd-C colimitation itself in the field have been unsuccessful thus far. Assaying for this type of limitation is difficult because of several reasons. First, there is the practical difficulty of manipulating carbon and the highly contamination-prone zinc simultaneously in experiments. Second, the effect that may be observed from Zn-C colimitation is likely to be a subtle one: because this is a type III colimitation, the increase in Zn abundance should improve the ability to acquire carbon, as has been observed in culture studies. This improvement in growth rate is likely to be much more subtle than the chronic iron limitation of diatoms in HNLC regions: the zinc–carbon colimitation laboratory experiments demonstrate that low Zn and low CO_2 can cause reduced growth rates that are ameliorated by increases in Zn (e.g., Morel et al. 1994, Tortell and Morel 2000). These data show that zinc deficiency causes a decrease in the efficiency of the CCM, resulting in suboptimum performance due to the loss of carbonic anhydrase activity, and hence the lack of Zn should result in a subtle decrease in growth rates and sensitivity to CO_2 that may be hard to detect with traditional incubation methods.

Iron and light colimitation may be one of the most important colimitations in the marine environment, having been observed in high-latitude marine environments (Maldonado et al. 1999; Boyd et al. 2001; Coale et al. 2004). Yet

the complexities of these "nutrients" makes their colimitation somewhat difficult to classify as a specific type of colimitation. They have been treated as a type I colimitation, using the multiplicative Eq. 2, in global marine ecosystem primary productivity models (Moore et al. 2004). Here we argue that they probably should be considered as a type III colimitation, with Fe affecting light acquisition. It is important to note that this is distinct from the converse situation of light affecting Fe acquisition, where our definition of type III colimitation involves the increase in the half-saturation constant for growth of one substrate as result of a decrease in environmental availability of a second substrate (e.g., Fe modulating K_{MLight}), and not the considerably more complex scenario of both substrates affecting the others' half-saturation constants for growth. This putative assignment for a type III colimitation of Fe on light is based on stoichiometric calculations and laboratory experiments that have demonstrated that iron limitation results in decreases in cellular chlorophyll likely from an inability to synthesize the iron-containing photosynthetic units (PSUs), in particular the iron-rich photosystem I (Raven 1988; Sunda and Huntsman 1997). Iron limitation should then decrease the capability for acquiring photons from the reduction in PSUs, as supported by measurements of photochemical quantum yield (Berman-Frank et al. 2001). We can consider the converse colimitation dependence, where light now influences the acquisition of iron, for example through reducing the energy available to the iron reductase. If this were to be true it would be difficult to parameterize since it suggests that both light and iron influence the acquisition of the other, rather than just one substrate controlling the acquisition of a second as described in Eq. 9. However, this specific cellular phenomenon has not yet, to our knowledge, been differentiated from a more general cellular response to light limitation. For example, most experiments of iron uptake and iron reductase activity in marine diatoms have been conducted in the dark to avoid photochemical reactions, suggesting sufficient residual cellular energy for iron acquisition without a continuous input of photons (Anderson and Morel 1982; Maldonado and Price 2001; Shaked et al. 2005). Indeed, these photochemical reactions with iron may also be responsible for the observed enhancement in iron uptake with increasing light observed in field studies (Maldonado et al. 1999) through increased bioavailability of iron via photochemical reduction of natural iron ligands. Although this distinction does not detract from the convincing evidence of colimitation of light and iron in the North Pacific and the Southern Ocean (Maldonado et al. 1999), it is important in our attempt to correctly classify and hence parameterize iron–light colimitation.

It is also known that modulations in light levels affect the cellular quotas for iron, where a decrease in light causes an increase in the iron cellular quota (Sunda and Huntsman 1997). However, this increase in quota is believed to be due to changes in growth rate caused by light and to not directly affect the cell's ability to acquire iron. For example, at a given iron concentration the uptake rate of inorganic iron at different light levels remains constant at the maximum rates permitted by physics and chemistry (kinetics of Fe transfer to surface uptake sites and available space on the cell's membrane), resulting in different quotas as cellular division changes (a phenomenon referred to as a biodilution effect, Sunda and Huntsman 1997). As a result, we conclude that there is evidence for a type III colimitation of Fe influencing the ability of the cell to acquire light, but not the converse colimitation order of light directly influencing Fe acquisition.

Finally, the potential for colimitation involving trace elements occurring in nature is likely to be crucially influenced by trace metal speciation, as demonstrated earlier with the simple PAPPE calculations. Situations could easily arise where a component of the phytoplankton community is limited by a different suite of nutrients from another group of phytoplankton because of differences in metal complexation chemistry. Indeed, such a scenario of ecological warfare through metal ligand production may have evolved through geologic time between the prokaryotes (especially the cyanobacteria) and the eukaryotic phytoplankton, as we have previously outlined (Saito et al. 2003). A possible modern example of this is the Costa Rica upwelling dome, where we have observed Fe-Co colimitation, and where all the cobalt in surface waters is strongly bound to strong organic ligands (Saito et al. 2005). In such a scenario, the cyanobacterial component of the community is limited by type I Fe-Co colimitation, while the diatoms appear to be type I/II- and perhaps III-limited as well (e.g., Fe-Zn/Co, C). Because the cyanobacteria do not substitute Zn for Co (type 0), and are believed to be able to access the cobalt bound by strong ligands (Saito et al. 2002), they have a distinct niche from diatoms that have type II Co-Zn substitution capabilities and are believed to be unable to access Co and Zn bound by strong ligands. This is consistent with the observations of Fe-Zn and Fe-Co stimulation of the productivity in the Costa Rica upwelling dome in 2005 (Franck et al. 2003; Saito et al. 2005).

In this manuscript we have organized the numerous pairs of colimitation into three distinct categories on the basis of their biochemical relationships (types II and III) or lack thereof (types 0 and I). We have also discussed how the concentrations of many nutrients and micronutrients in the oceans are potentially close to colimiting, but that our predictive ability is largely clouded by the large uncertainties surrounding both the diversity of the chemical species of each nutrient and the diversity of biological strategies phytoplankton use to acquire those various chemical forms. Although the 3D representations of colimitations presented here clearly differentiate the three types, the mathematical descriptions are intended primarily as examples. There is a tension between the theoretically based Michaelis–Menten enzyme kinetics, derived from specific chemical reactions, and the empirical Monod growth equation and the permutations used here, which may be consistent with observations, but are not based in theory because they essentially approximate the aggregate of all cellular reactions. An obvious useful continuation of this study would be to recast these types of colimitation using the Droop equations that incorporate cellular elemental quotas, which, while still an empirical treatment, might

better allow for the compartmentalization of cellular quotas in different biochemical functions. Developing an understanding of the factors controlling primary production in the oceans is important for global carbon cycle modeling. We hope this study provides some of the language and the impetus to develop new strategies with which to address the complexities of this problem.

References

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

Armbrust, E. V., and others. 2004. The genome of the diatom *Thalassiosira pseudonana*: Ecology, evolution and metabolism. Science **306:** 79–86.

Arrigo, K. R. 2005. Marine microogranisms and global nutrient cycles. Nature **437:** 349–355.

Azam, F., T. Fenchel, J. G. Field, J. S. Gray, L. A. Meyer-Reil, and F. Thingstad. 1983. The ecological role of water-column microbes in the sea. Mar. Ecol. Prog. Ser. **10:** 257–263.

Bates, N. R., A. F. Michaels, and A. H. Knap. 1996. Seasonal and interannual variability of oceanic carbon dioxide species at the U.S. JGOFS Bermuda Atlantic Time-series Study (BATS) site. Deep-Sea Res. II **43:** 347–383.

Benitez-Nelson, C. R. 2000. The biogeochemical cycling of phosphorus in marine systems. Earth-Sci Rev. **51:** 109–135.

Berg, J. M., and Y. Shi. 1996. The galvanization of biology: A growing appreciation for the roles of zinc. Science **271:** 1081–1085.

Berman-Frank, I., J. T. Cullen, Y. Shaked, R. Sherrell, and P. G. Falkowski. 2001. Iron availability, cellular quotas, and nitrogen fixation in *Trichodesmium*. Limnol. Oceanogr. **46:** 1249–1260.

Bertrand, E. M., and others. 2007. Vitamin B_{12} and iron colimitation of phytoplankton growth in the Ross Sea. Limnol. Oceanogr. **52:** 1079–1093.

Bishop, J. K. B., S. E. Calvert, and M. Y. S. Soon. 1999. Spatial and temporal variability of POC in the northeast Subarctic Pacific. Deep-Sea Res II. **46:** 2699–2733.

Boyd, P. W. C., G. R. DiTullio, F. B. Griffiths, D. A. Hutchins, B. Queguiner, P. N. Sedwick, and T. W. Trull. 2001. Control of phytoplankton growth by iron supply and irradiance in the subantarctic Southern Ocean: Experimental results from the SAZ project. J. Geophys. Res. **106:** 31573–31584.

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

Buitenhuis, E. T., K. R. Timmermans, and H. J. W. De Baar. 2003. Zinc–bicarbonate colimitation of *Emiliania huxleyi*. Limnol. Oceanogr. **48:** 1575–1582.

Burmaster, D. 1979. The continuous culture of phytoplankton: Mathematical equivalence among three steady-state models. Am. Nat. **113:** 123–134.

Coale, K. H., and others. 2004. Southern Ocean iron enrichment experiment: Carbon cycling in high- and low-Si waters. Science **304:** 408–414.

Cullen, J. T., T. W. Lane, F. M. M. Morel, and R. M. Sherrell. 1999. Modulation of cadmium uptake in phytoplankton by seawater CO_2 concentration. Nature **402:** 165–167.

de Baar, H. J. W. 1994. von Liebig's Law of the Minimum and plankton ecology (1899–1991). Prog. Oceanogr. **33:** 347–386.

Droop, M. R. 1973. Some thoughts on nutrient limitation in algae. J. Phycol. **9:** 264–272.

———. 1974. The nutrient status of algal cells in continuous culture. J. Mar. Biol. Assoc. U.K. **54:** 825–855.

Dupont, C. L., S. Yang, B. Palenik, and P. E. Bourne. 2006. Modern proteomes contain putative imprints of ancient shifts in trace metal geochemistry. Proc. Natl. Acad. Sci. USA **103:** 17822–17877.

Dyhrman, S. T., P. D. Chappell, S. T. Haley, J. W. Moffett, E. D. Orchard, J. B. Waterbury, and E. A. Webb. 2006. Phosphonate utilization by the globally important marine diazotroph *Trichodesmium*. Nature **439:** 68–71.

———, E. Webb, D. M. Anderson, J. Moffett, and J. Waterbury. 2002. Cell specific detection of phosphorus stress in *Trichodesmium* from the Western North Atlantic. Limnol. Oceanogr. **47:** 1823–1836.

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

Falkowski, P. G. 1997. Evolution of the nitrogen cycle and its influence on the biological sequestration of CO_2 in the ocean. Nature **387:** 272–274.

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

Harrison, P. J. 2002. Station Papa time series: Insights into ecosystem dynamics. J. Oceanogr. **58:** 259–264.

Hein, M., and K. Sand-Jensen. 1997. CO_2 increases oceanic primary productivity. Nature **388:** 526–527.

Howarth, R. W., and J. J. Cole. 1985. Molybdenum availability, nitrogen limitation and phytoplankton growth in natural waters. Science **229:** 653–655.

Ichikawa, T. 1982. Particulate organic carbon and nitrogen in the adjacent seas of the Pacific Ocean. Mar. Biol. **68:** 49–60.

John, S. G., R. W. Geis, M. A. Saito, and E. A. Boyle. 2007. Zinc isotope fractionation during high-affinity and low-affinity zinc transport by the marine diatom *Thalassiosira oceanica*. Limnol. Oceanogr. **52:** 2710–2714.

Karl, D. M., and K. Yanagi. 1997. Partial characterization of the dissolved organic phosphorus pool in the oligotrophic North Pacific Ocean. Limnol. Oceanogr. **42:** 1398–1405.

Klausmeier, C. A., E. Litchman, and S. A. Levin. 2004. Phytoplankton growth and stoichiometry under multiple nutrient limitation. Limnol. Oceanogr **49:** 1463–1470.

Landry, M. R., J. Constantinuo, M. Latasa, S. L. Brown, R. R. Bidigare, and M. E. Ondrusek. 2000. Biological response to iron fertilization in the eastern equatorial Pacific (IronEx II). III. Dyamics of phytoplankton growth and microzooplankton grazing. Mar. Ecol. Prog. Ser. **201:** 57–72.

Lane, T. W., M. A. Saito, G. N. George, I. J. Pickering, R. C. Prince, and F. M. M. Morel. 2005. A cadmium enzyme from a marine diatom. Nature **435:** 42.

Legovic, T., and A. Cruzado. 1997. A model of phytoplankton growth on multiple nutrients based on the Michaelis-Menten-Monod uptake, Droop's growth and Liebig's law. Ecol. Model. **99:** 19–31.

Liebig, J. V. 1855. Principles of agricultural chemistry with special reference to the late researches made in England. Dowden, Hutchinson & Ross.

Maldonado, M. T., P. Boyd, P. J. Harrison, and N. M. Price. 1999. Co-limitation of phytoplankton growth by light and Fe during winter in the NE subarctic Pacific Oean. Deep Sea Res. II **46:** 2475–2485.

———, and N. M. Price. 1996. Influence of N substrates on iron requirements of marine centric diatoms. Mar. Ecol. Prog. Ser. **141:** 161–172.

———, AND N. M. PRICE. 2001. Reduction and transport of organically bound iron by *Thalassiosira oceanica* (Bacillariophyceae). J. Phycol. **37:** 298–309.

MANN, E. L., AND S. W. CHISHOLM. 2000. Iron limits the cell division rate of *Prochlorococcus* in the Eastern Equatorial Pacific. Limnol. Oceanogr. **45:** 1067–1076.

MARTIN, J. H., AND S. E. FITZWATER. 1988. Iron deficiency limits phytoplankton growth in the north-east Pacific subarctic. Nature **331:** 341–343.

———, R. M. GORDON, S. FITZWATER, AND W. W. BROENKOW. 1989. VERTEX: Phytoplankton/iron studies in the Gulf of Alaska. Deep-Sea Res. **36:** 649–680.

MILLS, M. M., C. RIDAME, M. DAVEY, J. L. ROCHE, AND R. J. GEIDER. 2004. Iron and phosphorus co-limit nitrogen fixation in the eastern Tropical Atlantic. Nature **429:** 292–294.

MONOD, J. 1942. La croissance des cultures bacteriennes. Hermann.

MOORE, J. K., S. C. DONEY, AND K. LINDSAY. 2004. Upper ocean ecosystem dynamics and iron cycling in a global three-dimensional model. Global Biogeochem. Cycles **18**, GB4028, doi:10.1029/2004GB002220.

MOREL, F. M. M. 1987. Kinetics of nutrient uptake and growth in phytoplankton. J. Phycol. **23:** 137–150.

———, A. J. MILLIGAN, AND M. A. SAITO. 2003. Marine bioinorganic chemistry: The role of trace metals in the oceanic cycles of major nutrients, p. 113–143. *In* H. D. H. K. K. Turekian [ed.], Treatise on geochemistry: Elsevier Science.

———, J. R. REINFELDER, S. B. ROBERTS, C. P. CHAMBERLAIN, J. G. LEE, AND D. YEE. 1994. Zinc and carbon co-limitation of marine phytoplankton. Nature **369:** 740–742.

———, AND OTHERS. 2002. Acquisition of inorganic carbon by the marine diatom *Thalassiosira weissflogii*. Funct. Plant Biol. **29:** 301–308.

NORTH, R. L., S. J. GUILDFORD, R. E. H. SMITH, S. M. HAVENS, AND M. R. TWISS. 2007. Evidence for phosphorus, nitrogen, and iron colimitation of phytoplankton communities in Lake Erie. Limnol. Oceanogr. **52:** 315–328.

O'HALLORAN, T. V., T. D. RAE, P. J. SCHMIDT, R. A. PUFAHL, V. C. CULOTTA, AND T. V. O'HALLORAN. 1999. Undetectable intracellular free copper: The requirement of a copper chaperone for superoxide dismutase. Science **284:** 805–808.

O'NEILL, R. V., D. L. DEANGELIS, J. J. PASTOR, B. J. JACKSON, AND W. M. POST. 1989. Multiple nutrient limitations in ecological models. Ecol. Model. **46:** 147–163.

PALENIK, B., AND F. M. M. MOREL. 1991. Amine oxidases of marine phytoplankton. Appl. Environ. Microbiol. **57:** 2440–2443.

PEERS, G., AND N. M. PRICE. 2004. A role for manganese in superoxide dismutases and growth of iron deficient diatoms. Limnol. Oceanogr. **49:** 1774–1783.

———, S. QUESNEL, AND N. M. PRICE. 2005. Copper requirements for iron acquisition and growth of coastal and oceanic diatoms. Limnol. Oceanogr. **50:** 1149–1158.

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

———, AND ———. 1991. Colimitation of phytoplankton growth by nickel and nitrogen. Limnol. Oceanogr. **36:** 1071–1077.

RAVEN, J. A. 1988. The iron and molybdenum use efficiencies of plant growth with different energy, carbon and nitrogen sources. Plant Phytol. **109:** 279–287.

———. 1990. Predications of Mn and Fe use efficiencies of phototrophic growth as a function of light availability for growth and of C assimilation pathway. New Phytol. **116:** 1–18.

REDFIELD, A. B., B. H. KETCHUM, AND F. A. RICHARDS. 1963. The influence of organisms on the composition of seawater, p. 26–77. *In* M. N. Hill [ed.], The sea, Vol. 2. Wiley Interscience.

RHEE, G.-Y. 1978. Effects of N : P atomic ratios and nitrate limitation on algal growth, cell composition, and nitrate uptake. Limnol. Oceanogr. **23:** 10–25.

RIEBESELL, U., D. A. WOLF-GLADROW, AND V. SMETACEK. 1994. Carbon dioxide limitation of marine phytoplankton growth rates. Nature **361:** 249–251.

ROBINSON, N. J., C. M. PROCTER, E. L. CONNOLLY, AND M. L. GUERINOT. 1999. A ferric-chelate reductase for iron uptake from soils. Nature **397:** 694–697.

RUE, E. L., AND K. W. BRULAND. 1995. Complexation of iron(III) by natural ligands in the Central North Pacific as determined by a new competitive ligand equilibration/adsorptive cathodic stripping voltammetric method. Mar. Chem. **50:** 117–138.

———, AND ———. 1997. The role of organic complexation on ambient iron chemistry in the equatorial Pacific Ocean and the response of a mesoscale iron addition experiment. Limnol. Oceanogr. **42:** 901–910.

SAITO, M. A. 2001. The biogeochemistry of cobalt in the Sargasso Sea. Ph.D. thesis. Massachusetts Institute of Technology-Woods Hole Oceanographic Institution.

———, AND T. J. GOEPFERT. 2008. Zinc-cobalt colimitation of *Phaeocystis antarctica*. Limnol. Oceanogr. **53:** 266–275.

———, AND J. W. MOFFETT. 2001. Complexation of cobalt by natural organic ligands in the Sargasso Sea as determined by a new high-sensitivity electrochemical cobalt speciation method suitable for open ocean work. Mar. Chem. **75:** 49–68.

———, AND ———. 2002. Temporal and spatial variability of cobalt in the Atlantic Ocean. Geochim. Cosmochim. Acta **66:** 1943–1953.

———, ———, S. W. CHISHOLM, AND J. B. WATERBURY. 2002. Cobalt limitation and uptake in *Prochlorococcus*. Limnol. Oceanogr. **47:** 1629–1636.

———, ———, AND G. DITULLIO. 2004. Cobalt and nickel in the Peru upwelling region: A major flux of cobalt utilized as a micronutrient. Global Biogeochem. Cycles **18**, GB4030, doi:4010.1029/2003GB002216.

———, G. ROCAP, AND J. W. MOFFETT. 2005. Production of cobalt binding ligands in a *Synechococcus* feature at the Costa Rica Upwelling Dome. Limnol. Oceanogr. **50:** 279–290.

———, D. SIGMAN, AND F. M. M. MOREL. 2003. The bioinorganic chemistry of the ancient ocean: The co-evolution of cyanobacteria and biogeochemical cycles at the Archean–Proterozoic boundary. Inorg. Chim. Acta **356:** 308–318.

SAMBROTTO, R. M., AND OTHERS. 1993. Elevated consumption of carbon relative to nitrogen in the surface ocean. Nature **363:** 248–250.

SEDWICK, P. N., G. R. DITULLIO, AND D. J. MACKEY. 2000. Iron and manganese in the Ross Sea, Antarctica: Seasonal iron limitation in Antarctic shelf waters. J. Geophys. Res **105:** 11321–11336.

SHAKED, Y., A. B. KUSTKA, AND F. M. M. MOREL. 2005. A general kinetic model for iron acquisition by eukaryotic phytoplankton. Limnol. Oceanogr. **50:** 872–882.

———, Y. XU, K. LEBLANC, AND F. M. M. MOREL. 2006. Zinc availability and alkaline phosphatase activity in *Emiliania huxleyi*: Implications for Zn-P co-limitation in the ocean. Limnol. Oceanogr. **51:** 299–309.

STERNER, R. W., AND J. J. ELSER. 2002. Ecological stoichiometry: The biology of elements from molecules to the biosphere. Princeton Univ. Press.

SUGIO, S., B. Y. HIRAOKA, AND F. YAMAKURA. 2000. Crystal structure of cambialistic superoxide dismutase from *Porphyromonas gingivalis*. Eur. J. Biochem. **267:** 3487–3495.

Sunda, W., and R. R. L. Guillard. 1976. The relationship between cupric ion activity and the toxicity of copper to phytoplankton. J. Mar. Res. **34:** 511–529.

———, and S. A. Huntsman. 1992. Feedback interactions between zinc and phytoplankton in seawater. Limnol. Oceanogr. **37:** 25–40.

———, and ———. 1995*a*. Cobalt and zinc interreplacement in marine phytoplankton: Biological and geochemical implications. Limnol. Oceanogr. **40:** 1404–1417.

———, and ———. 1995*b*. Iron uptake and growth limitation in oceanic and coastal phytoplankton. Mar. Chem. **50:** 189–206.

———, and ———. 1997. Interrelated influence of iron, light, and cell size on marine phytoplankton growth. Nature **390:** 391–392.

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

Tabares, L. C., C. Bittel, N. Carrillo, A. Bortolotti, and N. Cortez. 2003. The single superoxide dismutase of *Rhodobacter capsulatus* is a cambialistic, manganese-containing enzyme. J. Bacteriol. **185:** 3223–3227.

Timmermans, K. R., and others. 2001. Co-limitation by iron and light of *Chaetoceros brevis*, *C. dichaeta* and *C. calcitrans* (Bacillariophyceae). Mar. Ecol. Prog. Ser. **217:** 287–297.

Tortell, P. D., and F. M. M. Morel. 2000. Inorganic carbon acquisition in coastal Pacific phytoplankton communities. Limnol. Oceanogr. **45:** 1485–1500.

Tripp, B. C., C. B. Bell, F. Cruz, C. Krebs, and J. G. Ferry. 2004. A role for iron in an ancient carbonic anhydrase. J. Biol. Chem **279:** 6683–6687.

Webb, E. A., J. W. Moffett, and J. B. Waterbury. 2001. Iron stress in open ocean cyanobacteria (*Synechococcus*, *Trichodesmium*, and *Crocosphaera*): Identification of the idiA protein. Appl. Environ. Microbiol. **67:** 5444–5452.

Wells, M. L., N. M. Price, and K. W. Bruland. 1994. Iron limitation and the cyanobacterium *Synechococcus* in equatorial Pacific waters. Limnol. Oceanogr. **39:** 1481–1486.

Winn, C. D., F. T. Mackenzie, C. J. Carrillo, C. L. Sabine, and D. M. Karl. 1994. Air–sea carbon dioxide exchange in the North Pacific Subtropical Gyre: Implications for the global carbon budget. Global Biogeochem. Cycles **8:** 157–163.

Wisniewski, R. J. 2006. Relating the biogeochemistries of zinc, cobalt, and phosphorus to phytoplankton activities in the sea. Ph.D. thesis. MIT-WHOI.

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

Wolfe-Simon, F., D. Grzebyk, O. Schofield, and P. G. Falkowski. 2005. The role and evolution of superoxide dismutases in algae. J. Phycol. **41:** 453–465.

Wong, C. S., N. A. D. Waser, Y. Nojiri, F. A. Whitney, J. S. Page, and J. Zeng. 2002. Seasonal cycles of nutrients and dissolved inorganic carbon at high and mid latitudes in the North Pacific Ocean during the Skaugran cruises: Determination of new production and nutrient uptake ratios. Deep-Sea Res. II **49:** 5317–5338.

Wu, J., and E. A. Boyle. 2002. Iron in the Sargasso Sea: Implications for the processes controlling dissolved Fe distribution in the ocean. Global Biogeochem. Cycles **16:** 331–337.

———, ———, W. Sunda, and L. Wen. 2001. Soluble and colloidal iron in the oligotrophic North Atlantic and North Pacific. Science. **293:** 847–849.

———, W. Sunda, E. A. Boyle, and D. M. Karl. 2000. Phosphate depletion in the western North Atlantic Ocean. Science **289:** 752–762.

Yee, D., and F. M. M. Morel. 1996. In vivo substitution of zinc by cobalt in carbonic anhydrase of a marine diatom. Limnol. Oceanogr. **41:** 573–577.

Received: 21 February 2007
Accepted: 11 June 2007
Amended: 21 July 2007