J. Phycol. **46,** 1–12 (2010)

DOI: 10.1111/j.1529-8817.2009.00756.x

REVIEW

IS THE GROWTH RATE HYPOTHESIS APPLICABLE TO MICROALGAE?[1]

Kevin J. Flynn[2]

Institute of Environmental Sustainability, Department of Pure and Applied Ecology, Wallace Building, Swansea University, Singleton Park, Swansea SA2 8PP, UK

John A. Raven

Division of Plant Sciences, University of Dundee at SCRI, Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA, UK

T. Alwyn V. Rees

Leigh Marine Laboratory, University of Auckland, P.O. Box 349, Warkworth, New Zealand

Zoe Finkel

Environmental Science Program, Mount Allison University, Avard-Dixon, 144 Main Street, Sackville E4L 1A7, Canada

Antonietta Quigg

Departments of Marine Biology and Oceanography, Texas A&M University at Galveston, 5007 Avenue U, Galveston, Texas 77551, USA

and John Beardall

School of Biological Sciences, PO Box 18, Monash University, Clayton, Victoria 3800, Australia

The growth rate hypothesis (GRH) asserts, from known biochemistry, that maintaining high growth rates requires high concentrations of ribosomes. Since ribosomes are rich in phosphorus (P), the GRH predicts a positive correlation between growth rate and P content; this correlation is observed in some organisms. We consider the application of the GRH to phytoplankton and identify several key problems that require further research before the hypothesis can be accepted for these organisms. There are severe methodological problems that confound interpretation of data for testing the GRH. These problems include the measurement of protein and nucleic acids (such that ratio of these components carries a high level of uncertainty), studies of steady-state versus dynamic systems, and the presentation of data per cell (especially as cell size varies with growth rate limitations) and the calculation of growth rates. In addition, because of the short generation times and rapid responses of these organisms to perturbations, ribosome and RNA content is expected to vary in response to (de)repression of various systems; content may increase on application of growth-limiting stress. Finally, that most phytoplankton accumulate P when not P stressed conflicts with the GRH. In consequence, the value of the GRH for any sort of predictive role in nature appears to be severely limited. We conclude that the GRH cannot be assumed to apply to phytoplankton taxa without first performing experimental tests under transient conditions.

Key index words: **growth rate hypothesis; nutrient limitation; phytoplankton; protein; ribosome; RNA**

Abbreviation: **GRH, growth rate hypothesis**

Biologists have long been concerned with understanding the rates of and limitations to growth because growth rate is a primary determinant of community trophic dynamics and ecosystem productivity. The work of Sterner and Elser (2002) has done much to rekindle interest in this area by highlighting the importance of element ratios (i.e., stoichiometry) in ecology. In essence an offshoot of physiological ecology (Calow 1999), ''ecological stoichiometry'' describes trophic dynamics as a function not only of the absolute quantities of elements available in the environment, but also the stoichiometric composition of biomass and, in particular, differences in elemental composition within and between trophic levels. In general terms, organisms or tissues with higher concentrations of nutrients tend to grow more rapidly (e.g., higher nitrogen [N] and/or [P] relative to carbon [C]). This link

[1]Received 4 February 2009. Accepted 12 June 2009.
[2]Author for correspondence: e-mail k.j.flynn@swansea.ac.uk.

between nutrient content and growth is of ecological importance because, for example, it implies a positive feedback between stoichiometry and nutrient regeneration: consumption of organisms of high nutrient content leads to high nutrient regeneration, thus helping to maintain the high nutrient content of the primary producers. Associated with the concept of ecological stoichiometry is the GRH (Sterner and Elser 2002), which attempts to establish a causal relationship between the growth rate and the elemental and macromolecular composition of biomass based on the stoichiometry and protein-synthesis rate of a ribosome. In the following study, we assess whether the GRH can be applied to phytoplankton.

The GRH. While never explicitly stated by Sterner and Elser (2002), the GRH implies two fundamental assumptions: (1) the rate of formation of peptide bonds by a ribosome occurs at a rate that is essentially fixed across diverse organisms and environments, and (2) the vast bulk of protein production is allocated to new growth, as opposed to maintenance during protein turnover (Allen and Gillooly 2009). Consequently, increases in the rate of protein synthesis during growth and development occur through increased biomass allocation to P-rich rRNA, which is responsible for protein synthesis at the level of translation. Since proteins constitute a large, if not the largest, macromolecular fraction of biomass, assumptions (1) and (2) above imply that the growth rate of a cell can be directly linked to the abundance of ribosomes. Interpolating further, since rRNA contains a significant fraction (sometimes the majority) of cellular P, increases in rRNA concentrations can affect not only the rate of protein synthesis, but also the overall C:N:P ratio of biomass. Accordingly, one may expect a causal link between cellular P content and growth rate. A positive relationship between nutrient (element) content and growth is qualitatively consistent with the Droop cell-quota model frequently used by phycologists (Flynn 2008). Vrede et al. (2004) draw upon phytoplanktonic examples in their discussion on the subject of the GRH, pointing out that better correlations for autotrophs are often found between increased growth and a decreased C:N (carbohydrate:protein) ratio than a decreased N:P (protein:RNA) ratio.

The GRH has its origins in studies conducted more than three decades ago, which focused mainly on nonphotolithotrophic organisms. These studies documented positive correlations between specific growth rate and rRNA concentrations for chemoorganotrophic bacteria (Caldwell et al. 1950), the colorless green algal flagellate *Polytomella* (Jeener 1953), a vertebrate eye lens (Dische et al. 1961), the photolithotrophic euglenoid alga *Euglena* (Cook 1963), amphipod and branchiopod crustaceans and a gastropod (Sutcliffe 1965), the colorless coccoid green alga *Prototheca* (Poynton 1973), the photolithotrophic coccoid green alga *Scenedesmus* (Rhee 1973), and a fungus (Sturani et al. 1973, Alberghina et al. 1975). These studies examined the growth rates of organisms and organs limited by a variety of resources, not just by P. The early algal work is considered in more detail below when we analyze data for algae.

Below we consider various aspects of the GRH and examine whether its assumptions and predictions are applicable to algae. This is an important matter because a means to robustly monitor the relative growth rate of organisms would be of great utility for laboratory as well as field researchers. We start by considering the methods used to measure ribosome, RNA, protein, and elemental content, and growth rates, because the reliability of data from these methods in turn affects the formulation, testing, and application of the GRH.

Methodological issues. To test the GRH, it is important that concentrations of proteins and ribosomes be measured as accurately as is possible. In practice, the variables actually measured are the total concentrations of protein and RNA. It is then assumed that ribosome densities are correlated with RNA concentrations, as expected given that ribosomal P typically accounts for the vast majority (~85%) of cellular P (Sterner and Elser 2002). If one further assumes that protein concentration (and hence N content) remains constant, and that most RNA occurs in P, one would expect an inverse correlation between cellular N:P and growth rate. This expectation brings the GRH back into the domain of ecological stoichiometry, from whence it emerged, as mentioned in the introduction.

1) Measurements of RNA and protein: Evaluating the GRH requires reliable measurements of cellular RNA and protein. Unfortunately, there are a number of methodological considerations that potentially compromise measurements of cellular RNA and protein levels. For RNA quantification, there are two common methods: orcinol determination of ribose and assays involving the binding of fluorescent compounds (Fara et al. 1996). The major problem with the orcinol method is interference by other sugars (e.g., Healey and Hendzel 1975). Consequently, the orcinol method tends to overestimate the amount of RNA unless this potential interference is taken into account. Moreover, the degree of interference within the same species of microalgae could vary with nutritional and environmental conditions depending on whether levels of interfering sugars increase or decrease. A very common response of phytoplankton under nutrient stress is the accumulation of sugars. Thus, this is a fundamental problem. The fluorescence-based methods appear to be more reliable but usually involve correcting for the dye binding to DNA (e.g., Fara et al. 1996).

With protein, there are a number of potential problems depending on the choice of assay method and protein standard. The two most commonly used spectrophotometric assays are the Lowry and Brad-

ford assays, which measure different properties of proteins (Berges et al. 1993, Barbarino and Lourenço 2005). Neither assay yields protein values that correspond to values computed more directly and reliably from the amino-acid composition of extracted proteins (Crossman et al. 2000, Barbarino and Lourenço 2005). A further issue with protein determination is the choice of calibration standard. Unfortunately, the reactivity of protein standards differs irrespective of the assay (Berges et al. 1993, Larson and Rees 1994). The Lowry assay gives higher protein values than the Bradford assay using BSA as the standard (Clayton et al. 1988, Berges et al. 1993, Crossman et al. 2000, Barbarino and Lourenço 2005). However, the Lowry assay may overestimate (Berges et al. 1993) or underestimate (Barbarino and Lourenço 2005) total phytoplankton protein. Consequently, the two most common assays for determining protein will rarely provide a reliable estimate of protein content.

The measured RNA:protein ratio thus depends on the choice of assays and standards. In most of the phytoplankton studies cited here, the orcinol and Lowry methods were used. Consequently, there is a tendency toward overestimating the RNA:protein in phytoplankton and underestimating the rate of protein synthesis per ribosome.

2) Elemental ratios: Rather than measuring protein and RNA, the determination of elemental C:N:P may be considered. In laboratory culture, this is relatively easy, although infrequently measured. However, in the field, the determination of elemental composition for phytoplankton is confounded by the presence of similar size particles that are collected concurrently with the cells. One option to address this issue would be to use microelemental analysis, which makes use of X-ray spectra from EM to allow for the elemental analysis of individual cells (Heldal et al. 2003, Twining et al. 2003). However, if one can obtain C:N:P, then immediately there is the option of employing the well-established quota-style analyses, rather than the GRH, to help estimate growth and establish growth-limiting factors. Measuring microalgal N:P alone is, as we shall see, of little use.

3) Growth rates: The seemingly simple activity of measuring growth rates is complicated in dynamic situations by asynchronous changes in metabolites, biomass, and cell numbers. This phenomenon results in C-specific, cell-specific, chlorophyll-specific (etc.) growth rates being dissimilar, and on occasion, potentially even being contradictory (Flynn 2006). For example, on exhaustion of external nutrient N, the N-specific growth rate becomes zero, while cell and C-specific growth continues as the N quota declines to its subsistence value and organic C is accumulated in cells or excreted. Protein synthesis, at least gross synthesis associated with turnover, also continues. While most phycologists and field workers count cells or measure chl, growth rate determination as implicated by the GRH should be N specific (relating to protein-specific growth). Making such measurements in the laboratory is nontrivial, but in the very conditions under which the GRH would (if it were accepted) be of most value, in the field, the transient nature of environmental conditions conspires to utterly confound its use. On the face of it, the GRH only appears applicable to steady-state situations.

Questioning the physiological basis of the GRH. At the base of the GRH is the assumption that the rate of protein synthesis by a ribosome is largely fixed. From first principles, it is possible to obtain the expected relationship.

The maximum in vivo rate of polypeptide chain elongation in eukaryotes has been estimated at 10 amino-acyl units per ribosome per second at 30°C (Karpinets et al. 2006, their tables 1, 3) and 5.5 amino-acyl units per ribosome per second at 22°C (Orlowski 1981). This suggests a Q_{10} of about 2 (see Rodriguez-Correa and Dahlberg 2008). The rate at 15°C (the assumed mean temperature of the ocean) is thus about 3.5 amino-acyl units per ribosome second. However, it is not clear what assumptions have been made in the calculations leading to the rates or polypeptide elongation given above; a rather lower value of 1.5 amino-acyl units per ribosome per second at 20°C can be calculated from data in Sterner and Elser (2002).

We make the assumption that eukaryotic marine phytoplankton have an N:P of 16:1 on the basis of the Redfield ratio, and that the same fraction of N is in the total cell protein as the fraction of P in rRNA (i.e., the atomic ratio of N in protein to P in rRNA is 16:1). Neither of these assumptions is robust, as the ratios vary over orders of magnitude for each (Geider and LaRoche 2002), but we continue with these values. Since the elements in ribosomes and proteins are given in mass terms, hereafter the mass-based Redfield ratio of N:P = 7.23 is used.

From Karpinets et al. (2006), there is 0.08 g $P \cdot g^{-1}$ dry RNA and 0.172 g $N \cdot g^{-1}$ dry protein. With half of the dry mass of eukaryotic ribosomes taken up by RNA and the rest by protein (Nieuwenhuysen et al. 1978), this P per RNA ratio corresponds to 0.04 g $P \cdot g^{-1}$ dry ribosomes. Slightly different values are given by Geider and LaRoche (2002), at 0.061 g $P \cdot g^{-1}$ dry RNA (=0.0305 g $P \cdot g^{-1}$ dry ribosomes) and 0.155 g $N \cdot g^{-1}$ dry protein. The highest value of 0.21 g $N \cdot g^{-1}$ dry protein was determined from their original data by Lourenço et al. (2002). The Karpinets et al. (2006) values are used subsequently. From these values, the ratio of ribosomes to protein can be computed as follows.

A mean relative molecular mass (weight) of 120 for amino-acyl units is assumed. The relative molecular mass of eukaryotic cytosolic ribosomes is 4×10^6 (Nieuwenhuysen et al. 1981). Thus, 1 g P in ribosomes [at 0.04 g $P \cdot g^{-1}$ dry ribosomes = (1/0.04)g dry ribosome] is equivalent to 3.76×10^{18}

ribosomes (4×10^6 g of dry ribosomes = 6.022×10^{23} ribosomes, thus 1/0.04 g ribosomes = $6.022 \times 10^{23}/4 \times 10^6$ ribosomes). Plastid and mitochondrial ribosomes are not considered separately from ribosomes in the cytosol.

For protein, 7.23 g N is equivalent to 42 g protein (using the conversion factor of Karpinets et al. 2006), or 0.35 mol amino-acyl units (i.e., 42.0 g protein divided by the relative molecular mass of amino-acyl units = 42.0/120 = 0.35). Taking the mass (Redfield) N:P ratio of 7.23, 0.35 mol amino-acyl units equates to 1 g P in ribosomes, which equates to 2.10×10^{23} amino-acyl units in total cell protein; so there are ($2.10 \times 10^{23}/3.76 \times 10^{18}$ =) 0.559×10^5 molecules of amino-acyl equivalents as total cell protein associated with each ribosome.

A specific growth rate of $1 \cdot d^{-1}$ is equivalent to $1.16 \times 10^{-5} \cdot s^{-1}$, so that the required rate of amino-acyl unit addition per ribosome is $(0.559 \times 10^5) \times (1.16 \times 10^{-5}) \cdot s^{-1}$ or $0.648 \cdot s^{-1}$ (i.e., almost 20% of the maximum in vivo rate of $3.5 \cdot s^{-1}$). For a specific growth rate of $1 \cdot week^{-1}$, the required rate of addition is $0.093 \cdot s^{-1}$, amounting to only ~3% of the maximum in vivo rate.

The above calculations do not allow for any increase in protein:rRNA with decreasing growth rate as required by the GRH, and as supported by some but by no means all of the data for phytoplankton (see below). More importantly, ribosomes are required not only for the formation of new proteins during growth and development, but also for the replacement of proteins lost through metabolic processes (Gillooly et al. 2005). Thus, if the fundamental assumption of the GRH is correct, ribosome densities should increase not only with the growth rate, but also with the rate of protein turnover, and hence with the metabolic rate (Gillooly et al. 2005). For organisms, such as phytoplankton, that grow in highly transient environments, with rapid changes in nutrients and light, significant (de)repression activity and protein turnover are expected in response to various stresses. Those stresses need not be growth limiting. Although not commonly considered as such, the proteins and enzymes for nitrate transport and assimilation are stress proteins; cells switching between ammonium and nitrate consumption would be expected to change their levels of transcription and ribosome content without necessarily changing their growth rate.

Translation makes new proteins and replaces degraded proteins. If a cell contains the quantity of protein, P, expressed in units of amino acids $\cdot$ cell^{-1}, then the rate of change in P is given by following equation.

$$\frac{dP}{dt} = S - dP - \mu P \quad (1)$$

where S is the synthesis (translation) rate (amino acids $\cdot$ cell$^{-1} \cdot s^{-1}$), d is the mean decay rate (weighted by the abundance $\cdot$ length) of proteins (s^{-1}), and μ is growth rate (s^{-1}). At steady state, $S = P(d + \mu)$.

Quigg and Beardall (2003) measured rates of protein turnover over a 10-fold range of light-limited growth rates (~0.1 to ~$1.2 \cdot d^{-1}$) for *Dunaliella tertiolecta* (Chlorophyceae) and *Phaeodactylum tricornutum* (Bacillariophyceae). Both algae had two pools of protein with respect to turnover. One pool, accounting for ~0.4% of the total protein, turned over more rapidly (2–4 turnovers $\cdot d^{-1}$) than did the other pool; the remainder of the protein turned over at 0.15–0.45 $\cdot d^{-1}$. Assuming two pools of protein, with long- and short-turnover times (d_L and d_S, respectively), accounting for 99.6% and 0.4% of protein, respectively, we obtain the following equation:

$$S = 0.996 \cdot P \cdot (\mu + d_L) + 0.004 \cdot P \cdot (\mu + d_S) \quad (2)$$

This simplifies to give equation 3.

$$S = P \cdot (\mu + 0.996 \cdot d_L + 0.004 \cdot d_S) \quad (3)$$

Considering protein synthesis in terms of the activity of ribosomes, if N_r is the number of ribosomes (ribosomes $\cdot$ cell^{-1}) and R_T is translation rate (amino acids $\cdot$ ribosome$^{-1} \cdot s^{-1}$), then we obtain equation 4.

$$S = N_r \cdot R_T \quad (4)$$

This, rearranged to solve for R_T, yields equation 5.

$$R_T = \frac{S}{N_r} = \frac{P \cdot (\mu + 0.996 \cdot d_L + 0.004 \cdot d_S)}{N_r} \quad (5)$$

Earlier in this section, we computed that 0.559×10^5 molecules of amino-acyl equivalents as total cell protein could be associated with each ribosome. Thus, setting $P/N_r = 0.559 \cdot 10^5$, we obtain R_T (as amino acids $\cdot$ ribosome$^{-1} \cdot s^{-1}$), as in the following equation:

$$R_T = 0.559 \cdot 10^5 \cdot (\mu + 0.966 \cdot d_L + 0.004 \cdot d_S) \quad (6)$$

The specific rate of protein synthesis, μ_P, is given in following equation.

$$\mu_P = \mu + 0.966 \cdot d_L + 0.004 \cdot d_S \quad (7)$$

Using estimates of μ, d_L, and d_S from Quigg and Beardall (2003) for *Dunaliella* and *Phaeodactylum*, and making the assumption that the sizes of the long- and short-turnover pools are the same for fast- and slow-growing cells, enables the generation of Figure 1. While turnover accounts for an increasing proportion of total protein synthesis as cell growth declines under light limitation, there is a good relationship ($R^2 = 0.97$) between growth rate and protein synthesis. As caveats, however, it should be noted that these data come from turbidostats (the relationship through transients is unknown), that the relationship does not pass through the origin, and that changes in protein turnover rates with

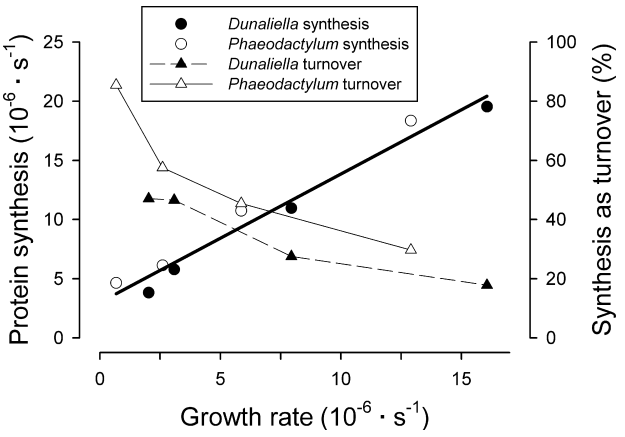

FIG. 1. Changes in gross protein synthesis with growth rate, and in the proportion of that synthesis accounting for protein turnover. Data used from Quigg and Beardall (2003) for the chlorophyte *Dunaliella tertiolecta* and diatom *Phaeodactylum tricornutum*, with calculations as described in the text associated with equations (1)–(7).

different nutrient stresses and temperature remain unknown. We also assume, in using equation (7) to generate Figure 1, that the rate of protein synthesis by a ribosome is fixed, that indeed $P/N_r = 0.559 \cdot 10^5$.

The idea that net growth correlates with the concentration of molecules (rRNA), assumes that all other factors (''substrates'') are nonlimiting. The relationship shown in Figure 1 is for light limitation. Just as the GRH requires that the rate of net protein synthesis relates to ribosome and rRNA content, so there is the implicit expectation that proteins (enzymes) are operating at a fixed (presumably high) fraction of their maximum specific activity. Cells that are growing at low rates, limited by nutrients, can have high concentrations of enzyme to maximize the cell-specific rate, while the enzyme-specific rate may be low. An example comes from the work of Beardall et al. (1991), which showed that the quotient of RUBISCO activity to RUBISCO protein is low in N-replete green microalgal cells, but increases when the growth rate decreases under N limitation. More generally, de facto, proteins with low (or zero) catalytic function act as N stores. This includes RUBISCO in many algal cells, the growth rate of which is limited by light (Sukenik et al. 1989, Lin and Carpenter 1997, Jenks and Gibbs 2000). Cryptophytes and many cyanobacteria contain much N as light-harvesting phycobiliproteins; under conditions of maximum synthesis (light limiting), their effective catalytic rate is also low.

Storage of N as a polypeptide is energy expensive, especially when the polypeptide is made on ribosomes (i.e., the means by which almost all polypeptides are synthesized in cells). Here, the theoretical minimum cost is assumed as four ATP converted to ADP per peptide bond produced, with values of 10.8 or more when all associated processes are taken into account (references in Scheurwater et al. 2000, Quigg and Beardall 2003). In contrast, peptide synthesis that does not involve ribosomes in the enzyme-catalyzed reaction [just in producing the relevant enzyme(s)] is used in the biosynthesis of the arginine-aspartate 1:1 copolymer cyanophycin that occurs in most cyanobacteria. Here, the minimum cost is experimentally determined as 1.3, and theoretically as 1.0, ATP converted to ADP per peptide bond formed (Aboulmagd et al. 2001). While the associated energy costs have not apparently been calculated for cyanophycin biosynthesis (i.e., the cost of producing each molecule of cyanophycin synthetase, the turnover rate of enzyme, and the enzyme's maximum specific reaction rate), the indirect involvement of ribosomes and mRNA means that these associated costs are lower in absolute terms than for protein synthesis using ribosomes.

Together with the increasing fraction of cell nitrogen accounted for by ribosomal nitrogen in more rapidly growing cells, nonribosomal protein decreases with increasing growth rate. This decrease in nonribosomal nitrogen means that the achieved specific reaction rate of a given enzyme (or transporter) in the slower-growing cells is decreased more than in proportion to the decrease in specific growth rate. The GRH focuses on the rate of protein synthesis required for a given specific growth rate without explicit consideration of the implications for the extent to which the proteins have achieved a low specific reaction rate, or indeed to what extent the synthesis repairs damage or really contributes to net growth.

Just as cells may contain proteins (enzymes) that are working at far below maximum capacity, the same is likely for ribosomes, although measurements are lacking. An organism whose change in composition with growth rate supports the GRH would require that more ribosomes are produced for any metabolic retooling that is needed to meet transient new resource supply situations. In contrast, an organism that carries ''excess'' ribosomes at low specific growth rates might show a more rapid response to the new conditions and thereby potentially gain an advantage in terms of a rapid increase in growth rate.

One may question whether possession of surplus ribosomes is more costly than carrying an excess of protein in organisms whose growth rate is constrained by resource supply. If this is so, then it could be possible to rationalize why (for a given cell size and total non-DNA macromolecule concentration) there are fewer ribosomes at low growth rates, independent of the validity of the GRH. The answer is only obvious when P is the limiting resource: ''excess'' ribosomes are much more costly in terms of P to synthesize at each cell division than is the same amount of N in protein, granted the very low P requirement for phosphorylation of proteins.

It is possible that excess rRNA operons in the genome (i.e., a greater number than are needed to support the rate of rRNA production used for steady-state growth) might be used in a rapid response to resource resupply. Such a rapid response, permitted either by a large content of ribosomes or the capacity for rapid production of ribosomes, could have selective advantage in competition among species for resources. In particular, this might have the effect of decreasing the lag phase, or the amount of time taken to begin growing by cells in stationary phase, which can be strongly selectively advantageous for cells growing under transient resource supply (Vasi et al. 1994).

To complicate matters further, protein turnover and gross protein synthesis are both difficult rates to measure in microalgae and other organisms (Quigg and Beardall 2003). Consequently, the GRH is generally evaluated using only data on the net rate of synthesis (i.e., growth rate), which is itself only (an unknown) proportional to the net rate of protein synthesis if protein concentrations per unit of biomass remain constant. The potential for the transient decoupling of changes in ribosome density and RNA content from growth rate is clear. Such concerns are not new; the use of RNA:DNA and similar ratios was suggested by Dortch et al. (1984, 1985) as indicators of growth rate in phytoplankton but criticized as being nonrobust under transient conditions (Flynn 1990).

Evidence for and against the application of the GRH to microalgae. The work of Sterner and Elser (2002) and Elser et al. (2003) considered the generality of the GRH primarily by reference to P-limited growth of organisms from different ecosystems. While the GRH appears to show promise under light-limited steady state (Fig. 1), a qualitative survey of the literature provides rather mixed support for the GRH in microalgae (Table 1). These data show a diversity of relationships between growth rate and RNA per cell, per-cell C, per-cell protein, or per-cell DNA (see also Table 2). For some of these data sets, there is a degree of parallelism between RNA content and growth rate, such that there is only a modest change in the specific reaction rate of rRNA in protein synthesis with changes in growth rate; this conforms to predictions of the GRH. At the other extreme, the RNA content does not appear to change with growth rate, so that the reaction rate of rRNA exactly parallels the changes in specific growth rate, in clear contradiction of the GRH.

Among prokaryotes, cyanobacteria show a diversity of relationships between RNA content and growth rate (Table 1). The most investigated oxygenic photolithotroph is *Synechococcus*, which encompasses substantial phylogenetic diversity. The earliest studies of this genus were conducted using the freshwater strain PCC 6301, then known as *Anacystis nidulans* (Mann and Carr 1974, Parrott and Slater 1980, Utkilen 1982). In general, studies of this taxon yielded positive relationships between RNA and growth rate when growth rates were varied by altering light, CO_2, and/or magnesium availability. Importantly, however, relationships between RNA and growth rate were exponential or sigmoidal; the relationship was not linear, as would be predicted by the GRH if protein concentrations were constant and the contribution of protein turnover to the gross rate of protein synthesis was negligible. Positive relationships between RNA and growth are also reported for the *Synechococcus* strain WH 8103, but not for WH 7803 (a non-phycoerythrin-containing strain) or WH 8101, which both exhibited the highest growth rates at intermediate RNA concentrations. Light-limited growth rates of *Prochlorococcus* were similarly related to RNA concentrations. Overall, these relationships suggest that the rate of protein synthesis per ribosome varies with light availability, which is inconsistent with the GRH assumption of constant ribosome kinetics. In addition, these studies did not assess the extent to which protein concentrations and rates of protein turnover varied with the light regime.

Among photolithotrophic eukaryotic algae, Cook (1963) determined that the RNA:protein ratio decreased with decreasing growth rate for *Euglena gracilis* (Euglenophyceae), but only at the lowest light-limiting irradiances, thus providing only some support for the GRH. Rhee (1973, 1978) reported that growth rate was a saturating function of the cellular RNA content, and a more nearly linear function of the cellular protein content, when growth rate of the freshwater green alga *Scenedesmus* sp. (Chlorophyceae) was varied by altering the availability of phosphate and nitrate. In both instances, Rhee's results showed a nonlinear increase in specific growth rate with RNA content, with some increase in the specific reaction rate of rRNA in protein synthesis with increasing growth rate. Schlesinger and Shuter (1981) examined effects of changes in PAR and temperature on growth rate and RNA:C ratio for four strains of freshwater green microalgae, namely, *Selenastrum minutum* (Chlorophyceae) and three species of *Scenedesmus*. In all instances, a light-induced increase in growth rate was paralleled by an increase in RNA:C, similar to many of the findings for cyanobacteria. For temperature, however, a decrease in growth rate with decreasing temperature was paralleled by an increase in RNA:C, consistent with an increased potential for protein synthesis on a cell basis; this could partly or wholly offset the decreased specific reaction rate of ribosomes at cooler temperatures.

Dortch et al. (1983) report RNA:DNA and DNA:C, and hence RNA:C, during ammonium and nitrate depletion of *Amphidinium carterae* (Dinophyceae), and during ammonium depletion of *Thalassiosira nordenskioldii* (Bacillariophyceae) in batch culture. The RNA:DNA and RNA:C decreased with decreasing growth rate, as did (when reported) total

TABLE 1. Summary of studies relevant to the growth rate hypothesis (GRH) in prokaryote and eukaryote microalgae.

Group	Organism	Environment	Limiting resource	Observation	GRH support	Reference
Cy	*Synechococcus* sp. PCC 6301[a]	fw	Light	Exponential increase in RNA with GR	Yes?	Mann and Carr (1974)
Cy	*Synechococcus* sp. PCC 6301[a]	fw	Light or CO_2	Sigmoidal increase in RNA with GR	Yes?	Parrott and Slater (1980)
Cy	*Synechococcus* sp. PCC 6301[a]	fw	Mg	Sigmoidal increase in RNA with GR	Yes?	Binder and Liu (1998) using data from Utkilen (1982)
Cy	*Synechococcus* sp. PCC6301	sea	Light	Exponential increase in RNA with GR	Yes?	Lepp and Schmidt (1998)
Cy	*Synechococcus* sp. WH8103	sea	Light	Increases	Yes	Lepp and Schmidt (1998)
Cy	*Synechococcus* sp. WH7803	sea	Light	RNA constant at low GR; linear up to 0.7 d^{-1}; decline at higher GR	No	Kramer and Morris (1990)
Cy	*Synechococcus* sp. WH7803	sea	?	RNA constant at low GR; linear at intermediate GRs; decline at higher GR(?)	No?	Worden and Binder (2003)
Cy	*Synechococcus* sp. WH8101	sea	Light	RNA constant at low GR; linear at intermediate GRs; decline at higher GR(?)	No	Binder and Liu (1998)
Cy	*Prochlorococcus* sp.	sea	?	RNA constant at low GR; linear at intermediate GRs; decline at higher GR(?)	No	Worden and Binder (2003)
Ch	*Polytomella caeca*	fw	Organic nutrients	RNA increase with GR	Yes	Jeener (1953)
Tre	*Prototheca zopfii*	fw	Organic nutrients	rRNA decreases with increasing GR	No	Poynton (1973); nonphotosynthetic
Eu	*Euglena gracilis*	fw	Light	RNA increase with GR	Yes	Cook (1963)
Ch	*Scenedesmus* sp.	fw	P and N	GR saturating function of RNA	No?	Rhee (1973, 1978)
Ch	*Selenastrum minutum*	fw	Light	Increase in RNA with increase in GR	Yes	Schlesinger and Shuter (1981)
Ch	*Scenedesmus* spp. (3 species)	fw	Light	Increase in RNA with increase in GR	Yes	Schlesinger and Shuter (1981)
Ch	*Selenastrum minutum*	fw	Temperature	Temperature down, decreases GR, increases RNA	No	Schlesinger and Shuter (1981)
Chl	*Scenedesmus* spp. (3 species)	fw	Temperature	Temperature down, decreases GR, increases RNA	No	Schlesinger and Shuter (1981)
Di	*Amphidinium carterae*	sea	Ammonium and nitrate	Decrease in RNA with decreasing growth	Yes	Dortch et al. (1983)
Bac	*Thalassiosira nordenskioldii*	sea	Ammonium	Decrease in RNA with decreasing growth	Yes	Dortch et al. (1983)
Di	*Amphidinium carterae*	sea	Light	No change in RNA with growth rate	No	Thomas and Carr (1985)

Bac, Bacillariophyceae; Cy, Cyanobacteria; Ch, Chlorophyceae; Di, Dinophyceae; Eu, Euglenophyceae; Tre, Trebouxiophyceae; fw, freshwater.
[a] *Anacystis nidulans.*

TABLE 2. Summary of data for growth rate versus RNA and protein per cell.

Organism	Cell volume (μm^3)[a]	Growth rate (d^{-1})[a]	fg RNA · cell^{-1} (late log)[b]	fg protein · cell^{-1} (late log)[b,c]	g RNA · g^{-1} protein (late log)[d]	fg RNA · μm^{-3} cell[d]	fg protein · μm^{-3} cell[d]	Net protein synthesis · RNA d^{-1} [d]
Synechococcus subsalsus (Cy)	2.3	1.00	0.2	11.9	0.016	0.08	5.25	62.5
Chlorella minutissima (Tre)	1.3	0.50	0.2	6.7	0.030	0.16	5.28	16.7
Dunaliella tertiolecta (Ch)	178.0	0.87	16.3	117.2	0.139	0.09	0..66	6.25
Tetraselmis gracilis (Pra)	640.0	0.78	8.0	574.8	0.014	0.01	0.90	55.7
Hilsea sp. (Cry)	190.0	0.64	22.6	246.3	0.092	0.12	1.30	6.96
Isochrysis galbana (Pry)	60.8	0.87	1.9	38.8	0.049	0.03	0.64	17.8
Nannochloropsis oculata (Eus)	13.3	0.64	0.3	19.3	0.016	0.02	1.45	40
Phaeodactylum tricornutum (Bac)	124.0	1.12	2.5	59.3	0.042	0.02	0.48	26.7
Skeletonema costatum (Bac)	110.0	1.20	12.1	144.2	0.084	0.11	1.31	14.3
Prorocentrum minimum (Dino)	1,395.0	0.65	33.0	1,423.4	0.023	0.02	1.02	28.3

Bac, Bacillariophyceae; Ch, Chlorophyceae; Cry, Cryptophyceae; Cy, Cyanobacteria; Dino, Dinophyceae; Eus, Eustigmatophyceae; Pra, Prasinophyceae; Pry, Prymnesiophyceae; Tre, Trebouxiophyceae.
[a,b,c]Lourenço et al. (2002, 1998, 2004); [d]calculated from columns 2–5.

cellular N. It is not clear whether the specific reaction rate of rRNA changes during N depletion. Other work on *A. carterae* (Thomas and Carr 1985), in this instance using light as the growth-rate-controlling resource, gave different results with no change in cell RNA or protein content with changing growth rate. There is clearly a decrease in specific reaction rate of rRNA in parallel with decreasing relative growth rate. The work of Laws et al. (1983) on *Thalassiosira weissflogii* (Bacillariophyceae) (with less detailed data for six other species of marine phytoplankton) also gave variable relationships between RNA and growth rate. To what extent differences in these results represent different culture techniques, that the organisms are also growing through transient shocks, into nutrient limitation, rather than in steady-state growth, is not clear. However one interprets it, the approach is not robust and rather conflicts with the cleaner relationship suggested by Figure 1.

Some close relatives of photolithotrophic eukaryotic algae are nonphotosynthetic; these apochlorotic organisms can only grow chemo-organotrophically. For the apochlorotic alga *Polytomella caeca* (Chlorophyceae), Jeener (1953) reported an increase in RNA per unit biomass with increasing growth rate by varying substrate supply, consistent with the GRH (Table 1). In contrast, Poynton (1973; see Karpinets et al. 2006) reported that the RNA:protein ratio was independent of the growth rate for *Prototheca zopfii* (Trebouxiophyceae), a colorless relative of *Chlorella*, when growth rate was varied by altering the composition of the chemo-organotrophic growth medium. Assuming a constant rate of protein turnover, this result implies that the specific reaction rate of rRNA, rather than increasing ribosome concentrations, increases with the growth rate; this is inconsistent with the GRH. It is a moot point as to whether data on these nonphotosynthetic organisms can be used in analyses of photosynthetic organisms, despite their phylogenetic relatedness to photosynthetic organisms.

The explanation for all the above-reported variability lays in the fact that the stoichiometry of light and nutrient supply, and whether the supply is limiting or saturating for growth, results in characteristic changes in macromolecular and stoichiometric changes in elemental composition (Finkel et al. 2006). In general, phytoplankton increase their relative investment in light-harvesting pigments when light limits growth rates (Richardson et al. 1983, Falkowski and LaRoche 1991, MacIntyre et al. 2002, Quigg et al. 2006). Likewise, investment in other processes (nutrient transport, synthesis of alkaline phosphatase, etc.) increases in response to nutrient stress. Furthermore, under transient conditions (which may include simple diurnal events), one may expect additional decoupling of synthesis and growth. The synthesis of different macromolecules has different energetic costs and elemental requirements (Raven 1984, 1988, 1990, Raven et al. 1999, Geider and LaRoche 2002, Quigg and Beardall 2003).

The most accurate analysis of microalgal cell protein content, for which there are also data for RNA content at different growth rates, is that of Lourenço et al. (1998, 2002, 2004) (data summarized in Table 2). Protein concentrations were estimated as the difference between free amino acids in cells and total amino acids in cells after protein hydrolysis. In these experiments, growth rates (d^{-1}) at a temperature of $23 \pm 2°C$ in the 12 h light phase and $20 \pm 1°C$ in the 12 h dark phase as determined by cell counts or fluorescence varied by only 2.4-fold range among the nine eukaryotic algal strains and one cyanobacterial strain. In contrast, values of RNA per cell volume varied 12-fold, and the RNA-specific rate of protein synthesis varied 10-fold (Table 2). Clearly, there is a much greater range of RNA-specific protein synthesis rates than of growth rates in this interspecies comparison involving nine classes from six divisions (phyla), and the ranking of species is quite different for the two parameters. Although these data relate only to

resource-saturated growth of the algae, the result, again, does not bode well for attempts to estimate the growth rate of natural assemblages of microalgae by reference to cellular RNA concentrations.

Turning to reports in which total P is used as a surrogate for rRNA, Ågren (2004) analyzed data on the growth rate and P content of the freshwater green planktonic microalga *Selenastrum* as well as seedlings of the dicotyledonous tree *Betula* and found very limited agreement with the GRH (see also Goldman et al. 1979, Elrifi and Turpin 1985, Flynn 2002, Gusewell 2004, Klausmeier et al. 2004, Knecht and Goransson 2004, Niklas 2006). Some earlier attempts to correlate growth rate and plant rRNA or P per unit biomass in vascular land plants were confounded by making the rRNA and/or P measurements on mature (nongrowing) leaves where roles of rRNA are in protein turnover, including repair of photodamaged D1 protein of PSII, rather than in net tissue growth (see Raven 1989). Similar problems would beset applications of similar methodologies to natural phytoplankton communities.

Although there is some evidence that N:P decreases in some species with decreases in growth rate as a result of low irradiance, the pattern is weak, especially when comparing across species (Fig. 2a). In general, there is a weak relationship between growth rate and N:P (Fig. 2b). The commonly used Droop model (see Flynn 2008) of nutrient-limited growth describes growth as a saturating function of internal nutrient concentration (expressed as nutrient:cell volume, or nutrient:cell carbon). The Droop model would predict, as do the observations on which it was based, a decrease in the ratio [limiting element]:[nonlimiting element] within biomass as the availability of the limiting nutrient decreases. In other words, if P is limiting growth, then there is an expectation that C:P and N:P would increase as growth rate decreases with decreasing availability of phosphate. If N is limiting, then there is an expectation that N:P would decrease as growth rate decreases with decreasing availability of nitrate. This is seen in the data of Elrifi and Turpin (1985), most of which came from steady-state systems, for the microalga *Selenastrum minutum* (Fig. 3). The relationship between whole-cell P and growth rate is thus very far from simple and clearly does not lend itself readily to interpretation along the lines of the GRH.

Certainly, there is not the unanimous support that would be required to consider using the GRH approach unquestioningly. Data only appear to match the GRH prediction for P-limiting conditions. As in marine waters, N rather than P is considered more likely limiting for microalgae (but see recent reviews by Howarth and Marino 2006, Elser et al. 2007), and in general terms, light is more likely limiting of microalgal growth, this lack of support for the GRH is rather damning. This is especially so as one needs to know which nutrient or factor is limiting to decide whether the GRH is applicable;

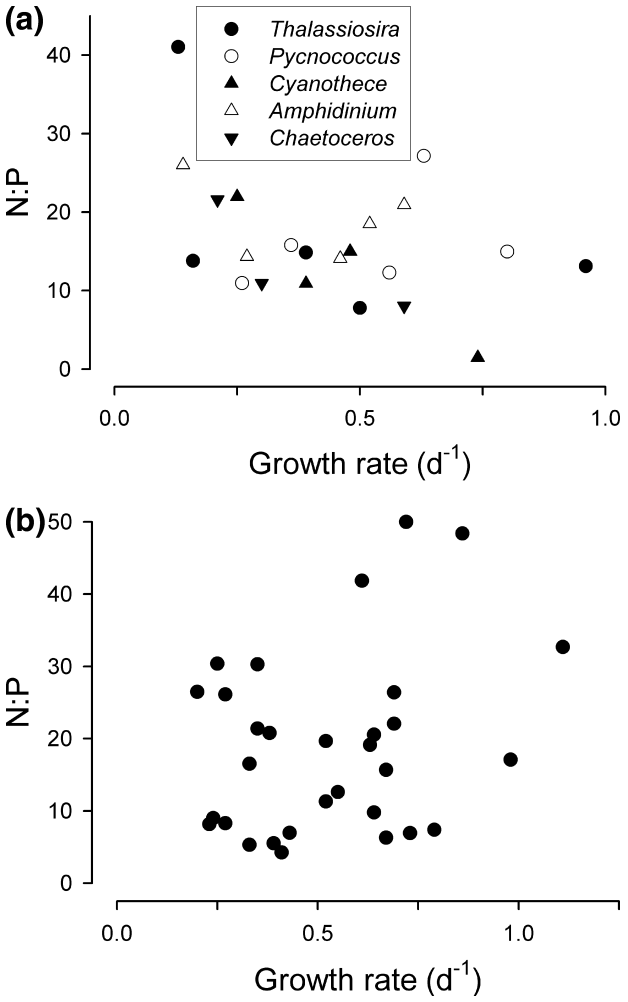

FIG. 2. Steady-state N:P as a function of growth rate: (a) within a species when irradiance limits growth (data from Finkel et al. 2006); (b) among species under irradiance and nutrient saturating conditions (data from Quigg et al. 2003, Ho et al. 2003, and A. Quigg, A. J. Irwin, and Z. V. Finkel unpublished data).

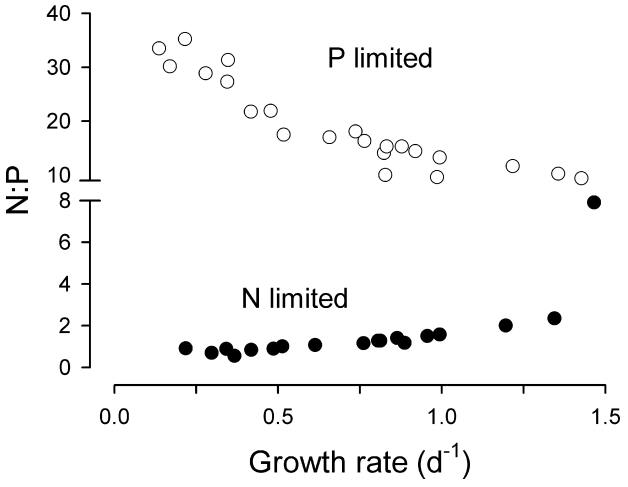

FIG. 3. Steady-state N:P as a function of growth rate for *Selenastrum minutum* when nitrate or phosphate limits growth (data derived from Elrifi and Turpin 1985).

determining what limits marine primary production has long tested marine scientists.

Conclusions. In its strictest form, the GRH assumes a specific reaction rate of rRNA in protein synthesis that is invariant with changes in specific growth rate of a given organism. This assumption is not widely supported by data on oxygenic photosynthetic organisms. The fundamental basis of the GRH, while appearing acceptable under certain conditions, also appears highly questionable in general terms, failing to account for transients between growth rate and ribosome, RNA and protein synthesis/concentration, or indeed for net versus gross protein synthesis under different conditions. There does not seem to be a unique, convincing, alternative explanation of the data consistent with the GRH, or of the data that are not consistent with the GRH. Indeed, Sterner and Elser (2002) acknowledge that there are problems in attempting to do so, noting that the lack of agreement of the GRH in many photosynthetic organisms, which they believe could result from the very large RUBISCO content (see Vrede et al. 2004).

From a real-world application view point (e.g., the suggestion of Worden and Binder 2003), there is an important facet to the GRH that requires careful consideration. Since fluctuations in rRNA will occur in response to the synthesis of stress proteins, and organisms in nature are confronted with changing stress patterns daily, if not hourly or more frequently, the GRH would need to be very robust to be accepted for use in most ecological applications. If it does not yield a clear prediction for growth rate, then arguably it should be rejected as the basis for a methodology for routine use. If it is not robust, then there is also a real risk that modellers and theoreticians will grasp it and use it in ways that are unsafe. For photoautotrophs, there is no such clear expression of the GRH. Accordingly, the GRH must be rejected for these organisms, and hence as a general hypothesis for biological processes.

This work was part of the Allometry and Stoichiometry in Aquatic Ecosystems Working Group 33, supported by the Australian Research Council New Zealand (ARC-NZ) Research Network on Vegetation Function. Contributions from Drew Allen, Jason Bragg, and Anthony Richardson are acknowledged gratefully. The University of Dundee is a registered Scottish charity, No: SC015096.

Aboulmagd, E., Oppermann-Sanio, F. B. & Steinbücherl, A. 2001. Purification of *Synechocystis* sp. strain PCC6308 cyanophycin synthetase and its characterization with respect to substrate and primer specificity. *Appl. Environ. Microbiol.* 67:2176–82.

Ågren, G. I. 2004. The C:N:P stoichiometry of autotrophs – theory and observations. *Ecol. Lett.* 7:185–91.

Alberghina, F. A. M., Sturani, E. & Gohlke, J. R. 1975. Levels and rates of synthesis of ribosomal ribonucleic acid, transfer ribonucleic acid, and protein in *Neurospora crassa* in different steady states of growth. *J. Biol. Chem.* 250:4381–8.

Allen, A. P. & Gillooly, J. F. 2009. Towards an integration of ecological stoichiometry and the metabolic theory of ecology to better understand nutrient cycling. *Ecol. Lett.* 12:369–84.

Barbarino, E. & Lourenço, S. O. 2005. An evaluation of methods for extraction and quantification of protein from marine macro- and microalgae. *J. Appl. Phycol.* 17:447–60.

Beardall, J., Roberts, S. & Millhouse, J. 1991. Effects of nitrogen limitation on inorganic carbon uptake and specific activity of ribulose-1.5-P_2 carboxylase in green microalgae. *Can. J. Bot.* 69:1146–50.

Berges, J. A., Fisher, A. E. & Harrison, P. J. 1993. A comparison of Lowry, Bradford and Smith protein assays using different protein standards and protein isolated from the diatom *Thalassiosira pseudonana. Mar. Biol.* 115:187–93.

Binder, B. J. & Liu, Y. C. 1998. Growth rate regulation of rRNA content of a marine *Synechococcus* (Cyanobacterium) strain. *Appl. Environ. Microbiol.* 64:3346–51.

Caldwell, P. C., MacKor, E. L. & Hinshelwood, C. 1950. The ribose nucleic acid content and cell growth of *Bact. lactis aerogenes. J. Chem. Soc.* 1950:993–1003.

Calow, P. [Ed.] 1999. *Blackwell's Concise Encyclopaedia of Ecology.* Blackwell Science, Oxford, UK, 152 pp.

Clayton, J. R., Dortch, Q., Thoresen, S. S. & Ahmed, S. I. 1988. Evaluation of methods for the separation and analysis of proteins and free amino acids in phytoplankton samples. *J. Plankton Res.* 10:341–58.

Cook, J. R. 1963. Adaptations in growth and division in *Euglena* effected by energy supply. *J. Protozool.* 10:436–44.

Crossman, D. J., Clements, K. D. & Cooper, G. J. S. 2000. Determination of proteins for studies of marine herbivory: a comparison of methods. *J. Exp. Mar. Biol. Ecol.* 244:45–65.

Dische, Z., Devi, A. & Zelminis, G. 1961. Influence of age and cataract formation on the ribonucleic acid of the lens. *Am. J. Ophthalmol.* 51:993–1003.

Dortch, Q., Clayton Jr., J. R., Thoresen, S. S. & Ahmed, S. I. 1984. Species differences in accumulation of nitrogen pools in phytoplankton. *Mar. Biol.* 81:237–50.

Dortch, Q., Clayton Jr., J. R., Thoresen, S. S., Cleveland, J. S., Bressler, S. L. & Ahmed, S. I. 1985. Nitrogen storage and use of biochemical indices to assess nitrogen deficiency and growth rate in natural plankton populations. *J. Mar. Res.* 43:437–64.

Dortch, Q., Roberts, T. L., Clayton Jr., J. R. & Ahmed, S. I. 1983. RNA/DNA ratio and DNA concentration as indicators of growth rate and biomass in planktonic marine organisms. *Mar. Ecol. Prog. Ser.* 13:61–71.

Elrifi, I. R. & Turpin, D. H. 1985. Steady-state luxury consumption and the concept of nutrient ratios: a study with phosphate and nitrate limited *Selenastrum minutum* (Chlorophyta). *J. Phycol.* 21:592–602.

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

Elser, J. J., Bracken, M. E. S., Cleland, E. E., Gruner, D. S., Harpole, W. S., Hillebrand, H., Ngai, J. T., Seabloom, E. W., Shurin, J. B. & Smith, J. E. 2007. Global analysis of nitrogen and phosphorus limitation of primary production in freshwater, marine, and terrestrial ecosystems. *Ecol. Lett.* 10:1135–42.

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

Fara, A., Berdalet, E. & Arin, L. 1996. Determination of RNA and DNA concentrations in natural plankton samples using thiazole orange in combination with DNase and RNase digestions. *J. Phycol.* 32:1074–83.

Finkel, Z. V., Raven, J. A., Reinfelder, J. R., Schofield, O. E. & Falkowski, P. G. 2006. Irradiance and elemental stoichiometry of marine phytoplankton. *Limnol. Oceanogr.* 51:2690–701.

Flynn, K. J. 1990. The determination of nitrogen status in microalgae. *Mar. Ecol. Prog. Ser.* 61:297–307.

Flynn, K. J. 2002. How critical is the critical N:P ratio? *J. Phycol.* 38:961–70.

Flynn, K. J. 2006. Mechanistic models of algal physiology. *In* Subba Rao, D. V. [Ed.] *Algal Cultures, Analogues of Blooms and Applications.* Science Publishers, Enfield, New Hampshire, pp. 501–32.

Flynn, K. J. 2008. The use, abuse, misconceptions and insights from quota model 40 years on. *Oceanogr. Mar. Biol. Annu. Rev.* 46:1–23.

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

Gillooly, J. F., Allen, A. P., Brown, J. H., Elser, J. J., Martinez del Rio, C., Savage, V. M., West, G. B., Woodruff, W. H. & Woods, H. A. 2005. The metabolic basis of whole-organism RNA and phosphorus content. *Proc. Natl. Acad. Sci. U. S. A.* 102:11923–7.

Goldman, J. C., McCarthy, J. J. & Peavey, D. G. 1979. Growth rate influence on the chemical composition of phytoplankton in oceanic waters. *Nature* 279:210–5.

Gusewell, S. 2004. N:P ratios in terrestrial plants: variation and functional significance. *New Phytol.* 164:243–66.

Healey, F. P. & Hendzel, L. L. 1975. Effect of phosphorus deficiency on two algae growing in chemostats. *J. Phycol.* 11:303–9.

Heldal, M., Scanlan, D. J., Noroland, S., Thingstad, F. & Mann, N. H. 2003. Elemental composition of single cells of various trains of marine *Prochlorococcus* and *Synechococcus* using X-ray microanalysis. *Limnol. Oceanogr.* 48:1732–43.

Ho, T.-Y., Quigg, A., Finkel, Z. V., Milligan, A. J., Wyman, K., Falkowski, P. G. & Morel, F. M. M. 2003. The elemental composition of some marine phytoplankton. *J. Phycol.* 39: 1145–59.

Howarth, R. W. & Marino, R. 2006. Nitrogen as the limiting nutrient for eutrophication in coastal marine ecosystems: evolving views over three decades. *Limnol. Oceanogr.* 51:364–76.

Jeener, R. 1953. Ribonucleic acid and protein synthesis in continuous cultures of *Polytomella caeca*. *Arch. Biochem. Biophys.* 43:381–8.

Jenks, A. & Gibbs, S. P. 2000. Immunolocalization and distribution of Form II Rubisco in the pyrenoid and chloroplast stroma of *Amphidinium carterae* and Form I Rubisco in the symbiont-derived plastids of *Peridinium foliaceum* (Dinophyceae). *J. Phycol.* 36:127–38.

Karpinets, T. V., Greenwood, D. J., Sams, C. E. & Ammons, J. T. 2006. RNA:protein ratio of the unicellular organisms as a characteristic of phosphorus and nitrogen stoichiometry and the cellular requirements of ribosomes for protein synthesis. *BMC Biol.* 4:30. doi: 10.1196/1741-7007-4-30.

Klausmeier, C. A., Litchman, E., Daufresne, T. & Levin, S. A. 2004. Optimum nitrogen-to-phosphorus stoichiometry of phytoplankton. *Nature* 429:171–4.

Knecht, M. R. & Goransson, A. 2004. Terrestrial plants require nutrients in similar proportions. *Tree Physiol.* 24:447–60.

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

Larson, T. R. & Rees, T. A. V. 1994. Arrest of cell division but not protein synthesis in sodium-deficient cells of the marine diatom *Phaeodactylum tricornutum* Bohlin. *Planta* 195:195–200.

Laws, E. A., Karl, D. M., Redalje, D. G., Jurick, R. S. & Winn, C. D. 1983. Variability in ratios of phytoplankton carbon and RNA to ATP and chlorophyll a in batch and continuous cultures. *J. Phycol.* 19:439–45.

Lepp, P. W. & Schmidt, T. M. 1998. Nucleic acid content of *Synechococcus* spp. during growth in continuous light and light-dark cycles. *Arch. Microbiol.* 170:201–7.

Lin, S. & Carpenter, E. J. 1997. Rubisco of *Dunaliella tertiolecta* is redistributed between the pyrenoid and the stroma as a light/shade response. *Mar. Biol.* 127:521–9.

Lourenço, S. O., Barbarino, E., Lanfer Marquez, U. M. & Aidar, E. 1998. Distribution of intracellular nitrogen in marine microalgae: basis for the calculation of specific nitrogen-to-protein conversion factors. *J. Phycol.* 34:798–811.

Lourenço, S. O., Barbarino, E., Lavin, P. L., Marque, U. M. L. & Aidar, E. 2004. Distribution of intracellular nitrogen in marine microalgae. Calculation on new nitrogen-to-protein conversion factors. *Eur. J. Phycol.* 39:17–32.

Lourenço, S. O., Barbarino, E., Mancini-Filho, K., Schinke, K. P. & Aidar, E. 2002. Effects of different nitrogen sources on the growth and biochemical profile of 10 marine microalgae in batch culture: an evaluation of aquaculture. *Phycologia* 41:158–68.

MacIntyre, H. L., Kana, T. M., Anning, T. & Geider, R. J. 2002. Photoacclimation of photosynthetic irradiance in response curves and photosynthetic pigments in microalgae and cyanobacteria. *J. Phycol.* 38:17–38.

Mann, N. & Carr, N. G. 1974. Control of macromolecular composition and cell division in the blue-green alga *Anacystis nidulans*. *J. Gen. Microbiol.* 83:399–405.

Nieuwenhuysen, P., de Voeght, F. & Clauwert, J. 1978. Ribonucleic acid and protein content of eukaryotic ribosomes isolated from *Artemia salina*. *Anal. Biochem.* 88:532–8.

Nieuwenhuysen, P., de Voeght, F. & Clauwert, J. 1981. The molecular weight of *Artemia* ribosomes, as determined from their refractive index increment and light-scattering intensity. *Biochem. J.* 197:689–93.

Niklas, K. J. 2006. Plant allometry, leaf nitrogen and phosphorus stoichiometry, and interspecific trends in annual growth rates. *Ann. Bot.* 97:155–63.

Orlowski, M. 1981. Growth-rate-dependent adjustment of ribosome function in the fungus *Mucor racemosus*. *Biochem. J.* 196:403–10.

Parrott, L. M. & Slater, J. H. 1980. The DNA, RNA protein composition of the cyanobacterium *Anacystis nidulans* grown in light- and carbon dioxide-limited chemostats. *Arch. Microbiol.* 127:53–8.

Poynton, R. O. 1973. Effect of growth rate on the macromolecular composition of *Prototheca zopfii*, a colorless alga which divides by multiple fission. *J. Bacteriol.* 113:203–11.

Quigg, A. & Beardall, J. 2003. Protein turnover in relation to maintenance metabolism at low photon flux in two marine microalgae. *Plant Cell Environ.* 26:693–703.

Quigg, A., Finkel, Z. V., Irwin, A. J., Rosenthal, Y., Ho, T.-Y., Reinfelder, J. R., Schofield, O., Taylor, F. J. R. & Falkowski, P. G. 2003. The evolutionary inheritance of elemental stoichiometry in marine phytoplankton. *Nature* 425:291–4.

Quigg, A., Kevekordes, K., Raven, J. A. & Beardall, J. 2006. Limitations on microalgal growth at very low photon flux densities: the role of energy slippage and H^+ leakage. *Photosynth. Res.* 88:299–310.

Raven, J. A. 1984. A cost-benefit analysis of photon absorption by photosynthetic unicells. *New Phytol.* 98:593–625.

Raven, J. A. 1988. The iron and molybdenum use efficiencies of growth with different energy, carbon and nitrogen sources. *New Phytol.* 109:279–87.

Raven, J. A. 1989. Fight or flight: the economics of repair and avoidance of photoinhibition of photosynthesis. *Funct. Ecol.* 3:5–19.

Raven, J. A. 1990. Predictions of Mn and Fe use efficiencies of phototrophic growth as a function of light availability for growth and C assimilation pathway. *New Phytol.* 116:1–18.

Raven, J. A., Evans, M. C. W. & Korb, R. E. 1999. The role of trace metals in photosynthetic electron transport in O_2-evolving organisms. *Photosynth. Res.* 60:111–49.

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

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

Richardson, K., Beardall, J. & Raven, J. A. 1983. Adaptation of unicellular algae to irradiance: an analysis of strategies. *New Phytol.* 93:157–91.

Rodriguez-Correa, D. & Dahlberg, A. E. 2008. Kinetic and thermodynamic studies of peptidyltransferase in ribosomes from the extreme thermophile *Thermus thermophilus*. *RNA* 14: 2314–8.

Scheurwater, I., Dünnebacke, M., Eising, R. & Lamber, H. 2000. Respiratory costs and rate of protein turnover in the roots of fast-growing (*Dactylis glomerata* L.) and a slow-growing (*Festuca ovina* L.) grass species. *J. Exp. Bot.* 51:1089–97.

Schlesinger, D. A. & Shuter, B. J. 1981. Patterns of growth and cell composition of freshwater algae in light-limited continuous cultures. *J. Phycol.* 17:250–6.

Sterner, R. W. & Elser, J. J. 2002. *Ecological Stoichiometry. The Biology of the Elements from Molecules to the Biosphere*. Princeton University Press, Princeton, New Jersey, 466 pp.

Sturani, E., Magnami, F. & Alberghina, F. A. 1973. Inhibition of ribosomal-RNA synthesis during a shift-down of growth in *Neurospora crassa*. *Biochim. Biophys. Acta* 319:165–273.

Sukenik, A., Bennett, J., Mortain-Bertrand, A. & Falkowski, P. G. 1989. Adaptation of the photosynthetic apparatus to irradiance in *Dunaliella tertiolecta*. *Plant Physiol.* 92:891–8.

Sutcliffe Jr., W. H. 1965. Growth estimates from ribonucleic acid content in some small organisms. *Limnol. Oceanogr.* 10S:R253–8.

Thomas, P. H. & Carr, N. G. 1985. The invariance of macromolecular composition with altered light limited growth-rate of *Amphidinium carteri* (Dinophyceae). *Arch. Microbiol.* 142:81–6.

Twining, B. S., Baines, S. B., Fisher, N. S., Maser, J., Vogt, S., Jacobsen, C., Tovar-Sanchez, A. & Sañudo-Wilhelmy, S. 2003. Quantifying trace elements in individual aquatic protist cells with a synchrotron x-ray fluorescence microprobe. *Anal. Chem.* 75:3806–16.

Utkilen, H. C. 1982. Magnesium-limited growth of the cyanobacterium *Anacystis nidulans*. *J. Gen. Microbiol.* 128:1849–62.

Vasi, F., Travisano, M. & Lenski, R. E. 1994. Long-term evolution in *Escherichia coli*. II. Changes in life-history traits during adaptation to a seasonal environment. *Am. Nat.* 144:432–56.

Vrede, T., Dobberfuhl, D. R., Kooijman, S. A. L. M. & Elser, J. J. 2004. Fundamental connections among organism C:N:P stoichiometry, macromolecular composition, and growth. *Ecology* 85:1217–29.

Worden, A. Z. & Binder, B. J. 2003. Growth regulation of rRNA content in *Prochlorococcus* and *Synechococcus* populations in oligotrophic environment. *J. Phycol.* 39:527–34.