

STOICHIOMETRY AND PLANKTONIC GRAZER COMPOSITION OVER GRADIENTS OF LIGHT, NUTRIENTS, AND PREDATION RISK

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Abstract. Mechanisms that explain shifts in species composition over environmental gradients continue to intrigue ecologists. Ecological stoichiometry has recently provided a new potential mechanism linking resource (light and nutrient) supply gradients to grazer performance via elemental food-quality mechanisms. More specifically, it predicts that light and nutrient gradients should determine the relative dominance of P-rich taxa, such as *Daphnia*, in grazer assemblages. We tested this hypothesis in pond mesocosms (cattle tanks) by creating gradients of resource supply and predation risk, to which we added diverse assemblages of algal producer and zooplankton grazer species. We then characterized the end-point composition of grazer assemblages and quantity and elemental food quality of edible algae. We found that somatically P-rich *Daphnia* only dominated grazer assemblages in high-nutrient, no-predator treatments. In these ecosystems, P sequestered in producers exceeded a critical concentration. However, other grazers having even higher body P content did not respond similarly. These grazers were often abundant in low-nutrient environments with poorer food quality. At face value, this result is problematic for ecological stoichiometry because body composition did not correctly predict response of these other species. However, two potential explanations could maintain consistency with stoichiometric principles: species could differentially use a high-P resource (bacteria), or body composition might not always directly correlate with nutrient demands of grazers. Although our data cannot differentiate between these explanations, both suggest potential avenues for future empirical and theoretical study.

Key words: *Daphnia*; grazers; light; nutrients; phosphorus; predation; predation-risk gradients; resource-supply gradients; species composition, explaining shifts in; stoichiometry; zooplankton.

INTRODUCTION

Ecologists remain challenged to mechanistically explain changes in species composition over environmental gradients. In planktonic systems, “resource-ratio” (Tilman 1982, Huisman and Weissing 1995, Grover 1997) and “keystone predation” (Leibold 1996, Grover 1997) models predict changes in species composition along productivity gradients. Ecological stoichiometry has produced a third hypothesis. It forecasts shifts in grazer composition over gradients of light and nutrient supply to ecosystems (Sternner et al. 1997, Sternner and Elser 2002). Specifically, it predicts that grazer species with high somatic phosphorus content should dominate high-nutrient, low-light ecosystems that produce good elemental food quality, while grazers

with low-nutrient bodies should dominate low-nutrient, high-light ecosystems where food quality is poorer.

These predictions emerge from several key premises. The first is that both producers (plants) and grazers are packages of carbon and elemental nutrients, such as nitrogen and phosphorus. However, producers and grazers often differ in the ratio at which, say, carbon (C) and phosphorus (P) are packaged. In the laboratory and nature, primary producers are plastic in their P content (Andersen 1997, Sternner et al. 1997, Elser et al. 2000, Hessen et al. 2002), and much of this variation may be driven by (relative) supply of light and nutrients to ecosystems (“light:nutrient hypothesis”; Sternner et al. 1997).

Second, this variation in nutrient content of producers, driven by relative supplies of light and nutrients, can impact growth rates of grazers such as *Daphnia*. In contrast to producers, *Daphnia* typically has high average P content, but its body composition is much less plastic (it is almost homeostatic; Andersen and Hessen 1991, DeMott et al. 1998). Thus, *Daphnia* requires algal food of high P content (or high “elemental quality”) to grow optimally. When *Daphnia* consumes phosphorus-poor food, imbalances between the nutrient content of its food and its own body become important—*Daphnia* growth and reproduction may become

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nutrient limited (Sterner 1993, 1994, Urabe and Sterner 1996, Sterner et al. 1998, Elser et al. 2001). Furthermore, these mismatches may be common in nature (Elser et al. 2000).

Third, the elemental composition of zooplankton varies among grazer taxa. Some genera have higher and some have lower P content than *Daphnia* (Andersen and Hessen 1991). Grazers with high P content generally have high maximal growth rates (reflecting their high ribosomal RNA content; Elser et al. 1996) and also should be sensitive to P content of their food. Grazers with lower body P content should be less prone to nutrient limitation but have lower maximal growth rates (Urabe and Watanabe 1992, DeMott et al. 1998, Sterner and Elser 2002). Thus, in ecosystems with high light supply relative to nutrients, species that grow well when food quality is poor should dominate herbivore assemblages. In contrast, high elemental food quality in ecosystems receiving high nutrient:light supply ratios should allow P-rich herbivores to grow efficiently and become dominant through their growth-rate advantage (Elser et al. 1996, Main et al. 1997).

In an enclosure experiment, Urabe et al. (2002) have shown supporting evidence for this hypothesis: both food quality and *Daphnia*'s dominance of a local grazer assemblage increased with nutrient additions to low-nutrient, lake mesocosms. This field experiment was encouraging because it involved multiple species of grazers and producers and was conducted in semi-natural conditions. However, it was a short-term, local experiment and documented response of a closed species assemblage to light and nutrient perturbations. Consequently, it did not allow for arrivals of new species from the regional species pool. These arrivals could have affected the long-term outcome of the experiment (Leibold et al. 1997). In fact, results from similar short-term, local experiments may depend sensitively on time allowed for colonization by new species and for extinction of inferior competitors (Leibold et al. 1997).

In this study we used a different experimental approach that incorporated regional species pools and longer time scales. In a series of replicated pond mesocosms, we manipulated nutrients (by increasing total supply and altering N:P ratios) and light (using shade cloth), but also varied predation risk of zooplankton by adding an invertebrate predator. Throughout the experiment, we periodically added diverse assemblages of producers and grazers collected from natural ponds spanning wide gradients of light and nutrient supply. Presumably, these introductions eliminated time needed for colonization by new species. Furthermore, we added a wide diversity of algal species that should have varied extensively in their interspecific resistance to grazing, digestibility, and competitive ability (Leibold 1989, Grover 1997, DeMott and Tessier 2002). It seems possible that such variability could weaken the light:nutrient hypothesis by leading to parallel changes in

plant composition. We added a very diverse assemblage of grazer species that varied not only in P content but also susceptibility to predators. Variability in resistance to predation is known to affect species composition over resource gradients (Leibold 1996, Grover 1997).

Ecological stoichiometry predicts (1) increases in P-related food quality (i.e., lower C:P ratios) of edible algal producers as the supply rate of nutrients increases and/or light availability decreases, and (2) dominance of P-rich grazers such as *Daphnia* spp. only in ecosystems containing high-quality food and low predation risk (if susceptible to predators). In this study we created a food-quality gradient, which was driven at least in part by experimentally manipulated supply of light and nutrients. We then documented dominance by P-rich *Daphnia* along this food-quality gradient. *Daphnia* dominated ecosystems containing good elemental food quality and low predation risk, but not in environments with poor food quality. Both of these findings support stoichiometric predictions. However, grazer taxa with even higher P content than *Daphnia* could dominate poor food-quality environments. We offer two explanations for these latter, counterintuitive results, implicating differential ability to eat bacteria and/or reexamination of a grazer's nutrient demands.

METHODS

Experimental setup, sampling, and sample analysis

We used a fully factorial experimental design to test the simultaneous effects of light availability, absolute nitrogen and phosphorus availability, relative N:P availability, and trophic structure on zooplankton community composition. During May–June 2000 we created gradients of light and nutrient supply and predation risk in 300-L cattle tanks. To each tank we added silica sand substrate, well water, and inorganic nitrogen (N) and phosphorus (P) (NaNO_3 and NaH_2PO_4 , respectively) to raise nutrient concentrations to target N:P ratios (Ratio; 5:1, 14:1, and 50:1) and supply (Supply; high was $10\times$ low) (see below). Also, we reduced light availability in half of the tanks (Light) by 90% using neutral shade cloth. We inoculated each mesocosm with diverse assemblages of algae and zooplankton at fortnightly intervals. These inoculae were collected along wide, natural light–nutrient gradients from local ponds (proximate to Kellogg Biological Station and within Barry and Middleville State Game Areas, Kalamazoo and Barry Counties, Michigan, USA). In plus-predator treatments (Trophic), we also added 12 adults of *Notonecta undulata*. Each cattle tank also received 30 *Physa* spp. snails and 30 *Rana catesbeiana* tadpoles to graze and remineralize nutrients bound in benthic algae. These grazers maintained relatively low periphyton growth on the walls of the mesocosm (median: 2.64 mg chl a/m^2 , interquartile range: 1.17–12.78 mg chl a/m^2 ; see Fig. 2 of Leibold and Wilbur [1992] for comparison without benthic grazers). Finally, we

added nutrients weekly to approximately maintain target levels (total N and total P levels of 14 and 700, 25 and 370, and 44 and 220 for 5:1, 14:1, and 50:1 Ratio treatments, respectively; “high” Supply treatments received 10× these levels) by assuming a 5%/day loss rate from the water column (M. A. Leibold and V. H. Smith, *unpublished data*). The factorial experimental design was

$$\text{Replicates}_{3-4}(\text{Ratio}_3 \times \text{Supply Level}_2 \times \text{Light}_2 \\ \times \text{Trophic Structure}_2). \quad (1)$$

We sampled at the end of summer to characterize final composition of grazer assemblages and algal stoichiometry. We collected 8.5-L samples using tube samplers and a fixed sampling regime during three sampling periods: 15–17 and 29–31 September and 19–21 October, but data from these samples were averaged before analyses. During each period the zooplankton samples were sieved through 88- μm Nitex mesh and preserved in acid Lugol’s solution. Zooplankton was identified and counted (Pennak 1978) to species for *Daphnia* and *Simocephalus* but to genus for all other taxa, and up to 25 individuals/species/sample were measured using a 40× dissecting scope and converted into dry mass using published length–mass regressions (McCauley 1984). Additionally, during the first sampling period only, we sieved water through 35- μm mesh for subsequent C:P analysis of the “edible” algal fraction (Cottingham 1999). We filtered edible seston (which is mostly algae but also contains any suspended detritus and bacteria) on pre-combusted, acid-rinsed GF/F filters. We then dried (60°C) one sample per mesocosm to measure C:N content (Carlo-Ebra auto-analyzer), and froze (–80°C) a second sample for particulate P measurements (APHA 1980, Prepas and Rigler 1982). We also measured biomass of “edible” (<35 μm) algae using chlorophyll *a* as a proxy (by extracting samples on GF/F filters in chilled ethanol [Webb et al. 1992] and by using narrow-band fluorimetry [Welschmeyer 1994]). To rank zooplankton taxa by body composition, we measured the particulate P content of 50–200 μg dry mass from each of the nine major zooplankton taxa using the methods of DeMott et al. (1998). Animals were collected from environments in which they were abundant. The *Daphnia* and *Simocephalus* samples were collected from 10 and 3 separate mesocosms, respectively; for other taxa, we often lumped animals from different mesocosms/treatments to collect enough dry mass for analyses.

Statistical analyses

We analyzed the response of seston stoichiometry using nonparametric ANOVA. Before analysis we first transformed edible C, P, and C:P to optimally reduce heteroscedasticity following Taylor (1961). Then we used Anderson’s (2001a, b) approach to partition variation, which is described for balanced designs. To

more readily implement her approach, we imposed balance on the experimental design (Eq. 1) by randomly eliminating one of the four replicates in each of the six high-nutrient, no-predator treatments, and then tested for significance of each factor using 9999 randomizations in Matlab 5.3 (MathWorks 1999). We set $\alpha = 0.0167$ (i.e., 0.05/3) to correct for the interrelated comparisons.

We analyzed L-shaped relationships between *Daphnia* abundance and C:P ratio and seston P (see *Results*) using tree regression (De’ath and Fabricius 2000). A regression-tree algorithm (SYSTAT 8.0; SPSS 1998) recursively split the explanatory variable into increasingly homogenous groups of *Daphnia* abundance. The final split point of the explanatory variable minimized within-group sum of squares.

The remaining analyses characterized response of zooplankton taxa to the experimental treatments. To rigorously test the hypothesis conveyed by our experimental design, we used a method for distance-based, nonparametric, multivariate analysis of variance as outlined in Anderson (2001a). This nonparametric approach combats problems with multivariate normality, particularly since rare species can often have skewed distributions dominated by zeros. Following the advice of Anderson (2001b), we permuted raw assemblage data 9999 times to produce approximate tests.

The “distance-based” aspect permitted variance partitioning built on appropriate distance measures. Traditional MANOVA implicitly uses Euclidean distance, which is often inappropriate for ecological data because zero values create a “species abundance paradox” (Legendre and Legendre 1998, Legendre and Gallagher 2001) where ecosystems that do not share species may be more similar than ecosystems that share species. Here, we first $\log(X + 1)$ transformed species abundance data to stabilize variances. Then, we used the metric Hellinger distance transformation of Legendre and Gallagher (2001) to create the distance matrix, which was later variance-partitioned and ordinated. We also show results of analyses based on the metric Chord distance and nonmetric Bray-Curtis distance, both of which may perform well with similar assemblage data (Legendre and Legendre 1998, Legendre and Gallagher 2001). For comparison, we also included results based on Euclidean distance.

To describe the response by grazer taxa, we used two complementary graphical approaches involving distance-based redundancy analysis (db-RDA). We first reduced dimensionality of species responses to experimental treatments (using Hellinger distance) and created two-dimensional biplots using the RDA routine of CANOCO 4.0 (ter Braak and Simaleur 1998). Biplots simultaneously show species response to all of treatments but only in two dimensions at a time. We focused the ordination on relationships between species and treatment vectors (using “RDA scaling type 2”; Legendre and Legendre 1998:586). To help further inter-

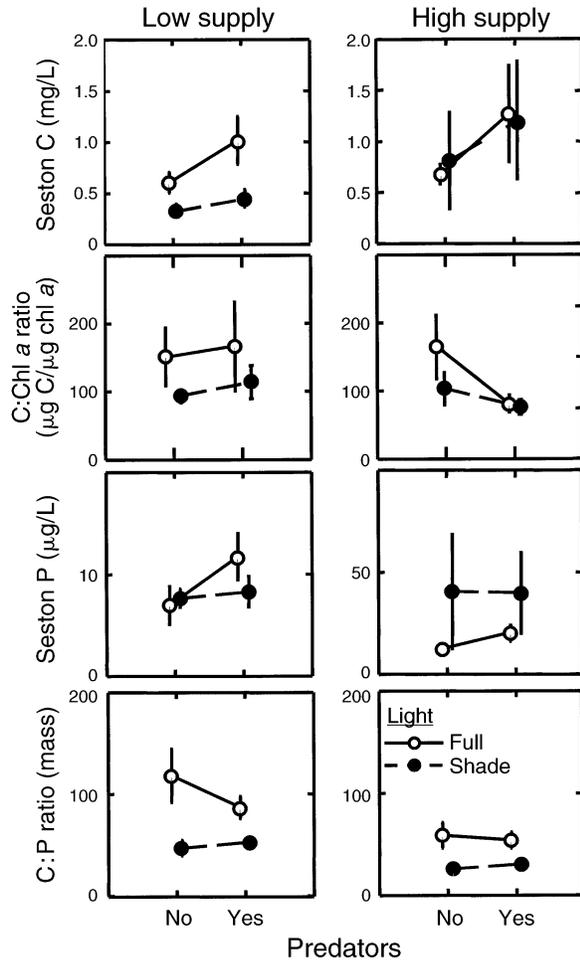


FIG. 1. Response of "edible" seston stoichiometry. Seston carbon (C) responded strongly to the light treatment, seston phosphorus (P) responded strongly to the nutrient-supply treatment, and the C:P ratio responded strongly to the supply and light gradients and weakly to the nutrient ratio gradient. The C:chl *a* ratios are low, indicating that most seston is not detritus. Points are means \pm 1 SE. Note the change in scale of seston P between low- and high-supply treatments.

pret the db-RDA results, we then calculated correlations between species abundance and experimental treatments using all of the orthogonal dimensions from the RDA. This approach shows how species responded to only individual treatments but in all dimensions. Our scaling choice implied that angles between a species vector (\mathbf{y}) and an experimental treatment vector (\mathbf{x}) reflected the correlation between them (r_{xy}). This correlation was estimated using

$$r_{xy} = \cos^{-1}(\theta) = \frac{\mathbf{x} \cdot \mathbf{y}}{|\mathbf{x}| |\mathbf{y}|} \quad (2)$$

where θ is the angle between \mathbf{x} and \mathbf{y} . We plotted correlations between 11 taxa for which we had P data (Fig. 4 and Andersen and Hessen 1991) and significant treatment vectors.

RESULTS

Seston stoichiometry responded to the light–nutrient–trophic treatments. Seston C was higher in unshaded than in shaded ecosystems (Light effect, $P = 0.0001$; Appendix A, Fig. 1), and consisted largely of algal matter, based on observed edible C:chl *a* ratios (Fig. 1; median = 81.3, interquartile range: 63.8–141.0; correlation of $\log(\text{chl } a) - \log(\text{C})$: Pearson's $r = 0.74$, $P < 0.0001$ after 99 999 randomizations, $N = 72$ mesocosms) which fall well within reported ranges for phytoplankton growth at various nutrient and light supplies (Shuter 1979, Geider et al. 1987, 1996). Seston P was greater in high-nutrient ecosystems than in low-nutrient ecosystems (Supply effect, $P < 0.0001$; Appendix A, Fig. 1). As a result, both light and nutrient supply drove the C:P ratio response (Appendix A, Fig. 1). Seston C:P ratios were higher (i.e., seston was less P rich) at low nutrient supply than at high nutrient supply (via a Supply effect on seston P; $P < 0.0001$) and also were higher at full light than at low light (via a Light effect on seston C, $P < 0.0001$). Although nonsignificant after correction for multiple comparisons ($P = 0.0354$, Appendix A), seston C:P ratios were generally lower at 5:1 than 50:1 N:P ratios.

Daphnia pulex (hereafter *Daphnia*) responded strongly to the nutrient-supply and predation-risk manipulations (Fig. 2). In low-P-supply mesocosms, *Daphnia* comprised <5% of zooplankton assemblage biomass in 34 of 36 ecosystems, and this response was

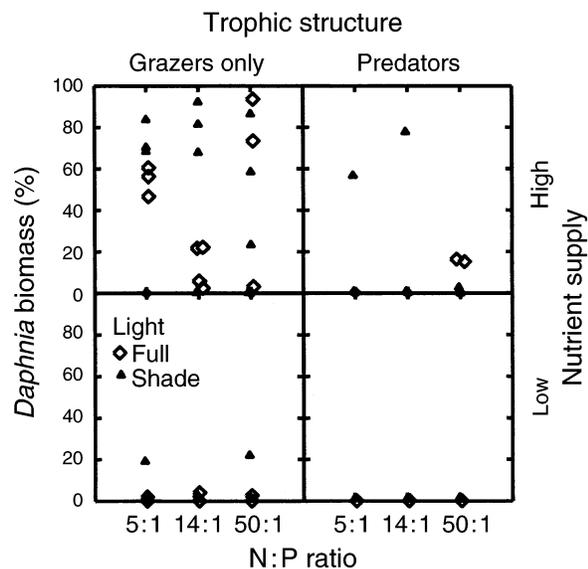


FIG. 2. Response of *Daphnia* to experimental treatments. An experimental trophic-manipulation treatment comprised "grazer-only" and "predator" treatments, while in the "nutrient-supply" treatment, high-supply mesocosms received 10 times the nutrient loading of low-supply mesocosms. Each point represents a separate mesocosm but is the mean of three sampling dates (no error bars shown). *Daphnia* biomass is on a percentage basis, relative to the crustacean zooplankton community (dry mass, $\mu\text{g/L}$).

insensitive to predator incidence (*Daphnia* were rare, i.e., <5% of assemblage biomass, in 16 of 18 mesocosms without predators, and in 18 of 18 mesocosms with predators). In contrast, *Daphnia* were more commonly dominant in high-nutrient systems. Without predators, *Daphnia* often (12 of 24 cases) comprised >50% of zooplankton assemblage biomass. However, in 7 of 24 of the no-predator tanks, *Daphnia* constituted <5% of total assemblage biomass. In ecosystems with high nutrient supply and with predators, *Daphnia* comprised >5% of assemblage biomass in only 4 of 18 cases.

In predator-free ecosystems, the relationship between *Daphnia* biomass and seston C:P ratios was L shaped (Fig. 3). At high C:P ratios (P-poor food), *Daphnia* biomass was low, whereas at low C:P ratios (P-rich food), *Daphnia* biomass ranged from low to high. Based on tree regression, a C:P ratio of 41.1 by mass (106.2 by moles) separated these regions. However, this response was likely driven by seston P, not seston C (Fig. 3). The response of *Daphnia* to seston P was also L shaped (or possibly T shaped). At low seston P, biomass of *Daphnia* was low, whereas past a threshold (7.5 μg particulate P/L), abundance of *Daphnia* ranged widely (Fig. 3). Generally, seston P fell below this threshold in low-nutrient mesocosms, whereas most high-nutrient mesocosms had higher seston P (Fig. 3). In contrast, we found no relationship between seston C and abundance of *Daphnia* (Fig. 3).

Response of the zooplankton assemblage

Before characterizing the response of the grazer assemblage, we first noted a gradient of body composition among major zooplankton taxa in the experiment (ANOVA of log-transformed P content; $F_{8,24} = 46.7$, $P < 0.0001$). Although *Daphnia* generally is given great attention due to its high P content, *Daphnia pulex* certainly did not have extreme C:P stoichiometry relative to several other co-occurring grazers. *Ceriodaphnia*, *Scapholebris*, and *Simocephalus* had nutrient content similar to or higher than *Daphnia*. Notably, these grazers are all in the same family (Daphnidae). Like *Daphnia*, *Diaphanasoma* and the cyclopoid copepod *Mesocyclops* also have intermediate nutrient content, while *Chydorus*, calanoid copepods, and ostracods also had low nutrient content (Fig. 4).

How did these zooplankton taxa respond to the light–nutrient–predation treatments? The distance-based (db), nonparametric (np) MANOVA results were similar based on Hellinger, Bray-Curtis, Chord, and Euclidean distances: Ratio ($P = 0.0019$), Supply ($P < 0.0001$), Light ($P < 0.0001$), and Trophic ($P < 0.0001$) treatments all shaped zooplankton assemblages (Appendix B). Additionally, three interactions were statistically significant: Ratio \times Supply ($P < 0.0021$), Ratio \times Light ($P = 0.0009$), and Supply \times Light ($P < 0.0001$) (Appendix B). Vectors coding for these seven significant terms in the db, np MANOVA model are

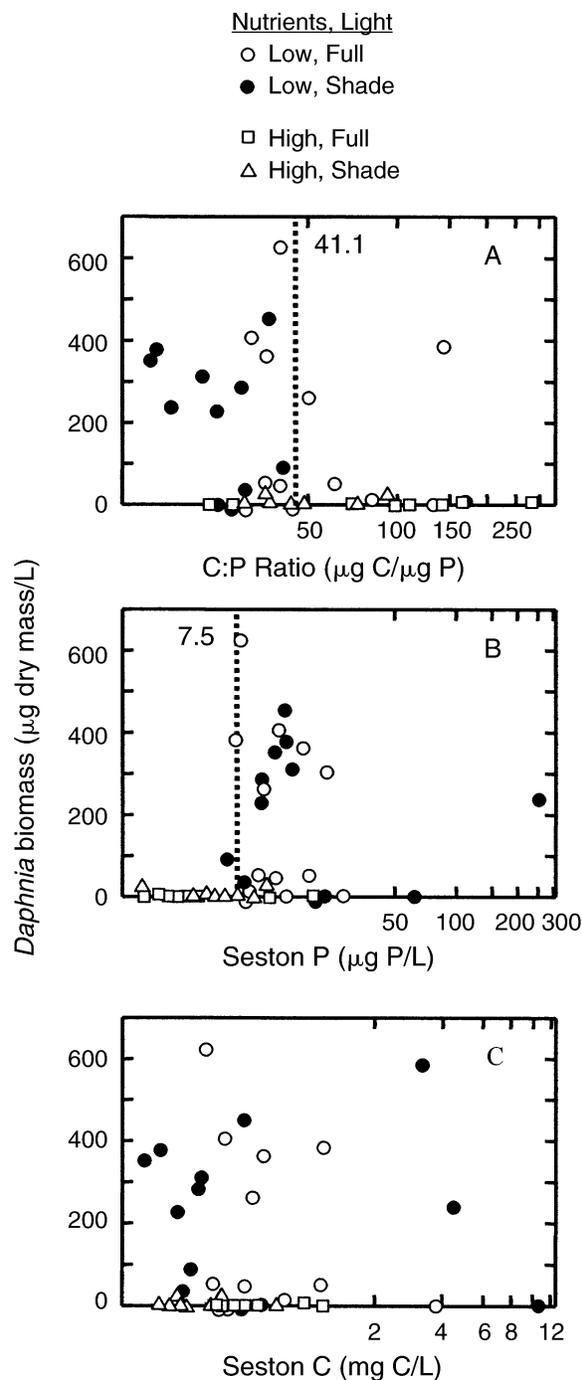


FIG. 3. Relationship between *Daphnia* biomass and “edible” (<35 μm) algal seston stoichiometry for algae + grazer treatments: (A) C:P ratio, (B) particulate P, (C) particulate C. Note the log-scale x-axis in (B) and (C). Vertical dashed lines represent values of C:P ratio (41.1 by mass), and seston phosphorus (7.5 μg P/L) that split *Daphnia* abundance into “lower” and “higher” abundance groups, based on tree regression.

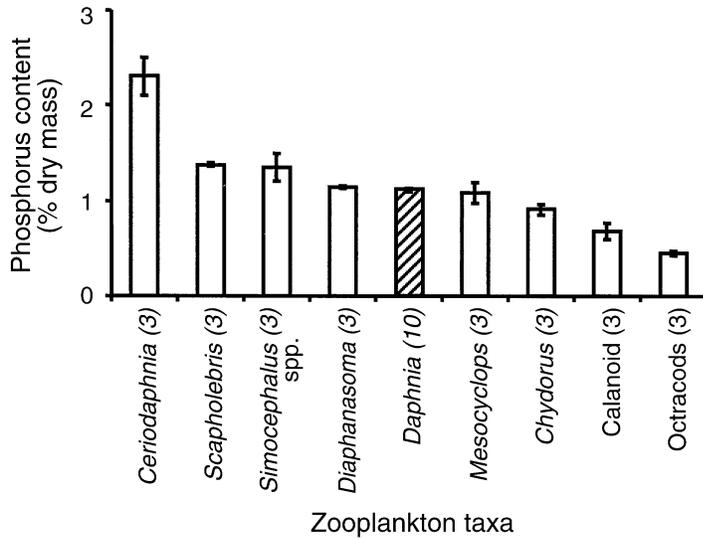


FIG. 4. Measurements of the phosphorus content trait (percentage P per unit dry mass) for nine abundant taxa in the experiment, ranked from highest to lowest P content. Numbers in parentheses near taxa names are sample sizes. Bars indicate means \pm 1 SE.

illustrated in the constrained ordination results (Figs. 5 and 6).

We used both the RDA biplots (Fig. 5) and derivative correlation calculations (Fig. 6) to interpret species responses. First, *Daphnia* responded most strongly to the high-nutrient (i.e., lower C:P ratio; Fig. 1, Appendix A) and low-predation treatments, but not strongly to the light treatments and nutrient-ratio treatments (which also affected seston C:P ratios; see also Fig. 1, Appendix A). In contrast, other P-rich taxa were more abundant in low-nutrient environments (*Ceriodaphnia*, *Scapholebris*, *Simocephalus vetulus*) and high-light environments (*Ceriodaphnia*, *Diaphanosoma*, *Scapholebris*, *Simocephalus vetulus*), especially in jointly high-light, low-nutrient environments (*Scapholebris* and *S. vetulus*; Supply \times Light interaction) or in high-light, high-nutrient environments (*Ceriodaphnia*, *S. serrulatus*, *Diaphanosoma*; Supply \times Light interaction). Furthermore, several of these taxa were more (*Ceriodaphnia*) or less (*Scapholebris*, *S. serrulatus*) proportionately abundant at 50:1 than at 14:1 and 5:1 ratio treatments, particularly in high-light environments (both Ratio \times Light interactions) and low-nutrient environments (both Ratio \times Supply interactions).

The constrained ordination also revealed that response of low-P species was idiosyncratic (Figs. 5 and 6). Calanoid copepods were most abundant at low nutrient supply (Supply effect) and at high predation intensity (Trophic effect). Two cladocerans, *Chydorus* and *Bosmina*, often responded oppositely; for instance, *Chydorus* was more abundant in high-nutrient (Supply effect) and low-light (Light effect) environments. In contrast, *Bosmina* was most abundant at high light and at 5:1 and 14:1 ratios (involving complex Ratio \times Supply and Ratio \times Light interactions), but was not affected by predator incidence. Both *Bosmina* and *Chydorus* were less abundant in 50:1 than 14:1 and 5:1 treatments (Ratio effect, Figs. 5 and 6).

Cyclopoid copepods responded uniquely. Several taxa (*Acanthocyclops*, *Diacyclops*, *Eucyclops*, and copepodites) were abundant in low-light environments with nutrients supplied at 50:1 ratios (Fig. 5). However, high correlations with the Ratio \times Supply (all four taxa) and Ratio \times Light (all but *Diacyclops*) interactions suggested context dependency of these responses (not shown). *Mesocyclops* was most abundant in the 5:1 and low-light treatments (Figs. 5 and 6).

DISCUSSION

We tested current stoichiometric theory linking supply of light and nutrients with changes in the species composition of grazer assemblages via elemental food-quality mechanisms. Our approach differs from previous tests of the "light:nutrient hypothesis" (Sterner et al. 1997, Hessen et al. 2002) because we allowed diverse assemblages of producers and grazer to respond to the light, nutrient, and predation treatments throughout the growing season. As a result, we characterized response of a regional species pool, and not local assemblages alone, to these gradients (Leibold et al. 1997).

The light–nutrient hypothesis predicts that the relative supplies of light and nutrients should drive variation in phosphorus content of edible phytoplankton. This link was observed in our experiment, as C:P ratio of algal seston was higher, and, subsequently, elemental food quality was worse, for grazers in fully lit and low-nutrient environments (Fig. 1). However, light and nutrients affected C:P ratios differently. Light availability affected C sequestered in algal tissue, while nutrient supply affected P sequestered in algal tissue (Fig. 1).

Daphnia responded to this gradient in food quality largely as predicted by the ecological stoichiometry—but with one important nuance. *Daphnia* dominated grazer assemblages only in predator-free ecosystems with high nutrient-supply rates (Fig. 2). In these en-

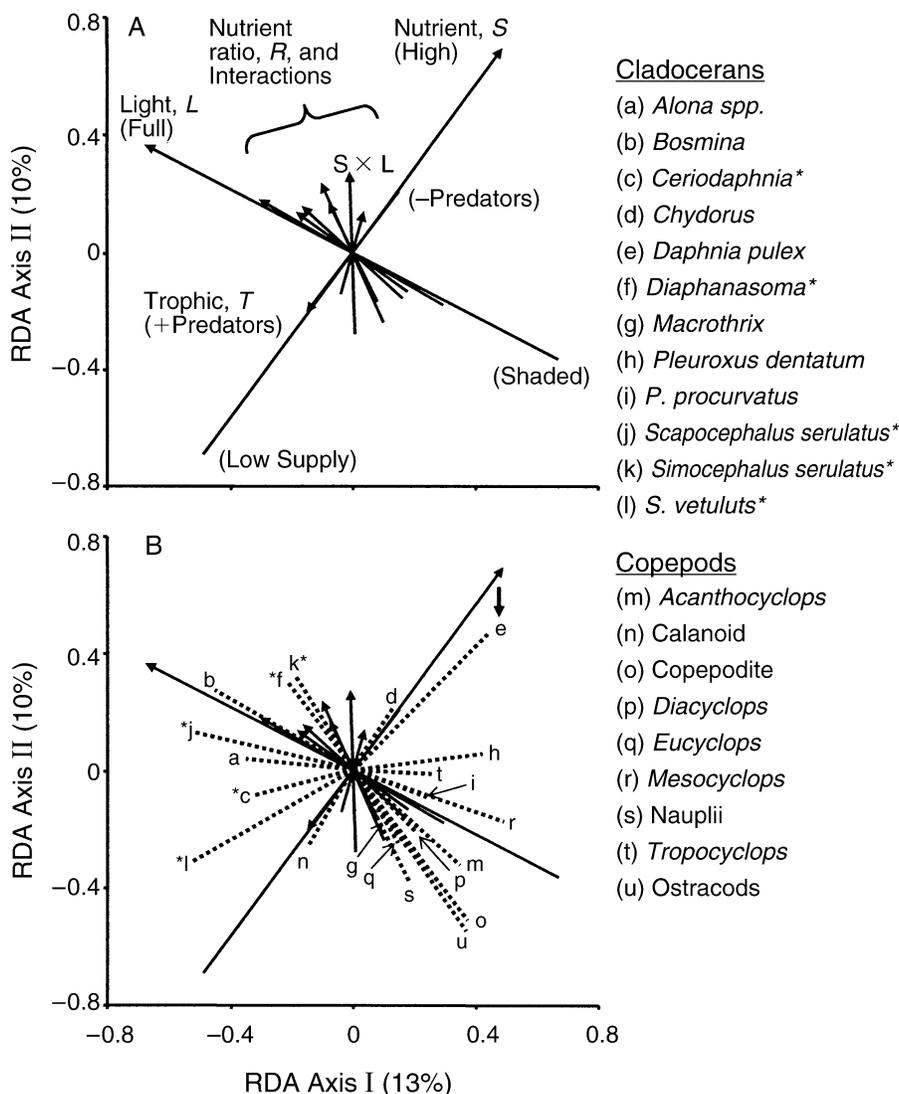


FIG. 5. Biplots of 21 taxon vectors (dashed lines) in relation to vectors representing the statistically significant (Appendix B) treatments (solid arrows). These two-dimensional results follow a constrained ordination using redundancy analysis (RDA; see *Methods* for details). The ordination is scaled so that the angles between taxon and treatment vectors reflect their correlations: acute angles imply strong positive response of taxon to a treatment; obtuse angles imply a strong negative response. For clarity, treatment vectors are separated from taxon vectors in panel (A); both sets of vectors are combined in panel (B). In the taxa key and in panel (B) the arrow points to *Daphnia*, and the stars mark five taxa with body P composition similar to or higher than *Daphnia*. Two vectors code for the Ratio treatment and each of its interactions because there were three levels (5:1, 14:1, 50:1). The percentage of variance explained by each of the first two RDA axes (analogous to the scaled eigenvalues of the principal components in PCA) is presented in parenthesis in each axis label.

vironments, food quality generally fell below a sestonic C:P ratio of 41.1 by mass (106 by moles; Fig. 3). Interestingly, this level is lower than critical C:P levels cited in laboratory and theoretical studies of *Daphnia*-only ecosystems (Urabe and Watanabe 1992, Andersen 1997, Sterner and Elser 2002). However, it appears that *Daphnia* responded to levels of sequestered P, not sequestered C (Fig. 3). In almost all high-nutrient ecosystems, sequestered algal P exceeded a threshold (7.5 μg particulate P/L) above which *Daphnia* could dominate grazer assemblages. Conversely, almost all low-

nutrient ecosystems fell below this particulate P threshold. In these systems, *Daphnia* was not an important component of grazer assemblages. Consequently, these results suggest that a threshold requirement of sequestered P, rather than a key C:P ratio, acted as a determinant of *Daphnia* dominance.

Predation risk was the other major determinant of abundance of *Daphnia*. Consistent with previous studies (Scott and Murdoch 1983, Arner et al. 1998), the presence of notonectid predators greatly reduced both the actual and the relative biomass of *Daphnia* in high-

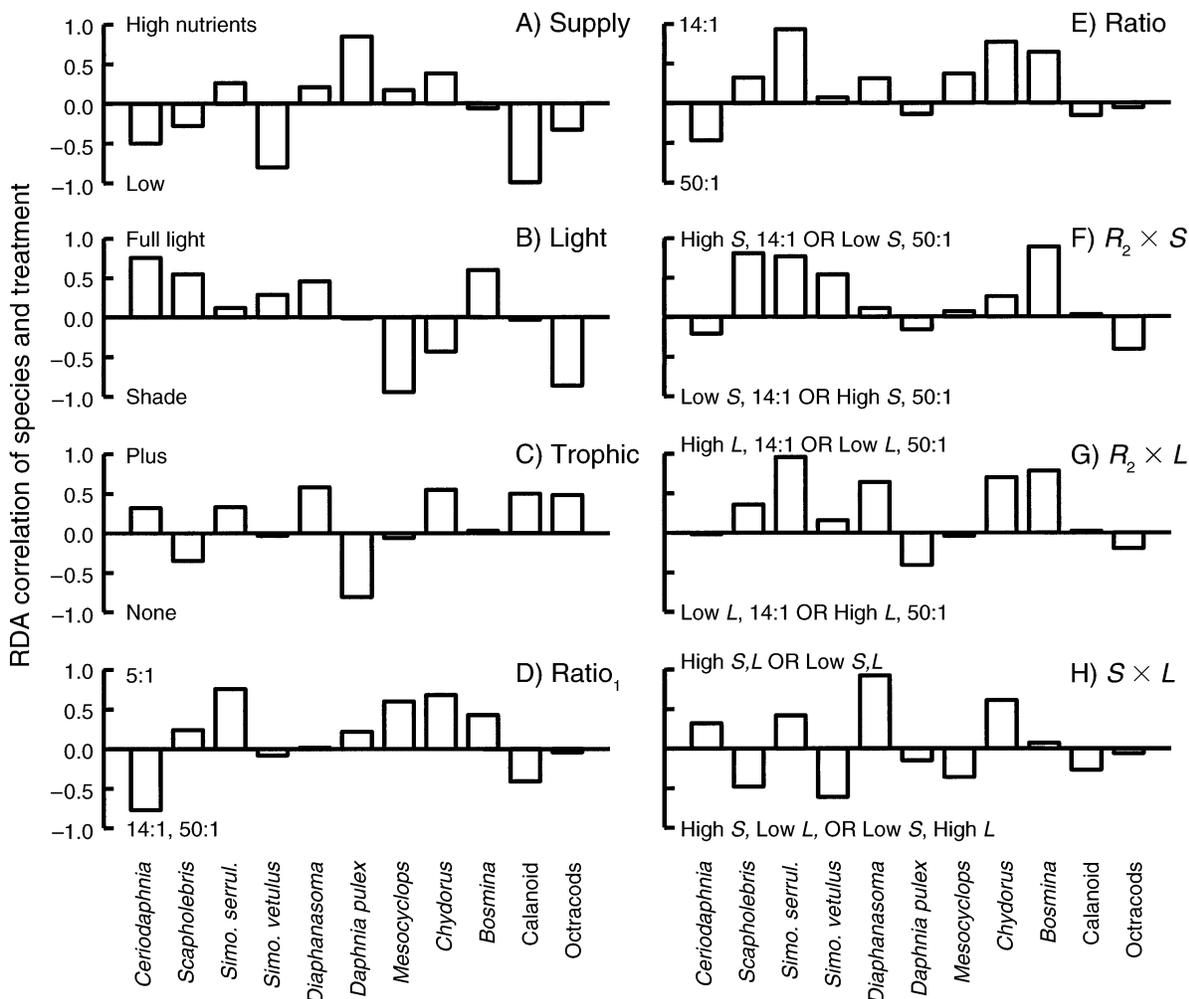


FIG. 6. Multidimensional correlations of 11 taxa for which phosphorus content measurements are available (see Fig. 3). The *Bosmina* ranking came from Andersen and Hessen (1991), and the two *Simocephalus* species (*serrulatus* and *vetulus*) were assumed to have similar body composition. Taxa are ranked from highest P content to lowest. Guides for interpreting strong negative and positive correlations are also provided with each panel. Two vectors represent the three levels of the Ratio (R) \times Supply (S) and Ratio (R) \times Light (L) treatments; therefore each Ratio vector has a subscript. Responses of the taxa to the two vectors representing both the Ratio (R) \times Supply (S) and Ratio (R) \times Light (L) treatments were very similar; hence, only one of the vectors in each interaction is shown.

nutrient ecosystems. However, at low nutrient-supply rates the role of predation in determining *Daphnia* dominance was negligible. In this treatment, low sequestered P apparently constrained *Daphnia* abundance—regardless of predator incidence. Superficially, this result resembles predictions of food-web theory, which ignores stoichiometry (e.g., Leibold 1996), but the mechanism here is different. In traditional theory, predators play a minor role at lower nutrient supply because predator biomass is low. Yet, in the case here, prey biomass (*Daphnia*) remained low regardless of predator incidence (likely) due to stoichiometric (P) constraints. Thus, both predation and food quality shaped zooplankton assemblages through effects on *Daphnia*, supporting the conceptual model of Elser et al. (1998) of stoichiometry–predation interactions.

Even in environments in which it could flourish (high nutrients, no predators), *Daphnia* did not always dominate zooplankton assemblages. In >25% of the high-nutrient, no-predator treatments, *Daphnia* was a minor or undetectable component of zooplankton assemblages (Fig. 2). This result suggests two possibilities: in high-nutrient ecosystems, relative abundance of *Daphnia* oscillated asynchronously among mesocosms, or alternative stable states existed. Because we only sampled the experiment at the end of the growing season, we cannot dismiss the oscillatory scenario (although all seven ecosystems had low *Daphnia* abundance during all three sampling dates). However, stoichiometrically explicit, nonlinear food-chain models (Andersen 1997, Loladze et al. 2000) can predict alternative stable states at high nutrient-supply rates. In these stoichio-

metrically explicit models, a grazer like *Daphnia* can either coexist with an edible plant, or it can be driven extinct due to poor food quality. In a model of stoichiometrically explicit grazer competition (Hall, *in press*), alternative stable states can emerge at intermediate resource supply.

For proponents of ecological stoichiometry, the general response of *Daphnia* is encouraging because it suggests a link between light:nutrient supply, food quality, and dominance by a P-rich grazer species. Yet, if this mechanism is truly general, other zooplankton taxa (*Ceriodaphnia*, *Scapholebris*, *Simocephalus*, *Diaphanosoma*; see Fig. 4) with high somatic P content should have responded similarly and consistently to the sestonic C:P gradient (Leibold 1998). However, they did not. In fact, several of these taxa were more abundant in low-nutrient or high-light ecosystems (Figs. 5 and 6). Both of these treatments provided poorer food quality (higher C:P) for grazing herbivores.

At face value, this inconsistency is problematic for stoichiometric theory because the proposed body-composition mechanism failed to correctly predict responses of other high-P species. There are at least two plausible explanations for these results. While they do not contradict stoichiometric principles, they do suggest areas needing further work. First, some grazers may occupy somewhat different feeding niches than *Daphnia*. For instance, *Scapholebris* typically feeds upon surface film, so measurements of nutrient content of "seston" from a water column may or may not adequately describe the nutrient content of its diet. However, documented examples of competition between *Daphnia* and *Ceriodaphnia* (Neill 1975, Lynch 1978, 1979, Smith and Cooper 1982), *Diaphanosoma* (Matee 1987), and *Simocephalus* (Grover et al. 2000) suggest diet high overlap between *Daphnia* and other grazers. Yet, zooplankton grazers can differentially use bacterial resources smaller than 1–2 μm . For instance, *Ceriodaphnia* and *Diaphanosoma* have finer-mesh filters than *Daphnia* (Pace et al. 1983). These differences could be important because bacteria typically have higher nutrient content than algae (Elser et al. 1996, Sterner et al. 1998, Sterner and Elser 2002), and thus could provide crucial sources of P for these grazers and not *Daphnia*. Unfortunately, we did not separate size fractions of seston into bacterial and non-bacterial components, so it is impossible to evaluate this hypothesis with results from our experiment. If bacteria provide important sources of P for some but not other grazers, however, future stoichiometrically explicit models of grazer competition should include bacteria as a food resource and as an interactor with phytoplankton. Furthermore, (in)ability to eat bacteria should be viewed as an important ecological trait of zooplankton in stoichiometric context.

A second, alternative explanation focuses upon determinants of nutrients demands of grazers. Currently, body composition of grazers has gained most attention

in stoichiometric experiments and theory (Sterner and Elser 2002), but recent models of stoichiometrically explicit competition among grazers (Loladze et al. 2004; Hall, *in press*) suggest that this emphasis may be too narrow. These models expand upon a theme that originated in early stoichiometric studies (e.g., Urabe and Watanabe 1992, Hessen 1992; also Sterner 1997, DeMott et al. 1998): other grazer traits, such as conversion efficiency and respiration rate, also determine nutrient demands, and hence competitive ability of grazers for nutrients. Thus, body composition of a grazer solely determines its competitive ability for nutrients only if all other traits are equal. Yet, all other traits among zooplankton species are not equal, and they can vary temporally within an ecosystem and among systems (e.g., Burns 1969, Arnold 1971, Romanovsky and Feniova 1985, Bengtsson 1987, DeMott 1989, Hu and Tessier 1995). Given these differences in traits, theory permits a grazer with higher P content (e.g., *Ceriodaphnia*) to outcompete a species with lower P content (e.g., *Daphnia*) in systems with nutrient-deficient food. Furthermore, if *Daphnia* is a superior C competitor to such a high P grazer (Gliwicz 1990), these models predict: (1) shifts in species composition from superior nutrient competitors (*Ceriodaphnia*) to superior C competitors (*Daphnia*) over a nutrient-supply gradient, and (2) alternative stable states of assemblage structure of grazers (i.e., response of *Daphnia* in high-nutrient, no-predation treatments; Fig. 2). Both theoretical predictions qualitatively match our otherwise-problematic results. Consequently, they could be explained by stoichiometrically explicit competition theory without invoking hypotheses involving bacteria.

The two main explanations proposed for our challenging experimental results (bacteria as food vs. determinants of competitive ability for nutrients) are not mutually exclusive. Instead, both hypotheses point to areas deserving attention in future stoichiometrically explicit experiments and theoretical efforts. Regardless of the specific explanation, we have documented shifts in grazer composition over resource supply and predation gradients that are consistent with stoichiometric mechanisms. Thus, like the keystone-predation and resource-ratio models, ecological stoichiometry can predict changes in grazer composition over environmental gradients. However, our empirical findings (and companion models) suggest that body composition of grazers should not necessarily be the focal trait in stoichiometric research. Instead, body composition should be combined with other physiologically and ecologically relevant traits to determine a grazer's competitive ability along food-quality gradients driven by resource supply.

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APPENDIX A

A table presenting results of nonparametric univariate analyses of edible seston C, P, and C:P ratio is available in ESA's Electronic Data Archive: *Ecological Archives* E085-068-A1.

APPENDIX B

A table presenting results of nonparametric, Hellinger distance-based MANOVA of zooplankton community-composition response to the treatments, using Anderson's (2001a, b) method, is available in ESA's Electronic Data Archive: *Ecological Archives* E085-068-A2.