Ecology, 85(5), 2004, pp. 1217–1229

FUNDAMENTAL CONNECTIONS AMONG ORGANISM C:N:P STOICHIOMETRY, MACROMOLECULAR COMPOSITION, AND GROWTH

TOBIAS VREDE,[1,5] DEAN R. DOBBERFUHL,[2,6] S. A. L. M. KOOIJMAN,[3] AND JAMES J. ELSER[2]

[1]*Department of Limnology, Evolutionary Biology Centre, Uppsala University, Norbyvägen 20, SE-752 36 Uppsala, Sweden*
[2]*Department of Biology, Arizona State University, Tempe, Arizona 85287 USA*
[3]*Department of Theoretical Biology, Institute of Ecological Sciences, Vrije Universiteit, de Boelelaan 1087, 1081 HV Amsterdam, The Netherlands*

Abstract. Whereas it is acknowledged that the C:N:P stoichiometry of consumers and their resources affects both the structure and the function of food webs, and eventually influences broad-scale processes such as global carbon cycles, the mechanistic basis for the variation in stoichiometry has not yet been fully explored. Empirical evidence shows that the specific growth rate is positively related to RNA concentration both between and within taxa in both unicellular and multicellular organisms. Since RNA is rich in P and constitutes a substantial part of the total P in organisms, a high growth rate is also connected with a high P content. We argue that the reason for this pattern is that the growth of all biota is closely linked with their protein synthesis rate, and thus with the concentration of ribosomal RNA. Dynamic energy budget theory supports the positive relationship between RNA and specific growth rate in microorganisms, whereas the predictions concerning multicellulars only partially agrees with the observed pattern. In a simple model of consumer growth, we explore the consequences of various allocation patterns of RNA, protein, carbohydrates/lipids, and other biochemical constituents on organism potential growth rate and C:N:P stoichiometry. According to the model the percentage of N and especially percentage of P per dry mass increases with increasing specific growth rate. Furthermore, the model suggests that macromolecule allocation patterns and thus N:P stoichiometry are allowed to differ substantially at low growth rates whereas the stoichiometry at high growth rates is much more constricted at low N:P. The model fits empirical data reasonably well, but it is also acknowledged that complex life cycles and associated physiological constraints may result in other patterns. We also use a similar approach of modeling organism growth from basic biochemical principles to illustrate fundamental connections among biochemical allocation and C:N stoichiometry in autotroph production, which is based on allocation patterns between carbohydrates and rubisco. Similar to the RNA–protein model, macromolecular composition and C:N ratios are more constrained at high than at low growth rates. The models and the empirical data together suggest that organism growth is tightly linked with the organisms' biochemical and elemental composition. The stoichiometry of growth impinges on nutrient cycles and carbon fluxes at the ecosystem level. Thus, focus on the biological basis of organism C:N:P stoichiometry can mechanistically connect growth strategy and biochemical and cellular mechanisms of biota to major ecological consequences.

Key words: autotroph production; C:N:P stoichiometry; consumer growth rate; dynamic energy budget theory; macromolecular allocation patterns; protein allocation; ribosomal RNA; rubisco.

INTRODUCTION

It is now well established that stoichiometric constraints are important in regulating organism growth and nutrient cycling in terrestrial as well as in aquatic food webs (Sterner and Elser 2002). In particular nitrogen (N) and phosphorus (P) have been considered important in this context because these elements are both structurally and functionally important in all organisms (Sterner 1995, Elser et al. 1996), and either N or P (or both) often limit primary production (Schindler 1977, Elser et al. 1990, Vitousek and Howarth 1991), bacterial production (Hessen et al. 1994, Elser et al. 1995, K. Vrede et al. 1999), and consumer growth (Gulati and DeMott 1997, Elser et al. 2000*a*, Sterner and Schulz 1998). Existing data indicate that autotrophs (algae and vascular plants) exhibit a wide variation in C:N:P ratios (Elser et al. 2000*a*, Nielsen et al. 1996, Hessen et al. 2004), and that much of this variation occurs within individual taxa as a response to environmental conditions (physiological plasticity). For example, it is well known that differences in the supply of P to phytoplankton result in widely differing internal P cell quota (Droop 1974, Andersen 1997). In contrast,

Manuscript received 26 April 2002; revised 4 June 2003; accepted 20 July 2003; final version received 26 August 2003. Corresponding Editor: R. M. Nisbet. For reprints of this Special Feature, see footnote 1, p. 1177.

[5] E-mail: tobias.vrede@ebc.uu.se

[6] Present address: St. Johns River Water Management District, Palatka, Florida, USA.

metazoans show muted intra-specific variation (physiological homeostasis) but can differ substantially in C:N:P stoichiometry across taxa, major life stages, and sex (Andersen and Hessen 1991, Hessen and Lyche 1991, Markow et al. 1999, Sterner and George 2000). The small variation in consumer stoichiometry relative to that of the prey, and potentially large elemental imbalances between producers and consumers, have implications both for the growth of the consumer and for nutrient recycling in food webs (Sterner and Hessen 1994, Elser and Urabe 1999). Consumers experiencing food with C:P or C:N ratios higher than what they demand will thus have low growth efficiencies for C, which eventually will lead to low consumer growth rate and biomass, and consequently also low grazing pressure on the autotrophs. High autotroph C:P or C:N ratios are therefore associated with slow turnover of primary producers and only a small percentage of the net primary production will be consumed by grazers in such systems (Cebrian et al. 1998, Cebrian 1999). Likewise, the N and P contents of detritus from terrestrial and aquatic autotrophs are positively related with the detrital decomposition rate (Enriquez et al. 1993), indicating that the substrate quality (in terms of C:N:P) affects the activity of decomposers. The C:N:P stoichiometry of consumers and their resources thus affects both the structure and the function of food webs, and will eventually influence broad-scale processes such as global carbon cycles (Hessen et al. 2004).

However, the mechanistic basis for the variation in stoichiometry of biota has not yet been fully explored (Falkowski et al. 2000). Therefore, a number of questions need to be considered: What are C, N, and P used for in organisms? Why does C:N:P stoichiometry differ among organisms? What relationships are there between important life-history traits such as maximum growth rate and C:N:P stoichiometry? What connections are there between biochemical composition, C:N:P stoichiometry, and growth of organisms? To address these questions, we present some empirical data as well as theoretical considerations regarding the relationship between RNA content and growth rate, and two simple models that relate the stoichiometry of organisms to their biochemical composition and growth rate. Our main assertion is that all biota share a common core of cellular–molecular machinery required for growth, and that this machinery imposes inexorable constraints on the organisms' elemental composition. Stoichiometric imbalances between resources and consumers cause the growth rate to decline because organisms have fundamental elemental and biochemical demands for building their biosynthesis machinery. Consequently, the stoichiometry of production impinges on the performance of various individuals and populations of diverse species as well as on large-scale nutrient and carbon cycles.

Growth Rate, RNA, and C:N:P Stoichiometry

It has been proposed that variation in C:N:P stoichiometry in invertebrate metazoa is driven by differences in allocation to P-rich ribosomal RNA (rRNA) to meet the protein-synthesis demands associated with differences in characteristic specific growth rates of particular taxa and/or life stages (Hessen and Lyche 1991, Elser et al. 1996, 2000*c*). We'll refer to this set of ideas as the ''growth-rate hypothesis'' (GRH). Two important assumptions of the GRH are (1) that there is a positive relationship between rRNA concentration and specific growth rate, and (2) that the P in rRNA makes up a significant fraction of the total P in nonvertebrate organisms. In order to assess the validity of the GRH we need to consider these assumptions.

Ribosomes are the structures where both structural and enzymatic proteins are synthesized, and since proteins are the most abundant macromolecules in both prokaryotes and eukaryotes (Alberts et al. 1983), the ribosomes constitute the core of the biosynthesis machinery in all cells. Ribosomes consist mainly of rRNA but also of structural proteins and are organized in a small sub-unit (30S or 40S), which decodes the genetic instructions brought to the ribosome by messenger RNA, and a large sub-unit (50S or 60S), which catalyzes the formation of peptide bonds between amino acids (Lafontaine and Tollervey 2001 [sub-units are classified and named after their size, as reflected by their sedimentation coefficient; ''S'' is a Svedberg unit, and 1S is equivalent to 1×10^{-13} sec]). Since protein synthesis rate to a large extent depends on the number of ribosomes rather than their efficiency (Nomura et al. 1984), a high growth rate should be closely related to the protein production rate and to the amount of rRNA in a cell. Since rRNA makes up approximately 75–80% of the total RNA in most cells (Brandhorst and McConkey 1974, Campana and Schwartz 1981), differences in cellular rRNA concentrations, and therefore also in the number of ribosomes per cell and protein synthesis capacity, should influence the total RNA concentration. Indeed, when data on growth rate are plotted as a function of total RNA per dry mass (DM) of organisms covering a wide range of diversity from bacteria to invertebrate metazoans, a positive interspecific relationship between RNA content and growth rate is obvious (Fig. 1A). Furthermore, in groups for which there are data on several species, e.g., insects, crustaceans, eukaryotic microorganisms, and bacteria, there is also a positive relationship between RNA concentration and growth rate within each group (Fig. 1A). RNA content varies with growth rate also on the intraspecific level, as exemplified by the cockroach (*Blatella*), the diatom *Thalassiosira*, and the bacterium *Escherichia coli* (Fig. 1B). In addition to the data shown in Fig. 1, similar relationships between RNA:DM, RNA:protein, or RNA:DNA with either growth rate or egg production rate have also been observed in

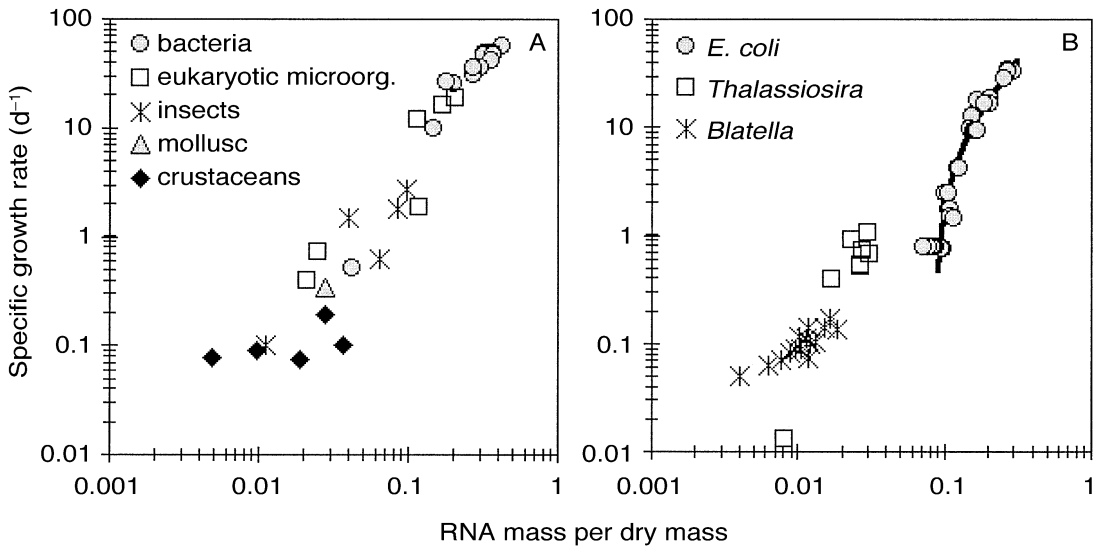

FIG. 1. Relationships between specific growth rate and RNA content (mass:mass ratio) of bacteria, eukaryotic microorganisms, and invertebrates (log–log scale). (A) Mean growth rate and RNA content for each species. (B) Growth rate and RNA content of three selected species. The line fitted to the *Escherichia coli* data is based on the DEB (dynamic energy budget) model. Data are from Leick (1968) (protein is assumed to be 60% of dry mass [Alberts et al. 1983]), Koch (1970), Sutcliffe (1970 [and references therein]), Dagg and Littlepage (1972), Healey and Hendzel (1975), Dortch (1983), and T. Vrede (*unpublished data*).

several other studies, including bacteria (Kato 1994), crustaceans (McKee and Knowles 1987, Saiz et al. 1998, Wagner et al. 1998, 2001), fish (Buckley 1984, Mathers et al. 1993), and mammals (Sutcliffe 1970). Although it seems safe to conclude that fast-growing organisms generally have a higher rRNA content than slow-growing organisms, there remains some scatter in the RNA vs. growth rate relationship. This scatter can at least partly be a result of differences in temperature between the experiments since protein synthesis is a temperature-dependent process, which is reflected by increasing growth rate with increasing temperature at the same RNA content (Buckley 1984, Saiz et al. 1998, Wagner et al. 2001).

The second key component of the GRH is that phosphorus in RNA represents a major component of total biomass P in the organism. RNA contains ~10% P by mass (depending to some extent on the proportion of C-G and A-U base pairs), which is high compared to most other biomolecules (Elser et al. 1996). Since RNA is one of the most abundant and P-rich biomolecules, a large P allocation to RNA is expected, particularly in organisms with high growth rate (Elser et al. 1996). As an extreme example, RNA can constitute as much as 30% of the dry mass in *E. coli* (Fig. 1B; Koch 1970), which translates into 3% P per dry mass in RNA alone. The P:DM ratio of rapidly growing *E. coli* has been reported to be 4.4% (Fagerbakke et al. 1996), which indicates that RNA actually is a major P fraction in this bacterium. Similarly, nucleic acids constitute the major pool of P in crustacean zooplankton species that have been examined (T. Vrede et al. 1999, Dobberfuhl 1999), and since RNA:DNA ratios of crustaceans often are much higher than 1 (Saiz et al. 1998, Wagner et al. 1998, Gorokhova and Kyle 2002, T. Vrede et al. 2002), P in RNA accounts for a significant fraction of the total P. Indeed, while the amount of data was limited, Dobberfuhl (1999) reported a positive relationship between RNA P content and total P content in some crustacean zooplankton taxa with a slope of ~1. A similar relationship between RNA P and total P content has been observed in the herbivorous insect *Sabinia setosa* (Schade et al. 2003). Together, these studies indicate that both intraspecific and interspecific variation in P content of animals can be explained by differences in allocation to RNA.

Thus, there is substantial empirical and theoretical support for the assumptions of the GRH regarding the positive relationship between rRNA concentration and specific growth rate, and that P in rRNA makes up a significant fraction of the total P in organisms. A high growth rate should thus be associated with a high P content. The positive interspecific relationship between growth rate and RNA concentration therefore suggests that important differences in life-history strategies should be reflected in organism P content. It is well known that bacteria, which in general have high growth rates, have very low C:P ratios, on average 50:1 (by atoms) for bacterioplankton (Fagerbakke et al. 1996) and 35:1 for exponentially growing non-nutrient-limited cultured marine bacteria (K. Vrede et al. 2002). These C:P ratios are substantially lower than those of more slowly growing organisms such as the cladoceran *Daphnia* (C:P 85:1) or the very slow-growing copepod

Acanthodiaptomus (C:P 212:1) (Andersen and Hessen 1991). In P-limited cultures of the cyanobacterium *Anabaena* and the green alga *Scenedesmus*, growth rate is correlated with both cellular RNA and P contents, and the increase in RNA can account for a large part of the increase in P (Healey and Hendzel 1975). There are also data indicating increased P content among and within cladoceran species with increasing growth rate (Main et al. 1997, DeMott et al. 1998, Elser et al. 2000*b*). In sum, the GRH offers a promising avenue for further inquiry that might connect cellular–genetic–biochemical mechanisms to ecological ramifications in diverse biota (Elser et al. 2000*c*). So, to better understand the fundamental nature of these connections we present two simple models of consumer and autotroph growth to examine the magnitude and nature of shifts in organism C:N:P ratios and growth rate that accompany major shifts in biochemical allocation patterns. But before doing so we will first take a closer look at the relationship between RNA content and specific growth rate of organisms within the more formal framework of dynamic energy budget (DEB) theory (Kooijman 2000).

The Relationship Between RNA and Specific Growth Rate in DEB Context

Whereas stoichiometric constraints on organism growth can be explicitly accounted for within the DEB (dynamic energy budget) theory (Kooijman et al. 2004), we will focus on the relationship between RNA and specific growth rate here. The simplest DEB model delineates two state variables in an organism: reserve and structure, which both are generalized compounds, i.e., mixtures of compounds that do not change in composition (the strong homeostasis assumption). The term ''reserve'' stands for compounds that will be used for metabolic purposes. Meanwhile, those compounds can have an active role in metabolism. The bulk of reserve and structure consists of lipids, carbohydrates, proteins, and nucleic acids, and any particular type of these compounds can belong to both reserve and structure. The only way to know the distribution between reserve and structure of each type of compound is to study changes in body composition as a function of growth rate, or during starvation. The higher the feeding rate, the larger the reserve density (i.e., the ratio of reserve and structure), the larger the growth rate. Thus, the observation that the RNA content increases with increasing specific growth rate (Fig. 1) means that the RNA content of reserve must exceed that of structure. The rate of RNA turnover in the reserve is completely determined by the reserve turnover rate. For isomorphs (i.e., organisms that do not change in shape during growth), this turnover rate is inversely proportional to the volumetric length of the structure (i.e., the cubic root of structure's volume), whereas for V1-morphs (i.e., organisms that change in shape during growth such that their structure's surface area is proportional to their volume) the reserve turnover rate is constant. The structure-specific maintenance is constant in the DEB model. Part of the maintenance is used for the turnover of compounds in the structure, while structure has a constant composition. This means that each compound in the structure can have its own turnover rate. Since the bulk of RNA both in reserve and in structure most likely is ribosomal, and it can be assumed that all ribosomes have the same turnover rate, it is natural to assume that the turnover rate of structure's RNA equals that of reserve's RNA. From the perspective of each RNA molecule it then does not matter whether it belongs to reserve or to structure. We are now ready to study how RNA content changes with specific growth rate in more detail, which in the context of DEB theory directly relates to the variation of reserve relative to structure.

If we compare individuals of the same species and with the same body size, but at different food (substrate) concentrations, we expect higher reserve densities and thus higher RNA contents at higher food concentrations. In multicellulars, the food intake (which is proportional to the surface area of the organism) must balance or exceed the maintenance cost (which is proportional to structure's volume). Many multicellulars retain their shape during growth (i.e., they are isomorphs), and thus their volume increases more rapidly than their surface area. Consequently, as they become bigger, they will eventually reach an asymptotic body size where food intake balances maintenance costs. This is the asymptotic body size, and it depends on the food concentration. If we select a body size smaller than the asymptotic one for the lowest food concentration, we expect to find specific growth rates that increase with the food concentration. Therefore we also expect that the RNA content increases with the specific growth rate when animals of the same species and size experience different food concentrations. Also in unicellulars there is an increase in RNA content along with increasing growth rate. The fraction of dry mass that is RNA can be modeled as

$$\frac{M_R}{M} = \frac{\theta_v M_V + \theta_e M_E}{M_V + M_E} = \frac{\theta_v + \theta_e f\omega_{Em}}{1 + f\omega_{Em}}$$

where the mass of RNA, M_R, is a fraction θ_v of the dry mass of structure M_V and a fraction θ_e of the dry mass of the reserve M_E; ω_{Em} is the maximum specific mass of the reserve, and f stands for the scaled functional response (which is 0 at no substrate and 1 at abundant substrate). Because unicellulars behave like V1-morphs almost irrespective of changes in shape during the cell cycle, it does not matter if we deal with a single individual or with many individuals (Kooijman 2000), and we can therefore fit the model to data on growth rate and RNA content on the population level. A least squares fit of the model to the data of Koch (1970) on *Escherichia coli* (Fig. 1B) indicate that $\theta_v = 0.084$,

$\theta_e = 0.61$, and $\omega_{Em} = 2.98$. Thus, RNA increases with specific growth rate, and most of the RNA is part of the reserves. In conclusion, within species of both multicellular and unicellular organisms, the RNA is expected to increase together with the specific growth rate with increasing food (substrate) concentration.

Another type of comparison is between individuals of the same multicellular species at the same food density, but at different body sizes. DEB theory delineates three basic life stages for multicellulars—embryo (which does not feed or reproduce), juvenile (which feeds, but does not reproduce), and adults. The reserve density, and therefore also the expected RNA content, is highest in the embryo stage, but constant in the juvenile and adult stages at constant food density. The specific growth rate, however, decreases with increasing size because a successively larger part of the reserves will be used for maintenance. Therefore we expect that RNA content is independent of the specific growth rate in juveniles and adults in this way of comparison. The empirical data for the cockroach *Blatella* (Fig. 1B), however, indicate an increase in RNA content with increasing specific growth rate. This may indicate that the strong homeostasis assumption is not valid, but may also be an effect of behavioral changes during growth, such that larger body sizes experience higher food densities, and therefore increase their feeding rate, reserves, RNA content, and specific growth rate.

When we compare different species of unicellulars grown at abundant food (substrate) density, DEB theory predicts that the specific growth rate increases with the unicellulars' reserve capacity. The RNA content is therefore expected to increase with the specific growth rate among species. The predicted positive relationship between specific growth rate and RNA content can be seen among both prokaryotic and eukaryotic microorganisms (Fig. 1A). At abundant food, the reserve density in multicellulars (which is constant in juveniles and adults of the same species) increases among species approximately with the volumetric length of the full-grown adult. The maximum specific growth rate, which is expected to occur at a body size of 4/27 of the maximum size, is proportional to the inverse of the volumetric length of the full-grown adult. If we assume that the composition of reserve is independent of maximum body size, and that RNA is more abundant in reserve than in structure, we expect a negative relationship between mass-specific RNA content and maximum specific growth rate among multicellular species. This covariation of parameter values among species, and their relationship with maximum body size, rests on the idea that maximum body size reflects the balance between the maximum food intake, which is coupled to surface area, and maintenance, which is coupled to volume. Although data are limited for this comparison, this may explain why the relationship between specific growth rate and RNA content apparently is weaker among multicellular species than among microorganisms (Fig. 1A).

In conclusion, DEB theory predicts that the RNA content of microrganisms increases with increasing specific growth rate both within and between species as well as with increasing food concentration. In multicellulars, on the other hand, we expect positive, negative, or no relationship between RNA content and specific growth rate, depending on what type of comparison we make.

A Model of Consumer Growth and C:N:P Stoichiometry

Another way of thinking about the relationship between RNA content and specific growth rate is to consider an organism's ribosomal RNA (rRNA) to be the protein-output machinery driving growth while the organism's protein biomass is the ''overhead'' that must be generated in order for the organism to grow at a balanced rate. Thus, the connections among growth and C:N:P stoichiometry will be mediated not only by RNA allocation but also by the organism's protein-allocation scheme. In such a scenario, increased protein allocation will tend to slow an organism's growth, as it represents a larger overhead that must be produced by the organism's rRNA catalytic capacity. In the following we summarize the formulation and predictions of a simple model of organism growth that uses basic information on relative allocation to major biochemical pools to predict organism growth and C:N:P stoichiometry from cellular ''first principles'' (Dobberfuhl 1999).

In the model, only pools of biomolecules that contain significant amounts of C, N, or P are considered; these include nucleic acids, proteins, chitin, adenylates (e.g., ATP [adenosine triphosphate]), phospholipids, triglycerides, and carbohydrates. These biomolecules comprise nearly all of the dry mass of cells so inclusion of any remaining biomolecules should not substantially alter calculated C, N, or P contents. DNA, chitin, adenylates, and phospholipids are fixed at characteristic values, as these pools are generally smaller and less variable than other N- or P-containing biomolecules (Elser et al. 1996, Sterner and Elser 2002). In contrast, protein and RNA contents are more variable and present in sufficient quantities that they could contribute most of the variation in N and P content within and among taxa (Båmstedt 1986, Sterner and Hessen 1994, Elser et al. 1996, Sterner and Elser 2002). Similarly, triglycerides and carbohydrates can vary widely as a function of ontogenetic stage and nutritional condition and can alter animal C content both within and among taxa (Elendt 1989, Sterner and Elser 2002). Therefore, each hypothetical organism contained basal allocations of DNA, chitin, adenylates, and phospholipids (all allocations are expressed as percentage of dry mass (DM)). Next, allocations of protein and RNA are assigned representing different life-history strategies so that protein varied from 30% to 70% (Skjoldal 1981,

TABLE 1. Symbols and parameter values used in the model of consumer growth.

Symbol	Model parameter	Value and unit	Reference
μ	Growth capacity	d^{-1}	
F	Fraction of total RNA allocated to rRNA	80%	Campana and Schwartz (1981)
α	Protein production rate per rRNA	3.3 μg protein·μg $rRNA^{-1}\cdot d^{-1}$ at 20°C	Sadava (1993)
M_r	Mass of RNA in individual ribosomes	4.53×10^{-12} μg rRNA $ribosome^{-1}$	Sadava (1993)
k_s	Protein synthesis rate	2.5×10^{-11} μg protein·$ribosome^{-1}\cdot d^{-1}$	Sadava (1993)
r	Protein retention efficiency	60%	Mathers et al. (1993)
t	Time	days	
G	Fractional allocation to lipids and carbohydrates	variable, ≥15% of DM	Elendt (1989)
R	Fractional allocation to RNA	variable, 1–20% of DM	McKee and Knowles (1987), Berberovic (1991)
T	Fractional allocation to protein	variable, 30–70% of DM	McKee and Knowles (1987), Berberovic (1991)
B	Fixed baseline fractional allocation to chitin, phospholipids, DNA, and adenylates	5% of DM chitin	Dittrich (1991), Jeuniaux and Voss-Foucart (1991), Mayzaud and Martin (1975)
		5% of DM phospholipids	Elendt (1989)
		1% of DM DNA	McKee and Knowles (1987), Berberovic (1991)
		0.75% of DM adenylates	Skjoldal (1981)
C_T	C content of protein	46% of DM	
C_R	C content of RNA	36% of DM	
C_B	C content of baseline chitin, phospholipids, DNA, and adenylates (biomass weighted average)	51% of DM	
C_G	C content of triglycerides (30%) and carbohydrates (70%) (biomass weighted average)	54.2% of DM	
N_T	N content of protein	17.2% of DM	Elser et al. (1996)
N_R	N content of RNA	15.5% of DM	Elser et al. (1996)
N_B	N content of baseline chitin, phospholipids, DNA, and adenylates (biomass weighted average)	6% of DM	Elser et al. (1996)
P_R	P content of RNA	9.2% of DM	Elser et al. (1996)
P_B	P content of baseline chitin, phospholipids, DNA, and adenylates (biomass weighted average)	4% of DM	Elser et al. (1996)

Note: DM = dry mass.

McKee and Knowles 1987, Berberovic 1991) while RNA independently varied from 1–20% (McKee and Knowles 1987). The rest of the organism's dry mass not allocated to the preceding biomolecules was allocated to a storage pool with a mixture of 30% triglycerides and 70% carbohydrates (Elendt 1989). This storage pool was not allowed to fall below 15% of DM, which is typical of well-fed daphnids (Elendt 1989). Thus, hypothetical organisms were constructed from the major biomolecules from which elemental contents (%C, %N, and %P per DM), N:P ratio, and growth capacity were calculated. For our purposes, ''growth capacity'' refers to an animal's maximum growth rate under resource-saturated conditions. Assuming balanced growth among all biochemical pools, growth capacity for various combinations of protein allocation T and RNA allocation R was calculated as

$$\mu = \ln[(T + \alpha FR) \times T^{-1}] \times t^{-1}$$

where μ is the specific growth rate of the protein pool, F is the fractional mass allocation of rRNA to total RNA, and t is time (parameter values appear in Table 1). The coefficient α in the equation above is given by

$$\alpha = k_s \times M_r^{-1} \times r$$

where k_s is the protein synthesis rate per ribosome, M_r is the mass of rRNA in individual ribosomes, and r is the protein retention efficiency (net protein biomass increment/total protein production). Elemental content for any allocation pattern of protein and RNA was calculated as

$$\%C = (T \times C_T) + (R \times C_R) + (B \times C_B) + (G \times C_G)$$

$$\%N = (T \times N_T) + (R \times N_R) + (B \times N_B)$$

$$\%P = (R \times P_R) + (B \times P_B)$$

where subscripts T, R, B, and G indicate fractional mass allocation of protein, RNA, fixed basal pools, and lipid/carbohydrate, respectively, and C, N, and P indicate

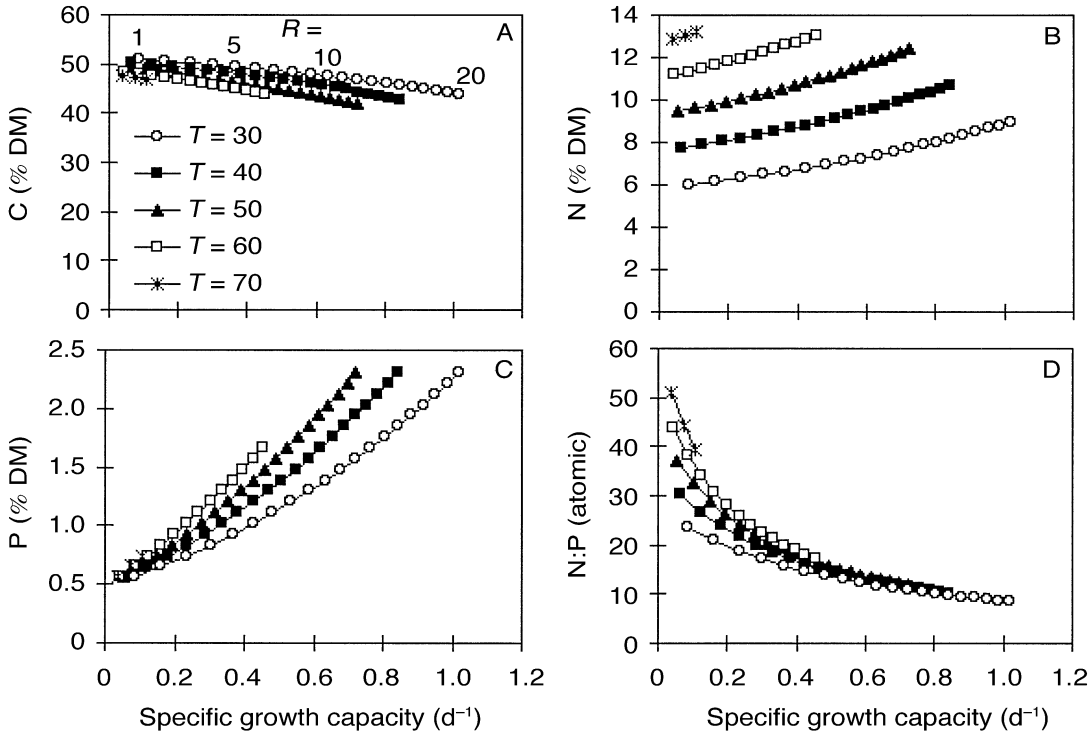

FIG. 2. Predictions of the RNA : protein model for (A) percentage of C per dry mass, (B) percentage of N per dry mass, (C) percentage of P per dry mass, and (D) body N:P ratio (atomic) as a function of specific growth capacity of a hypothetical metazoan consumer. The curves show the relationships between elemental composition and growth capacity at altered RNA allocations (R = 1–20% of dry mass) and at various fixed protein allocations (T = 30–70% of dry mass). The 60% and 70% protein curves are truncated since simultaneously high protein and RNA allocations would exceed 100% of dry mass.

the carbon, nitrogen and phosphorus content of these classes of molecules.

The model makes several predictions regarding how elemental contents (%C, %N, %P) should vary with changes in biochemical allocations associated with different life-history strategies. At a given protein allocation, %C will tend to decrease slightly as growth rate increases (Fig. 2A), though these changes are small relative to total body C content and may not be biologically meaningful. Similarly, N content at a fixed protein allocation is predicted to increase slightly with increasing growth rate (Fig. 2B), reflecting the similarity in % N of major biomolecules (protein and RNA) that vary in the model. Also note from this figure that increased protein allocation at a given level of RNA allocation lowers growth capacity. In contrast to predictions of small changes in C and N contents, the model predicts that %P should increase strongly with increasing growth capacity for all levels of protein allocation (Fig. 2C), reflecting the key role of P-rich RNA in the growth process. Therefore, body N:P ratio should decrease with increasing growth capacity (Fig. 2D). Furthermore, the model predicts that body N:P should not only be generally higher at low growth capacities, but also more variable. Thus, there appear to be many ways (biochemically and thus in terms of elemental composition) to grow slowly but only a limited set of ways to grow rapidly. These patterns tell us that there is a specific pattern of coupling of C, N, and P during secondary production. Specifically, the process of animal production becomes increasingly P intensive as the specific rate of production increases.

To test the predictions that as growth rate increases %N should increase slightly, %P should increase substantially, while the N:P ratio should decline, we used data from Main et al. (1997), Carillo et al. (2001) and DeMott (1998), who measured elemental contents and growth rates of crustacean zooplankton species. Model prediction curves were generated for organisms with 30% and 50% protein allocation, values bracketing literature values for cladoceran species (McKee and Knowles 1987, Berberovic 1991) and fixed basal allocation at values typical of crustaceans (Mayzaud and Martin 1975, Skjoldal and Båmstedt 1977, Skjoldal 1981, Elendt 1989, Dittrich 1991, Jeuniaux and Voss-Foucart 1991). These curves essentially form a prediction envelope within which the empirical data are expected to lie if the model is quantitatively accurate. It is apparent that the model predicts both growth rate and elemental composition for the cladocerans and the copepodites and adult copepods reasonably well (Fig. 3). The %N of the cladocerans and the copepodites and adult copepods increased slightly (Fig. 3A), while %P increased more strongly (Fig. 3B), with increasing

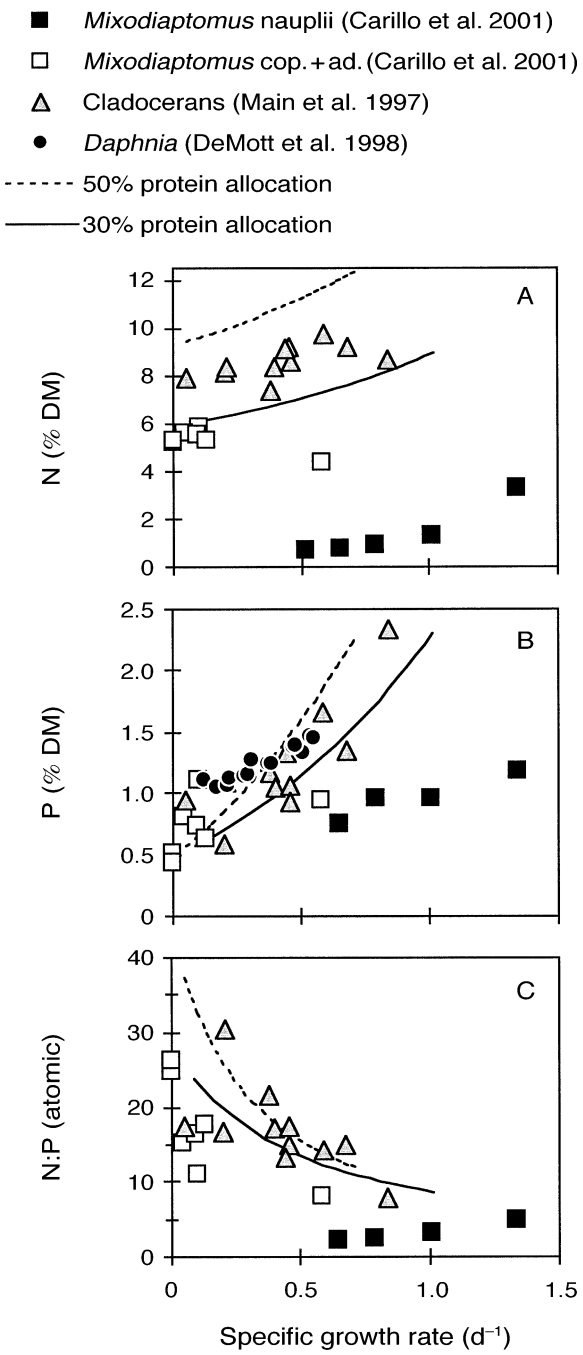

FIG. 3. Observed and predicted relationships between elemental composition and specific growth rate in crustacean zooplankton species: (A) percentage of N per dry mass in relation to specific growth rate; (B) percentage of P per dry mass in relation to specific growth rate; and (C) body N:P ratio (atomic) in relation to specific growth rate. Solid and dashed lines indicate predicted values of elemental composition and growth rate for 30% and 50% protein allocation, respectively.

growth rate. Body N:P ratio also followed the predicted relationship, decreasing and becoming less variable with increasing growth capacity (Fig. 3C). On the other hand, the model did not fit to the data on the copepod nauplii very well. Although both %P and in particular %N are lower than the model predictions, there is an increase in both parameters with increasing growth rate as predicted by the model, and the general trend across all life stages is a decreasing N:P ratio as predicted by the model. The low N and P content of the nauplii may be explained by the complex life cycle of the copepods, which includes the metamorphosis from nauplius larva to copepodite and that may have, as a consequence, significantly altered constraints on the biochemical composition. One of the assumptions in the model is that the growth is balanced, i.e., the macromolecular composition does not change over time. This assumption is not valid if the allocation to proteins and/or carbohydrates and lipids varies in a systematic way during the life cycle. It should also be noted that model predictions were not the result of calibration to values of any particular zooplankton species, suggesting that incorporating species- or stage-specific values for model parameters would increase the accuracy and predictive capacity of the model.

These results also suggest that hypothetical organisms can be assembled from biochemical building blocks to match and predict measured properties of real organisms. Starting with a small number of biomolecules containing C, N, and P, we constructed chemically and biologically realistic ''organisms'' that grew at biologically accurate rates and had elemental content consistent with real organisms. Note that the model should also hold for non-metazoans, such as bacteria, algae, and vascular plant tissue under conditions in which luxury uptake and storage of nutrient elements is not occurring. Thus, these results indicate that the relative allocations to protein and RNA are important parameters in determining the elemental content (especially %P) and growth capacity of an organism and therefore in influencing the role and success of an animal in an ecosystem context due to the influence of body elemental composition on trophic dynamics and nutrient recycling.

Note however that the model involves two assumptions about the kinetics of growth that may influence the degree to which such strong associations as we show are generally observed. First, the percentage of synthesized protein retained as new biomass, *r*, may vary (Hawkins et al. 1986, Sugden and Fuller 1991, McCarthy et al. 1994) and in fact such changes may be important in maintaining balanced growth in which protein biomass accrues at the same rate as other biochemical pools. Second, while the maximum rate of amino-acid polymerization, k_s, by a ribosome is a characteristic largely determined by the physical chemistry of ribosome and tRNA (transfer RNA) interactions, the realized rate of protein synthesis per ribosome may

vary for different species, nutritional conditions, ontogeny, or tissue types (Jensen and Pedersen 1990). This implies that if in fact mineral P shortages are a common constraint on rapid-growth-rate species (Sterner and Hessen 1994, Sterner and Elser 2002), natural selection might act to increase protein synthesis rate per ribosome (and thus per unit P required) to maintain a given growth rate while minimizing allocation to P-rich rRNA. Altering the amino-acid polymerization rate per ribosome can change growth capacity without affecting elemental contents. Thus, there may be some latitude to adjust growth capacity without changing elemental content. This could in part, explain some degree of the scatter seen in the relationships between growth rate and %P and N:P ratio (Fig. 3).

The C:N Stoichiometry of Autotroph Production—Rubisco and Carbohydrates

The RNA–protein model described above was designed to elucidate the coupling between growth and C:N:P stoichiometry in animals assuming that the primary biochemical pools of interest were rRNA and protein. The apparent success of this approach in illuminating the stoichiometric coupling of C, N, and P in metazoa suggests that a similar conceptual approach might be useful in understanding how growth and C:N:P are connected in autotrophs due to the core biochemistry associated with primary production. In the above example for metazoans, we considered rRNA as the ''productive machinery'' of growth and protein as the cellular overhead that must be produced to attain a certain growth rate. While C:N:P stoichiometry is also likely associated with the RNA-related mechanics of growth in autotrophs (Rhee 1973, Elrifi and Turpin 1985) as just mentioned, another important focus of analysis for autotrophic organisms would be on carbohydrate dynamics. In the following we adapt the approach just described to illuminate C:N stoichiometry in autotrophs. Now, the biomass ''overhead'' is carbohydrate allocation and the ''productive machinery'' is photosynthesis investment. To represent the latter, we will use allocation to ribulose bisphosphate carboxylase (rubisco), the enzyme that drives the dark reactions of photosynthesis. Rubisco represents a dominant pool of N in autotroph biomass and thus is perhaps the most abundant protein on Earth. For example, rubisco can comprise as much as 60% of leaf protein (Groot and Spiertz 1991) and can contain 75% of leaf N (Stocking and Ongun 1962). These contributions underlie the well-known association between autotroph photosynthesis capacity and biomass N content (Marshall and Porter 1991). Thus, rubisco N is considered to be a dominant but variable pool of N in an autotroph. In this analysis our goal is to consider how allocations to carbohydrates (as when terrestrial plants must allocate to cellulose for structural support) and to rubisco jointly affect potential growth of autotroph biomass and its C:N stoichiometry. Since biomass C:N is closely connected to ''nitrogen-use efficiency'' (Vitousek 1982), in doing so we can illustrate some of the biochemical mechanisms underlying a major determinant of N cycling in ecosystems.

At the outset we note that this approach is not likely to be strictly accurate, or least precisely analogous to the analysis performed above for rRNA and protein, as the carbohydrate output of photosynthesis is not deployed solely as carbohydrate or carbohydrate polymers. Instead, a considerable amount of the output must also be shunted into biosynthesis pathways for synthesis of other macromolecules, such as lipids, proteins, and nucleic acids. Thus, we would expect that the approach will overestimate growth rate relative to observed values. Nevertheless, the analysis may be heuristic in illustrating how major allocation patterns have ramifications for growth and elemental composition.

We constructed the model by first establishing a ''core biochemistry'' of autotroph biomass made up of reasonable mixtures of carbohydrate (30% of the core), protein (35%), nucleic acid (6%), ATP (0.75%), phospholipid (12%), and neutral lipid (16%). Thus, the ''core stoichiometry'' of autotroph biomass has a C:N:P of 113:14:1. As in the RNA–protein model described above, ''model autotrophs'' were then constructed having a given value of allocation to carbohydrate (ranging from 10 to 85%) in combination with an allocation to rubisco (ranging from 2.5 to 60%) (Groot and Spiertz 1991, Falkowski and Raven 1997). In no case was the core stoichiometry allowed to have a share of less than 5% of total biomass, and for carbohydrate–rubisco combinations for which their summed allocations were less than 95%, the remainder of the biomass was assumed to be comprised of biomass having the core stoichiometry described earlier. For each combination of allocations to carbohydrate, rubisco, and core stoichiometry, the C:N of the biomass was calculated based on the relative allocations and the known elemental combination of the biomolecules involved. Finally, the maximum specific growth rate of the carbohydrate pool was calculated following the strategy described above for the rRNA–protein model. To do so, allocations were converted to actual biomass values and the actual biomass of rubisco enzyme was calculated. Then, the gross rate of photosynthetic carbohydrate production was calculated using a known value for photosynthetic output of rubisco enzymes (60 mol $CO_2 \cdot mol^{-1}$ enzyme$\cdot s^{-1}$; Falkowski and Raven 1997), corrected for a respiratory loss of 30%, which is typical for respiration of recently fixed carbon (Ryle 1984). Potential specific growth rate (μ_p) of the carbohydrate biomass was calculated based on this rate of production relative to the total carbohydrate present:

$$\mu_p = \ln[(C_I + C_N) \cdot C_I^{-1}] \cdot t^{-1}$$

where C_I is the initial carbohydrate present (the variable allocation plus the carbohydrate in the ''core''), C_N is the net carbohydrate produced according to the partic-

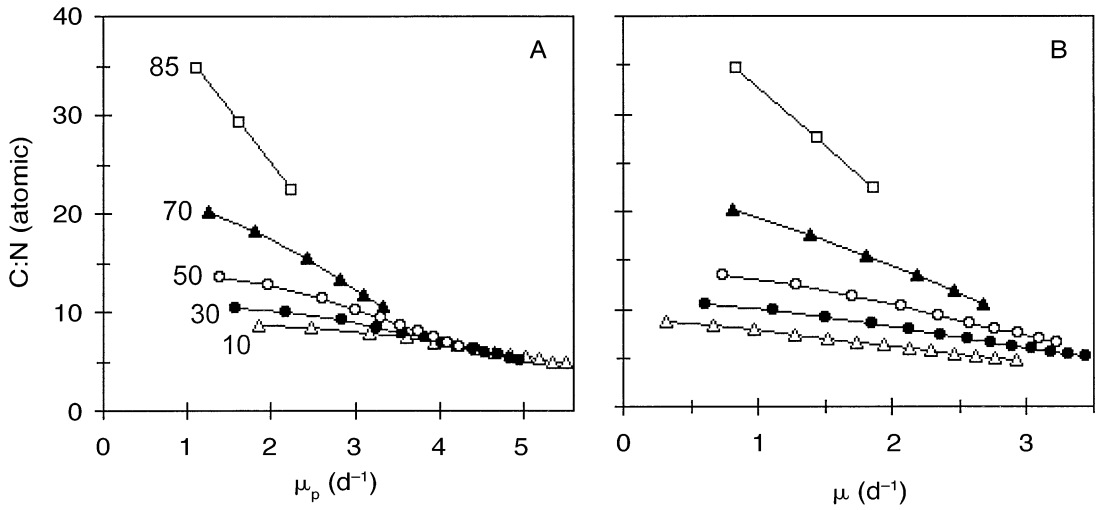

FIG. 4. Predictions of the carbohydrate:rubisco model about biomass C:N ratio (atomic) and specific growth rate in a hypothetical phototroph. Curves representing fixed allocation levels to carbohydrate (ranging from 10% to 85%) are shown. For a fixed carbohydrate allocation level, growth rate increases as rubisco allocation increases from 2.5% to 65%. Some curves are truncated because of the limitation that combined rubisco plus carbohydrate allocations could not exceed 95%. (A) Potential growth rate of the carbohydrate biomass (μ_p) assuming 30% respiratory losses. (B) Potential growth rate of the biomass (μ) assuming balanced growth (no change in relative allocation to different biomolecules) and respiratory loss of 50%.

ular rubisco allocation, and *t* is the time interval assumed. Once biomass C:N and growth rate were calculated for each feasible combination of carbohydrate and rubisco allocations, C:N was plotted against growth rate for different values of rubisco allocation holding carbohydrate allocation constant.

The model reveals trends similar to those resulting from the RNA–protein model discussed above, namely that biomass C:N ratio decreases (N content increases) with increasing growth rate (Fig. 4A). Rapidly growing biomass had uniformly low C:N ratio, which increased as growth rate declined for lower allocation values to rubisco. In general, the slope of the increase in C:N with decreasing growth was greater for high than low carbohydrate allocations, reflecting the fact that when rubisco and carbohydrate allocations were both low, the remainder of the biomass was attributed to the ''core,'' which had low C:N. Another instructive point emerging from Fig. 4A is that autotrophs with low total allocation to carbohydrate can exhibit a wide range in μ_p while autotrophs with major allocation to carbohydrate do not have such flexibility and have a relatively low maximum μ_p. The model also suggests that slowly growing autotroph biomass can have highly variable C:N, similar to the RNA–protein model in which slowly growing animals exhibited a wide range of N:P values (Fig. 2D).

Relative to observed variation in autotroph C:N and growth rate, the model is of mixed performance. Actual values of biomass C:N in freshwater seston (presumably algae dominated) in nature range from 5 to 20 with a mean of 10 while values for terrestrial foliage range from 5 to 90 with a mean of 36 (Elser et al. 2000*a*), values that are not unreasonable relative to predicted values shown in Fig. 4A. Thus, the assumed combination of biochemical allocations employed in the model seem appropriate in that they result in reasonable values of biomass C:N when their elemental contents are summed. In combination with the observations in Elser et al. (2000*a*), these calculations indicate that the average leaf in nature must not have an allocation to rubisco of more than ~10%, as the resulting C:N of such an allocation is 36 (assuming conservatively that all non-rubisco biomass is carbohydrate). Such knowledge may assist in constraining ecosystem models that couple C and N dynamics. However, as expected, the model considerably overestimates growth rate, as real growth rates of autotroph biomass generally range from 0.01 to 2.0 d^{-1} (Nielsen et al. 1996) but the model predicted values exceeding 5 d^{-1} for combinations with high rubisco and low carbohydrate allocations. As mentioned above this mismatch is not unexpected, given that μ_p is unlikely to be closely related to overall μ because recently fixed C must be allocated to pathways other than carbohydrate accumulation. This allocation to other pathways will also inevitably cause larger respiration losses. The 30% respiratory losses that were used in the model are typical for the short-term respiration of recently fixed C in higher plants, while the total respiratory losses may be 50% or even higher in the longer term (Ryle 1984). An alternative way of calculating growth rate and C:N stoichiometry is to use a respiratory loss of 50% at the same time as the constraint that the growth is balanced is included in the model (previously the increment in biomass consisted only of

carbohydrates). A growth rate calculated in that way should resemble the autotrophs' overall growth rate rather than the carbohydrate growth rate, and thus be more comparable to empirical data, although it still does not account for the changes in RNA concentrations (and %P) that should also be linked with growth rate. The resulting output of the model resembles the previous results, but with the difference that the calculated growth rates are substantially lower for any given C:N ratio (Fig. 4B). Even though the convergence at low C:N at high growth rates is less pronounced in the modified model, it is evident that there is room for only modest variation in C:N stoichiometry at high growth rates. Compared with the data of Nielsen et al. (1996), the modified model approaches the observed range in growth rates of autotrophs. Regardless of specific aspects related to the quantitative fit of the model to observed values, we argue that the most important message from the model is its qualitative predictions: generally decreasing C:N with increasing growth rate along with wide variation in C:N at low growth but tightly constrained C:N when specific growth rates are rapid.

Relevance of the C:N:P Stoichiometry of Production in Relation to Carbon Sequestration

The RNA–protein and rubisco–carbohydrate models above are admittedly simplistic with much cellular–molecular realism missing, and more sophisticated models connecting nutrient use to biochemical allocation, productivity, and growth do exist (e.g., Shuter 1979, Geider et al. 1998, Kooijman 2000, Hanegraaf and Muller 2001). However, we believe that the simplicity of the models presented above enhances their heuristic value. Together, these models suggest the existence of fundamental constraints on C:N:P stoichiometry and growth rate that are established by the allocation scheme of major biomolecules. For example, there seem to be many biochemical allocation patterns and stoichiometric relationships that allow slow growth, whereas there is only a limited set of stoichiometric and biochemical allocation patterns that permit rapid growth. Empirical evidence reviewed above, as well as the model predictions, show that strong interdependencies exist among organism growth strategy, biochemical investment, and biomass elemental composition. Thus, rapid growth requires that there is a good match between resource and consumer stoichiometry. In terms of C:P ratios in plants and herbivores, a close match is apparently not the general rule, neither in aquatic nor in terrestrial habitats, suggesting that stoichiometric constraints on herbivore growth are common (Elser et al. 2000*a*).

These interdependencies represent fundamental constraints on evolutionary and ecological dynamics driven by the biochemical requirements of the cellular machinery required for contrasting life strategies. One example of a situation where we suggest these constraints are important is when the nutrient availability for autotrophs is changed, due to either natural or anthropogenic environmental changes. Environmental nutrient supply affects the nutrient content and biochemical composition and growth rate of the autotrophs. Nutrient-limited autotrophs can thus produce new biomass with very high C:N and C:P ratios, albeit slowly. Due to the limited ability of herbivores to adjust their stoichiometry, there is a trade-off between growth rate and P demand in herbivores. Therefore, increasing autotroph C:nutrient ratios will likely result in herbivore species shifts from ''*r*-strategists'' with high P demand and high growth rates to ''*K*-strategists'' with low P demand and low growth rates, and the herbivore community will become characterized by low herbivore growth rates and biomass, and low community grazing rates. Also, higher trophic levels will be negatively affected by the inefficient transfer of organic matter across the autotroph–herbivore interface. In such a scenario, a large fraction of the primary production is expected to become especially nutrient-poor detritus. Furthermore, detritus with high C:nutrient ratios will be degraded slowly by microbes with their own potentially high nutrient demands associated with their protein-synthesis machinery, decelerating nutrient recycling rates and eventually affecting the C sequestration rate. Thus, the stoichiometric constraints on organism biochemical composition and growth reviewed above have important consequences also at large scales: they influence the structure and function throughout food webs and alter carbon fluxes in ecosystems or even globally (Cebrian 1999, Hessen et al. 2004). Focus on organism C:N:P stoichiometry can thus mechanistically connect growth strategy and biochemical and cellular mechanisms of biota to major ecological consequences. Success in making these connections may be closer than is generally believed. What will be required are innovative interactions among ecologists, evolutionary biologists, physiologists, and cell biologists. Furthermore, the models suggest inexorable connections between the very chemistry of life at the biochemical level and the fate of organic matter in the biosphere. A stoichiometric perspective can be a powerful tool for forging those connections.

Literature Cited

Alberts, B., D. Bray, J. Lewis, M. Raff, K. Roberts, and J. D. Watson. 1983. Molecular biology of the cell. Garland Publishing, New York, New York, USA.

Andersen, T. 1997. Pelagic nutrient cycles: herbivores as sources and sinks. Springer-Verlag, Berlin, Germany.

Andersen, T., and D. O. Hessen. 1991. Carbon, nitrogen, and phosphorus content of freshwater zooplankton. Limnology and Oceanography **36**:807–814.

Båmstedt, U. 1986. Chemical composition and energy content. Pages 1–58 *in* E. D. S. Corner and S. C. M. O'Hara, editors. The biological chemistry of marine copepods. Clarendon, Oxford, UK.

Berberovic, R. 1991. Biomass composition of two sympatric *Daphnia* species. Dissertation. University of Konstanz, Konstanz, Germany.
Brandhorst, B. P., and E. H. McConkey. 1974. Stability of nuclear RNA in mammalian cells. Journal of Molecular Biology **85**:451–463.
Buckley, L. 1984. RNA-DNA ratio: an index of larval fish growth in the sea. Marine Biology **80**:291–298.
Campana, T., and L. M. Schwartz. 1981. RNA and associated enzymes. Pages 877–944 *in* L. M. Schwartz and M. M. Azar, editors. Advanced cell biology. Van Nostrand Reinhold, New York, New York, USA.
Carrillo, P., M. Villar-Argaiz, and J. M. Medina-Sanchez. 2001. Relationship between N:P ratio and growth rate during the life cycle of calanoid copepods: an in situ measurement. Journal of Plankton Research **23**:537–547.
Cebrian, J. 1999. Patterns in the fate of production in plant communities. American Naturalist **154**:449–468.
Cebrian, J., M. Williams, J. McClelland, and I. Valiela. 1998. The dependence of heterotrophic consumption and C accumulation on autotrophic nutrient content in ecosystems. Ecology Letters **1**:165–170.
Dagg, M. J., and J. L. Littlepage. 1972. Relationship between growth rate and RNA, DNA, protein, and dry weight in *Artemia salina* and *Euchaeta elongata*. Marine Biology **17**: 162–170.
DeMott, W., R. D. Gulati, and K. Siewertsen. 1998. Effects of phosphorus-deficient diets on the carbon and phosphorus balance of *Daphnia magna*. Limnology and Oceanography **43**:1147–1161.
Dittrich, B. 1991. Biochemical composition of the parasitic amphipod *Hyperia galba* in relation to age and starvation. Journal of Comparative Physiology B, Biochemical, Systemic, and Environmental Physiology **161**:441–449.
Dobberfuhl, D. R. 1999. Elemental stoichiometry in crustacean zooplankton: phylogenetic patterns, physiological mechanisms, and ecological consequences. Dissertation. Arizona State University, Tempe, Arizona, USA.
Dortch, Q., T. L. Roberts, J. R. Clayton, Jr., and S. I. Ahmed. 1983. RNA/DNA ratios and DNA concentations as indicators of growth rate and biomass in planktonic marine organisms. Marine Ecology Progress Series **13**:61–71.
Droop, M. R. 1974. The nutrient status of algal cells in continuous culture. Journal of the Marine Biological Association of the United Kingdom **54**:825–855.
Elendt, B.-P. 1989. Effects of starvation on growth, reproduction, survival and biochemical composition of *Daphnia magna*. Archiv für Hydrobiologie **116**:415–433.
Elrifi, I. R., and D. H. Turpin. 1985. Steady-state luxury consumption and the concept of optimum nutrient ratios: a study with phosphate and nitrate limited *Selenastrum minutum* (Chlorophyta). Journal of Phycology **21**:592–602.
Elser, J. J., D. R. Dobberfuhl, N. A. Mackay, and J. H. Schampel. 1996. Organism size, life history, and N:P stoichiometry. BioScience **46**:674–684.
Elser, J. J., W. F. Fagan, R. F. Denno, D. R. Dobberfuhl, A. Folarin, A. Huberty, S. Interlandi, S. S. Kilham, E. McCauley, K. L. Schulz, E. H. Siemann, and R. W. Sterner. 2000*a*. Nutritional constraints in terrestrial and freshwater food webs. Nature **408**:578–580.
Elser, J. J., E. R. Marzolf, and C. R. Goldman. 1990. Phosphorus and nitrogen limitation of phytoplankton growth in the freshwaters of North America: a review and critique of experimental enrichments. Canadian Journal of Fisheries and Aquatic Sciences **47**:1468–1477.
Elser, J. J., W. J. O'Brien, D. R. Dobberfuhl, and T. E. Dowling. 2000*b*. The evolution of ecosystem processes: growth rate and elemental stoichiometry of a key herbivore in temperate and arctic habitats. Journal of Evolutionary Biology **13**:845–853.
Elser, J. J., B. L. Stabler, and P. R. Hassett. 1995. Nutrient limitation of bacterial growth and rates of bacterivory in lakes and oceans: a comparative study. Applied and Environmental Microbiology **9**:105–110.
Elser, J. J., R. W. Sterner, E. Gorokhova, W. F. Fagan, T. A. Markow, J. B. Cotner, J. F. Harrison, S. E. Hobbie, G. M. Odell, and L. J. Weider. 2000*c*. Biological stoichiometry from genes to ecosystems. Ecology Letters **3**:540–550.
Elser, J. J., and J. Urabe. 1999. The stoichiometry of consumer-driven nutrient recycling: theory, observations, and consequences. Ecology **80**:735–751.
Enriquez, S., C. M. Duarte, and K. Sandjensen. 1993. Patterns in decomposition rates among photosynthetic organisms—the importance of detritus C:N:P content. Oecologia **94**:457–471.
Fagerbakke, K. M., M. Heldal, and S. Norland. 1996. Content of carbon, nitrogen, oxygen, sulphur and phosphorus in native aquatic and cultured bacteria. Aquatic Microbial Ecology **10**:15–27.
Falkowski, P. G., and J. A. Raven. 1997. Aquatic photosynthesis. Blackwell Science, Oxford, UK.
Falkowski, P., et al. 2000. The global carbon cycle: A test of our knowledge of earth as a system. Science **290**:291–296.
Geider, R. J., H. L. MacIntyre, and T. M. Kana. 1998. A dynamic regulatory model of phytoplanktonic acclimation to light, nutrients, and temperature. Limnology and Oceanography **43**:679–694.
Gorokhova, E., and M. Kyle. 2002. Analysis of nucleic acids in *Daphnia*: development of methods and ontogenetic variations in RNA-DNA content. Journal of Plankton Research **24**:511–522.
Groot, J. J. R., and J. H. J. Spiertz. 1991. The role of nitrogen in yield formation and achievement of quality standards in cereals. Pages 227–247 *in* J. R. Porter and D. W. Lawlor, editors. Plant growth: interactions with nutrition and environment. Cambridge University Press, Cambridge, UK.
Gulati, R. D., and W. R. DeMott. 1997. The role of food quality for zooplankton: remarks on the state-of-art, perspectives and priorities. Freshwater Biology **38**:753–768.
Hanegraaf, P. P. F., and E. B. Muller. 2001. The dynamics of the macromolecular composition of biomass. Journal of Theoretical Biology **212**:237–251.
Hawkins, A. J. S., B. L. Bayne, and A. J. Day. 1986. Protein turnover, physiological energetics and heterozygosity in the blue mussel, *Mytilus edulis*: the basis of variable age-specific growth. Proceedings of the Royal Society (London) B **229**:161–176.
Healey, F. P., and L. L. Hendzel. 1975. Effect of phosphorus deficiency on two algae growing in chemostats. Journal of Phycology **11**:303–309.
Hessen, D. O., G. I. Ågren, T. R. Anderson, J. J. Elser, and P. de Reuter. 2004. Carbon sequestration in ecosystems: the role of stoichiometry. Ecology **85**:1179–1192.
Hessen, D. O., and A. Lyche. 1991. Inter- and intraspecific variations in zooplankton element composition. Archiv für Hydrobiologie **121**:343–353.
Hessen, D. O., K. Nygaard, K. Salonen, and A. Vähätalo. 1994. The effect of substrate stoichiometry on microbial activity and carbon degradation in humic lakes. Environment International **20**:67–76.
Jensen, K. F., and S. Pedersen. 1990. Metabolic growth rate control in *Escherichia coli* may be a consequence of subsaturation of the macromolecular biosynthetic apparatus with substrates and catalytic components. Microbiological Reviews **54**:89–100.
Jeuniaux, C., and M. F. Voss-Foucart. 1991. Chitin biomass and production in the marine environment. Biochemical Systematics and Ecology **19**:347–356.

Kato, K. 1994. Planktonic bacterial DNA and RNA synthesis from algal extracellular products in a eutrophic lake. FEMS Microbiology Ecology **15**:291–298.
Koch, A. L. 1970. Overall controls on the biosynthesis of ribosomes in growing bacteria. Journal of Theoretical Biology **28**:203–231.
Kooijman, S. A. L. M. 2000. Dynamic energy and mass budgets in biological systems. Second edition. Cambridge University Press, Cambridge, UK.
Kooijman, S. A. L. M., T. Andersen, and B. W. Kooi. 2004. Dynamic energy budget representations of stoichiometric constraints on population dynamics. Ecology **85**:1230–1243.
Lafontaine, D. L. J., and D. Tollervey. 2001. The function and synthesis of ribosomes. Nature Reviews Molecular Cell Biology **2**:514–520.
Leick, V. 1968. Ratios between contents of DNA, RNA and protein in different micro-organisms as a function of maximal growth rate. Nature **217**:1153–1155.
Main, T. M., D. R. Dobberfuhl, and J. J. Elser. 1997. N:P stoichiometry and ontogeny of crustacean zooplankton: a test of the growth rate hypothesis. Limnology and Oceanography **42**:1474–1478.
Markow, T. A., B. Raphael, D. Dobberfuhl, C. M. Breitmeyer, J. J. Elser, and E. Pfeiler. 1999. Elemental stoichiometry of *Drosophila* and their hosts. Functional Ecology **13**:78–84.
Marshall, B., and J. R. Porter. 1991. Concepts of nutritional and environmental interactions determining plant productivity. Pages 99–124 *in* J. R. Porter and D. W. Lawlor, editors. Plant growth: interactions with nutrition and environment. Cambridge University Press, Cambridge, UK.
Mathers, E. M., D. F. Houlihan, I. D. McCarthy, and L. J. Burren. 1993. Rates of growth and protein-synthesis correlated with nucleic-acid content in fry of rainbow trout, *Oncorhynchus mykiss*—effects of age and temperature. Journal of Fish Biology **43**:245–263.
Mayzaud, P., and J. L. M. Martin. 1975. Some aspects of the biochemical and mineral composition of marine plankton. Journal of Experimental Marine Biology and Ecology **17**:297–310.
McCarthy, I. D., D. F. Houlihan, and C. G. Carter. 1994. Individual variation in protein turnover and growth efficiency in rainbow trout, *Oncorhynchus mykiss* (Walbaum). Proceedings of the Royal Society (London) B **257**:141–147.
McKee, M. J., and C. O. Knowles. 1987. Levels of protein, RNA, DNA, glycogen and lipid during growth and development of *Daphnia magna* Straus (Crustacea: Cladocera). Freshwater Biology **18**:341–351.
Nielsen, S. L., S. Enriquez, C. M. Duarte, and K. Sand-Jensen. 1996. Scaling maximum growth rates across photosynthetic organisms. Functional Ecology **10**:167–175.
Nomura, M., R. Gourse, and G. Baughman. 1984. Regulation of the synthesis of ribosomes and ribosomal components. Annual Review of Biochemistry **53**:75–117.
Rhee, G.-Y. 1973. Continuous culture study of phosphate uptake, growth rate and polyphosphate in *Scenedesmus* sp. Journal of Phycology **9**:495–506.
Ryle, G. J. A. 1984. Respiration and plant growth. Pages 3–16 *in* J. M. Palmer, editor. The physiology and biochemistry of plant respiration. Cambridge University Press, Cambridge, UK.
Sadava, D. E. 1993. Cell biology: organelles, structure and function. Jones and Bartlett, Boston, Massachusetts, USA.
Saiz, E., A. Calbet, A. Fara, and E. Berdalet. 1998. RNA content of copepods as a tool for determining adult growth rates in the field. Limnology and Oceanography **43**:465–470.
Schade, J. D., M. Kyle, S. E. Hobbie, W. F. Fagan, and J. J. Elser. 2003. Stoichiometric tracking of soil nutrients by a desert insect herbivore. Ecology Letters **6**:96–101.
Schindler, D. W. 1977. Evolution of phosphorus limitation in lakes. Science **195**:260–262.
Shuter, B. 1979. A model of physiological adaptation in unicellular algae. Journal of Theoretical Biology **78**:519–552.
Skjoldal, H. R. 1981. ATP concentration and adenylate energy charge of tropical zooplankton from waters inside the Great Barrier Reef. Marine Biology **62**:119–123.
Skjoldal, H. R., and U. Båmstedt. 1977. Ecobiochemical studies on the deep-water pelagic community of Korsfjorden, Western Norway. Adenine nucleotides in zooplankton. Marine Biology **42**:197–211.
Sterner, R. W. 1995. Elemental stoichiometry of species in ecosystems. Pages 240–252 *in* C. G. Jones and J. H. Lawton, editors. Linking species and ecosystems. Chapman and Hall, New York, New York, USA.
Sterner, R. W., and J. J. Elser. 2002. Ecological stoichiometry. Princeton University Press, Princeton, New Jersey, USA.
Sterner, R. W., and N. B. George. 2000. Carbon, nitrogen, and phosphorus stoichiometry of cyprinid fishes. Ecology **81**:127–140.
Sterner, R. W., and D. O. Hessen. 1994. Algal nutrient limitation and the nutrition of aquatic herbivores. Annual Review of Ecology and Systematics **25**:1–29.
Sterner, R. W., and K. L. Schulz. 1998. Zooplankton nutrition: recent progress and a reality check. Aquatic Ecology **32**:261–279.
Stocking, C. R., and A. Ongun. 1962. The intracellular distribution of some metallic elements in leaves. American Journal of Botany **49**:284–289.
Sugden, P. H., and S. J. Fuller. 1991. Regulation of protein turnover in skeletal and cardiac muscle. Biochemical Journal **273**:21–37.
Sutcliffe, W. H. 1970. Relationship between growth rate and ribonucleic acid concentration in some invertebrates. Journal of the Fisheries Research Board Canada **27**:606–609.
Vitousek, P. 1982. Nutrient cycling and nutrient use efficiency. American Naturalist **119**:553–572.
Vitousek, P. M., and R. W. Howarth. 1991. Nitrogen limitation on land and in the sea—how can it occur. Biogeochemistry **13**:87–115.
Vrede, K., T. Vrede, A. Isaksson, and A. Karlsson. 1999. Effects of nutrients (phosphorus, nitrogen, and carbon) and zooplankton on bacterioplankton and phytoplankton—a seasonal study. Limnology and Oceanography **44**:1616–1624.
Vrede, K., M. Heldal, S. Norland, and G. Bratbak. 2002. Elemental composition (C, N, P) and cell volume of exponentially growing and nutrient-limited bacterioplankton. Applied and Environmental Microbiology **68**:2965–2971.
Vrede, T., T. Andersen, and D. O. Hessen. 1999. Phosphorus distribution in three crustacean zooplankton species. Limnology and Oceanography **44**:225–229.
Vrede, T., J. Persson, and G. Aronsen. 2002. The influence of food quality (P:C ratio) on RNA:DNA ratio and somatic growth rate of *Daphnia*. Limnology and Oceanography **47**:487–494.
Wagner, M. M., R. G. Campbell, C. A. Boudreau, and E. G. Durbin. 2001. Nucleic acids and growth of *Calanus finmarchicus* in the laboratory under different food and temperature conditions. Marine Ecology Progress Series **221**:185–197.
Wagner, M., E. Durbin, and L. Buckley. 1998. RNA:DNA ratios as indicators of nutritional condition in the copepod *Calanus finmarchichus*. Marine Ecology Progress Series **162**:173–181.