Annual Review of Marine Science

Ecological Stoichiometry of Ocean Plankton

Allison R. Moreno[1] and Adam C. Martiny[1,2]

[1]Department of Ecology and Evolutionary Biology, University of California, Irvine, California 92697; email: amartiny@uci.edu

[2]Department of Earth System Science, University of California, Irvine, California 92697

Annu. Rev. Mar. Sci. 2018. 10:6.1–6.27

The *Annual Review of Marine Science* is online at marine.annualreviews.org

https://doi.org/10.1146/annurev-marine-121916-063126

Keywords

Redfield ratio, C:N:P, growth rate hypothesis, translation-compensation hypothesis, direct control, state factor

Abstract

Marine plankton elemental stoichiometric ratios can deviate from the Redfield ratio (106C:16N:1P); here, we examine physiological and biogeochemical mechanisms that lead to the observed variation across lineages, regions, and seasons. Many models of ecological stoichiometry blend together acclimative and adaptive responses to environmental conditions. These two pathways can have unique molecular mechanisms and stoichiometric outcomes, and we attempt to disentangle the two processes. We find that interactions between environmental conditions and cellular growth are key to understanding stoichiometric regulation, but the growth rates of most marine plankton populations are poorly constrained. We propose that specific physiological mechanisms have a strong impact on plankton and community stoichiometry in nutrient-rich environments, whereas biogeochemical interactions are important for the stoichiometry of the oligotrophic gyres. Finally, we outline key areas with missing information that is needed to advance understanding of the present and future ecological stoichiometry of ocean plankton.

INTRODUCTION

In the early part of the twentieth century, Alfred Redfield demonstrated that the elemental compositions of surface plankton are uniformly similar to the ratios of dissolved nutrients in the deep ocean and defined the Redfield molar ratio of 106C:16N:1P (Redfield 1958). He proposed three hypotheses to explain this similarity: (*a*) It is merely a coincidence, (*b*) phytoplankton have the ability to change their stoichiometry to match the environmental supply, and (*c*) phytoplankton control the ocean chemistry through the remineralization of exported material. Based on the available data, the first hypothesis seemed unlikely. Redfield proposed that microorganisms set the concentrations of nutrients in the deep ocean through their ability to fix C and N and thus supported the third hypothesis (Augueres & Loreau 2015, Lenton & Klausmeier 2007).

Recent studies have demonstrated systematic variation in the particulate C:N:P across ocean regions and seasons, challenging the paradigm of a constant plankton elemental (Redfield) ratio (**Figure 1**). The variation in community C:N:P across ocean regions has been observed using two separate approaches: directly measuring the elemental stoichiometry of particulate organic matter (Martiny et al. 2013a,b) and using inverse models to indirectly infer this ratio from inorganic nutrient fields (DeVries & Deutsch 2014, Teng et al. 2014, Weber & Deutsch 2010). The C:N, C:P, and N:P ratios of particulate organic matter are below or near Redfield proportions in high-latitude and equatorial upwelling regions but commonly above Redfield proportions in the oligotrophic gyres (**Figure 1**). This pattern is also observed across seasons, with the three ratios higher in the summer and fall (warm and nutrient depleted) and lower in the winter and spring (colder and nutrient replete) at many sites (Martiny et al. 2016b, Singh et al. 2015, Talarmin et al. 2016). In addition, there appear to be some regional differences in ratios across the oligotrophic gyres: C:P is highest in the North Atlantic gyre, next highest in the North Pacific gyre, and only slightly above Redfield proportions in the Southern Hemisphere oligotrophic gyres (**Figure 1**). The C:N ratio also shows differences among oligotrophic gyres, with the highest ratio in the

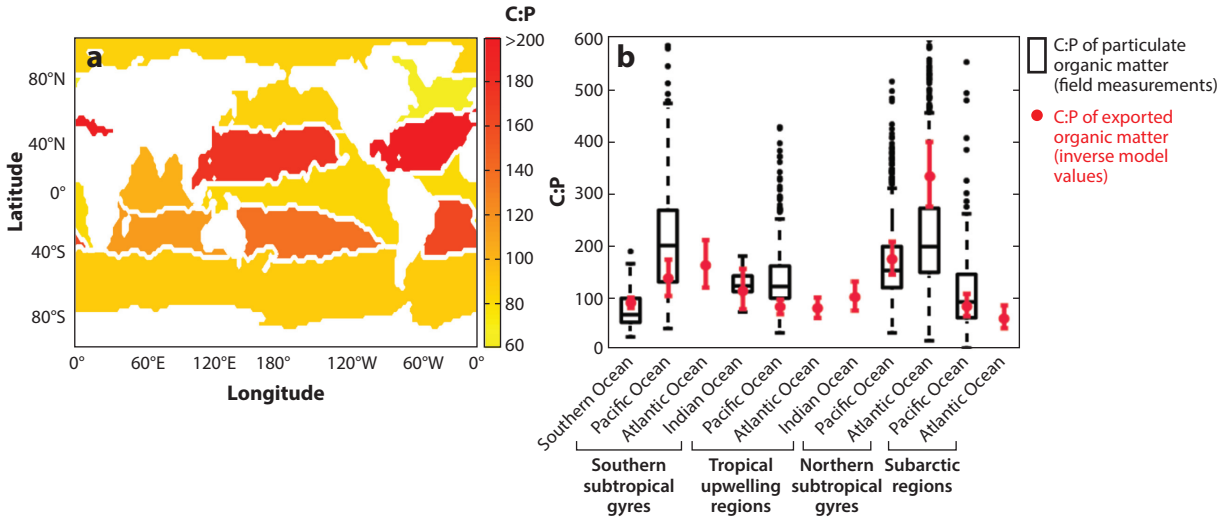

Figure 1

Regional variation in the elemental composition of exported organic matter and surface particulate organic matter. (*a*) C:P of exported matter across 11 regions classified based on the surface phosphate concentration (Teng et al. 2014). (*b*) A comparison of the C:P of exported organic matter (based on inverse model values) and the surface C:P of particulate organic matter (based on field measurements) of these 11 regions (Martiny et al. 2013a,b).

South Atlantic and eastern North Atlantic and the lowest in the North Pacific and western North Atlantic. This raises the question, which mechanism(s) can explain the observed field patterns?

Our review builds on past excellent summaries of this topic (e.g., Flynn et al. 2010, Geider & La Roche 2002, Sterner & Elser 2002). First, we aim to include recent research and modeling work. Second, we aim to put forward plausible mechanisms describing the observed variation in elemental stoichiometry among marine plankton lineages, regions, and seasons. To achieve this, we borrow a framework from ecology covering direct control and state factor mechanisms (Chapin et al. 2012). A direct control mechanism provides an immediate connection between an environmental condition and the cellular or community C:N:P through regulation, acclimation, and selection/adaptation. External state factors control the ecosystem structure and function and associated community stoichiometry through external biogeochemical feedbacks. In this review, we introduce some general concepts about acclimation versus adaptation and provide an overview of how the detailed molecular biochemistry of a cell can be linked to the stoichiometry. We then examine the stoichiometric outcomes based on physiological changes in growth rate, temperature, nutrient limitation, light, C source, interactions between said factors, and finally lineage identity via adaptation. Then we discuss how state factors via ocean nutrient cycles and biogeochemical feedbacks may lead to differences in the overall nutrient supply and associated elemental stoichiometry of ocean regions. Finally, we outline key areas with missing information that is needed to advance understanding of the present and future ecological stoichiometry of ocean plankton, communities, and particulate matter. We use examples of marine plankton but incorporate observations from other systems to support and illustrate the broader applications of our synthesis.

ACCLIMATION VERSUS ADAPTATION

Many hypotheses of ecological stoichiometry interchange processes of acclimation and adaptation when describing the elemental stoichiometric outcome of environmental variation; however, these processes are not synonymous. Acclimation is the physiological process that modifies the cellular biochemistry and elemental requirements in response to an environmental change and occurs within an organism's lifetime. Adaptation is the evolutionary process and occurs intergenerationally, leading to lineage-specific changes in physiological capabilities and elemental composition. Here, changes in the stoichiometry of a community occur through selection. Many ecological stoichiometry models blend the two responses and predict the same effect of an environmental change within (acclimation) and across (adaptation) organisms (**Figure 2*a***). An example is temperature, which regulates the enzyme kinetics and physiology of an organism. In addition, there are clear differences in plankton diversity across a gradient of temperature. As a result, temperature affects the elemental composition of marine communities owing to a combination of changes in physiology and diversity (Hall et al. 2008). These contrasting responses may not lead to the same outcome if the underlying biochemical mechanism differs (**Figure 2*b*,*c***). For instance, a heat shock response is not the same as shifting the optimal temperature for growth. Understanding when acclimative and adaptive responses differ is crucial for properly extrapolating from experimental studies of single lineages to whole communities.

BIOCHEMICAL COMPONENTS

To link biochemical regulation with the elemental composition of cells, we must consider the molecular components underlying the cellular C, N, and P pools and their stoichiometry (Geider & La Roche 2002, Sterner & Elser 2002). Molecules rich in C include carbohydrates and lipids. Peptidoglycan and chitin are two carbohydrates that do contain N, but the specific carbohydrate

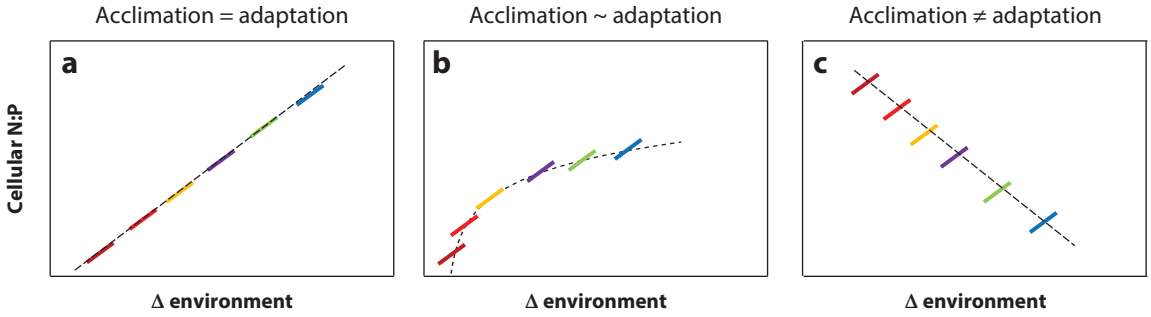

Figure 2

Influence of acclimation and adaptation processes on the relationship between an environmental change and the elemental composition. (*a*) In the first scenario, the environmental impacts on the N:P ratio in a cell that occur via acclimation and adaptation are aligned, and understanding the individual response provides a model for how communities are affected. (*b*) In the second scenario, the two processes are related but lead to a nonlinear interaction. (*c*) In the third scenario, the outcome of a physiological adjustment of the cellular chemistry is the opposite of how a cell adapts to a different environment; in this scenario, physiological models may be misguided when predicting the elemental stoichiometry across ocean gradients. Each colored line represents a different species being examined within an experiment or study.

chemistry in marine plankton is unconstrained. Lipids are also rich in C, but membrane-bound lipids often contain polar head groups with phosphate and/or N (Van Mooy & Fredricks 2010). Molecules rich in N include proteins and photosynthetic components. Protein is the most abundant cellular macromolecule and constitutes on average 32% of the cellular dry weight in phytoplankton (Finkel et al. 2016) and up to 63% in heterotrophic bacteria (Simon & Azam 1989). Thus, protein N is expected to correlate closely with the overall N cell quota (although there is some disagreement about the exact relationship) (Finkel et al. 2016, Lourenço et al. 2004). For phytoplankton, photosynthetic components such as chlorophyll and other associated pigments can also be important N pools. Molecules rich in P include membrane lipids, polyphosphates, and nucleic acids. Phospholipids are relatively P rich (39C:0.8N:1P) but are often a lesser part (<10%) of the P quota in marine organisms (Mouginot et al. 2015, Van Mooy & Devol 2008). Inorganic polyphosphates are an enigmatic component of the P pool, serving as a store of both P and energy (Kornberg et al. 1999). The absolute amount of inorganic P in a marine organism is poorly constrained but could be an important control on the P quota (Daines et al. 2014). Nucleic acids are relatively low in C but contain high N and especially P and have a C:N:P ratio of 9.5:3.7:1 (Sterner & Elser 2002). The ribosome is a key component of cellular biosynthesis and contains a substantial fraction of cellular proteins and RNA (Geider & La Roche 2002, Sterner & Elser 2002). As such, biosynthesis is viewed as a key P-rich and to some extent N-rich process in relation to C. Thus, many ecological stoichiometry hypotheses describing C:P and N:P are based on the regulation of biosynthetic capacity and associated requirement for P.

PHYSIOLOGICAL MODELS FOR ELEMENTAL STOICHIOMETRY

The Growth Rate Hypothesis

The growth rate hypothesis (GRH) states that differences in organismal C:N:P ratios are generated by allocation changes in biosynthetic machinery and associated P-rich ribosomes (Elser et al. 2000). The hypothesis is based on three presumptions: (*a*) Cells achieve a higher growth rate by increasing the abundance of ribosomes containing P-rich rRNA, (*b*) P allocated to rRNA

constitutes the majority of the P cell quota, and (*c*) the P cell quota controls the C:P and N:P of an organism. Thus, the N and C cell quotas should display less or no variation at different growth rates. There are two variants of this hypothesis—namely, that the physiological regulation of the ribosomes of an individual organism is due to either (*a*) acclimation or (*b*) adaptive differences in allocation strategies that result in different C:N:P ratios among lineages (Elser et al. 2000). Thus, it is key to distinguish the two. Here, we first discuss the acclimation variant, which seeks to test the relationship between cellular components (RNA and ribosomes) and growth rate but not the absolute abundance of said components, as it relates to the three presumptions of the GRH. As will become apparent, it is important to keep track of the factor controlling growth (nutrients, temperature, light, etc.).

There is strong experimental support for the first presumption, because increased growth rate generally correlates with an increase in the RNA content within many lineages (as shown in **Table 1**) (Elser et al. 2003). A clear relationship between ribosome counts and growth rate is well established in the model organism *Escherichia coli* (Gausing 1982, Schaechter et al. 1958). A similar positive relationship has also been detected in many marine lineages. The marine cyanobacteria *Synechococcus* sp. WH8101 and WH8102 have a positive relationship between RNA and growth rate under P-limited conditions (Garcia et al. 2016, Van Mooy & Devol 2008). A meta-analysis of diverse plankton found that the freshwater zooplankton species *Daphnia pulicaria* and *Daphnia galeata*, *Drosophila melanogaster*, *E. coli*, and lake bacteria demonstrate a clear linear relationship between RNA content and growth rate (Elser et al. 2003). Note that all species, with the exception of the freshwater zooplankton species, were grown under P-limited conditions; when grown in environments with sufficient P, *D. pulicaria*, *Daphnia pulex*, *E. coli*, and lake bacteria did not statistically show any relationship between RNA content and growth rate (Elser et al. 2003). Under

Table 1 Relationship of RNA to growth rate in diverse species

Species	Controlling mechanism	Relationship	Reference(s)
Escherichia coli	Replete	Positive linear	Gausing 1982, Schaechter et al. 1958
Daphnia pulicaria	Replete	No relationship	Elser et al. 2003
Daphnia pulex	Replete	No relationship	Elser et al. 2003
Escherichia coli	Replete	No relationship	Elser et al. 2003
Lake bacteria	Replete	No relationship	Elser et al. 2003
Freshwater zooplankton	Replete	No relationship	Elser et al. 2003
Daphnia pulicaria	P limited	Positive linear	Elser et al. 2003
Daphnia galeata	P limited	Positive linear	Elser et al. 2003
Drosophila melanogaster	P limited	Positive linear	Elser et al. 2003
Escherichia coli	P limited	Positive linear	Elser et al. 2003
Lake bacteria	P limited	Positive linear	Elser et al. 2003
Synechococcus sp. WH8101	P limited	Positive linear	Van Mooy & Devol 2008
Synechococcus sp. WH8102	P limited	Positive linear	Garcia et al. 2016
Synechococcus sp. WH8102	P limited	Positive linear	Garcia et al. 2016
Escherichia coli	Temperature (0°C to 25°C)	Positive linear	Broeze et al. 1978, Schaechter et al. 1958
Pseudomonas fluorescens	Temperature (20°C and 24°C)	Positive linear	Chrzanowski & Grover 2008
Pseudomonas fluorescens	Temperature (14°C)	Negative linear	Chrzanowski & Grover 2008
Scenedesmus	Temperature (5°C and 25°C)	Negative linear	Rhee & Gotham 1981
Prochlorococcus	Light	Positive linear	Lin et al. 2013

N-limited conditions, *Synechococcus* sp. WH8102 does show a linear relationship between RNA and growth rate (Garcia et al. 2016).

In temperature-limited organisms, the link between growth rate and rRNA content is less clear. If temperature controls the growth rate in *E. coli*, there is little increase in RNA (Broeze et al. 1978, Schaechter et al. 1958). When grown in chemostats with temperature as the controlling factor (14°C, 20°C, and 24°C), the freshwater bacterium *Pseudomonas fluorescens* displays a similar linear relationship between RNA content and growth rate, but only under medium- and high-temperature conditions (Chrzanowski & Grover 2008). At 14°C, *P. fluorescens* showed a statistically significant negative linear relationship with RNA content and growth rate. At 20°C and 24°C, the relationship appears to be quadratic: RNA is high at low growth rates (i.e., 0.03 h^{-1}), has a minimum at a growth rate of 0.10 h^{-1}, and then increases at high growth rates (i.e., 0.13 h^{-1}). In the phytoplankton *Scenedesmus*, there is even a negative relationship between cellular RNA-P and growth controlled by temperature (Rhee & Gotham 1981). These results provide mixed support for higher RNA with growth under changing temperature conditions, but the data set is limited.

There is good support for a positive link between rRNA content and growth in marine phytoplankton when light controls growth (Flynn et al. 2010). Multiple strains of *Synechococcus* and *Prochlorococcus* show a three-phase relationship with growth rate: At low growth rates, rRNA content remain relatively constant; at intermediate growth rates, rRNA increases; and at extremely high growth rates, rRNA plateaus (Binder & Liu 1998, Kramer & Morris 1990, Worden & Binder 2003). However, another study of *Prochlorococcus* found a linear positive relationship (Lin et al. 2013). Thus, there appears to be some differentiation among closely related ecotypes. However, the experiments demonstrate a positive relationship between growth rate and rRNA content for most light-controlled conditions.

There are fewer experimental data supporting the second presumption in the GRH, which states that rRNA-P is the dominant fraction of the P cell quota. In a study of *E. coli*, RNA-P was commonly the majority of the overall P quota (Cotner et al. 2006). However, marine (phyto)plankton data addressing this question are scarce. Rhee (1973) quantified P allocation into RNA-P, DNA-P, lipids, and poly-P of the freshwater algae *Scenedesmus* across growth rates under P-limiting conditions (**Figure 3**).

Rhee's work showed an increase in RNA-P as well as total P cell quota with growth rate and thus appears to support the GRH. However, other P pools increased with growth as well, and rRNA-P constituted only 20–30% of the P cell quota. A similar pattern was observed in marine *Synechococcus* sp. WH8102 (Garcia et al. 2016) and heterotrophic bacteria (Chrzanowski & Grover 2008). Again, RNA-P and total P increased with growth, but only 30% of the total P could be attributed to nucleic acids. Thus, rRNA-P may be only a partial contributor to the overall P cell quota, requiring a better empirical understanding of how other P pools (i.e., polyphosphates, phospholipids, and DNA) are regulated to fully predict how growth affects the P cell quota.

The third presumption in the GRH is that the P cell quota is the main control on C:P and N:P. Multiple experiments have shown that the C and N cell quotas are also sensitive to the growth rate, as many heterotrophic microbial lineages increase cell size with growth (Schaechter et al. 1958, Vadia & Levin 2015). Additional support comes from the finding that the C and N cell quotas of phytoplankton increase up to 100% with growth (Garcia et al. 2016, Goldman et al. 1979, Laws & Bannister 1980). However, the exact change is dependent on the factor limiting growth (P source, N source, or light). Thus, it is important to consider changes in other quotas when predicting the outcome of growth on the elemental stoichiometry of marine plankton, and we have an incomplete understanding of how non-RNA biochemical pools respond to changing growth rates.

Overall, there is support for the underlying biochemical mechanism in the GRH of increasing rRNA with growth under some conditions across marine plankton lineages. There is also support

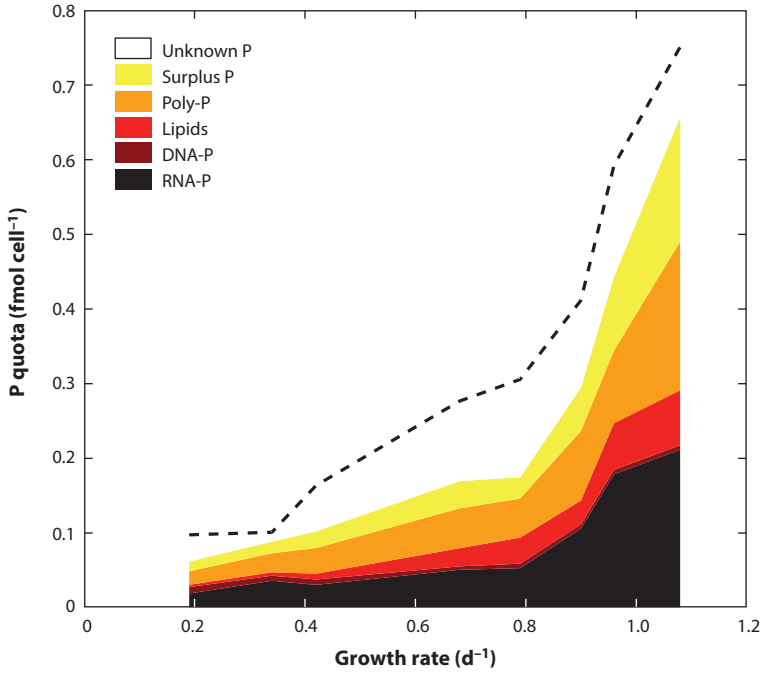

Figure 3

Breakdown of total P quota with increased growth rate. The dashed line represents the total P quota, and the colored regions represent the distribution of P among different cellular components. Data are from Rhee (1973).

that changes in the P quota exert control on C:P and N:P. However, it appears—based on only a few studies—that RNA-P is typically a minor component of the overall P quota. If RNA-P is only a minor component of (and does not control) the P quota, the association between C:P or N:P and growth rate under P limitation could simply be due to a nutrient limitation effect on all P pools, including storage. This conclusion would severely limit the applicability of the GRH for marine systems, and we further examine interactions between multiple factors and the GRH below (see the section titled Interactions).

Temperature

Temperature plays a key role in regulating cellular biochemical processes and elemental composition across all taxa. The relationship between temperature and phytoplankton N:P and C:P stoichiometry has been predicted to be positive based on decreasing cellular allocation to ribosomes at elevated temperatures. We term this idea the translation-compensation hypothesis, which states that as growth rate is held constant, organisms growing at higher temperature will have higher C:P and N:P ratios. The biochemical reasoning for this hypothesis is that the ribosome-specific protein synthesis rate is temperature sensitive (Hochachka & Somero 1984, Toseland et al. 2013, Yvon-Durocher et al. 2015). As such, cells require higher ribosome content at colder temperatures than they do at warmer temperatures to achieve the same growth rate. Similarly to the GRH, this hypothesis relies on the assumption that the ribosome content is an important control on the overall cellular P pool.

There is considerable experimental support for the hypothesis that ribosome efficiency changes in response to temperature across a range of organisms (Broeze et al. 1978, Farewell & Neidhardt 1998, Toseland et al. 2013). The next question is, what happens to the ribosome concentration when the temperature changes? To answer this question, it is essential to keep track of growth rate. In a batch culture experiment, a temperature increase led to an increasing growth rate in *E. coli* but no clear changes in the RNA (or presumably the ribosome content) (Yun et al. 1996). In a chemostat experiment where growth was kept constant, the RNA content was negatively correlated with temperature in *E. coli* and *Aerobacter* (Tempest & Hunter 1965, Yun et al. 1996). However, another study of *E. coli* observed a more complex link between temperature and RNA under a constant growth rate (Cotner & Wetzel 1992). Toseland et al. (2013) observed that the protein synthesis rates and ribosome counts of two phytoplankton species were sensitive to temperature and that phytoplankton growing in high-latitude environments increased their transcription of translationally linked genes, suggesting that their production of ribosomes was greatest at low temperatures. Thus, there is mixed support for a downregulation of RNA with temperature under constant growth.

A limitation of the translation-compensation hypothesis is that temperature also affects many other cellular processes (e.g., represented by the Q_{10} factor) (Geider 1987). Thus, the outcome is driven by a rebalancing of allocations to all processes. For example, temperature influences the expression and function of photosynthetic proteins, including Rubisco (Mackey et al. 2013, Maxwell et al. 1994), whose activity can limit C fixation (Davison 1991). The Q_{10} of photochemistry, including all photochemical processes, ranges from 1.0 to 2.08 (Raven & Geider 1988) but can be higher. For example, the Q_{10} for the carboxylase activity of Rubisco from both *Phaeodactylum tricornutum*, a temperate diatom, and *Nitzschia kerguelensis*, a cold-water diatom, is 2.66 (Raven & Geider 1988). These changes in photosynthetic C fixation machinery could mitigate the decline in RNA-P predicted by the translation-compensation hypothesis. Other examples include the temperature impact on lipid and carbohydrate content, which influences the cellular C quota (Henderson & Mackinlay 1989, Thompson et al. 1992, Zhu et al. 1997).

Several studies have examined how temperature influences the cellular nutrient quotas under constant growth. In *E. coli*, increasing temperature led to increased C and N and decreased P quotas (Cotner et al. 1997). Under nutrient-limited growth and a fixed growth rate, the P quota declined in *Scenedesmus* sp. and *Asterionella formosa*, as predicted by the translation-compensation theory (Rhee & Gotham 1981). The C and N quotas also declined, but the slopes were dependent on nutrient availability (replete or limited by N or P), leading to a complex stoichiometric outcome.

The majority of experiments that examine the influence of temperature on cellular nutrient quotas do not control for growth rate, making it challenging to determine the independent effects of temperature and growth rate on elemental composition. Thus, temperature can simultaneously change the growth rate and the elemental composition, but separating the two can be difficult. The P quota in *Prochlorococcus* under nutrient-replete conditions and varying growth increased slightly with temperature (Martiny et al. 2016b); however, the C and N quotas also changed positively. Quotas were also positively affected in *Synechococcus* sp. CCMP1334 and *Prochlorococcus* sp. CCMP1986 (Fu et al. 2007) as well as in some larger phytoplankton. *Thalassiosira pseudonana*, *Pavlova tricornutum*, and *Pavlova lutheri* demonstrated a U-shaped pattern in the C and N quotas with increasing temperature (Thompson et al. 1992). *Chaetoceros calcitrans* showed a slight variation in the cellular C and N quotas, whereas *Isochrysis galbana* under an exponential growth rate demonstrated highly variable C and N quotas with increased temperature (Thompson et al. 1992). Berges et al. (2002) grew *T. pseudonana* in batch culture at three different temperatures and found that, as the temperature increased, there was an increase in the C quota but relatively no change

in the N quota. In summary, it is clear that temperature affects all three cell quotas, but only a few studies provide direct support for the translation-compensation hypothesis.

Temperature can lead to direct effects on stoichiometry. In *E. coli*, the elemental ratios behave as predicted by the translation-compensation hypothesis. By contrast, *P. fluorescens* showed little systematic change in C:N:P with temperature. In the only temperature-dependence study in phytoplankton under constant growth, N:P and C:P declined, were constant, or even increased with temperature, depending on the condition (Yvon-Durocher et al. 2015). The effect of temperature on elemental stoichiometry has also been quantified without controlling for growth rate. Fu et al. (2007) and Martiny et al. (2016b) observed high variability in C:P and N:P in closely related strains of *Prochlorococcus*, but this variability did not obey a uniform trend. In a single study of *Synechococcus* sp. CCMP1334, there was a decrease in C:N and C:P, whereas temperature changes led to a decrease of ∼20% in C:N and an increase of ∼25% in C:P in *Chaetoceros wighamii* (Spilling et al. 2015).

Temperature has a complex effect on cellular allocations and elemental stoichiometry. Laboratory experiments suggest that ribosomal biosynthesis has a high Q_{10}, providing support for the translation-compensation hypothesis. However, we currently have a limited molecular biological understanding of how temperature leads to a system-wide reallocation of metabolic networks in most marine phytoplankton. Thus, the translation-compensation hypothesis singles out an individual process with a clear temperature dependence but also relies on the weakly supported assumption that other processes are less dependent on temperature. For example, plankton C and N quotas often change with temperature, although systematic mechanisms explaining these observations are lacking. Additionally, our knowledge of the influence of temperature on elemental stoichiometry has been negatively affected by a paucity of experimental studies that control for the influence of temperature on growth rates. Thus, our current understanding of the influence of temperature on elemental stoichiometry is inadequate, and we cannot at this point identify a uniform effect.

Nutrient Limitation

A clear effect of nutrient limitation (i.e., the nutrient supply ratio) on cellular elemental stoichiometry has been documented extensively but shown to be dependent on the specific element limiting growth. Nutrient limitation has historically been described by a simple empirical hyperbolic relationship between cell quotas and growth rate (Droop 1968). The Droop model assumes that cell resources are partitioned into two basic cellular components: a structural pool and a storage pool. The structural pool is linked to the lineage, whereas the storage pool is controlled by specific nutrient uptake rates that determine the growth physiology. In model studies that have tracked multiple nutrients and their stoichiometry, the cellular stoichiometry is predicted to match the environmental supply at low growth rates (the "you are what you eat" phase), and as the rate of nutrient input and associated growth increase, the cellular stoichiometry converges on an optimal ratio (the "you eat what you need" phase) (Klausmeier et al. 2004, Persson et al. 2010). At maximum growth rate, the elemental stoichiometry reaches an organism-specific single value (Bi et al. 2012, Klausmeier et al. 2004, Rhee & Gotham 1980, Sterner & Elser 2002). Thus, both the ratio and rate of the nutrient supply influence the elemental stoichiometry. As discussed above, in a balanced system, the latter is directly tied to the cellular growth rate and the associated growth rate effects on stoichiometry. In this section, we discuss only the impact of the nutrient supply ratio and restrict our attention to studies that specifically control for the effect of nutrient supply on growth rate. This should make it possible to disentangle the influence of the nutrient supply ratio from that of the growth rate.

A non-Droop line of reasoning for how nutrients influence cellular allocations derives from molecular biology. Under P stress, most lineages induce a series of proteins in the phosphate regulon to increase inorganic P uptake or access P bound to organic molecules (Torriani-Gorini 1987, Wanner 1993). The proteins directly involved in transporting phosphate include an outer membrane porin (*pho*E), a phosphate-binding protein (*pst*S), and an ABC transporter (*pst*ABC). To access organically bound P, cells can to varying degrees induce enzymes that cleave phosphoesters (e.g., *pho*A or *pho*X), phosphordiesters (e.g., *pho*D), phosphonates (phn), and so on. Some of these proteins—especially *pho*A, *pho*E, and *pst*S—are highly induced under P stress in both heterotrophic bacteria and phytoplankton lineages (Martiny et al. 2006, Torriani-Gorini 1987). Assuming a constant growth rate, such induction of P acquisition proteins can greatly increase the total protein content of the cell, as seen in *Scenedesmus* (Rhee 1978).

Cells may also respond to stress by reducing P pools, which can be accomplished via several molecular mechanisms, including substituting P located in lipids (Mouginot et al. 2015; Van Mooy et al. 2006, 2009) or drawing down inorganic P storage. The mechanism for substituting lipids has been well documented, but phospholipids may contribute only up to 10% of total P (Mouginot et al. 2015; Van Mooy & Devol 2008; Van Mooy et al. 2006, 2009). There is more uncertainty associated with the regulation of cellular inorganic P (e.g., polyphosphate) accumulation (Kornberg et al. 1999). This pool may not dominate the overall particulate P in marine environments (Diaz et al. 2016), although some studies show inorganic P as a large fraction of the cellular P quota (Rhee 1973). Most stoichiometric models assume that polyphosphates serve primarily as nutrient storage, but polyphosphate has a dual role in microbial metabolism because it can serve as both energy and P storage. The energy storage role is closely tied to growth physiology, but the absolute level of polyphosphate can vary extensively among organisms, as many other compounds can store energy (Mino et al. 1998). In cells where polyphosphates serve primarily as energy storage, we would expect a stronger regulation by growth physiology. However, the regulation of polyphosphates is largely uncharacterized in abundant marine phytoplankton lineages, and the concentration shows an unexplained negative correlation with P availability in marine environments (Diaz et al. 2016, Martin et al. 2014). Thus, there may be unknown interactions between growth rate and nutrient limitation that influence cellular P pools.

The molecular N mirrors to some extent the P stress response. Under N stress, cells can induce a series of proteins facilitating increased N uptake (Herrero et al. 1985, Tolonen et al. 2006). This includes upregulation of ammonia transport and utilization of alternative N sources, such as nitrite, nitrate, urea, and organically bound N (e.g., amino acids or nucleotides). However, it appears that, in contrast to P, N acquisition proteins are induced to a lower level and lead to a smaller change in protein content (Rhee 1978). The degree of N storage in many marine phytoplankton is poorly understood. Lineages may store N in pigment molecules (e.g., cyanophycin and phycocyanin) or amino acids, and N stress influences the cellular pigment content (Caperon & Meyer 1972, Collier & Grossman 1992, Geider et al. 1998, Harrison et al. 1976, Rhee 1978). In addition, some diatoms can store inorganic forms in the vacuole (Conover 1975). However, we lack data for N storage and allocation patterns across many marine lineages, including most of the dominant phytoplankton lineages in the ocean.

There is evidence that nutrient limitation affects the cellular biochemical makeup beyond simple partitioning between structural and storage components. The capacity of excess uptake of nutrients and the degree to which specific proteins are regulated vary both among and within phytoplankton lineages (Martiny et al. 2006, Tolonen et al. 2006) and depend on the limiting nutrient (Agren 2004, Bi et al. 2012). To capture some of the dynamics, models describing the elemental stoichiometry are just starting to consider such complex responses (Bonachela et al. 2013), but there are many unknowns. Studies have shown that the P cell quota is highly sensitive

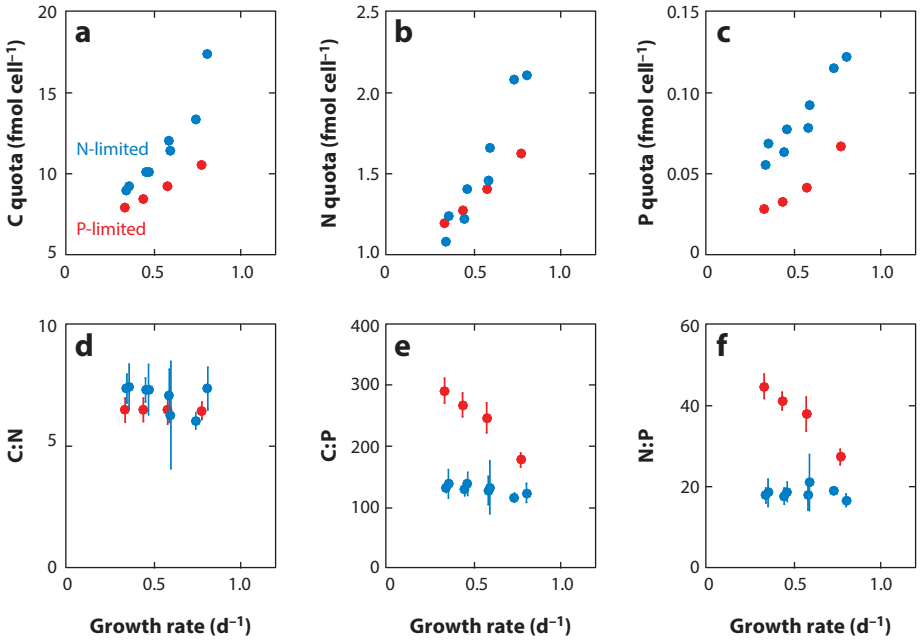

Figure 4

Interactive effects of nutrient limitation and growth on cellular quotas and ratios (Garcia et al. 2016). Red circles represent P-limited conditions, and blue circles represent N-limited conditions.

to P limitation. At a fixed growth rate, more than a quadrupling in the P cell quota can occur when shifting from P to N limitation in both large and small phytoplankton types (Elrifi & Turpin 1985, Geider & Osborne 1989, Leonardos & Geider 2004b, Mouginot et al. 2014, Rhee 1978) (**Figure 4**). The N quota can also be sensitive to N limitation, but the degree varies among lineages. In *Scenedesmus*, the N quota varies fivefold between N and P limitation under a constant growth rate (Rhee 1978). By contrast, *Synechococcus* exhibits only a small variation in N quota (Garcia et al. 2016, Mouginot et al. 2015). A shift in N source under constant growth rates appears to have little impact on cellular N quotas, and the P quota seems to be largely invariant under N limitation and constant growth (Goldman et al. 1979).

The fact that cellular quotas are affected by the availability of inorganic nutrients leads to questions of whether the sizes of the N or P quotas are equally sensitive to nutrient limitation and whether a differential response could drive overall stoichiometric changes. In a large meta-analysis, Moore et al. (2013) proposed that P quotas are most sensitive to nutrient limitation. This may be because P storage is less costly owing to its simple format (inorganic chain) and takes up less cellular space than N storage. If this idea is correct, we should expect more variation in C:P and N:P than in C:N. In support, the P quota is more variable in size in small phytoplankton, such as marine *Synechococcus* and the small diatom *Chaetoceros muelleri* (Garcia et al. 2016, Leonardos & Geider 2004b) (**Figure 4**). However, it is less clear whether this also applies to large phytoplankton (which may possibly have a larger storage capacity for both N and P). Here, several studies have observed wide variation in both N and P quotas depending on the type of nutrient limitation (Cotner et al. 2006, Goldman et al. 1979, Lynn et al. 2000, Rhee 1978). Because only a few studies have carefully controlled growth rates while studying nutrient limitation, it is unclear whether

such a distinction between large and small phytoplankton types is robust, but the possibility is intriguing (Caperon & Meyer 1972, Laws & Bannister 1980).

Few studies have directly examined the impact of nutrient limitation on the C cell quota and cell size, but this additional effect on C can affect the overall C:nutrient ratios (Flynn 2008). For some lineages (*Scenedesmus* and *Stephanodiscus minutulus*), cells and C quotas are larger under P limitation than they are under N limitation (Lynn et al. 2000, Rhee 1978). By contrast, *Synechococcus* and *C. muelleri* show less variation in cell size and C quota across limitation types (Garcia et al. 2016, Geider et al. 1996, Leonardos & Geider 2004b, Mouginot et al. 2015) (**Figure 4*a***). Finally, Harrison et al. (1976) saw that a switch from Si to N limitation leads to a large decline in the C and N quotas.

The impact of nutrient limitation on C:N:P under constant growth largely follows the expected outcomes from the analysis of individual quotas. P limitation uniformly leads to increased C:P and N:P across lineages (**Figure 4*d–f***). This is driven partly by a decline in the P quota, but the outcome is amplified by increases in the protein content and overall cell size in many phytoplankton (**Figure 4**). The outcome of N limitation is more varying. For lineages with a sensitive N quota (e.g., *Scenedesmus*), the elemental ratios behave according to the findings of Klausmeier et al. (2004), with a larger C:N and lower N:P under N limitation. However, *Synechococcus*, *C. muelleri*, and *Dunaliella* do not show this behavior, and the C:N:P can be largely constant in the N-limited range of nutrient supply ratios (Garcia et al. 2016, Goldman et al. 1979, Mouginot et al. 2015) (**Figure 4**). Thus, it appears that the elemental stoichiometry of phytoplankton is highly sensitive to P limitation, but the response is lineage dependent for N limitation.

So far, we have discussed only limitation by the two major nutrients, P and N. However, other nutrients, such as Fe and Si, also influence the growth rate and possibly the C:N:P stoichiometry of phytoplankton. In a study of the diatom *Thalassiosira weissflogii*, growth rate increased as expected with increasing Fe availability (Price 2005). This increase in Fe and growth rate led to increasing C:P and N:P but flat C:N. By contrast, studies of *Synechococcus*, *Prochlorococcus*, and *Pseudo-nitzschia* showed the opposite relationship with Fe availability and stoichiometry (Cunningham & John 2017, Marchetti & Harrison 2007). Si limitation may not affect the elemental quotas and stoichiometry beyond changing the growth rate. In *S. minutulus*, the C and N quotas were similar to those in fast-growing cells and cells growing under P limitation (Lynn et al. 2000). Furthermore, the C:N was similar to P limitation patterns and unlimited growth, and the C:P was similar to N limitation patterns and unlimited growth. Thus, Si and Fe may influence the elemental stoichiometry either directly or via a control on growth rate, but there are no data that allow differentiation of these two effects.

Light

Light availability can lead to phytoplankton photoacclimation and associated changes in cellular allocation strategies (Falkowski & LaRoche 1991, Leonardos & Geider 2004b). The main biological components involved are the biosynthetic apparatus (P rich), light-harvesting apparatus (N rich), and energy storage reserves (C rich). At a fixed growth rate, cells can be nutrient limited at high light and light limited at low light. Under high light and nutrient limitation, the cellular light-harvesting apparatus is downregulated in order to minimize the risk of photooxidative stress (Geider et al. 1996). Furthermore, energy reserves are high (C-rich lipids and polysaccharides) (Kromkamp 1987). Under low light, the photosynthetic apparatus increases in size for light harvesting, and C storage compounds decrease. Thus, we expect a strong positive impact of light on C:N and a lesser positive effect on C:P. However, if P is the main limiting nutrient at high light, the P quota will be additionally affected and modify the proposed effects.

There is support for the overall mechanism in phytoplankton of low storage C but high pigment under low light and high storage C but low pigment under high light in cultures (Behrenfeld et al. 2002, Leonardos & Geider 2004a) and field populations (Bouman et al. 2006). Because light-harvesting proteins can constitute 18–50% of cellular proteins and lipids and carbohydrates constitute 32–43% of cellular biomass, changes in these two pools should affect the C and N quotas. In support of this idea, Leonardos & Geider (2004a) found that the N quota was ∼50% lower under high light than it was under low light in *C. muelleri* across a range of nutrient supply ratios. A similar pattern occurred in the cryptophyte *Rhinomonas reticulata*, but only under N limitation; under P limitation, the N quota was similar under both high and low light (Leonardos & Geider 2005, Thompson et al. 1991).

The effect of light on C:N:P can be viewed in two ways: One can either (*a*) identify the effect at a given growth rate and nutrient supply ratio or (*b*) examine the impact on the optimal C:N:P (i.e., C:N:P at μ_{max}, sometimes also called critical C:N:P). From the first perspective, C:N is positively related to light availability in several large phytoplankton lineages (Leonardos & Geider 2004a) and less varying in others (MacIntyre et al. 2002). C:P is high under high light for *Amphidinium carterae*, whereas N:P decreases with increased light for *T. weissflogii*, *Cyanothece* sp., and *A. carterae* (Finkel et al. 2006). From the second perspective, the optimal N:P is negatively correlated with light across a range of phytoplankton lineages (Thrane et al. 2016). Thus, the impact of light on stoichiometry has a clear mechanistic basis that is well supported by observations.

Light availability can also influence diel changes in cell quotas and elemental stoichiometry, as many cellular processes vary over a daily cycle in marine organisms (Olson et al. 1986, Waldbauer et al. 2012, Zinser et al. 2009) and communities (Ottesen 2014). Photosynthetic and C fixation proteins are expressed during the day, cell division proteins near sunset, and carbohydrate metabolism (glycogen catabolism and pentose phosphate pathway) proteins at night. This leads to diel variability in cellular components such as nucleic acids, pigments, and protein concentrations (Lopez et al. 2016, Matallana-Surget et al. 2014, Vaulot et al. 1995). Under a constant growth rate, the cellular C and N (and to a lesser extent P) quotas also exhibited diel cycling in *Synechococcus* (Lopez et al. 2016). Here, the C quota followed fixation rates and exhibited strong cycling, with a daily maximum before sunset and a low in the early morning. The N and P quotas showed lower amplitude and reached maxima earlier in the light period. There was also an interaction with growth physiology, whereby oscillations were stronger at a high growth rate. As a result, C:N and C:P were highest at the end of the light period, whereas N:P showed limited oscillation. The diatom *Skeletonema* exhibited a similar cycling for the C and N quotas as well as C:N (Anning et al. 2000). Thus, we expect that diel variation in elemental stoichiometry will occur in marine communities.

Carbon Source

The ability to fix CO_2 (autotrophy) as opposed to assimilating organic C (heterotrophy) may also affect cellular allocation strategies. Heterotrophic organisms constitute a large fraction of overall marine biomass (Gasol et al. 1997) and thus an important contribution to the combined C:N:P of a community. It is hypothesized that heterotrophic organisms are mostly C limited and thus frugal with C (Godwin et al. 2016, Goldman & Peavey 1979, Tezuka 1990). Contributing to C limitation is the fact that at least half of the assimilated C is respired during growth (i.e., the yield or carbon use efficiency). In most marine regions, this should lead to a nutrient limitation of phytoplankton and a C limitation of heterotrophic organism. The outcome is low C:N and C:P in many heterotrophic organisms, such as bacteria, zooplankton, and possibly mixotrophs (Cotner et al. 2006, Fagerbakke et al. 1996, Zimmerman et al. 2014). In this way, C availability could behave as a nutrient and act like the N and P limitation presented above (Meunier et al. 2012, Sterner & Elser 2002).

The exact underlying biochemical mechanism for how C limitation influences molecular pathways and macromolecules is not clearly elucidated. The simplest argument is that heterotrophic organisms under C limitation downregulate C storage molecules (Anderson & Dawes 1990, Holme & Palmsterna 1956). There may also be an upregulation of resource acquisition enzymes targeting complex C sources (Allison & Vitousek 2005, Arnosti et al. 2011), which would lead to a currently unconstrained increase in the N quota. Considerable experimental evidence indicates that heterotrophic bacteria exhibit a nutrient-like behavior for C acquisition. Studies have shown that specific bacteria lineages as well as whole communities display clear changes in cellular C:P along a gradient of C to P limitation (Godwin & Cotner 2015, Godwin et al. 2016, Makino et al. 2003). Marine bacteria at exponential growth under non-nutrient-limitation conditions also exhibit a broad range of C:P ratios, from 35:1 (Vrede et al. 2002) to 80:1 (Zimmerman et al. 2014), and C:N ratios from 3.8:1 to 4.5:1 (Fagerbakke et al. 1996). Despite the variation, these ratios are generally lower than those observed in phototrophs. Another group of heterotrophic plankton in the ocean is the zooplankton. These are larger organisms and perhaps more homeostatic in their biomass composition and less sensitive than phytoplankton and bacteria to C limitation (Malzahn et al. 2010, Meunier et al. 2012). Thus, the available data and models suggest that the ratio of chemoheterotrophic to photoautotrophic organisms could have a negative influence on C:P and C:N (Talmy et al. 2016).

Interactions

In addition to understanding the individual effects, we also need to consider the interactive effects of environmental factors. Two types of interactions require special attention: interactions with growth rate and interactions with nutrient limitation. As alluded to above, an organism's growth rate can have a strong modulating effect on the specific impact of an environmental factor (Hillebrand et al. 2013). This could be due to the presence of storage molecules and options for metabolic flexibility (Klausmeier et al. 2004), but many other molecular responses are possible. Thus, we need to consider these interactive terms when predicting the impact of environmental changes on the elemental stoichiometry of plankton.

The GRH states that differences in organismal C:N:P ratios are generated by variations in allocation strategies that increase the abundance of ribosomes with growth (Elser et al. 2000) (**Figure 5*a***). As described above, the effect of growth rate on stoichiometry likely depends on whether temperature, a specific nutrient, or light controls growth (**Figure 5*b***). If temperature is the growth-limiting factor, we would predict a (nearly) neutral response, as cellular processes simply run faster. Under N limitation, we have seen that organisms with limited N storage, such as *Synechococcus* and heterotrophic bacteria, show a slight (or no) increase in N:P or C:P with growth rate (the opposite of the response predicted by the GRH) (Garcia et al. 2016, Goldman et al. 1979). However, a different pattern is seen in larger organisms capable of elevated N storage (Caperon & Meyer 1972, Laws & Bannister 1980). When light is controlling growth, we expect slightly negative relationships among light, growth, and N:P. Here, C:N and C:P might show the opposite trend owing to C storage. Finally, we expect to see a strong negative relationship for N:P and growth when P is the controlling factor (Hillebrand et al. 2013). Thus, depending on the factor controlling growth and possibly the ability to store N, we predict unique relationships between C:N:P and growth rate.

The specific impact of an environmental factor is also expected to be modulated by nutrient limitation and associated reduced allocation of this element. A chemostat experiment showed that *Scenedesmus* sp. and *A. formosa* N and P quotas increased with decreasing temperature (Rhee et al. 1981). Under nutrient limitation (both N- and P-limited conditions, separately), this relationship

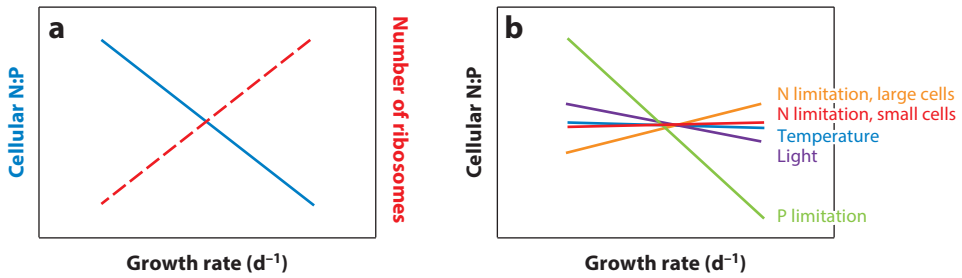

Figure 5

The link between cellular biochemical composition and N:P. (*a*) The growth rate hypothesis (GRH) prediction based on a higher number of ribosomes (*dashed line*), high P quota, and lower N:P (*solid line*). (*b*) A modified GRH that considers the factor limiting growth.

continued to hold true with increased temperature in *Scenedesmus*. However, the N-limited samples showed a higher cellular N quota, whereas the P-limited samples showed no difference in cellular P quota under nutrient-sufficient samples. In *A. formosa*, the opposite trend occurs: Under N limitation, the cellular N quota did not differ with increased temperature, and under P limitation, the cellular P quota was higher than it was in samples grown under nutrient-sufficient conditions (Rhee et al. 1981).

Nutrient limitation also has a large impact on the relationship between light intensity and stoichiometry. Increased N:P ratios and decreased C:P ratios should correspond with decreased light. A laboratory experiment found that the N quota under N limitation was higher in low light than in high light and showed a similar relationship under P limitation (Leonardos & Geider 2004a). The P quota under N limitation was higher in low light than in high light, with a linear negative trend, but under P limitation, the P quota was not dependent on the light level (Leonardos & Geider 2004a). C:N increased during a transition from low-light, high-nutrient conditions to high-light, low-nutrient conditions in the same region (Geider et al. 1998). This is common in regions that experience spring blooms: Low-light, high-nutrient conditions occur before a bloom begins, and high-light, low-nutrient conditions occur after it has ended. However, the light-nutrient hypothesis is a good predictor with a strong positive relationship between the light:nutrient ratio and C:P ratio (Dickman et al. 2006). In sum, we find that both growth physiology and nutrient availability can have a large modulating effect on how a specific environmental factor influences C:N:P.

THE ROLE OF ADAPTATION AND PHYLOGENY IN CONTROLLING C:N:P IN MARINE PLANKTON

In the above sections, we have described how acclimation mechanisms can lead to variation in C:N:P. However, there is also considerable variation in C:N:P across taxa (Finkel et al. 2016, Geider & La Roche 2002, Ho et al. 2003, Zimmerman et al. 2014). Two main categories of mechanisms lead to taxon-specific C:N:P. The first, called acclimation extensions, follows the previously discussed acclimation mechanisms but now as the outcome of adaptation. The second category, called biological uniqueness, is based on the idea that the biochemical diversity among plankton is immense and can influence the cellular composition in a variety of ways.

One can apply acclimation-extension ideas to all physiological hypotheses (i.e., growth rate, temperature optimum, nutrient uptake capabilities, etc.). The adaptive GRH variant proposes that fast-growing lineages have lower N:P than slow-growing ones (Elser et al. 2000). There is support

for this hypothesis across large gradients in growth rate and organism size (Elser et al. 2000), but the hypothesis does not readily extend to marine phytoplankton (Edwards et al. 2012).

An adaptive extension of the translation-compensation hypothesis can also be evaluated for organisms with different optimal temperatures, but there are few data for such tests (Yvon-Durocher et al. 2015). The only study to our knowledge to test the adaptive version showed that the stoichiometric variation among high- and low-temperature-adapted ecotypes did not support an acclimation-extension version of the translation-compensation hypothesis (Martiny et al. 2016b). However, translation-related proteins appear to be expressed at higher relative proportions in cold-water plankton communities than in warm-water communities (Toseland et al. 2013). Thus, translation-related macromolecules may be more common in cold-water-adapted lineages, but the impact on stoichiometric outcome is unclear. There is evidence for adjustments of pigment concentrations to light availability (Chisholm et al. 1975, Geider 1987, Moore et al. 1998, Rocap et al. 1999). For nutrient availability, there is clear evidence for molecular adaptation, but the effect on cell quotas and stoichiometry is completely unknown (Martiny et al. 2006). Thus, there is support for a general adaptive mechanism for how light and nutrients affect cellular stoichiometry, but there are no data to support or refute such adaptive stoichiometric models for how temperature or interactions will have an effect.

All adaptive models have a strong covariance with phytoplankton cell size, as small cell types such as marine cyanobacteria dominate in warm, high-light, and low-nutrient environments (i.e., the oligotrophic gyres), whereas large cell types such as diatoms blooms in cold, low-light, and high-nutrient environments (and therefore are prevalent at high latitudes). This has led to a concept of slow-growing resource survivalists with high C:P and N:P and fast-growing bloomers with low C:P and N:P (Arrigo 2005, Klausmeier et al. 2004). Survivalists, such as *Prochlorococcus* and *Synechococcus*, dominate the stable, low-nutrient waters at low latitudes; have a low resource minimum; and have a high proportion of N-rich resource acquisition machinery, such as enzymes and photosystems. Elevated C:P and N:P in small cyanobacteria are supported by both laboratory and field studies. In the laboratory, *Prochlorococcus* and *Synechococcus* consistently have high C:P and N:P (Bertilsson et al. 2003, Garcia et al. 2016, Martiny et al. 2016a). Cell sorting also showed that *Prochlorococcus* and *Synechococcus* field populations have higher C:P and N:P than coexisting larger picoeukaryotic phytoplankton (Baer et al. 2017, Martiny et al. 2013b). Bloomers, such as diatoms, are normally larger and have a higher proportion of P-rich biosynthesis machinery. Diatoms in the Southern Ocean have lower N:P than coexisting slower-growing *Phaeocystis* (Arrigo et al. 1999, Weber & Deutsch 2010). These studies support the idea that bloomers and survivalists have unique stoichiometries. However, recent in situ estimates of *Prochlorococcus* and *Synechococcus* show high growth rates in tropical waters that rival those of many larger phytoplankton types (Hunter-Cevera et al. 2016, Liu et al. 1998, Ribalet et al. 2015). Thus, a concept of slow-growing, high-temperature, nutrient uptake specialists versus fast-growing bloomers does not fully capture the ecological roles of marine plankton communities and their predicted stoichiometric regulation.

The second category of taxon-specific C:N:P, biological uniqueness, is based on phylogenetically constrained elemental composition resulting from the myriad of biochemical behaviors found in marine plankton (Ho et al. 2003). Nearly all studies of both autotrophic and heterotrophic organisms have shown considerable differences of macromolecules, cell quotas, and ratios even among closely related marine taxa (Finkel et al. 2016, Geider & La Roche 2002, Zimmerman et al. 2014). The question is whether there are higher levels of phylogenetic organization that allow for an association of ratios with specific taxa, but limited data are available to fully evaluate this question.

LINKING MECHANISMS TO OBSERVED PATTERNS OF STOICHIOMETRIC VARIABILITY

What mechanisms control the elemental stoichiometry in marine communities? The strong latitudinal covariance of light, temperature, nutrient availability, and biodiversity makes it difficult to answer the question at this point. Several papers have made competing claims for temperature, nutrient availability, and biodiversity as the dominating control on elemental stoichiometry (Galbraith & Martiny 2015, Martiny et al. 2013a, Yvon-Durocher et al. 2015). With the current data, it is impossible to statistically separate the effects of these factors on the latitudinal gradients in C:N:P because all factors provide a fit to the regional patterns of stoichiometric variability (Martiny et al. 2013a, Toseland et al. 2013, Yvon-Durocher et al. 2015) (**Figure 1**). Thus, we need geographically (and environmentally) more diverse field observations to further tease apart these competing claims.

It is unlikely that irradiance has a large effect on the horizontal variation in C:N:P of marine phytoplankton because such a model cannot explain the lower ratios in equatorial upwelling zones. However, irradiance could influence vertical shifts in elemental ratios. In support of an impact of irradiance, C:N does increase with depth, although this pattern could also be driven by temperature and nutrient recycling (Martiny et al. 2013b, Schneider et al. 2003). However, we currently have a limited understanding of any vertical changes in C:P and N:P within the photic zone, and more work is needed to understand column variability for C:N:P.

Based on current field observations, we propose a hypothesis that variation in community (all plankton, including autotrophic and heterotrophic species) C:N:P within different low-nutrient ocean gyres is driven mainly by the nutrient supply ratio, sourced from a combination of vertical and N fixation inputs. Plankton "are what they eat," which suggests that a biogeochemical state factor mechanism controlling the total input of N and P should describe the stoichiometry in this biome. However, owing to a lack of N storage in small plankton types, we should never observe lower than Redfield proportions in C:P and N:P and little variation in C:N. N fixation resupplies bioavailable N into these regions, tying into the fluctuations of N:P ratios. The patterns of N fixation rate and phosphate concentration suggest that N fixation rates are highest and P concentrations lowest in the North Atlantic subtropical gyre, followed by the North Pacific subtropical gyre (Lomas et al. 2010, Wu et al. 2000). By contrast, N fixation rates are lower (and P higher) in the three Southern Hemisphere oligotrophic gyres (Mather et al. 2008, Moutin et al. 2007, Sohm et al. 2011). Based on our hypothesis and these apparent patterns of N fixation and P availability, we predict that C:P and N:P are highest in the North Atlantic, followed by the North Pacific subtropical gyres, and lowest in the Southern Hemisphere (but above Redfield proportions). In support of this prediction, the C:P and N:P ratios are greatest in the North Atlantic subtropical gyre, followed by the North Pacific gyre (**Figure 1**). By contrast, C:P and N:P are only slightly above Redfield proportions in the Southern Hemisphere gyres (**Figure 1**). The hypothesis would further suggest that the influence of Fe availability through N fixation on the N:P supply ratio (Mather et al. 2008) could serve as an important control on the elemental ratios in the oligotrophic gyres. Such variation in nutrient supply ratio and degree of P limitation would lead to a good fit between C:P and N:P and ambient phosphate concentration (Galbraith & Martiny 2015).

In nutrient-rich high-latitude environments, we hypothesize that plankton "eat what they need" and suggest that a direct control mechanism is most applicable in such biomes. In the absence of (or at least reduced) nutrient limitation, factors such as light, temperature, and unique lineage-dependent ratios may all influence the particulate organic matter ratios. Field and inverse model studies have suggested that, in the Southern Ocean, diatoms may have lower C:P and N:P than *Phaeocystis* (Arrigo et al. 1999, Weber & Deutsch 2010). This is an intriguing observation but

has not been fully explored in laboratory experiments. Such laboratory experiments would enable a disentanglement of the controls by individual environmental factors and controls by lineage-specific behaviors. Limited data are available on the elemental ratios in most high-latitude regions, but these observations suggest that the biogeography of specific plankton lineages could influence the elemental ratios.

IMPLICATIONS FOR OCEAN BIOGEOCHEMISTRY

A variable C:N:P of plankton and particulate organic matter has broad biogeochemical implications, including for our understanding of nutrient limitation, the regulation of N fixation, C export, and ecosystem and food web model predictions that are tuned with Redfield stoichiometry. The N:P ratio of 16:1 is typically used to differentiate between N and P limitation; phytoplankton are said to be N limited when N:P < 16:1 and P limited when N:P > 16:1 (Falkowski 1997, Geider & La Roche 2002, Tyrrell 1999). This concept becomes more fluid when different lineages have unique elemental compositions and N- and P-stressed cells coexist in the same water parcel (Alexander et al. 2015, Martiny et al. 2013a). There is also much debate between geochemists and biologists about the ultimate limiting nutrient for primary production: Geochemists argue that P is the ultimate limiting nutrient because of biological fixation of N, but biologists can demonstrate that many phytoplankton communities increase activity and biomass following N addition (Moore et al. 2013). Tyrrell (1999) tried to resolve this argument and presented a model suggesting that N is a proximate limiting nutrient and P is the ultimate limiting nutrient because of competition between regular phytoplankton and growth-penalized N fixers. However, his conclusion is dependent on the notion of a static elemental composition of phytoplankton communities. If the true elemental composition varies among lineages and environmental conditions, then the difference between proximate and ultimate limiting nutrients becomes more fluid.

Variation in the elemental composition of total marine communities can also influence our understanding of how N gain and loss processes are regulated and geographically distributed. The biological source and sink of N reserves are N fixation and denitrification. N fixation is a costly process that requires a large amount of Fe, whereas denitrification is a process favored in low-oxygen conditions. Deviations from the Redfield ratio in the relative concentrations of dissolved nitrate and phosphate have been used to map out regions of denitrification and N fixation. Based on this metric, Deutsch et al. (2001, 2007) suggested that gain and loss processes of N are spatially coupled in that upwelled waters that experience denitrification promote N fixation once the N-depleted waters are advected offshore. The spatial co-occurrence of N gain and loss processes was unexpected because in situ studies have suggested that water column denitrification occurs mostly in upwelling zones, whereas N fixation occurs mostly in the oligotrophic gyres (Sohm et al. 2011). Later studies have examined the impact of phytoplankton variable stoichiometry on the N cycling patterns and found that the interpreted spatial distribution is highly dependent on the assumed cellular elemental ratio (Deutsch & Weber 2012, Mills & Arrigo 2010). Allowing for high N:P and C:P in plankton growing in the gyres resulted in a much stronger correspondence between observed and predicted N fixation patterns (Weber & Deutsch 2012). The elemental stoichiometry of total marine communities also influences the proportions of electron donors and acceptors in oxygen minimum zones (Babbin et al. 2014) and associated N loss pathways (i.e., the dominance of denitrification versus anammox). Thus, knowing what sets the elemental composition of marine plankton and communities will likely affect our understanding of how marine N gain and loss processes are controlled.

The elemental compositions of marine plankton and communities may be important for how C export and the biological pump are regulated, both now and under future climate change

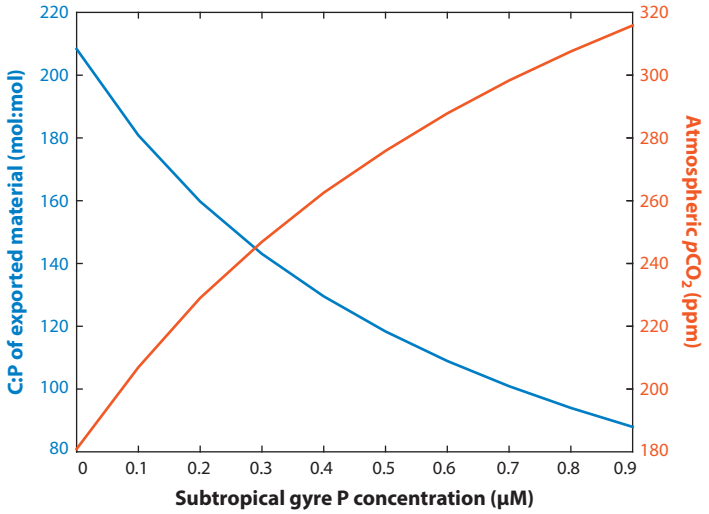

Figure 6

Linking subtropical gyre nutrient availability, the elemental composition of surface particles and export flux, and atmospheric pCO_2. To a first order, the C:P elemental composition of surface-ocean particles can be linked to nutrient availability (Martiny et al. 2013a). Based on this relationship, a simplified box-model simulation demonstrates how varying the C:P of exported material from low-latitude regions can lead to substantial long-term changes in atmospheric pCO_2 (Galbraith & Martiny 2015).

conditions. The canonical view of C export is that rates are low in the gyres because of their low nutrient inputs and high in temperate and upwelling regions because of their high nutrient inputs. If one considers the surface ocean as a water parcel box and a constant C:P of particles, a mass balance dictates that C export must equal the nutrient influx times a C:P of 106:1 (Dugdale & Goering 1967, Tyrrell 1999). However, detailed studies using different metabolite mass balances and inverse models do not support higher C export fluxes in mid-latitude ecosystems than in low-latitude ecosystems, instead showing little variation in export rates between these biomes (Emerson 2014, Richardson & Jackson 2007, Teng et al. 2014). Higher C:P of exported material, however, can result in significant C export despite increased ocean stratification and reduced nutrient inputs. As was demonstrated recently using a simplified box-model simulation, variations in the C:P of phytoplankton and exported material can have a substantial impact on long-term changes in C export and atmospheric CO_2 level (Galbraith & Martiny 2015) (**Figure 6**). Such studies lend support to the conclusion that the elemental composition of exported material can have a tangible impact on future predicted C export and possible long-term ocean feedbacks to atmospheric CO_2 (Sterner 2015).

CONCLUSIONS AND OUTSTANDING ISSUES

It has become clear that the upper-ocean plankton and particulate organic matter elemental ratios are not constant but instead display spatial and temporal differences. The quantification of C:N:P, and especially of particulate organic P, is geographically biased, and many regions have been either sparsely sampled or not sampled at all (Martiny et al. 2013a). Thus, we need a much richer (geographically and environmentally) data set to better determine the field patterns of C:N:P and test specific models. Despite deficiencies in data coverage, there is good experimental evidence that nutrient limitation (especially P) exerts a strong influence on the elemental composition of

marine plankton (Garcia et al. 2016, Goldman et al. 1979, Mouginot et al. 2015, Rhee 1978). We hypothesize that the nutrient supply ratio is the primary control in the oligotrophic gyres. If our hypothesis is correct, a clear understanding of the ultimate controls on the supply of N versus P is critical for understanding differences in C:N:P across the oligotrophic gyres. By contrast, lineage differences in the elemental composition and physiological responses to light or temperature may control the elemental composition in high-latitude environments.

It has become apparent that interactions between growth rate and specific environmental factors are important in setting the elemental composition. Thus, experiments that control growth rate (e.g., using chemostats) are critical for disentangling this interaction. Unfortunately, few such experiments have been performed, and the ones available have a disproportionate impact on current hypotheses. Furthermore, most work has been done on organisms that are not necessarily representative of a natural marine community and are rare or absent in the open ocean, and we need more experiments using abundant marine lineages. A parallel issue is that we currently have a limited understanding of the in situ growth rate for most marine phytoplankton lineages. For example, a temperature increase could lead to either increased growth rates (Eppley 1972, Sherman et al. 2016) or a reallocation of cellular machinery, as suggested by the translation-compensation hypothesis (Toseland et al. 2013), and the outcomes of these two scenarios will lead to distinct patterns of C:N:P. These issues leave many gaps in knowledge.

The GRH has been an important guiding hypothesis for understanding ecological stoichiometry. However, it has become apparent that phosphate bound to RNA may not always be a dominant P pool, and we predict that changes in RNA will have a limited influence on C:P and N:P in many marine environments (Zimmerman et al. 2014). Nevertheless, the impact of a changing growth rate on C:N:P strongly depends on the factor controlling growth. Thus, changes in growth rates across ocean regions could be a strong moderator on how other factors influence the elemental stoichiometry and are still important to contend with.

Many stoichiometric hypotheses are based on singling out the control of a single biochemical function (e.g., ribosomes and translation). However, most environmental factors affect multiple biochemical pathways. Various "-omics" techniques may prove valuable in addressing this issue, as -omics approaches can evaluate system-wide impacts. Furthermore, we need to quantify how specific environmental conditions affect macromolecular pools (Finkel et al. 2016) and, in particular, how inorganic phosphate pools are regulated. Such data would further facilitate an integrated view of cellular reallocations in response to environmental changes and enable us to move from hypotheses centered on single biochemical pathways to system-wide effects. This is already occurring with exciting new trait-based models, but they suffer from many biochemical unknowns (Daines et al. 2014).

There is clear evidence that biological processes control the C:N:P of marine communities, leading to non-Redfield proportions in many regions and seasons, and such processes will likely have a large impact on our understanding of many ocean biogeochemistry concepts. We propose that a linear relationship between phosphate concentration and C:P (and N:P) is a good first approximation for capturing the broad regional variation in these ratios (Galbraith & Martiny 2015). However, more sophisticated models are needed to cover the interactions among biogeochemical feedbacks, environmental conditions, growth physiology, and the biological uniqueness of marine plankton.

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.

ACKNOWLEDGMENTS

We thank David Talmy at the Massachusetts Institute of Technology, George Hagström at Princeton University, and Alyse Larkin at the University of California, Irvine, for many helpful comments. This work was supported by funding from the National Science Foundation Graduate Research Fellowship Program to A.R.M. and from the National Science Foundation Biological Oceanography Program (grants OCE-1046297 and OCE-1559002) to A.C.M.

LITERATURE CITED

Agren GI. 2004. The C:N:P stoichiometry of autotrophs—theory and observations. *Ecol. Lett.* 7:185–91

Alexander H, Jenkins BD, Rynearson TA, Dyhrman ST. 2015. Metatranscriptome analyses indicate resource partitioning between diatoms in the field. *PNAS* 112:E2182–90

Allison SD, Vitousek PM. 2005. Responses of extracellular enzymes to simple and complex nutrient inputs. *Soil Biol. Biochem.* 37:937–44

Anderson AJ, Dawes EA. 1990. Occurrence, metabolism, metabolic role, and industrial uses of bacterial polyhydroxyalkanoates. *Microbiol. Rev.* 54:450–72

Anning T, Macintyre HL, Pratt SM, Sammes PJ, Gibb S, Geider RJ. 2000. Photoacclimation in the marine diatom *Skeletonema costatum*. *Limnol. Ocean*. 45:1807–17

Arnosti C, Steen AD, Ziervogel K, Ghobrial S, Jeffrey WH. 2011. Latitudinal gradients in degradation of marine dissolved organic carbon. *PLOS ONE* 6:8–13

Arrigo KR. 2005. Marine microorganisms and global nutrient cycles. *Nature* 437:349–55

Arrigo KR, Robinson DH, Worthen DL, Dunbar RB, DiTullio GR, et al. 1999. Phytoplankton community structure and the drawdown of nutrients and CO_2 in the Southern Ocean. *Science* 283:365–67

Augueres AS, Loreau M. 2015. Regulation of Redfield ratios in the deep ocean. *Glob. Biogeochem. Cycles* 29:254–66

Babbin AR, Keil RG, Devol AH, Ward BB. 2014. Organic matter stoichiometry, flux, and oxygen control nitrogen loss in the ocean. *Science* 344:406–8

Baer SE, Lomas MW, Terpis KX, Mouginot C, Martiny AC. 2017. Stoichiometry of *Prochlorococcus*, *Synechococcus*, and small eukaryotic populations in the western North Atlantic Ocean. *Environ. Microbiol.* 19:1568–83

Behrenfeld MJ, Marañón E, Siegel DA, Hooker SB. 2002. Photoacclimation and nutrient-based model of light-saturated photosynthesis for quantifying oceanic primary production. *Mar. Ecol. Prog. Ser.* 228:103–17

Berges JA, Varela DE, Harrison PJ. 2002. Effects of temperature on growth rate, cell composition and nitrogen metabolism in the marine diatom *Thalassiosira pseudonana* (Bacillariophyceae). *Mar. Ecol. Ser.* 225:139–46

Bertilsson S, Berglund O, Karl DM, Chisholm SW. 2003. Elemental composition of marine *Prochlorococcus* and *Synechococcus*: implications for the ecological stoichiometry of the sea. *Limnol. Oceanogr.* 48:1721–31

Bi R, Arndt C, Sommer U. 2012. Stoichiometric responses of phytoplankton species to the interactive effect of nutrient supply ratios and growth rates. *J. Phycol.* 48:539–49

Binder BJ, Liu YC. 1998. Growth rate regulation of rRNA content of a marine *Synechococcus* (cyanobacterium) strain. *Appl. Environ. Microbiol.* 64:3346–51

Bonachela JA, Allison SD, Martiny AC, Levin SA. 2013. A model for variable phytoplankton stoichiometry based on cell protein regulation. *Biogeosciences* 10:4341–56

Bouman HA, Ulloa O, Scanlan DJ, Zwirglmaier K, Li WK, et al. 2006. Oceanographic basis of the global surface distribution of *Prochlorococcus* ecotypes. *Science* 312:918–21

Broeze RJ, Solomon CJ, Pope DH. 1978. Effects of low temperature on in vivo and in vitro protein synthesis in *Escherichia coli* and *Pseudomonas fluorescens*. *J. Bacteriol.* 134:861–74

Caperon J, Meyer J. 1972. Nitrogen-limited growth of marine phytoplankton—I. Changes in population characteristics with steady-state growth rate. *Deep-Sea Res. Oceanogr. Abstr.* 19:601–18

Chapin FS, Matson PA, Vitousek PM. 2012. *Principles of Terrestrial Ecosystem Ecology*. New York: Springer

Chisholm SW, Stross RG, Nobbs PA. 1975. Light/dark-phased cell division in *Euglena gracilis* (Z) (*Euglenophyceae*) in PO_4-limited continuous culture. *J. Phycol.* 11:367–73

Chrzanowski TH, Grover JP. 2008. Element content of *Pseudomonas fluorescens* varies with growth rate and temperature: a replicated chemostat study addressing ecological stoichiometry. *Limnol. Oceanogr.* 53:1242–51

Collier JL, Grossman AR. 1992. Chlorosis induced by nutrient deprivation in *Synechococcus* sp. strain PCC 7942: Not all bleaching is the same. *J. Bacteriol.* 174:4718–26

Conover SAM. 1975. Partitioning of nitrogen and carbon in cultures of the marine diatom *Thalassiosira fluviatilis* supplied with nitrate, ammonium, or urea. *Mar. Biol.* 32:231–46

Cotner JB, Ammerman JW, Peele ER, Bentzen E. 1997. Phosphorus-limited bacterioplankton growth in the Sargasso Sea. *Aquat. Microb. Ecol.* 13:141–49

Cotner JB, Makino W, Biddanda BA. 2006. Temperature affects stoichiometry and biochemical composition of *Escherichia coli*. *Microb. Ecol.* 52:26–33

Cotner JB, Wetzel RG. 1992. Uptake of dissolved inorganic and organic phosphorus compounds by phytoplankton and bacterioplankton. *Limnol. Oceanogr.* 37:232–43

Cunningham BR, John SG. 2017. The effect of iron limitation on cyanobacteria major nutrient and trace element stoichiometry. *Limnol. Oceanogr.* 62:846–58

Daines SJ, Clark JR, Lenton TM. 2014. Multiple environmental controls on phytoplankton growth strategies determine adaptive responses of the N:P ratio. *Ecol. Lett.* 17:414–25

Davison IR. 1991. Environmental effects on algal photosynthesis: temperature. *J. Phycol.* 27:2–8

Deutsch C, Key RM, Sarmiento JL, Ganachaud A. 2001. Denitrification and N_2 fixation in the Pacific Ocean. *Glob. Biogeochem. Cycles* 15:483–506

Deutsch C, Sarmiento JL, Sigman DM, Gruber N, Dunne JP. 2007. Spatial coupling of nitrogen inputs and losses in the ocean. *Nature* 445:163–67

Deutsch C, Weber T. 2012. Nutrient ratios as a tracer and driver of ocean biogeochemistry. *Annu. Rev. Mar. Sci.* 4:113–41

DeVries T, Deutsch C. 2014. Large-scale variations in the stoichiometry of marine organic matter respiration. *Nat. Geosci.* 7:890–94

Diaz JM, Bjorkman KM, Haley ST, Ingall ED, Karl DM, et al. 2016. Polyphosphate dynamics at Station ALOHA, North Pacific subtropical gyre. *Limnol. Oceanogr.* 61:227–39

Dickman EM, Vanni MJ, Horgan MJ. 2006. Interactive effects of light and nutrients on phytoplankton stoichiometry. *Oecologia* 149:676–89

Droop MR. 1968. Vitamin B_{12} and marine ecology. IV. The kinetics of uptake, growth and inhibition in *Monochrysis lutheri*. *J. Mar. Biol. Assoc. UK* 48:689–733

Dugdale RC, Goering JJ. 1967. Uptake of new and regenerated forms of nitrogen in primary productivity. *Limnol. Oceanogr.* 12:196–206

Edwards K, Thomas M, Klausmeier CA, Litchman E. 2012. Allometric scaling and taxonomic variation in nutrient utilization traits and maximum growth rate of phytoplankton. *Limnol. Oceanogr.* 57:554–66

Elrifi IR, Turpin DH. 1985. Steady-state luxury consumption and the concept of optimum nutrient ratios—a study with phosphate and nitrate limited *Selenastrum minutum* (Chlorophyta). *J. Phycol.* 21:592–602

Elser JJ, Acharya K, Kyle M, Cotner J, Makino W, et al. 2003. Growth rate–stoichiometry couplings in diverse biota. *Ecol. Lett.* 6:936–43

Elser JJ, Sterner RW, Gorokhova E, Fagan WF, Markow TA, et al. 2000. Biological stoichiometry from genes to ecosystems. *Ecol. Lett.* 3:540–50

Emerson S. 2014. Annual net community production and the biological carbon flux in the ocean. *Glob. Biogeochem. Cycles* 28:14–28

Eppley RW. 1972. Temperature and phytoplankton growth in the sea. *Fish. Bull.* 70:1063–85

Fagerbakke KM, Heldal M, Norland S. 1996. Content of carbon, nitrogen, oxygen, sulfur and phosphorus in native aquatic and cultured bacteria. *Aquat. Microb. Ecol.* 10:15–27

Falkowski PG. 1997. Evolution of the nitrogen cycle and its influence on the biological sequestration of CO_2 in the ocean. *Nature* 387:272–75

Falkowski PG, LaRoche J. 1991. Acclimation to spectral irradiance in algae. *J. Phycol.* 27:8–14

Farewell A, Neidhardt FC. 1998. Effect of temperature on in vivo protein synthetic capacity in *Escherichia coli*. *J. Bacteriol.* 180:4704–10

Finkel ZV, Follows MJ, Liefer JD, Brown CM, Benner I, Irwin AJ. 2016. Phylogenetic diversity in the macromolecular composition of microalgae. *PLOS ONE* 11:e0155977

Finkel ZV, Quigg A, Raven JA, Reinfelder JR, Schofield OE, Falkowski PG. 2006. Irradiance and the elemental stoichiometry of marine phytoplankton. *Limnol. Oceanogr.* 51:2690–701

Flynn KJ. 2008. Use, abuse, misconceptions and insights from quota models—the Droop cell quota model 40 years on. *Oceanogr. Mar. Biol.* 46:1–23

Flynn KJ, Raven JA, Rees TA V, Finkel Z, Quigg A, Beardall J. 2010. Is the growth rate hypothesis applicable to microalgae? *J. Phycol.* 46:1–12

Fu FX, Warner ME, Zhang YH, Feng YY, Hutchins DA. 2007. Effects of increased temperature and CO_2 on photosynthesis, growth, and elemental ratios in marine *Synechococcus* and *Prochlorococcus* (Cyanobacteria). *J. Phycol.* 43:485–96

Galbraith ED, Martiny AC. 2015. A simple nutrient-dependence mechanism for predicting the stoichiometry of marine ecosystems. *PNAS* 112:8199–204

Garcia NS, Bonachela JA, Martiny AC. 2016. Interactions between growth-dependent changes in cell size, nutrient supply and cellular elemental stoichiometry of marine *Synechococcus*. *ISME J.* 10:2715–24

Demonstrates the variation in cellular quotas and elemental stoichiometry under nutrient-limited conditions.

Gasol JM, del Giorgio PA, Duarte CM. 1997. Biomass distribution in marine planktonic communities. *Limnol. Oceanogr.* 42:1353–63

Gausing K. 1982. Regulation of ribosome synthesis in *E. coli*. *Trends Biochem. Sci.* 7:65–67

Geider RJ. 1987. Light and temperature dependence of the carbon to chlorophyll a ratio in microalgae and cyanobacteria: implications for physiology and growth of phytoplankton. *New Phytol.* 106:1–34

Geider RJ, La Roche J. 2002. Redfield revisited: variability of C:N:P in marine microalgae and its biochemical basis. *Eur. J. Phycol.* 37:1–17

Geider RJ, Macintyre HL, Kana TM. 1996. A dynamic model of photoadaptation in phytoplankton. *Limnol. Oceanogr.* 41:1–15

Formulates a dynamic photoacclimation phytoplankton model that results in a better understanding of biochemical allocation and C:N:P.

Geider RJ, MacIntyre HL, Kana TM. 1998. A dynamic regulatory model of phytoplanktonic acclimation to light, nutrients, and temperature. *Limnol. Oceanogr.* 43:679–94

Geider RJ, Osborne BA. 1989. Respiration and microalgal growth: a review of the quantitative relationship between dark respiration and growth. *New Phytol.* 112:327–41

Godwin CM, Cotner JB. 2015. Aquatic heterotrophic bacteria have highly flexible phosphorus content and biomass stoichiometry. *ISME J.* 9:2324–27

Godwin CM, Whitaker EA, Cotner JB. 2016. Growth rate and resource imbalance interactively control biomass stoichiometry and elemental quotas of aquatic bacteria. *Ecology* 98:820–29

Goldman JC, McCarthy JJ, Peavey DG. 1979. Growth-rate influence on the chemical composition of phytoplankton in oceanic waters. *Nature* 279:210–15

Goldman JC, Peavey DG. 1979. Steady-state growth and chemical composition of the marine chlorophyte *Dunaliella tertiolecta* in nitrogen-limited continuous cultures. *Appl. Environ. Microbiol.* 38:894–901

Hall EK, Neuhauser C, Cotner JB. 2008. Toward a mechanistic understanding of how natural bacterial communities respond to changes in temperature in aquatic ecosystems. *ISME J.* 2:471–81

Harrison PJ, Conway HL, Dugdale RC. 1976. Marine diatoms grown in chemostats under silicate or ammonium limitation. I. Cellular chemical composition and steady state growth kinetics of *Skeletonema costatum*. *Mar. Biol.* 35:177–86

Henderson RJ, Mackinlay EE. 1989. Effect of temperature on lipid composition of the marine cryptomonad *Chroomonas salina*. *Phytochemistry* 28:2943–48

Herrero A, Flores E, Guerrero MG. 1985. Regulation of nitrate reductase cellular levels in the cyanobacteria *Anabaena variabilis* and *Synechocystis* sp. *FEMS Microbiol. Lett.* 26:21–25

Hillebrand H, Steinert G, Boersma M, Malzahn A, Meunier CL, et al. 2013. Goldman revisited: Faster-growing phytoplankton has lower N:P and lower stoichiometric flexibility. *Limnol. Oceanogr.* 58:2076–88

Ho TY, Quigg A, Finkel Z V, Milligan AJ, Wyman K, et al. 2003. The elemental composition of some marine phytoplankton. *J. Phycol.* 39:1145–59

Hochachka PW, Somero GN. 1984. *Biochemical Adaptation*. Princeton, NJ: Princeton Univ. Press

Holme T, Palmsterna H. 1956. On the glycogen in *Escherichia coli B*; its synthesis and breakdown and its specific labeling with ^{14}C. *Acta Chem. Scand.* 10:1557–62

Hunter-Cevera KR, Neubert MG, Olson RJ, Solow AR, Shalapyonok A, Sosik HM. 2016. Physiological and ecological drivers of early spring blooms of a coastal phytoplankter. *Science* 354:326–29

Klausmeier CA, Litchman E, Levin SA. 2004. Phytoplankton growth and stoichiometry under multiple nutrient limitation. *Limnol. Oceanogr.* 49:1463–70

Kornberg A, Rao NN, Ault-Riche D. 1999. Inorganic polyphosphate: a molecule of many functions. *Annu. Rev. Biochem.* 68:89–125

Kramer JG, Morris I. 1990. Growth regulation in irradiance limited marine *Synechococcus* sp. WH 7803. *Arch. Microbiol.* 154:286–93

Kromkamp J. 1987. Formation and functional significance of storage products in cyanobacteria. *N. Z. J. Mar. Freshw. Res.* 21:457–65

Laws EA, Bannister TT. 1980. Nutrient- and light-limited growth of *Thalassiosira fluviatilis* in continuous culture, with implications for phytoplankton growth in the ocean. *Limnol. Oceanogr.* 25:457–73

Lenton TM, Klausmeier CA. 2007. Biotic stoichiometric controls on the deep ocean N:P ratio. *Biogeosciences* 4:353–67

Leonardos N, Geider RJ. 2004a. Effects of nitrate: phosphate supply ratio and irradiance on the C:N:P stoichiometry of *Chaetoceros muelleri*. *Eur. J. Phycol.* 39:173–80

Leonardos N, Geider RJ. 2004b. Responses of elemental and biochemical composition of *Chaetoceros muelleri* to growth under varying light and nitrate: phosphate supply ratios and their influence on critical N:P. *Limnol. Oceanogr.* 49:2105–14

Leonardos N, Geider RJ. 2005. Elemental and biochemical composition of *Rhinomonas reticulata* (Cryptophyta) in relation to light and nitrate-to-phosphate supply ratios. *J. Phycol.* 41:567–76

Lin Y, Gazsi K, Lance VP, Larkin AA, Chandler JW, et al. 2013. In situ activity of a dominant *Prochlorococcus* ecotype (eHL-II) from rRNA content and cell size. *Environ. Microbiol.* 15:2736–47

Liu H, Campbell L, Landry MR, Nolla HA, Brown SL, Constantinou J. 1998. *Prochlorococcus* and *Synechococcus* growth rates and contributions to production in the Arabian Sea during the 1995 southwest and northeast monsoons. *Deep-Sea Res. II* 45:2327–52

Lomas MW, Burke AL, Lomas DA, Bell DW, Shen C, et al. 2010. Sargasso Sea phosphorus biogeochemistry: an important role for dissolved organic phosphorus (DOP). *Biogeosciences* 7:695–710

Lopez JL, Garcia NS, Talmy D, Martiny AC. 2016. Diel variability in the elemental composition of the marine cyanobacterium *Synechococcus*. *J. Plankton Res.* 38:1052–61

Lourenço SO, Barbarino E, Lavín PL, Lanfer Marquez UM, Aidar E. 2004. Distribution of intracellular nitrogen in marine microalgae: calculation of new nitrogen-to-protein conversion factors. *Eur. J. Phycol.* 39:17–32

Lynn SG, Kilham SS, Kreeger DA, Interlandi SJ. 2000. Effect of nutrient availability on the biochemical and elemental stoichiometry in the freshwater diatom *Stephanodiscus minutulus* (Bacillariophyceae). *J. Phycol.* 36:510–22

MacIntyre HL, Kana TM, Anning T, Geider RJ. 2002. Photoacclimation of photosynthesis irradiance response curves and photosynthetic pigments in microalgae and cyanobacteria. *J. Phycol.* 38:17–38

Mackey KRM, Paytan A, Caldeira K, Grossman AR, Moran D, et al. 2013. Effect of temperature on photosynthesis and growth in marine *Synechococcus* spp. *Plant Physiol.* 163:815–29

Makino W, Cotner JB, Sterner RW, Elser JJ. 2003. Are bacteria more like plants or animals? Growth rate and resource dependence of bacterial C:N:P stoichiometry. *Funct. Ecol.* 17:121–30

Malzahn AM, Hantzsche F, Schoo KL, Boersma M, Aberle N. 2010. Differential effects of nutrient-limited primary production on primary, secondary or tertiary consumers. *Oecologia* 162:35–48

Marchetti A, Harrison PJ. 2007. Coupled changes in the cell morphology and elemental (C, N, and Si) composition of the pennate diatom *Pseudo-nitzschia* due to iron deficiency. *Limnol. Oceanogr.* 52:2270–84

Martin P, Dyhrman ST, Lomas MW, Poulton NJ, Van Mooy BAS. 2014. Accumulation and enhanced cycling of polyphosphate by Sargasso Sea plankton in response to low phosphorus. *PNAS* 111:8089–94

Demonstrates latitudinal gradients found in marine phytoplankton stoichiometry.

Martiny AC, Coleman ML, Chisholm SW. 2006. Phosphate acquisition genes in *Prochlorococcus* ecotypes: evidence for genome-wide adaptation. *PNAS* 103:12552–57

Martiny AC, Ma L, Mouginot C, Chandler JW, Zinser ER. 2016a. Interactions between thermal acclimation, growth rate, and phylogeny influence *Prochlorococcus* elemental stoichiometry. *PLOS ONE* 11:e0168291

Martiny AC, Pham CTA, Primeau FW, Vrugt JA, Moore JK, et al. 2013a. Strong latitudinal patterns in the elemental ratios of marine plankton and organic matter. *Nat. Geosci.* 6:279–83

Martiny AC, Talarmin A, Mouginot C, Lee JA, Huang JS, et al. 2016b. Biogeochemical interactions control a temporal succession in the elemental composition of marine communities. *Limnol. Oceanogr.* 61:531–42

Martiny AC, Vrugt JA, Primeau FW, Lomas MW. 2013b. Regional variation in the particulate organic carbon to nitrogen ratio in the surface ocean. *Glob. Biogeochem. Cycles* 27:723–31

Matallana-Surget S, Derock J, Leroy B, Badri H, Deschoenmaeker F, Wattiez R. 2014. Proteome-wide analysis and diel proteomic profiling of the cyanobacterium *Arthrospira platensis* PCC 8005. *PLOS ONE* 9:e99076

Mather RL, Reynolds SE, Wolff GA, Williams RG, Torres-Valdes S, et al. 2008. Phosphorus cycling in the North and South Atlantic Ocean subtropical gyres. *Nat. Geosci.* 1:439–43

Maxwell DP, Falk S, CG T, Huner NPA, Maxwell DP, et al. 1994. Growth at low temperature mimics high-light acclimation in *Chlorella vulgaris*. *Plant Physiol.* 105:535–43

Meunier CL, Haafke J, Oppermann B, Boersma M, Malzahn AM. 2012. Dynamic stoichiometric response to food quality fluctuations in the heterotrophic dinoflagellate *Oxyrrhis marina*. *Mar. Biol.* 159:2241–48

Mills MM, Arrigo KR. 2010. Magnitude of oceanic nitrogen fixation influenced by the nutrient uptake ratio of phytoplankton. *Nat. Geosci.* 3:412–16

Mino T, van Loosdrecht MCM, Heijnen JJ. 1998. Microbiology and biochemistry of the enhanced biological phosphate removal process. *Water Res.* 32:3193–207

Moore CM, Mills MM, Arrigo KR, Berman-Frank I, Bopp L, et al. 2013. Processes and patterns of oceanic nutrient limitation. *Nat. Geosci.* 6:701–10

Moore LR, Rocap G, Chisholm SW. 1998. Physiology and molecular phylogeny of coexisting *Prochlorococcus* ecotypes. *Nature* 393:464–67

Mouginot C, Kawamura R, Matulich KL, Berlemont R, Allison SD, et al. 2014. Elemental stoichiometry of Fungi and Bacteria strains from grassland leaf litter. *Soil Biol. Biochem.* 76:278–85

Mouginot C, Zimmerman AE, Bonachela JA, Fredricks H, Allison SD, et al. 2015. Resource allocation by the marine cyanobacterium *Synechococcus* WH8102 in response to different nutrient supply ratios. *Limnol. Oceanogr.* 60:1634–41

Moutin T, Karl DM, Duhamel S, Rimmelin P, Raimbault P, et al. 2007. Phosphate availability and the ultimate control of new nitrogen input by nitrogen fixation in the tropical Pacific Ocean. *Biogeosciences* 5:95–109

Olson RJ, Vaulot D, Chisholm SW. 1986. Effects of environmental stresses on the cell cycle of two marine phytoplankton species. *Plant Physiol.* 80:918–25

Ottesen EA. 2014. Multispecies diel transcriptional oscillatins in open ocean heterotrophic bacterial assemblages. *Science* 53:1689–99

Persson J, Fink P, Goto A, Hood JM, Jonas J, Kato S. 2010. To be or not to be what you eat: regulation of stoichiometric homeostasis among autotrophs and heterotrophs. *Oikos* 119:741–51

Price NM. 2005. The elemental stoichiometry and composition of an iron-limited diatom. *Limnol. Oceanogr.* 50:1159–71

Raven JA, Geider RJ. 1988. Temperature and algal growth. *New Phytol.* 110:441–61

Redfield AC. 1958. The biological control of the chemical factors in the environment. *Am. Sci.* 46:205–21

Rhee G-Y. 1973. A continuous culture study of phosphate uptake, growth rate and polyphosphate in *Scenedesmus* sp. *J. Phycol.* 9:495–506

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

Rhee G-Y, Gotham IJ. 1980. Optimum N:P ratios and coexistence of planktonic algae. *J. Phycol.* 16:486–89

Rhee G-Y, Gotham IJ. 1981. The effect of environmental factors on phytoplankton growth: temperature and the interactions of temperature with nutrient limitation. *Limnol. Oceanogr.* 26:635–48

Rhee G-Y, Gotham IJ, Chisholm SW. 1981. Use of cyclostat cultures to study phytoplankton ecology. In *Continuous Cultures of Cells*, Vol. 2, ed. PH Calcott, pp. 159–86. Boca Raton, FL: CRC

Ribalet F, Swalwell J, Clayton S, Jiménez V, Sudek S, et al. 2015. Light-driven synchrony of *Prochlorococcus* growth and mortality in the subtropical Pacific gyre. *PNAS* 112:8008–12

Richardson TL, Jackson GA. 2007. Small phytoplankton and carbon export from the surface ocean. *Science* 315:838–40

Rocap G, Moore LR, Chisholm SW. 1999. Molecular phylogeny of *Prochlorococcus* ecotypes. In *Marine Cyanobacteria*, ed. L Charpy, AWD Larkum, pp. 107–16. Monaco: Inst. Océanogr.

Provides the most comprehensive analysis of P-containing biological components and their abundance within organisms.

Schaechter M, Maaloe O, Kjeldgaard NO. 1958. Dependency on medium and temperature of cell size and chemical composition during balanced grown of *Salmonella typhimurium*. *J. Gen. Microbiol.* 19:592–606

Schneider B, Schlitzer R, Fischer G, Nothig EM. 2003. Depth-dependent elemental compositions of particulate organic matter (POM) in the ocean. *Glob. Biogeochem. Cycles* 17:1032

Sherman E, Moore J, Primeau F, Tanouye D. 2016. Temperature influence on phytoplankton community growth rates. *Glob. Biogeochem. Cycles* 30:550–59

Simon M, Azam F. 1989. Protein content and protein synthesis rates of planktonic marine bacteria. *Mar. Ecol. Prog. Ser.* 51:201–13

Singh A, Baer SE, Riebesell U, Martiny AC, Lomas MW. 2015. C:N:P stoichiometry at the Bermuda Atlantic Time-series Study station in the North Atlantic Ocean. *Biogeosciences* 12:6389–403

Sohm JA, Webb EA, Capone DG. 2011. Emerging patterns of marine nitrogen fixation. *Nat. Rev. Microbiol.* 9:499–508

Spilling K, Ylostalo P, Simis S, Seppala J. 2015. Interaction effects of light, temperature and nutrient limitations (N, P and Si) on growth, stoichiometry and photosynthetic parameters of the cold-water diatom *Chaetoceros wighamii*. *PLOS ONE* 10:e0126308

Sterner RW. 2015. Ocean stoichiometry, global carbon, and climate. *PNAS* 112:8162–63

Discusses the basis of elemental stoichiometry as well as the drivers and effects on ecosystems.

Sterner RW, Elser JJ. 2002. *Ecological Stoichiometry: The Biology of Elements from Molecules to the Biosphere*. Princeton, NJ: Princeton Univ. Press

Talarmin A, Lomas MW, Bozec Y, Savoye N, Frigstad H, et al. 2016. Seasonal and long-term changes in elemental concentrations and ratios of marine particulate organic matter. *Glob. Biogeochem. Cycles* 30:1699–711

Talmy D, Martiny AC, Hill C, Hickman AE, Follows MJ. 2016. Microzooplankton regulation of surface ocean POC:PON ratios. *Glob. Biogeochem. Cycles* 30:311–32

Tempest D, Hunter JR. 1965. The influence of temperature and pH value on the macro-molecular composition of magnesium-limited and glycerol-limited *Aerobacter aerogenes* growing in a chemostat. *J. Gen. Microbiol.* 41:267–73

Teng Y-C, Primeau FW, Moore JK, Lomas MW, Martiny AC. 2014. Global-scale variations of the ratios of carbon to phosphorus in exported marine organic matter. *Nat. Geosci.* 7:895–98

Tezuka Y. 1990. Bacterial regeneration of ammonium and phosphate as affected by the carbon:nitrogen:phosphorus ratio of organic substrates. *Microb. Ecol.* 19:227–38

Thompson PA, Guo MX, Harrison PJ. 1992. Effects of variation in temperature. I. On the biochemical composition of eight species of marine phytoplankton. *J. Phycol.* 28:481–88

Thompson PA, Harrison PJ, Parslow JS. 1991. Influence of irradiance of cell volume and carbon quota for ten species of marine phytoplankton. *J. Phycol.* 27:351–60

Thrane JE, Hessen DO, Andersen T, Hillebrand H. 2016. The impact of irradiance on optimal and cellular nitrogen to phosphorus ratios in phytoplankton. *Ecol. Lett.* 19:880–88

Tolonen AC, Aach J, Lindell D, Johnson ZI, Rector T, et al. 2006. Global gene expression of *Prochlorococcus* ecotypes in response to changes in nitrogen availability. *Mol. Syst. Biol.* 2:53

Torriani-Gorini A. 1987. The birth and growth of the Pho regulon. In *Phosphate Metabolism and Cellular Regulation in Microorganisms*, ed. A Torriani-Gorini, FG Rothman, S Silver, A Wright, E Yagil, pp. 3–11. Washington, DC: Am. Soc. Microbiol.

Combines metatranscriptomics and a cellular allocation strategy model to illustrate the impact of temperature on N:P.

Toseland A, Daines SJ, Clark JR, Kirkham A, Strauss J, et al. 2013. The impact of temperature on marine phytoplankton resource allocation and metabolism. *Nat. Clim. Change* 3:979–84

Tyrrell T. 1999. The relative influences of nitrogen and phosphorus on oceanic primary production. *Nature* 400:525–31

Vadia S, Levin PA. 2015. Growth rate and cell size: a re-examination of the growth law. *Curr. Opin. Microbiol.* 24:96–103

Van Mooy BAS, Devol AK. 2008. Assessing nutrient limitation of *Prochlorococcus* in the North Pacific subtropical gyre by using an RNA capture method. *Limnol. Oceanogr.* 53:78–88

Van Mooy BAS, Fredricks HF. 2010. Bacterial and eukaryotic intact polar lipids in the eastern subtropical South Pacific: water-column distribution, planktonic sources, and fatty acid composition. *Geochim. Cosmochim. Acta* 74:6499–516

Van Mooy BAS, Fredricks HF, Pedler BE, Dyhrman ST, Karl DM, et al. 2009. Phytoplankton in the ocean use non-phosphorus lipids in response to phosphorus scarcity. *Nature* 458:69–72

Van Mooy BAS, Rocap G, Fredricks HF, Evans CT, Devol AH. 2006. Sulfolipids dramatically decrease phosphorus demand by picocyanobacteria in oligotrophic marine environments. *PNAS* 103:8607–12

Vaulot D, Marie D, Olson RJ, Chisholm SW. 1995. Growth of *Prochlorococcus*, a photosynthetic prokaryote, in the equatorial Pacific Ocean. *Science* 268:1480–82

Vrede K, Heldal M, Norland S, Bratbak G. 2002. Elemental composition (C, N, P) and cell volume of exponentially growing and nutrient-limited bacterioplankton. *Appl. Environ. Microbiol.* 68:2965–71

Waldbauer JR, Rodrigue S, Coleman ML, Chisholm SW. 2012. Transcriptome and proteome dynamics of a light-dark synchronized bacterial cell cycle. *PLOS ONE* 7:e43432

Wanner BL. 1993. Gene regulation by phosphate in enteric bacteria. *J. Cell. Biochem.* 51:47–54

Weber TS, Deutsch C. 2010. Ocean nutrient ratios governed by plankton biogeography. *Nature* 467:550–54

Suggests the importance of community structure in understanding the variation in elemental stoichiometry.

Weber TS, Deutsch C. 2012. Oceanic nitrogen reservoir regulated by plankton diversity and ocean circulation. *Nature* 489:419–22

Worden AZ, Binder BJ. 2003. Application of dilution experiments for measuring growth and mortality rates among *Prochlorococcus* and *Synechococcus* populations in oligotrophic environments. *Aquat. Microb. Ecol.* 30:159–74

Wu J, Sunda W, Boyle EA, Karl DM. 2000. Phosphate depletion in the western North Atlantic Ocean. *Science* 289:759–62

Yun HS, Hong J, Lim HC. 1996. Regulation of ribosome synthesis in *Escherichia coli*: effects of temperature and dilution rate changes. *Biotechnol. Bioeng.* 52:615–24

Yvon-Durocher G, Dossena M, Trimmer M, Woodward G, Allen AP. 2015. Temperature and the biogeography of algal stoichiometry. *Glob. Ecol. Biogeogr.* 24:562–70

Zhu CJ, Lee YK, Chao TM. 1997. Effects of temperature and growth phase on lipid and biochemical composition of *Isochrysis galbana* TK1. *J. Appl. Phycol.* 9:451–57

Zimmerman AE, Martiny AC, Lomas MW, Allison SD. 2014. Phosphate supply explains variation in nucleic acid allocation but not C:P stoichiometry in the western North Atlantic. *Biogeosciences* 11:1599–611

Zinser ER, Lindell D, Johnson ZI, Futschik ME, Steglich C, et al. 2009. Choreography of the transcriptome, photophysiology, and cell cycle of a minimal photoautotroph, *Prochlorococcus*. *PLOS ONE* 4:e5135