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Coupling of growth rate and body stoichiometry in *Daphnia*: a role for maintenance processes?

MARCIA KYLE,* KUMUD ACHARYA,* LAWRENCE J. WEIDER,† KAREN LOOPER‡ AND JAMES J. ELSER*

*School of Life Sciences, Arizona State University, Tempe, AZ, U.S.A.

†Department of Zoology, University of Oklahoma Biological Station, Kingston, OK, U.S.A.

‡Department of Chemistry, Southeastern Oklahoma State University, Durant, OK, U.S.A.

SUMMARY

1. The growth rate hypothesis predicts positive relationships among growth rate (μ), body RNA (%RNA of dry mass) and body *P* (%P of dry mass) contents.
2. We tested this within- and across-species by growing five species/clones of *Daphnia* (*Daphnia magna*, *Daphnia pulex*, *Daphnia galeata* and two isolates of *Daphnia pulicaria*) with different combinations of food quantity and stoichiometric food quality.
3. Within each species, positive correlations among μ, %RNA and %P were seen and across species there was a strong association between%RNA and %P, consistent with the growth rate hypothesis. However, coupling of growth to %RNA and to %P differed for different species. In particular, the %RNA–μ and %P–μ relationships had similar slopes but considerably different *y*-intercepts (i.e.%P or %RNA at zero growth), with *D. pulicaria* and *D. galeata* having higher intercepts than *D. magna* and especially *D. pulex*. As a result of these displacements, the relative rankings of the species on the basis of %P and %RNA did not correspond to their rankings based on μ.
4. These findings suggest that within a narrow clade (e.g. the daphnids), interspecific differences in body P content may reflect not growth rate-related RNA allocation but instead the amount of RNA required for support of maintenance processes.

Keywords: carbon, *Daphnia*, growth rate hypothesis, phosphorus, RNA

Introduction

Understanding the flows and cycling of energy and chemical elements in pelagic ecosystems is an area of considerable interest in limnology and oceanography. Such flows are largely driven by the activities of planktonic biota, including bacterioplankton, phytoplankton, and micro- and macro- zooplankton (Gaedke, Hochstadter & Straile, 2002). Recent developments in the field of ecological stoichiometry have highlighted the importance of elemental imbalance (e.g. differences in the elemental composition of food items and consumers) in regulating both trophic dynamics and consumer-driven nutrient recycling in planktonic food webs (Sterner & Elser, 2002). In testing the ideas of ecological stoichiometry, much emphasis has been placed on understanding the factors affecting the growth rate of *Daphnia* and its dependence on algal food quality. It has been shown that body growth rate correlates well with algal phosphorus (*P*) content when food P content is below threshold levels (Urabe & Watanabe, 1992; Sterner, 1993; Gulati & DeMott, 1997). At the same time, P addition experiments have shown that at least some of this growth alteration is due directly to P deficiency (DeMott, Gulati & Siewertsen, 1998; Elser, Hayakawa

Correspondence: Marcia Kyle, School of Life Sciences, Arizona State University, Tempe, AZ 85287, U.S.A.
E-mail: mkyle@asu.edu
Present address: Kumud Acharya, Division of Hydrologic Sciences, Desert Research Institute, Las Vegas, NV 89119, U.S.A.

& Urabe, 2001; Boersma & Kreutzer, 2002). Furthermore, nutrient recycling rates and ratios have been closely linked to the elemental composition of both food and consumer (Elser & Urabe, 1999).

While variation in C : N : P ratios in the photoautotrophic organisms that make up the base of pelagic food webs is generally understood as being driven by physiological plasticity associated with the severity of phytoplankton nutrient limitation (Sterner & Elser, 2002), variation also exists in the C : P and N : P ratios of animal biomass (Andersen & Hessen, 1991). These variations have been viewed primarily as taxon- or stage-specific differences, as it has commonly been observed, for example, that the taxon *Daphnia* has consistently lower C : P and N : P ratios than other crustacean zooplankton species. To explain this variation, the growth rate hypothesis has been proposed (Fig. 1a; Sterner & Elser, 2002). The growth rate hypothesis attributes interspecific variation in body P content (%P, expressed relative to dry mass) in zooplankton to differences in their maximal growth rates (μ_{max}) due to the increased contents of P-rich ribosomal RNA (%RNA) required to meet the high protein synthesis demands of high growth rate. Implicit in the growth rate hypothesis is the assumption that among species there is a uniform physiological coupling of RNA allocation with μ (i.e. a common slope in a plot of %RNA versus μ) and a uniform fixed background level of P and RNA (i.e. a common intercept). Under these assumptions, physiological (intraspecific) and interspecific relationships should follow a single common function, as illustrated in Fig. 1.

Indeed, strong couplings between μ, RNA and P have been documented in various daphnids (DeMott *et al.*, 1998; Vrede, Andersen & Hessen, 1999; Acharya, Kyle & Elser, 2004), the bacterium *Escherichia coli* (Makino *et al.*, 2003), cyanobacteria (Lepp & Schmidt, 1998), yeast (Aiking & Tempest, 1976), algae (Rhee, 1978) and other biota (Elser *et al.*, 2003). While there are consistent general patterns within species and groups of species, considerable variation in the relationships of RNA and P contents with growth does exist among taxa. That is, the couplings among growth, P content and RNA content do not appear to be quantitatively equivalent across taxa. For example, in the study of Elser *et al.* (2003) involving data for microorganisms, crustaceans and insects, there was considerable variation among the taxa in the level of growth achieved for a given allocation to RNA (and thus to P). The microbes were particularly divergent in this regard, achieving growth rates that were two to three times higher than the metazoans at equivalent

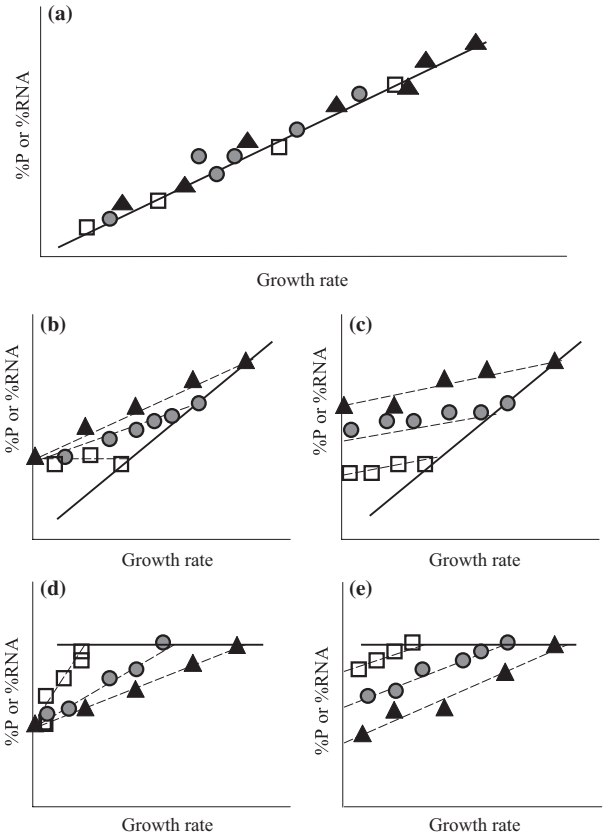

Fig. 1 Possible scenarios for the relationships between growth rate (μ), %phosphorus (P) and %RNA. In each panel, different species are indicated by different symbols (square, triangle, circle) while dotted lines indicate the physiological coupling for each species. The solid line indicates the predicted relationship between maximum RNA or P contents and maximum growth rate (μ_{max}). (a) The original concept of the growth rate hypothesis showing a situation in which each species exhibits similar physiological variation in P versus growth and RNA versus growth relationships (i.e. a universal growth function with similar slopes and intercepts for each species) but the three species differ in their maximum growth rates. Thus, a positive relationship between μ_{max} and maximum P and RNA contents across species is predicted. Panels (b) through (e) illustrate different potential scenarios. (b, c) In these scenarios, positive cross-species relationships of maximum P or RNA contents with μ_{max} are predicted but the species differ in their physiological slopes (panel b) or in their intercepts (panel c). (d, e) In these scenarios, no differences in maximum P or RNA contents occur despite differences in μ_{max} due to phylogenetic or ecophysiological constraints but the species differ in the slopes (panel d) or the intercepts (panel e) of their physiological coupling of RNA or P to growth rate.

RNA levels. The mechanisms for such differences are not clear. One possibility may involve 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 growth rates per unit RNA (and P) that the microbes exhibit. Another possibility is that the observed variation among taxa is due to differences in protein retention and turnover associated with body maintenance. More work is needed to clarify why there is a significant variation in RNA–P–growth couplings.

This led us to consider how different assumptions about underlying RNA metabolism might lead to different expectations about μ–RNA–P relationships. Panel (a) in Fig. 1 depicts the growth rate hypothesis as originally described while panels (b–e) show several possible scenarios regarding how%RNA and %P might vary physiologically with μ within species to result in different maximal values for each taxon. In one set of scenarios (panels b,c), we assume that there is a penalty associated with evolution of higher μ_{max}, in which either the 'efficiency' of RNA or P use (growth increment per unit P or RNA invested) decreases or the maintenance costs (RNA or P contents at zero growth rate) increase with increased μ_{max}. In another set of scenarios (panels d,e), we assume that evolution of higher growth rate is operating within ecological or phylogenetic constraints on maximum P or RNA contents and propose that higher μ might be achieved by altering either 'efficiency' (slope) or 'maintenance' (intercept). The scenarios illustrate that the particular physiological or evolutionary mechanisms that impinge on the coupling of growth, RNA or P may have different effects on the observed relationships among these variables and may help to understand how body stoichiometry is determined in diverse biota.

To evaluate which scenario or scenarios may be operating in *Daphnia*, we tested the growth rate hypothesis using five species/clones of *Daphnia* (*Daphnia magna*, *Daphnia pulex*, *Daphnia galeata* and two isolates of *Daphnia pulicaria*). Food quality and quantity were manipulated to generate variation in μ, RNA and P for each species/clone. The data provide strong support for the growth rate hypothesis within each species but not across species. Furthermore, the quantitative relationships among the variables differed for the species involved. In particular, the relationships appeared to differ most strongly in their intercepts (rather than their slopes), suggesting a situation consistent with the scenarios shown in Fig. 1d,e and thus implicating maintenance processes in determining body P content within this clade.

Methods

We used a strain of *Scendesmus obliquus* (Turpin) Kützing originating from the Max Planck Institute for Limnology (Plön, Germany) as the food source for the various experiments. This green alga was cultured in chemostats using COMBO, an artificial medium (Kilham *et al*., 1998), modified to result in P-sufficient or P-limited algal growth. Medium for P-sufficient *Scenedesmus* (high quality) contained 1000 μmol L^{-1} N and 50 μmol L^{-1} *P* (molar N : P ratio 20) and was supplied at a dilution rate of 1.0 day^{-1}. Medium for P-limited *Scenedesmus* (low quality) contained 5 μmol L^{-1} P and 500 μmol L^{-1} N (N : P 100) supplied at a dilution rate of 0.12 day^{-1}.

To test the growth rate hypothesis across a wide range of growth rates, we manipulated food abundance and quality to induce a range of physiological growth rates in each species of *Daphnia* (Table 1). Food quality, indexed by algal C : P ratio, ranged around 100 (by moles) for high quality, to around 1000 for low quality treatments. Food quantity was manipulated in various treatments across a range from 0.1 to 1.6 mg C L^{-1}. The concentrations and C : N : P ratios of algae from the chemostats were monitored throughout each experimental run. Algal samples were collected on precombusted GF/F filters and were dried in an oven at 60 °C prior to analysis for C and N contents (using Perkin–Elmer model 2400 elemental analyzer; Perkin-Elmer, Wellesley, MA, U.S.A.) and P content (using persulphate oxidation followed by the acid molybdate technique; APHA, 1998).

Growth experiments

Laboratory experiments were conducted on a large number of neonates of *D. galeata*, *D. pulex*, *D. magna* and two isolates of *D. pulicaria* (identified hereafter as Biwa and BL) of approximately the same age, size and condition. All *Daphnia* used in this experiment were from long-standing laboratory stocks isolated by

Table 1 *Scenedesmus* spp. quantity (mg C l^{-1}) and quality (C : P) supplied to five clones/species of *Daphnia*

Daphnia species	Quantity	Quality	Growth rate	*n*
Daphnia pulex	0.1–0.2	1022	0.49 (0.02)	29
	0.4–1.6	1022	0.60 (0.01)	39
	0.1–0.2	122	0.55 (0.01)	33
	0.4–1.6	122	0.72 (0.02)	39
Daphnia magna	0.1–0.2	1067	0.21 (0.01)	43
	0.4–1.6	1067	0.35 (0.02)	47
	0.1–0.2	145	0.25 (0.01)	42
	0.4–1.6	145	0.53 (0.01)	46
Daphnia pulicaria (BL)	0.1–0.2	997	0.19 (0.01)	42
	0.4–1.6	997	0.29 (0.02)	56
	0.1–0.2	144	0.30 (0.01)	48
	0.4–1.6	144	0.44 (0.02)	57
Daphnia pulicaria (Biwa)	0.1–0.2	981	0.11 (0.02)	9
	0.4–1.6	981	0.29 (0.02)	12
	0.1–0.2	112	0.38 (0.01)	11
	0.4–1.6	112	0.58 (0.02)	12
Daphnia galeata	0.1–0.2	1059	0.08 (0.02)	9
	0.4–1.6	1059	0.37 (0.02)	11
	0.1–0.2	120	0.26 (0.02)	11
	0.4–1.6	120	0.59 (0.02)	11

Mean growth rates (day^{-1}) and standard errors are given for each combination of quantity and quality treatment for the five species/clones with total number of animals measured for each combination (*n*).

several laboratories. *Daphnia galeata* and *D. pulicaria* (Biwa) were collected from Lake Biwa by J. Urabe, *D. pulex* and *D. pulicaria* (BL) were collected in Oklahoma by L. Weider and K. Looper, and *D. magna* was obtained from R. Sterner. All cultures were acclimated to growing in a modified animal COMBO for many generations. From each species, several individuals were grown individually in approximately 200-mL COMBO medium supplied with high concentrations (>1.5 mg C L^{-1}) of P-rich chemostat algae until they were actively reproducing after several clutches. Post-third-clutch neonates from these animals were then grown to maturity by the same method, but individually in jars containing COMBO medium and supplied with high quantities of high-quality algae daily. Every other day, each individual was transferred by pipette to a clean culture jar until it produced its third clutch. At the start of each experiment, neonates (within 24 h of birth) from the third and subsequent clutches of these individual animals were then pooled and used for the growth experiments. For each treatment combination nine to 57 animals were used for each treatment combination. Each experiment lasted 3 days.

Growth rate was estimated by measuring the rate of change of animal body mass using video image analysis (Acharya *et al.*, 2004). Individual animals were placed in small plastic Petri dishes and media was removed until animals were stationary and laterally positioned. Using a MagnaFire™ SP camera (Optronics, Goleta, CA, U.S.A.) mounted on a Leica Wild™ M3Z microscope (Heerbrugg, Switzerland) images of each animal were captured at 25× and animals were immediately re-suspended in N- and P-free COMBO medium and monitored for any physical effects from the procedure before being transferred to 70-mL jars (one individual per jar) containing the appropriate food suspension. These bottles were slowly rotated using a plankton wheel to maintain the algae in suspension. Food and media were replenished every 24 h by pipette transferring the *Daphnia* into fresh media. All growth experiments were conducted at a constant temperature (25 °C) and a light : dark cycle of 14 h : 10 h. Final measurement of body size was made after 72 h using the same approach as the initial measurements. From each photograph, the body outline (without tail spine) was traced using Image-Pro™ Express software (Media-Cybernetics, Silver Spring, MD, U.S.A.) to obtain a measurement of body area. Initial and final body area measurements were converted to body dry mass (see below) for calculation of growth rate (μ: in units of day^{-1}). After the final image capture, animals were transferred to sample containers and either dried (P samples) or placed in liquid nitrogen (RNA samples) and stored at −80 °C for later analysis (see below). RNA was analysed on individual animals while P was analysed on small groups (two to four individuals) from each treatment. A minimum of two replicate samples was run for RNA and P analyses.

Mass–area regression

Simultaneous with the experiments just described, a second experiment was performed on each species for both high or low food quality (C : P) levels to establish the relationship between body area (from image analysis) and body mass. From the large cohort of *Daphnia* neonates from which the other experimental animals were collected, three to four replicates consisting of eight to 10 animals each were pooled in 500-mL jars containing food and media. A few animals were randomly photographed and placed

immediately on preweighed tins, dried at 60 °C and weighed using a Mettler-Toledo microbalance (0.1 μg). Individual animal dry mass and average area were determined by dividing either total dry mass or total area determined by image analysis by the number of animals in the sample. Animals remaining in the 500-mL jars were transferred daily to new food and media, similar to the experimental animals. After 72 h all the animals were photographed and animals were randomly pooled into three replicates groups and then placed onto preweighed tins, dried at 60 °C and weighed. A regression analysis was performed for the average dry mass and the average image area measurements, including data from both the initial and final samples. A separate regression was performed for each food quality treatment for each species. All the mass–area regressions were linear ($R^2 > 0.9$, $P < 0.001$). Once initial and final dry masses of an individual were determined, its specific growth rate (μ, d^{-1}) was calculated as $\ln(m_e/m_i)/t$, where m_e is body dry mass at the end of the growth period, m_i is its initial mass and t is the duration of the growth period (3 days).

Chemical analyses

The P contained in individual 3-day-old animals was determined using colorimetric analysis after persulphate digestion (APHA, 1998) modified for low volume extractions, and converted to P content (% of dry mass, %P) using the body area–dry mass regression appropriate for the food quality experimental treatment. The amount of RNA contained in individual animals was determined using the microfluorometric method of Kyle *et al*. (2003) and converted to content measures (% of dry mass, %RNA) using the appropriate food quality body area–dry mass regression for each species. The P content due to RNA (%RNA–P) was calculated by assuming that RNA contains 8.6% P by mass (Sterner & Elser, 2002).

Statistical analyses

Effects of food quality and quantity were tested for each species by analysis of variance (ANOVA) following tests for homoscedasticity and normality. Regression analysis was used to examine the associations between %P or %RNA and μ, and between %P and %RNA–P. Following this, analysis of covariance (ANCOVA) was used to test for interspecific differences in the slope relationships of %P or %RNA with μ and of %P with %RNA–P.

Results

Analysis of variance indicated that for all species, food quality and quantity had a significant effect on μ (Table 2). In addition, both the quality and the quantity of the algae significantly affected %P for all species except one clone of *D. pulicaria*, which was only affected by food quantity, not quality. The influences of algal quality and quantity on %RNA were less obvious. Both food quality and quantity had significant effects on *D. pulex* and *D. galeata* %RNA, while food quantity alone affected *D. pulicaria* (BL) %RNA. For *D. magna* and *D. pulicaria* (Biwa), RNA content was not affected by either quality or quantity treatments.

To more closely examine the associations among μ, RNA and P, regression analysis was performed for each species. There was a significant correlation between %P and μ, %RNA and μ, and between %P and %RNA–P for each species except *D. galeata* (Table 3). These associations were relatively strong even though juvenile *Daphnia* exhibit ontogenetic variation in growth rate during the first days of life (DeMott, 2003) and our %P and %RNA data reflect an end-point determination on 3-day-old animals while the determinations of μ reflect growth processes accumulated over the previous 3 days. If closer-to-instantaneous determinations of μ had been made,

Table 2 Results of ANOVA testing the effects of food quality and quantity on growth rate, %P and %RNA for five *Daphnia* species/clones

	Growth rate		%P		%RNA	
Daphnia species	Quality	Quantity	Quality	Quantity	Quality	Quantity
Daphnia pulex	0.0063	0.0002	<0.0001	0.0069	0.025	0.0012
Daphnia magna	<0.0001	0.0014	<0.0001	0.0002	NS	NS
Daphnia pulicaria (BL)	<0.0001	<0.0001	NS	0.011	NS	0.0108
Daphnia pulicaria (Biwa)	0.023	0.014	0.042	0.042	NS	NS
Daphnia galeata	<0.0001	<0.0001	0.0009	0.0012	0.0024	0.0058

Table 3 Results of regression analyses testing relationships between %P or %RNA and μ, and between %P with %RNA–P for five *Daphnia* species/clones

Regression		*Daphnia* species	Slope	Int.	$P<$	R^2
P content	Growth rate	*Daphnia galeata*	0.95	0.82	0.09	0.82
		Daphnia magna	1.79	0.37	0.006	0.74
		Daphnia pulex	2.04	−0.31	0.005	0.75
		Daphnia pulicaria (Biwa)	1.25	0.74	0.01	0.98
		Daphnia pulicaria (BL)	1.04	0.95	0.02	0.70
RNA content	Growth rate	*D. galeata*	4.85	1.90	0.2	0.62
		D. magna	5.30	0.98	0.015	0.65
		D. pulex	8.47	−1.70	0.0004	0.89
		D. pulicaria (Biwa)	7.96	2.12	0.04	0.91
		D. pulicaria (BL)	6.16	2.63	0.005	0.81
P content	RNA–P content	*D. galeata*	1.84	0.55	0.029	0.94
		D. magna	2.71	0.29	0.026	0.59
		D. pulex	2.44	0.17	0.009	0.70
		D. pulicaria (Biwa)	1.66	0.44	0.01	0.97
		D. pulicaria (BL)	1.85	0.52	0.003	0.84

closer associations between chemical composition and growth rate may have been observed.

Inspection of the regressions for the four species suggested that, for each combination of variables, there was more variation in the intercepts than in the slopes (Figs 2–4). To evaluate this, ANCOVA was performed. The main effect of species was significant for both %P and %RNA ($P < 0.0001$) as was the effect of the covariate (μ). However, the species–μ interaction was non-significant ($P = 0.41$), indicating that the slopes of the %RNA–μ and %P–μ relationships were uniform for these species but that the relationships differed in the intercept. We also performed ANCOVA to examine possible species differences in %P–%RNA–P relationships using %RNA as the covariate. Similar to the growth ANCOVA results, there was no significant species–covariate interaction ($P > 0.41$), indicating a similar coupling of P to RNA across species. However, the main effects of the covariate and of species were both significant ($P < 0.0001$ for the covariate, $P < 0.03$ for species), indicating differences among species in the intercept of their %P versus %RNA–P relationships.

The *y*-intercepts of %P–μ and %RNA–μ relationships indicate RNA and P contents at zero growth rate and thus the differences among species in these intercepts suggest that the species differ in the background or maintenance levels of both %P and %RNA. Similarly, the significant difference among species in the intercepts of the %P–%RNA–P

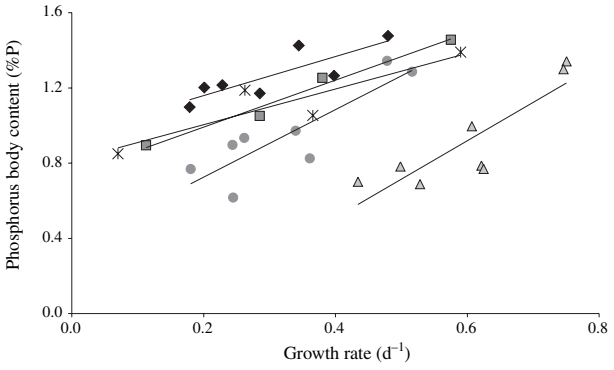

Fig. 2 Relationships between body phosphorus content (%P) and growth rate for five species/clones of *Daphnia*: *Daphnia pulex* (triangle), *Daphnia magna* (circle), *Daphnia pulicaria* (Lake Biwa clone; square), *Daphnia pulicaria* (BL clone; diamond) and *Daphnia galeata* (star).

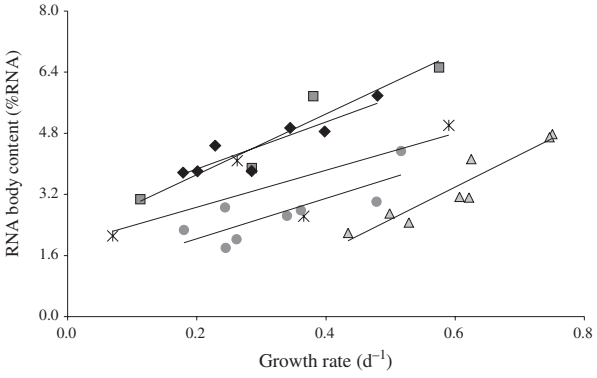

Fig. 3 Relationships between body RNA content (%RNA) and growth rate for five species/clones of *Daphnia*: *Daphnia pulex* (triangle), *Daphnia magna* (circle), *Daphnia pulicaria* (Lake Biwa clone; square), *Daphnia pulicaria* (BL clone; diamond) and *Daphnia galeata* (star).

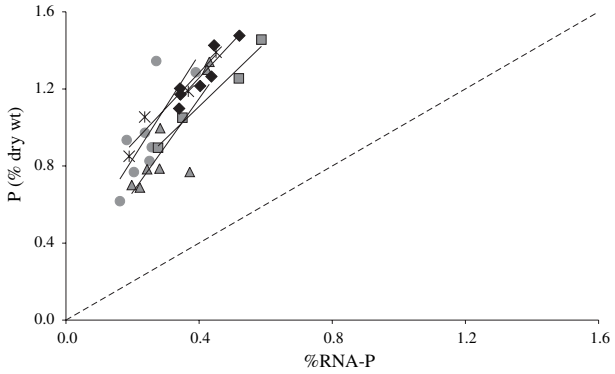

Fig. 4 Relationships between body phosphorus content (%P) and %RNA–P for five species/clones of *Daphnia*: *Daphnia pulex* (triangle), *Daphnia magna* (circle), *Daphnia pulicaria* (Lake Biwa clone; square), *Daphnia pulicaria* (BL clone; diamond) and *Daphnia galeata* (star). %RNA–P was calculated by multiplying the %RNA data by 0.086, the fraction of RNA mass contributed by *P* (Sterner & Elser, 2002). The solid lines indicate the regression lines for each species fit to all data points while the broken line indicates the 1 : 1 line. Displacement above the 1 : 1 lines indicates the contribution of non-RNA–P to overall P content.

relationships indicates that the species differ in their content of non-RNA phosphorus.

However, these ANCOVA analyses tell little of the among-species variations at maximum growth rate (μ_{max}). To examine this, one-way ANOVA was used to evaluate differences among species in μ_{max}, maximum P content ($\%P_{max}$) and maximum RNA content ($\%RNA_{max}$). In this analysis we confined the data considered to the treatment for each species in which P-sufficient algae were supplied at high concentration. Consistent with scenario e (Fig. 1), the species effect was significant for μ_{max} ($P = 0.046$) but not for $\%P_{max}$ ($P = 0.96$) or $\%RNA_{max}$ ($P = 0.79$).

Discussion

Prior work on *Daphnia* has shown that growth rate varies with both algal quantity and quality (Sterner, 1993; Boersma & Kreutzer, 2002), as shown by our data. Similarly, the finding that consumer %P is affected by the quality and quantity of the food source is not unusual in light of other studies (DeMott *et al*., 1998; Vrede, Persson & Aronsen, 2002). However, the more surprising finding of our study is that food quality and quantity did not have a consistent effect on %RNA in this set of species. This suggests that there is another possible control over body RNA content not explained by growth-related effects driven by food availability or food C : P ratio (food quality).

The generally strong intraspecific (physiological) couplings among μ, %RNA and %P that we observed provide strong support for the growth rate hypothesis (Table 2, Figs 2–4). However, in contradiction to the original formulation of the growth rate hypothesis, there was no clear association among μ_{max}, RNA_{max} and P_{max} for this set of species. This is perhaps not unexpected, given previous observations of μ_{max} and %P in a previous study of more *Daphnia* species (DeMott & Pape, 2005) and the very limited range of maximum growth rates exhibited within the relatively narrow *Daphnia* clade. While this set of species seems to have universal %P–μ, %RNA–μ and %P–%RNA couplings (i.e. similar slopes), they do not share a common 'background level' of RNA or *P* (similar to scenario e in Fig. 1). Thus, this suggests that *Daphnia* evolve different growth rates within potential phylogenetic or ecological constraints not by increasing their maximal allocations to RNA (and thus to P) but instead by altering the levels of RNA and P they must allocate to non-growth-related processes ('maintenance').

Consistent with the findings of Elser *et al*. (2003), this set of *Daphnia* species exhibited a strong positive relationship between %P and %RNA–P (Fig. 4). However, unlike the aggregated data for multiple species in that study, the slope of this relationship for *Daphnia* is considerably steeper (1.92 versus 0.97). What this means is that as RNA content increases with increasing growth rate, there is a pool of non-RNA–P that increases even more rapidly. This implies that, in *Daphnia* at least, rapid growth rate is even more P intensive than can be explained by the increased demand for RNA alone. The species also differed overall in the relative importance of this non-RNA–P. The form of this growth-dependent, non-RNA–phosphorus pool is not clear; perhaps this P is associated with short nucleic acids, single nucleotides or nucleotide precursors that are not detected by our fluorometric RNA-binding method.

A potentially important result from our analysis is that, while the species studied were quite similar in how RNA and P variations were coupled to growth (similar slopes in content versus μ relationships), they differed significantly in the background or maintenance levels of RNA and P that they carry, as reflected in significant differences among species in the intercepts of the content versus growth relationships. Our

data cannot resolve the causes of these differences and more investigation is needed. One possibility is that some species might have high overall rates of protein turnover and remodelling (Hawkins, 1991). In this scenario, high levels of RNA (and thus P) are needed for protein synthesis despite relatively modest growth rate. For example, differences in maintenance energy expenditures among individual mussels were shown to be determined by differences in dominant proteolytic enzymes and pathways used by different individuals (Hawkins & Day, 1996). Thus, differences in overall protein synthesis demands (and thus RNA levels) may be set not only by growth-related demands but also by potential differences among species in the relative importance and pathways of protein retention and reclamation in overall metabolism. To our knowledge, no empirical studies of protein turnover have yet been performed for *Daphnia*, despite the importance of assumptions about protein turnover for emerging stoichiometric models of *Daphnia* growth and metabolism (Vrede *et al*., 2004; Anderson *et al*., 2005).

Life histories vary widely among taxa. Different patterns of allocation of resources to various activities are linked with important life history traits such as development rate, size at maturity and reproductive output (Dudycha & Lynch, 2004). To increase its growth rate an individual must either raise its resource intake or, if the resource supply is limited, decrease its metabolic losses of the limiting resource (Gurney *et al*., 2003). However, organisms live in an environment with highly variable food supplies and evolve within developmental constraints. Therefore, within a phylogenetic group there may be a fundamental framework around which trait variation is organised (Dudycha & Lynch, 2004). Furthermore, the biochemical processes that generate key life history traits (e.g. pace of development) must occur within fundamental biochemical and stoichiometric constraints, such as the limits set by the couplings among growth, RNA and P shown in our study. This suggests that a relatively simple set of rules govern how metabolic processes impinge on the evolutionary ecology of zooplankton life history strategies.

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