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REPORT

Growth rate–stoichiometry couplings in diverse biota

J. J. Elser[1]*, K. Acharya[1], M. Kyle[1], J. Cotner[2], W. Makino[2], T. Markow[3], T. Watts[3], S. Hobbie[2], W. Fagan[4], J. Schade[1], J. Hood[2] and R. W. Sterner[2]

[1]School of Life Sciences, Arizona State University, Tempe, AZ 85287, USA

[2]Department of Ecology, Evolution, and Behavior, University of Minnesota, St Paul, MN 55108, USA

[3]Center for Insect Science, University of Arizona, Tucson, AZ 85721, USA

[4]Department of Biology, University of Maryland, College Park, MD 20742, USA

**Correspondence: E-mail: j.elser@asu.edu*

Abstract

Biological stoichiometry provides a mechanistic theory linking cellular and biochemical features of co-evolving biota with constraints imposed by ecosystem energy and nutrient inputs. Thus, understanding variation in biomass carbon : nitrogen : phosphorus (C : N : P) stoichiometry is a major priority for integrative biology. Among various factors affecting organism stoichiometry, differences in C : P and N : P stoichiometry have been hypothesized to reflect organismal P-content because of altered allocation to P-rich ribosomal RNA at different growth rates (the growth rate hypothesis, GRH). We tested the GRH using data for microbes, insects, and crustaceans and we show here that growth, RNA content, and biomass P content are tightly coupled across species, during ontogeny, and under physiological P limitation. We also show, however, that this coupling is relaxed when P is not limiting for growth. The close relationship between P and RNA contents indicates that ribosomes themselves represent a biogeochemically significant repository of P in ecosystems and that allocation of P to ribosome generation is a central process in biological production in ecological systems.

Keywords

Growth, phosphorous, RNA, stoichiometry.

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INTRODUCTION

Food webs, the pathways of energy and material flows as a result of trophic interactions in ecosystems (Elton 1927), constitute one of the most complex phenomena in modern biology (Pimm *et al.* 1991). This complexity is further enhanced because the 'nodes' comprising food web networks represent individual organisms of diverse species, each individual a complex biochemical system and each species a product of ongoing evolutionary change (Holt 1995). Understanding these interactions is one of the major themes in the emerging science of 'biocomplexity' (Michener *et al.* 2001). Indeed, the discovery of generalizable mechanistic pathways linking levels of biological organization from the genome to the ecosystem is an important focus of this new field of endeavour (Michener *et al.* 2001; Cottingham 2002). Understanding energy and material flows in food webs require elucidating the biochemical constraints and evolutionary forces operating on these components. Ecological stoichiometry is the study of the balance of energy and multiple chemical elements in ecological interactions (Sterner & Elser 2002); its extension to biological phenomena at other levels of organization (individual metabolism, cellular allocation and genetic change) can be referred to as 'biological stoichiometry' (Elser *et al.* 2000c). A focal idea in biological stoichiometry is the growth rate hypothesis (GRH, hereafter), which proposes that elevated demands for increased allocation to P-rich ribosomal RNA under rapid growth drives variation in the P content (and thus C : P and N : P ratios) of many biota (Elser *et al.* 2000a,c). The GRH therefore connects the evolution of a major life-history character (growth or development rate) to ecological impacts on trophic dynamics and nutrient cycling mediated by biomass C : N : P stoichiometry (Sterner & Elser 2002). Preliminary data supporting the GRH have been presented (Main *et al.* 1997; Vrede *et al.* 1998; Gorokhova *et al.* 2002) but are taxonomically restricted, dominated by data for the microcrustacean *Daphnia*. No multi-taxon analyses of a potential tripartite coupling among growth, RNA content and P content have yet been reported. In this study, we integrate and analyse diverse data from a variety of ecological, evolutionary, developmental and physiological settings to determine the conditions under which growth, RNA allocation and P content are closely associated and under which these parameters are decoupled. Our data show extremely tight and generalizable coupling among growth

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rate, RNA allocation and P content in biota ranging from microbes to weevils. This close coupling is observed for conditions under which organisms are growing maximally during development or at reduced growth rates under P-limitation. However, this close coupling is broken when growth is determined by other factors, such as starvation or limitation of growth by nitrogen.

METHODS

Our study involves a synthesis of published and unpublished data from studies that paired measurements of growth rates with biochemical and/or P contents. Growth rates were expressed as exponential rates of change: $\mu\ (d^{-1}) = \ln (M_2/M_1)/dt$, where M_2 and M_1 are dry masses at time periods 2 and 1, respectively, and dt is the time interval (in days) between observations. While it is probable that different taxa exhibit somewhat different temperature responses, not all taxa considered have been characterized for their temperature response surface. Therefore, all growth rates were corrected to 25°C using a single Q_{10} adjustment factor (2.5) representative of previously reported values for microbes and Metazoans. Methods of analysis for samples of larvae of the swimming crab (Lim & Hirayama 1991), the copepod (Carrillo *et al.* 2001), interspecific comparisons of freshwater zooplankton species (Main *et al.* 1997; Dobberfuhl 1999), microbes (Makino & Cotner 2003; Makino *et al.* 2003), and field observations of mesquite-feeding weevils (Schade *et al.* 2003) can be obtained in the original reports. Data for physiological variation in the freshwater crustacean *Daphnia* (*D. galeata*, *D. pulicaria*, *D. pulex*) are yet to be published; these studies followed the culturing methods used elsewhere (Main *et al.* 1997; Dobberfuhl 1999) and the protocols of biochemical and elemental analysis reported for the other taxa. In general P was determined by colorimetric analysis on dried materials oxidized to release organically bound PO_4 (APHA 1992). As data for RNA can be unreliable when compared across studies because of the heterogeneity of methods, all RNA values shown here were generated with a closely cross-calibrated fluorometric method newly developed for application to small samples (Gorokhova & Kyle 2002; Kyle *et al.* 2003) as a part of a coordinated ecoevolutionary study of biological stoichiometry (Elser *et al.* 2000c). The contribution of P present in RNA to total dry mass (%RNA-P) was directly calculated, as P contributes 9% of the total mass of nucleic acid molecules (Sterner & Elser 2002). Mass determinations for calculations of growth rate, RNA content and P content were generally made on separate and independent samples, reducing the possibility of artefacts because of autocorrelations. Furthermore, we have tested for possible autocorrelation artefacts driven by dry weight corrections in our *Drosophila* and *Daphnia* data sets by plotting total RNA-P per animal vs. total P per animal in paired samples across ontogeny or food conditions. These data retain the close association between RNA-P and total-P seen in the dry weight-corrected data and thus the tight correlations we report here do not reflect influences of autocorrelated data structures.

Data for ontogenetic variation (*Drosophila*, *Portunus*, *Mixodiaptomus*) involved intensive study of animals during larval development prior to maturation as adults (*Mixodiaptomus* data include values for adults as well) or further metamorphosis (*Portunus*). Data for interspecific comparisons of zooplankton taxa reflected observations for animals over a time period encompassing most of the juvenile period (Main *et al.* 1997). Data for *Escherichia coli* (Makino *et al.* 2003) and lake bacteria (Makino & Cotner 2003) involved laboratory chemostats operated at different dilution rates and fed with mixtures of organic and inorganic resources at C : P ratios ranging from 9.3 to 933. Data shown in Fig. 1 for physiological variation in *D. pulicaria* and *D. galeata* are for animals fed the green alga *Scenedesmus acutus* at limiting (0.25 mg C L^{-1}) or high (1.5 mg C L^{-1}) quantities at varying C : P from low (C : P ~100 by atoms) to high (C : P ~1000). Data shown in Fig. 2 for *D. pulicaria* involve animals fed high quantities (1.5 mg C L^{-1}) of *S. acutus* at varying C : N ratios from low (C : N ~6) to high (C : N ~18). Data in Fig. 2 for *D. pulex* are for animals raised at different temperatures (5, 10, 15, 20°C) and given *S. acutus* with high C : P ratio (*c.* 944) or low C : P ratio (*c.* 123) but presented at very low concentration (0.1 mg C L^{-1}) near the starvation threshold. Paired data on P content and RNA contents of the mesquite-feeding weevil *Sibinia setosa* (growth measurements were not made) were obtained for individuals sampled during different seasons in which rainfall caused significant differences in the P content of mesquite leaves that were transmitted to weevils in the form of increased P and RNA contents (Schade *et al.* 2003).

RESULTS

Our data for 10 taxa of microbes and Metazoans reveal close covariation of biomass P and RNA contents with temperature-corrected specific growth rate (μ) and tight correlations between total P content and P content contributed by RNA (Fig. 1). The data documenting these correlations are diverse, involving examinations of ontogenetic variation during planktonic larval development of the swimming crab *Portunus tribuberculatus*, interspecific differences in juvenile crustacean zooplankton growing maximally, physiological variation in *Daphnia* driven by effects of food abundance and stoichiometric food quality, physiological variation in the bacterium *E. coli* because of P-limitation, ontogenetic development of larval fruitfly *Drosophila melanogaster*, *in situ* ontogenetic development of the copepod *Mixodiaptomus laciniatus*, ecophysiological

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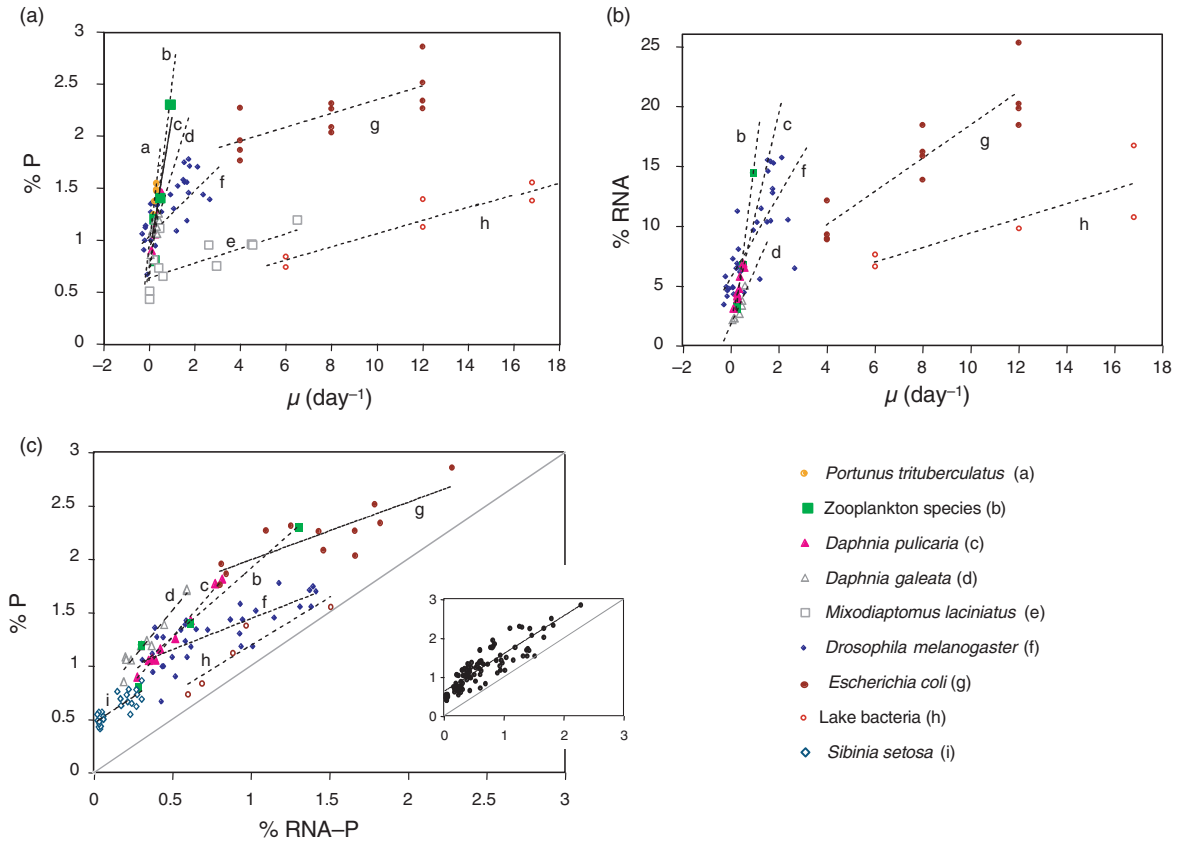

Figure 1 Diverse species exhibit coupling of biomass P content (% of dry mass) and biomass RNA content (% of dry mass) with specific growth rate (μ) and of total P content with RNA-P content. In some cases (*Daphnia pulicaria*, *D. galeata*) regression lines were extrapolated beyond the data to facilitate labelling. All relationships shown were statistically significant (one at a level of $P < 0.10$) and correlation parameters and statistics are summarized in Table 1. (a) P content (*y*) vs. growth rate (*x*): line a, larvae of the swimming crab *Portunus tribuberculatus* during ontogeny; line b, different species of crustacean zooplankton; line c, the freshwater crustacean *D. pulicaria* grown under P-limitation; line d, the freshwater crustacean *D. galeata* grown under P-limitation; line e, the freshwater copepod *Mixodiaptomus laciniatus* during ontogeny; line f, the fruit fly *Drosophila melanogaster* during ontogeny; line g, the enteric bacterium *Escherichia coli* grown under P-limitation; line h, for mixed lake bacterial assemblages grown under P-limitation. (b) RNA content (*y*) vs. growth rate (*x*): line b, different species of crustacean zooplankton; line c, *D. pulicaria*; line d, *D. galeata*; line f, *D. melanogaster*; line g, *E. coli*; line h, mixed lake bacterial assemblages. (c) Biomass P content (*y*) vs. P content because of RNA: line b, different species of crustacean zooplankton; line c, *D. pulicaria*; line d, *D. galeata*; line f, *D. melanogaster*; line g, *E. coli*; line h, mixed lake bacterial assemblages; line j, field-sampled individuals of the mesquite-feeding weevil *Sibinia setosa*. The inset shows the fit to the aggregated data for %P and %RNA-P.

variation exhibited by mixed communities of freshwater microbes under P-limitation in chemostats, and *in situ* ecophysiological variation presented by herbivorous weevils during interannual variation in food quality. Within taxa, each relationship had a statistically significant slope ($P < 0.05$; $P = 0.09$ for RNA content vs. μ for lake bacteria) and was generally tight (r^2 values ranging from 0.46 to 0.99, 11 of 21 relationships had $r^2 > 0.80$). Each also exhibited the expected positive correlations between both RNA and P contents and μ and between total P content with P contributed by RNA. Furthermore, in each case the P contained in RNA involved a significant percentage of total biomass P (mean: 49 ± 1.9% SE).

In contrast to these results, we observed uncoupling of growth, RNA and P contents when a subset of our heterotrophic study organisms (*Daphnia*, *E. coli*, and lake microbes) were grown under limitation by constituents other than P (Fig. 2). When *D. pulicaria* was fed high C : N algae (thus inducing protein/N-limitation), no significant relationships emerged for any combination of μ, RNA content, and P content ($P > 0.17$; Fig. 2). Similarly, when *D. pulex* was grown with different qualities of food (P-rich vs. low in P) at various temperatures but with food concentration at very low starvation levels (0.1 mg C L^{-1}), P content and RNA content were uncorrelated with growth rate ($P > 0.30$) but a relative weak correlation between P

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Table 1 Summary of regression parameters (slope, intercept) and statistical results for relationships presented in Fig. 1

Dependent variable (y)	Independent variable (x)	Taxon	Slope	Intercept	P-value	r^2
P content	Growth rate	*Portunus tribuberculatus*	1.88	0.91	<0.01	0.95
		Several zooplankton species	1.84	0.56	<0.01	0.93
		Daphnia pulicaria	1.32	0.72	<0.0001	0.98
		Daphnia galeata	0.73	0.90	<0.01	0.73
		Mixodiaptomus laciniatus	0.071	0.67	<0.03	0.46
		Drosophila melanogaster	0.22	1.12	<0.0001	0.51
		Escherichia coli	0.066	1.68	<0.01	0.57
		Lake bacteria assemblages	0.063	0.43	<0.01	0.87
RNA content	Growth rate	Several zooplankton species	15.9	−0.55	<0.01	0.99
		Daphnia pulicaria	8.69	1.92	<0.0001	0.91
		Daphnia galeata	4.53	1.74	<0.02	0.65
		Drosophila melanogaster	3.44	5.66	<0.0001	0.55
		Escherichia coli	1.39	4.52	<0.0001	0.84
		Lake bacteria assemblages	0.61	3.3	<0.09	0.69
P content	RNA-P content	Several zooplankton species	1.29	0.62	<0.03	0.94
		Daphnia pulicaria	1.69	0.42	<0.0001	0.70
		Daphnia galeata	1.78	0.63	<0.0001	0.94
		Drosophila melanogaster	0.58	0.86	<0.0001	0.62
		Escherichia coli	0.54	1.45	<0.0001	0.70
		Lake bacteria assemblages	0.90	0.29	<0.0001	0.85
		Sibinia setosa	1.00	0.46	<0.0001	0.67
		All data combined	0.97	0.62	<0.0001	0.78

and RNA contents was observed ($P < 0.05$, $r^2 = 0.50$; Fig. 2c). Finally, when *E. coli* was grown on substrates with very low C : P ratio (9.3), P content retained a positive relationship with μ (Fig. 2a; statistics were not computed because only three data points were available) but RNA content was similar for bacteria growing at the two higher growth rates (Fig. 2b). Thus, there was no association of total P content with RNA-P content (Fig. 2c). Similarly, coupling among these parameters was disrupted when lake bacteria were grown on substrates having a low C : P ratio of 93: P and RNA contents showed no relationship with μ (Fig. 2a,b) and were not intercorrelated (Fig. 2c).

DISCUSSION

The results presented in Fig. 1 constitute very strong support for the GRH in diverse settings: interspecific (different species of zooplankton; line b in Fig. 1), physiological (*Daphnia* and microbes growing under P-limitation; lines c, d, g and h), ontogenetic (*Drosophila*, *Diaptomus*, *Portunus* lines a, e and f), and ecological (*Sibinia*; line j). However, the couplings among growth, P content, and RNA content are not quantitatively equivalent across taxa. For example, there was considerable variation among the taxa considered in the level of growth achieved for a given allocation to RNA (and thus to P). The microbes considered were particularly divergent in this regard, achieving temperature-corrected growth rates that were two to three times higher than the metazoans considered at equivalent RNA levels. At least some of this variation likely reflects differences among taxa in the actual temperature dependence of growth rate. As Q_{10} values for growth were not determined in these studies, we applied a single Q_{10} correction (2.5) to all of the studies using 25°C as a standard. If, for example, the actual Q_{10} factor for *Mixodiaptomus* is >2.5, then the comparatively low growth rates they present relative to the microbes considered would simply be an artefact of an improper correction (but see below). The only way to resolve this question, however, would be to directly determine Q_{10} values for a comparable set of taxa for which growth rates along with RNA and P contents are also determined. However, the differences in growth performance per unit RNA or P for the different taxa considered are probably real, as some of the taxa included were raised at *c.* 25°C (e.g. lake bacteria, *D. melanogaster*, *D. pulicaria*, *D. galeata*) and thus did not require Q_{10} correction but nevertheless showed considerable differences in growth rate per unit RNA (and P). It is interesting to speculate about possible mechanisms for such differences. One possibility is that there are fundamental differences in the functional capability of different ribosomes: for example, prokaryotic ribosomes are more RNA-rich than eukaryotic ribosomes and appear to have higher protein translation rates (Sterner & Elser 2002). This would help to account for considerably higher temperature-corrected growth rates per unit RNA (and P) that the microbes exhibit

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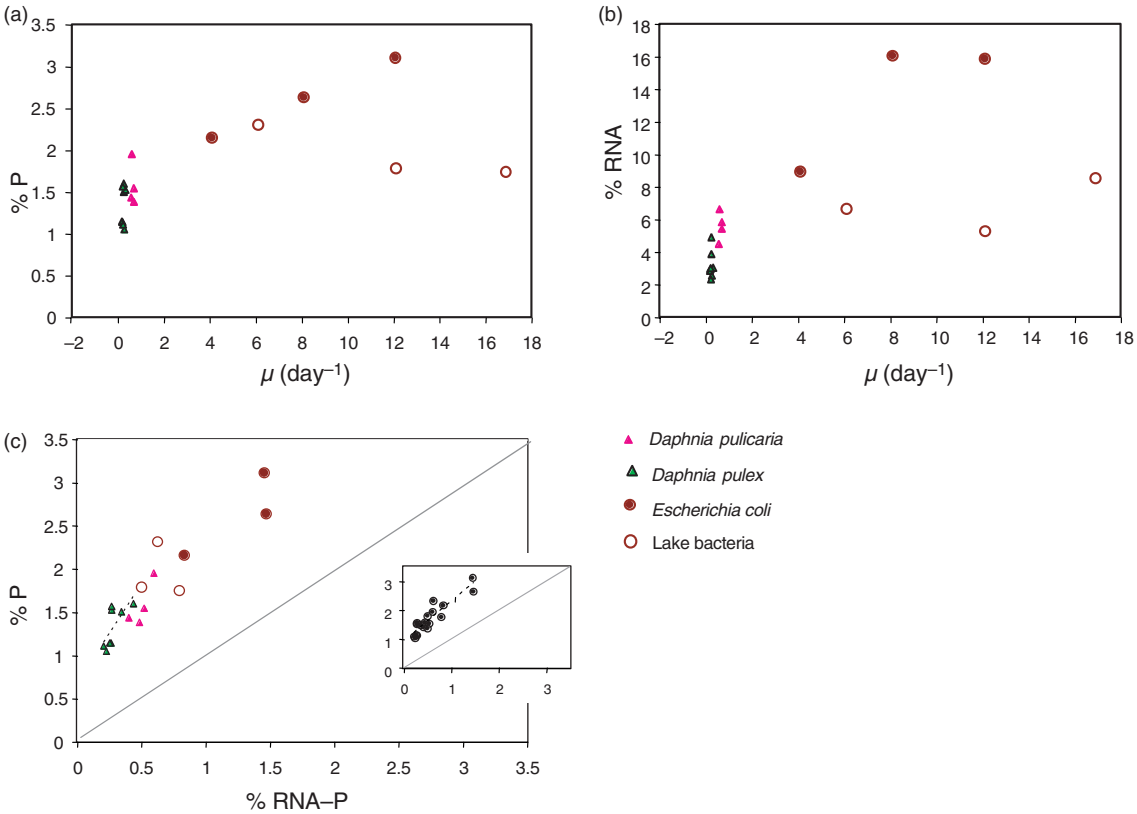

Figure 2 When organisms are grown under non-P-limiting physiological conditions, coupling of P content (panel a) and RNA content (panel b) with growth rate is broken. *Daphnia pulicaria* under N-limitation; *D. pulex* at starvation food levels; lake microbes and *Escherichia coli* grown at low substrate C : P ratio (93 for lake microbes; 9.3 for *E. coli*). Although total biomass P content and P content because of RNA were not closely associated within any of these individual studies (panel c; a relatively weak association was seen in the *D. pulex* data), a strong relationship between P content and RNA-P content nevertheless existed for all data combined (inset; $y = 1.37x + 0.97$; $P < 0.001$, $r^2 = 0.85$).

relative to the metazoans in our study. Another possible factor that may contribute to this observed variation among taxa is differences in protein retention and turnover. That is, in some taxa much protein output from a ribosome may be given over to remodelling or replacement of existing proteins, themselves being degraded, while in other taxa a higher percentage of synthesized protein is directly accrued into new biomass. Detailed studies of protein turnover and fate would be required to address this possibility.

There were also differences in how total biomass P content was associated with RNA content. For example, the field-isolated lake bacteria achieve high μ per unit RNA and per unit P. These bacteria also exhibited an unusually high percentage of total biomass P contributed by RNA (mean: 82%, range: 70–97%). Thus, these 'wild' microbes seem to invest little P in biochemicals beyond their RNA, consistent with observations that some bacteria, under P-limited growth, can replace membrane phospholipids, another major structural component containing P, with lipids lacking in P such as teichuronic acids (Lahooti & Harwood 1999), potentially providing competitive advantage under P scarcity (Vadstein 2000). In some cases (*Drosophila*, P-limited *E. coli*), the P content vs. RNA-P content relationship had a slope <1, indicating that RNA contributed a greater fraction of biomass P as RNA content (and, generally, growth rate itself) increased. In contrast, for the crustaceans studied, the slope was >1, indicating that, as RNA content increased with μ, non-RNA P increased disproportionately. The biochemical form of this non-RNA P is unclear but one possibility is that a significant pool of P-rich nucleotide precursors (NTPs, also discussed below) is generated in support of ribosome biosynthesis but these NTPs are not reactive with the nucleic acid-binding fluorochromes used in our assay. If true, for some taxa rapid growth would then be even more P-intensive than envisioned by the mechanisms of the GRH.

The overall coupling between biomass P content and P content contributed by RNA across all the taxa and

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conditions encompassed was quite close ($r^2 \sim 80\%$; Fig. 1c). Furthermore, the slope of the relationship was essentially 1.0 (0.97 ± 0.05 SE), indicating that at the broad scale of comparison across multiple taxa of aquatic and terrestrial Metazoa and microbes and diverse conditions of interspecific, ontogenetic and physiological variation, altered allocation to RNA is the primary determinant of variation in biomass P content. These relationships should extend to other taxa not included in our study. For example, a close coupling of growth and RNA content was reported in a study that included other species of microbes and insects (Sutcliffe 1970) and similar arguments for a close association among growth, RNA content and P content in P-limited phytoplankton have recently been made (Geider & La Roche 2002). However, an association of P and RNA contents is not expected for vertebrate animals, given the major contribution of mineral P in bones to their overall P content (Sterner & Elser 2002).

In general, the growth conditions involved in the studies contained in Fig. 1 were such that ribosome biogenesis would be expected to constrain growth and thus P would not accumulate in biomass pools other than ribosomal RNA. However, under unbalanced growth where P might accrue in storage pools or where ribosome biogenesis itself is not rate-limiting for growth, couplings among μ, RNA and P might be broken, as has been shown in photoautotrophs such as algae (Rhee 1973). This is what we observed in Fig. 2. Thus, at low substrate C : P ratio, the association of P content with growth rate was apparently broken for *E. coli* and all components of the hypothesized coupling were disrupted for lake bacteria. Similarly, for *Daphnia* growing at extremely low food levels (severe energy limitation) or under protein/N limitation, no correlations of RNA and P content with growth rate were seen. This decoupling is similar to what is observed in algae when measures are made of a non-limiting nutrient and growth varies because of limitation by another resource. For example, algal P content increases as growth slows under nitrogen or light limitation (Rhee 1973, 1978; Goldman *et al.* 1979). Such changes are generally interpreted as a sign of storage or 'luxury uptake'. The form of this non-RNA P is unclear. It is well known that algae, bacteria and even higher plants can store excess P as polyphosphate but formation of polyphosphate has not been demonstrated for Metazoa. One possibility is that this 'extra' P accumulates as NTPs, given that formation of mature ribosomes is likely inhibited in response to overall growth limitation by N or energy. Given that *Daphnia* apparently can store physiologically significant amounts of P during brief periods of feeding on P-rich foods (Sterner & Schwalbach 2001), identifying the biochemical form of this excess P may help in understanding how herbivorous animals integrate temporal or spatial variation in dietary P content. Regardless of this question, combining our findings with these previous studies of P storage, the most general message is that under non-P-limiting conditions the tripartite coupling of growth, RNA content and P content is broken for both heterotrophic and autotrophic organisms. Nevertheless, when data from these three studies were combined, a relatively close correlation between total biomass P-content and RNA-P content was retained ($P < 0.0001$, $r^2 = 0.85$; Fig. 2c), although its slope (1.37) was not *c.* 1. In addition, the intercept of this relationship was higher than that in Fig. 1c (0.97 vs. 0.62). This shows that, under conditions in which growth is uncoupled from RNA and P contents, organisms carry a larger fraction of their P outside RNA. As pointed out by Makino *et al.* (2003) in the context of their comparison of growth-dependent vs. substrate-dependent variations in biomass C : N : P stoichiometry in *E. coli* (these are the same data included in our study), such data may help in interpreting the sources of stoichiometric variation in heterotrophs in nature. Our data indicate that heterotrophic organisms such as metazoans and, apparently, microbes according to Makino *et al.*'s paper, exhibit strong but not perfect stoichiometric homeostasis and thus most variation in biomass P content (and thus C : P and N : P ratios) seen for heterotrophic organisms under natural conditions is probably a reflection of variation in actual growth rate, as decoupling of P content from RNA allocation and growth rate requires relatively extreme resource supply conditions (very low substrate C : P, high food C : N with low N : P, extremely low food levels).

Our data have additional ecological and evolutionary implications. First, they support the idea (Elser *et al.* 2000c; Sterner & Elser 2002) that evolution of rapid growth rate can encounter a stoichiometric constraint because of the disproportionate increases in P intake required to support ribosome biogenesis. Knowledge of such trade-offs may help in understanding the complex interactions of resource supply and consumer regulation in mediating biodiversity impacts on ecosystem function (Worm *et al.* 2002). This is because trade-offs linked to allocation of limiting resources provide an axis along which species diversification might occur in evolutionary time and along which species sorting and patterns of coexistence might emerge in ecological time. This has been suggested in a new stoichiometric model of species interaction among consumers, in which it is shown that two consumer species may stably coexist on a single prey item (in apparent violation of the competitive exclusion principle), provided that two potentially limiting currencies are tracked in the model and that the consumers exhibit a trade-off of intrinsic growth rate and nutrient (P) requirement (Loladze *et al.* 2003). Second, the close correlation between RNA and P contents indicates that the expression of ribosomal genes has significant direct and indirect effects on ecosystem processes. As living organisms can sequester significant fractions of circulating P in ecosystems e.g. *c.* 33% of water column P in *Daphnia* during periods of high

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abundance (Elser *et al.* 2000b); *c.* 50% of particulate P in bacterial biomass on average in oligotrophic lakes (Biddanda *et al.* 2001) and on average *c.* 50% of organismal P is in rRNA (Fig. 1c), increased expression of rDNA immediately leads to production of a biogeochemically significant pool of P in ecosystems. The fact that significant fractions of P are associated with highly labile nucleic acid pools (RNA, DNA) may also help to explain as to why P cycles more efficiently than N in the water column (Clark *et al.* 1998) and in soils (Schlesinger 1997). In addition, because production of RNA is directly coupled to growth (Fig. 1b), rDNA expression is indirectly connected to the processes of primary production (by autotrophs) and secondary production (by microbes and Metazoa) that are the trophic foundation of food webs. This implies that a better understanding of the structure, regulation and evolution of the genes encoding for rRNA will significantly inform studies at the interface of genomics, molecular biology and ecology as the field of biocomplexity emerges.

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REFERENCES

APHA (1992) *Standard Methods for the Examination of Water and Wastewater*, 18th edn. American Public Health Association/ Water Environment Federation, Washington, D.C., 1100 pp.

Biddanda, B., Ogdahl, M. & Cotner, J. (2001). Dominance of bacterial metabolism in oligotrophic relative to eutrophic waters. *Limnol. Oceanogr.*, 46, 730–739.

Carrillo, P., Villar-Argaiz, M. & Medina-Sanchez, J.M. (2001). Relationship between N:P ratio and growth rate during the life cycle of copepods: an *in situ* measurement. *J. Plankton Res.*, 23, 537–547.

Clark, L.L., Ingall, E.D. & Benner, R. (1998). Marine phosphorus is selectively remineralized. *Nature*, 393, 426.

Cottingham, K.L. (2002). Tackling biocomplexity: the role of people, tools, and scale. *BioScience*, 52, 793–799.

Dobberfuhl, D.R. (1999). *Elemental Stoichiometry in Crustacean Zooplankton: Phylogenetic Patterns, Physiological Mechanisms, and Ecological Consequences*. PhD Dissertation, Arizona State University, Tempe, AZ.

Elser, J.J., Dowling, T., Dobberfuhl, D.A. & O'Brien, J. (2000a). The evolution of ecosystem processes: ecological stoichiometry of a key herbivore in temperate and arctic habitats. *J. Evol. Biol.*, 13, 845–853.

Elser, J.J., Sterner, R.W., Galford, A.E., Chrzanowski, T.H., Findlay, D.L., Mills, K.H. *et al.* (2000b). Pelagic C:N:P stoichiometry in a eutrophied lake: responses to a whole-lake food-web manipulation. *Ecosystems*, 3, 293–307.

Elser, J.J., Sterner, R.W., Gorokhova, E., Fagan, W.F., Markow, T.A., Cotner, J.B. *et al.* (2000c). Biological stoichiometry from genes to ecosystems. *Ecol. Lett.*, 3, 540–550.

Elton, C. (1927). *Animal Ecology*. Sidgwick and Jackson Ltd, London.

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

Goldman, J.C., McCarthy, J.J. & Peavey, D.G. (1979). Growth rate influence on the chemical composition of phytoplankton in oceanic waters. *Nature*, 279, 210–215.

Gorokhova, E. & Kyle, M. (2002). Analysis of nucleic acids in *Daphnia*: development of methods and ontogenetic variations in RNA–DNA content. *J. Plankton Res.*, 24, 511–522.

Gorokhova, E., Dowling, T.A., Weider, L.J., Crease, T. & Elser, J.J. (2002). Functional and ecological significance of rDNA IGS variation in a clonal organism under divergent selection for production rate. *Proc. R. Soc. Lond. B*, 269, 2373–2379.

Holt, R.D. (1995). Linking species and ecosystems: where's Darwin? In: *Linking Species and Ecosystems* (eds Jones, C.G. & Lawton, J.H.). Chapman and Hall, New York, NY, pp. 273–279.

Kyle, M., Watts, T., Schade, J. & Elser, J.J. (2003). A microfluorometric method for quantifying RNA and DNA in terrestrial insects. *J. of Insect Science*, 3.1, 1–7.

Lahooti, M. & Harwood, C.R. (1999). Transcriptional analysis of the *Bacillus subtilis* teichuronic acid operon. *Microbiology – UK*, 145, 3409–3417.

Lim, B.K. & Hirayama, K. (1991). Growth and elemental composition (C,N,P) during larval developmental stages of mass-cultured swimming crab *Portunus trituberculatus*. *Mar. Ecol. Prog. Ser.*, 78, 131–137.

Loladze, I., Kuang, Y., Elser, J.J. & Fagan, W.F. (2003). Coexistence of two predators on one prey mediated by stoichiometry. *J. Theor. Biol.*, in press.

Main, T., Dobberfuhl, D.R. & Elser, J.J. (1997). N:P stoichiometry and ontogeny in crustacean zooplankton: a test of the growth rate hypothesis. *Limnol. Oceanogr.*, 42, 1474–1478.

Makino, W. & Cotner, J.B. (2003). C:N:P stoichiometry of a heterotrophic bacterial community in a freshwater lake: implications for substrate- and growth-dependent variations. *Aquat. Microb. Ecol.*, in press.

Makino, W., Cotner, J.B., Sterner, R.W. & Elser, J.J. (2003). Are bacteria more like plants or animals? Growth rate and substrate dependence of bacterial C:N:P stoichiometry. *Funct. Ecol.*, 17, 121–130.

Michener, W.K., Baerwald, T.J., Firth, P., Palmer, M.A., Rosenberger, J.L., Sandlin, E.A. *et al.* (2001). Defining and unraveling biocomplexity. *BioScience*, 51, 1018–1023.

Pimm, S.L., Lawton, J.H. & Cohen, J.E. (1991). Food web patterns and their consequences. *Nature*, 350, 669–674.

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

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

Schade, J., Kyle, M., Hobbie, S., Fagan, W. & Elser, J.J. (2003). Stoichiometric tracking of soil nutrients by a desert insect herbivore. *Ecol. Lett.*, 6, 96–101.

Schlesinger, W.H. (1997). *Biogeochemistry: An Analysis of Global Change*. Academic Press, San Diego, CA.

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Sterner, R.W. & Elser, J.J. (2002). *Ecological Stoichiometry: The Biology of Elements from Molecules to the Biosphere*. Princeton University Press, Princeton, NJ.

Sterner, R.W. & Schwalbach, M. (2001). Diel integration of food quality by *Daphnia*: Luxury consumption by a freshwater planktonic herbivore. *Limnol. Oceanogr.*, 46, 410–416.

Sutcliffe, W.H. (1970). Relationship between growth rate and ribonucleic acid concentration in some invertebrates. *J. Fish. Res. Bd. Can.*, 27, 606–609.

Vadstein, O. (2000). Heterotrophic, planktonic bacteria and cycling of phosphorus: phosphorus requirements, competitive ability, and food web interactions. *Adv. Microb. Ecol.*, 16, 115–167.

Vrede, T., Andersen, T. & Hessen, D.O. (1998). Phosphorus distribution in three crustacean zooplankton species. *Limnol. Oceanogr.*, 44, 225–229.

Worm, B., Lotze, H.K., Hillebrand, H. & Sommer, U. (2002). Consumer versus resource control of species diversity and ecosystem functioning. *Nature*, 417, 848–851.

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