

## N:P stoichiometry and ontogeny of crustacean zooplankton: A test of the growth rate hypothesis

**Abstract**—Recent studies have hypothesized that differences in elemental composition of zooplankton (N:P ratio) are related to differences in specific growth rate, as increased growth rate requires greater ribosomal RNA, which should result in increased %P and decreased N:P. This hypothesis was tested interspecifically using zooplankton taxa with different specific growth rates and intraspecifically using different ontogenetic stages within single species. Among all five species, growth rate was positively correlated with %N and %P and negatively correlated with N:P. However, %N increased only from ~8% to ~9% while %P varied fourfold across the observed range of growth rates, indicating that changes in %P drive the negative relationship between growth rate and N:P. Intraspecific comparisons showed that growth rate was positively related to %P and negatively related to N:P for *Daphnia lumholtzi*, but growth rate was unrelated to %P and N:P for *D. magna* and *D. obtusa*. Mean %P, %N, and N:P differed significantly among *Daphnia* spp. and among populations of *Scapholebris mucronata* and *Bosmina longirostris*. These data strongly support the growth rate hypothesis as an explanation for differences in body N:P in zooplankton and indicate that the biological stoichiometry of a rapid growth rate life-history is phosphorus intensive.

Recent studies have shown that different zooplankton taxa have different body N:P ratios (Andersen and Hessen 1991; Hessen and Lyche 1991), and it now appears that body stoichiometry of crustacean zooplankton is an important determinant of the effects of consumers on pelagic ecosystems (Sterner et al. 1992; Elser and Hassett 1994; Urabe 1995; Urabe et al. 1995). In particular, body N:P directly determines ratios of limiting nutrients (N:P) recycled by elementally homeostatic consumers (Sterner 1990; Urabe 1993; Urabe et al. 1995). When feeding on food of a given N:P ratio, high N:P consumers (e.g. calanoid copepods) will recycle N and P at low ratios, differentially retaining N and releasing P (Sterner 1990), whereas low N:P taxa (such as *Daphnia*) in contrast should generate high recycled N:P (Urabe 1993). Such processes are likely responsible for prior observations of shifts in the identity (N vs. P) of the primary limiting nutrient for phytoplankton growth following alterations of food web structure (Elser et al. 1988; Sterner et al. 1992). Zooplankton N:P is also important in understanding effects of food quality on secondary production in aquatic ecosystems. Specifically, zooplankton with low body N:P ratios and high P demands for growth (e.g. *Daphnia* spp.) are particularly sensitive to the P content of their food and suffer decreased growth and reproduction when consuming food with low P content (Sterner and Hessen 1994).

Why does body stoichiometry (N:P ratio) differ among consumer taxa? It has been hypothesized that interspecific differences in body N:P are related to differences in their

specific growth rate (Sterner 1995; Elser et al. 1996). This hypothesis states that slowly growing species (i.e. freshwater copepods) have a lower content of P-rich RNA and thus a lower P composition (as a percentage of dry weight; %P), while rapidly growing species (i.e. *Daphnia*) require a greater ribosomal RNA content for rapid protein synthesis and consequently have higher %P and lower body N:P. Thus, differences in body stoichiometry among consumer taxa may be explained by differential biochemical investments resulting from species-specific differences in specific growth rate. To test this hypothesis, one can compare a variety of zooplankton species having different characteristic specific growth rates. The growth rate hypothesis can also be tested by considering intraspecific variation, as many taxa undergo substantial shifts in specific growth rate during ontogeny. To test this hypothesis both inter- and intraspecifically, we measured specific growth rate, %N, and %P in various size classes of the cladocerans *Daphnia magna*, *Daphnia obtusa*, *Daphnia lumholtzi*, and in populations of *Scapholebris mucronata* and *Bosmina longirostris*. Based on the growth rate hypothesis, we predicted that body N:P should be negatively related to specific growth rate both among different zooplankton taxa and among ontogenetic stages within single zooplankton taxa. This pattern we predict will be primarily due to differences in body %P among animals given that P composition, and not N composition, differs most strongly among biologically significant molecules and organelles (Elser et al. 1996).

Populations of laboratory-raised *D. magna*, *D. obtusa*, *D. lumholtzi*, *S. mucronata*, and *B. longirostris* were cultured in 20-liter tanks at 24°C for several months prior to the experiments. All cultures were fed a 50:50 (by dry weight, DW) mixture of yeast and *Scenedesmus acutus* every 2–3 d. Food was added at high, saturating levels (~2 mg DW liter<sup>-1</sup> for *Scenedesmus*) to assure that animals were growing at their full growth potential. *Daphnia* populations were size-fractionated into three to four size classes. *D. magna* were divided into four size groups using 750, 1,000, and 1,350- $\mu$ m Nitex screen while *D. obtusa* were divided into three groups using 560- and 750- $\mu$ m Nitex screen. *D. lumholtzi* experienced high mortality during screening, so animals were hand-picked into a large size group and a small size group. Only adults were chosen for the large size group, and care was taken to select similar-sized animals for the small size group.

For each species, 30–40 individual animals from each size fraction were then hand-picked into replicate 1-liter jars. Three randomly selected jars from each size fraction were split into equal aliquots to obtain initial values of DW, %N, and %P. For initial DW determination, all animals from each aliquot were placed onto a preweighed, precombusted glass-

fiber filter, which was dried overnight at 60°C and reweighed on a microbalance ( $\pm 1 \mu\text{g}$ ). The total initial DW for each jar was then estimated by summing the DW from the two replicate filters, and the mean weight per individual was calculated as the summed DW/no. of animals per jar. Animals in the remaining jars were incubated for 2 d at 25°C and fed ad libitum with yeast and *S. acutus*, a diet previously found to yield best growth. After this 2-d incubation, the remaining jars were sampled for determination of final DW and %N and %P by placing the animals onto preweighed, precombusted glass-fiber filters that were then analyzed as described above. *S. mucronata* and *B. longirostris* were too small to divide on the basis of size, so replicate samples were taken from exponentially growing laboratory populations over a 2-d period (25°C, ad libitum food) for determination of growth rate. Samples were taken at 1-d intervals, preserved using Lugol's solution, and measured (body length) under a dissecting microscope.

Initial and final DWs for individuals from the *S. mucronata* and *B. longirostris* populations were calculated according to Rosen's (1981) length-weight relationship for *Scapholebris kingi* (Eq. 1) and *B. longirostris*

$$\ln(w) = 2.8713 + 3.079 \ln(L) \quad (1)$$

$$\ln(w) = 4.9344 + 4.849 \ln(L), \quad (2)$$

where  $\ln(w)$  is the logarithm of the DW estimate ( $\mu\text{g}$ ) and  $L$  is the total length from the head to the base of the tail spine (mm). For all species, specific growth rate ( $\mu$ , per d) was calculated as

$$\mu = \frac{1}{2} \ln(\text{final/initial biomass}). \quad (3)$$

For *Daphnia* species, the "biomass" values in Eq. 3 are total biomass estimates for *Daphnia* in each jar on day 0 or day 2, including both individual growth and production of young. For *S. mucronata* and *B. longirostris*, biomass values are total population biomass values on day 0 or day 2, as estimated from population counts and microscopic determinations of average animal biomass; these data thus also reflect individual growth plus production of offspring but omit potential mortality, which is assumed to be negligible in an exponentially growing population. While most of the increase in biomass seen in both methods likely reflects somatic growth rather than the production of young, we do not think reproductive growth confounds our results, as the ability of cladocerans to rapidly produce large numbers of young probably involves investment in P-rich ribosomes and should be reflected in body N:P stoichiometry. Thus, brood production is included in our growth estimates, but young were not separated from adults prior to elemental composition analysis.

For all %P determinations, replicate samples of the reweighed, dried animals were analyzed using persulfate oxidation followed by analysis of orthophosphate using the acid molybdate technique (APHA 1992), while %N content was determined using a Perkin-Elmer model 2400 elemental analyzer.

All %N, %P, and N:P data were log transformed before statistical analysis to stabilize variances. Intraspecific comparisons were performed via one-way ANOVA with ele-

Table 1. Dry weights (DW; mean and SE) for different size classes of the five species used. Size classes for *Daphnia magna* and *Daphnia obtusa* are based on mesh sizes while *Daphnia lumholtzi* was hand-picked. For *Bosmina longirostris* and *Scapholebris mucronata* animals were not size fractionated.

Species	Size class ( $\mu\text{m}$ )	DW ( $\mu\text{g}$ )	SE
<i>Bosmina longirostris</i>	All	0.34	0.03
<i>Scapholebris mucronata</i>	All	0.92	0.03
<i>Daphnia lumholtzi</i>	Small	8.84	0.29
	Large	35.87	1.60
<i>Daphnia magna</i>	<750	16.81	0.72
	750–1,000	33.62	2.69
	1,000–1,350	63.75	5.37
	>1,350	131.46	6.49
<i>Daphnia obtusa</i>	<560	3.18	1.86
	560–750	10.85	0.67
	>750	22.18	0.94

mental composition (%N, %P, N:P) as the dependent variable and size class as the independent variable. Interspecific comparisons were made using a one-way ANOVA with species as the independent variable and elemental composition (%N, %P, N:P) as the dependent variable. The Bonferroni-Dunn test was applied for individual pairwise comparisons. Additionally, elemental composition (%N, %P, N:P) was regressed against growth rate for all size classes across species. Finally, estimates of mean individual body size ( $\mu\text{g}$  DW) for animals of each species within each size fraction (Table 1) were regressed against mean individual growth rate and elemental content.

Analysis of variance revealed that differences in %N among size classes were statistically significant for all *Daphnia* species (all  $P < 0.03$ ). However, these differences were small and were not strongly correlated with growth rate within a given species (Fig. 1A). Differences in %P among size classes were also statistically significant for all *Daphnia* species (all  $P < 0.05$ ). In contrast to %N, %P was strongly related to specific growth rate within *D. lumholtzi* ( $r^2 = 0.89$ ,  $P = 0.002$ ) but was unrelated for *D. obtusa* and *D. magna* (Fig. 1B). Differences in body N:P among size classes were statistically significant for all three *Daphnia* species (all  $P < 0.003$ ). Within species, body N:P also was negatively correlated with specific growth rate for *D. lumholtzi* ( $r^2 = 0.88$ ,  $P = 0.003$ ), but no relationship was observed for *D. magna* and *D. obtusa* (Fig. 1C).

When the data for all species and size classes were considered together, differences among species in %N, %P, and N:P were strong (all  $P < 0.0001$ ). There was a significant, positive relationship between %N and specific growth rate across all species ( $r^2 = 0.38$ ,  $P = 0.04$ ; Fig. 1A). However, %N increased only from ~8% to ~9% across the observed range of growth rate, a change that likely has little biological or ecological significance. In contrast to %N, %P varied fourfold across the observed range of growth rates and was strongly and positively correlated with specific growth rate ( $r^2 = 0.65$ ,  $P = 0.002$ ; Fig. 1B). Thus, the observed decline of body N:P with specific growth rate ( $r^2 = 0.37$ ,  $P = 0.04$ ; Fig. 1C) primarily reflects changes in %P. Finally, no sig-

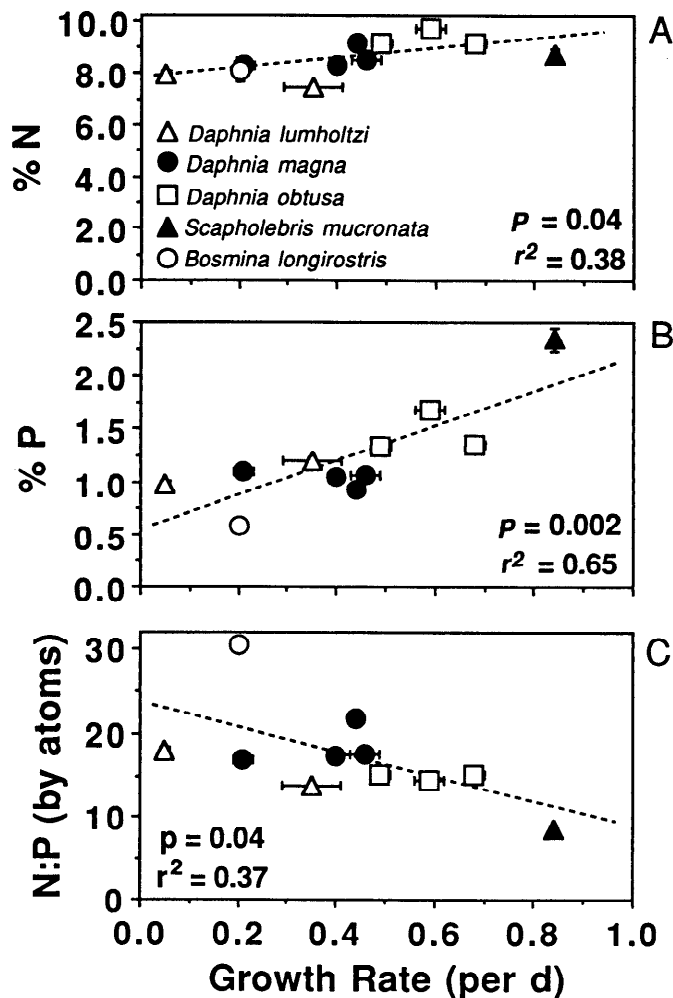


Fig. 1. Relationship between specific growth rate and (A) %N, (B) %P, and (C) N:P in five species of zooplankton. Data points represent mean values for growth rate and %N, %P, and N:P from replicate samples for each size fraction in *D. lumholtzi* (two size groups), *D. magna* (four size groups), and *D. obtusa* (three size groups), and for replicate samples from populations of *S. mucronata* and *B. longirostris*. Error bars represent  $\pm 1$  SE. The line represents the best fit from least-squares regression.

nificant relationship was found between body size ( $\mu\text{g DW}$ ) and specific growth rate ( $r^2 = 0.18$ ,  $P = 0.19$ ; Fig. 2), %N ( $r^2 = 0.06$ ,  $P = 0.47$ ; Fig. 3A), %P ( $r^2 = 0.06$ ,  $P = 0.48$ ; Fig. 3B), and N:P ( $r^2 = 0.01$ ,  $P = 0.75$ ; Fig. 3C) among species.

Although we did not separate eggs from adults during elemental analyses, our elemental ratios do not diverge much from Hessen (1992), who estimated that for *D. magna*, egg elemental content (N:P) was lower than adult elemental content. Because our estimate of adult + egg N:P ratio ( $\sim 17:1$  in largest size fraction of *D. magna*) is consistent with Hessen's estimate of egg N:P ratio (15.5:1), we do not think that the N:P ratio of our largest size category is an overestimate.

Our intraspecific results for *D. lumholtzi* indicate that elemental composition can change significantly during ontogeny in cladoceran taxa, supporting the hypothesis that dif-

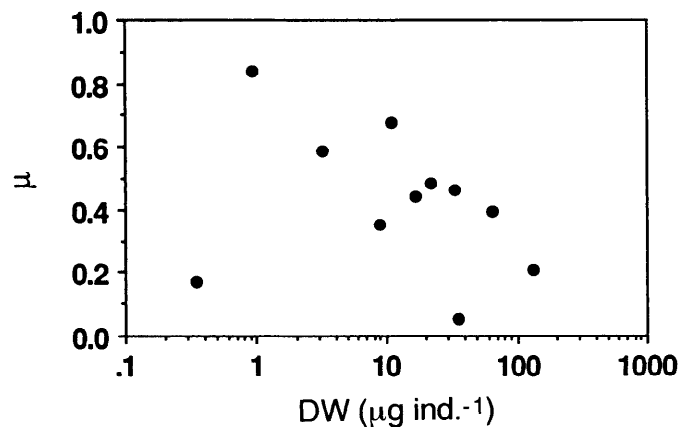


Fig. 2. Specific growth rate ( $\mu$ ) vs. mean dry weight (DW,  $\mu\text{g ind.}^{-1}$ ) in five species of zooplankton. Data represent mean values of DW ( $\mu\text{g}$ ) and growth rate for each size class of five species of zooplankton.

ferences in elemental content are related to specific growth rate. During ontogeny, both body size and specific growth rate covaried with elemental content within a species. Generally, there is a strong association between specific growth rate and body size across a very wide range of body size (Peters 1983). However, because we found no significant relationship between body size and specific growth rate, we conclude that elemental composition is more important than body size as a determinant of specific growth rate among these taxa. Elser et al. (1996) pointed out that biomolecules and cellular organelles vary far more in %P than in %N, and thus rapidly growing stages should tend to have a higher ribosomal RNA content, a higher %P, and a lower N:P ratio than do slowly growing stages. Our intraspecific results for *D. lumholtzi* support this idea, as %P varied much more between ontogenetic stages in these taxa than did %N. Our results for *D. lumholtzi* are also consistent with findings by McKee and Knowles (1987), who showed strong changes in RNA content during ontogeny. Although some of our intraspecific results indicate that N:P ratio can differ between ontogenetic stages, these changes were not general to all taxa (e.g. *D. magna* and *D. obtusa*). Additionally, within a species, body N:P of animals was not tightly correlated with specific growth rate at different life stages. This result may reflect decoupling of N:P and growth rate in the species studied. Alternatively, the nonsignificant correlations may reflect the fact that the range of variation of specific growth rate manifested during ontogeny within these species was relatively modest. Due to the lack of a relationship between growth rate and N:P within these species, we suggest that it is necessary to consider the effects of specific life-history strategies, aside from specific growth rate, on elemental content within individual species.

Our interspecific results covered a wider range of specific growth rates and strongly support our hypothesis that body N:P is linked to specific growth rate. To our knowledge, these data represent the first direct test of the growth rate hypothesis of Elser et al. (1996). Across all species tested, specific growth rate was a strong predictor of elemental composition. For example, *B. longirostris* had the second lowest

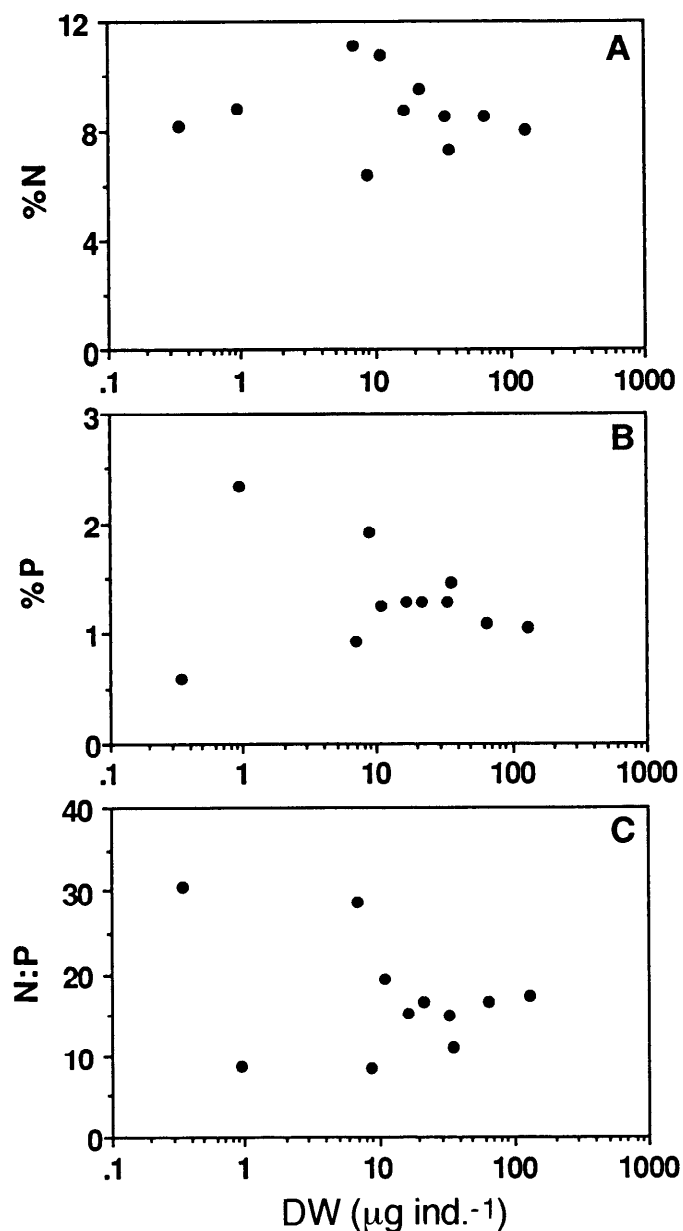


Fig. 3. Dry weight (DW) vs. (A) %N, (B) %P, and (C) N:P ratio in five species of zooplankton. Data points represent mean values of DW ( $\mu\text{g}$ ) and elemental content for each size class of five species of zooplankton.

growth rate and the highest N:P ratio, whereas *S. mucronata* had the highest growth rate and the lowest N:P ratio. These results support the idea that different life-history strategies among consumer species necessarily demand differential acquisition of biologically important elements (especially P), likely resulting in contrasting effects of those consumer species on nutrient recycling via food-consumer N:P stoichiometry (Elser et al. 1996).

The interspecific differences in body N:P that we document here are similar to those reported by Andersen and Hessen (1991) and Hessen and Lyche (1991): *Daphnia* species generally had low N:P while *Bosmina* had high N:P.

As argued by Sterner (1990) and Sterner et al. (1992), and directly demonstrated by Urabe et al. (1995), these differences imply that shifts in zooplankton community structure will generate changes in the relative rates of consumer-driven recycling of N and P. However, our intraspecific results indicate that it is not sufficient to know only the species composition of the zooplankton community to make inferences about the likely nature of the consumer-driven N:P recycling regime (i.e. % *Daphnia* or % calanoid copepods). For example, Elser et al. (1995) documented shifts in the relative importance of P or N limitation of phytoplankton growth in Castle Lake, examining shifts in N or P limitation associated with seasonal changes in zooplankton community N:P estimated by assuming all individuals of a given species had the same N:P. These associations were generally strong for two of the study years but not for the third. It is possible that the years differed in the age and/or size distribution of important zooplankton species, preventing an accurate assessment of the actual N:P of the zooplankton communities present. Ontogenetic dependence of body %P implies that rapidly growing stages of *Daphnia* or other cladocerans, which have higher P demands for growth and body maintenance compared with animals that grow more slowly, will recycle at a higher N:P ratio. Thus it may also be necessary to characterize the population age structure of dominant zooplankton taxa to accurately characterize the potential recycling regime in a given system.

Intra- and interspecific differences in body N:P content may also have implications for P-based food quality constraints on low N:P taxa. Rapidly growing animals with a low body N:P ratio will have especially high P demands for growth and thus are potentially most likely to be constrained by poor food quality (low algal P content; Urabe and Watanabe 1992; Sterner and Hessen 1994). Thus, body N:P ratios may reflect the degree of zooplankton sensitivity to P-based food quality. For example, a survey of temperate lakes found a mean seston N:P of  $\sim 40$  by moles (Elser and Hassett 1994). For zooplankton with body N:Ps substantially below 40 (e.g. *Daphnia*, *Scapholebris*), the resulting elemental imbalance between food and consumer may create P-based food quality constraints. As argued by Hessen (1992), this may also help to explain why some taxa fail to establish in some lakes.

Furthermore, our data on ontogenetic variation in body stoichiometry may have important implications for the phenomenon of intraspecific facilitation (sensu Sommer 1992) under P-limited herbivore growth as mediated by consumer-driven nutrient recycling feedbacks. Sommer (1992) showed that strongly P-limited chemostats were noninvasible by *Daphnia* seed populations but that *Daphnia* could invade slightly less P-limited chemostats, achieving high densities and driving algal biomass to low levels. Sommer argued that in these experiments *Daphnia* overcame constraints imposed by poor quality food by improving food quality (lowering food C:P) as *Daphnia* populations increased in density, lowering algal cell density (and thus P demand) while recycling P. In a series of field experiments, Urabe (1995) showed that experimenter-induced increases in *Daphnia* density lowered seston C:P, primarily by increasing the per capita supply of P to algae. The ability of a *Daphnia* population to establish

under poor food quality conditions thus appears to hinge on whether a demographic bottleneck can be overcome, permitting grazing and nutrient recycling to improve food quality. Our data show that early life stages of *Daphnia* have particularly high P composition and low body N:P and thus may be particularly sensitive to the effects of food quality. This suggests that the demographic constraint on population establishment under poor mineral nutrition may operate primarily via juvenile growth and maturation rates. However, most consideration of effects of food quality on *Daphnia* populations to date have focused on late juveniles and adults and thus may underestimate the potential stoichiometric constraints on *Daphnia* success. Thus, P-limitation of *Daphnia* populations (or other low N:P taxa) may be even more common than previously suggested.

We have presented and tested a hypothesis to explain observed variation in zooplankton body N:P. Our data indicated that specific growth rate is strongly correlated with %P and N:P within and especially between species. These data provide strong support for the cellular-based mechanisms linking specific growth rate and elemental composition proposed by Elser et al. (1996). These relationships also suggest provocative links between evolutionarily derived traits (i.e. specific growth rate) and ecosystem-level phenomena associated with food-web dynamics.

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