

## Consumer growth linked to diet and RNA-P stoichiometry: Response of *Bosmina* to variation in riverine food resources

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### Abstract

Feeding experiments were performed with riverine *Bosmina* to investigate how their somatic growth rate responded to variation in food resources arising from changing hydrodynamic conditions. Experimental manipulations of food quality and quantity were achieved by diluting riverine suspended particulate matter (seston) to obtain a range of food concentrations and by amending natural seston with laboratory-grown *Scenedesmus acutus*. *Bosmina* experienced food limitation during periods of elevated discharge when the P content of seston was low (C:P > 600). Growth rates covaried with body RNA and P contents ( $R^2 = 0.96$  and  $0.86$ , respectively; content expressed as percent of dry mass), which is consistent with the hypothesis that high growth rates require increased allocation to P-rich ribosomal RNA and that high seston C:P induces P limitation in riverine *Bosmina*. P limitation arises in riverine consumers of riverine seston when hydrologic conditions favor terrestrial inputs, sediment resuspension, and low algal productivity, thereby resulting in seston fractions dominated by P-poor materials. During low discharge, riverine seston was P-rich relative to *Bosmina* requirements and growth rates were decoupled from body P content. *Bosmina* RNA content was strongly and linearly related to growth over a broad range of resource conditions, suggesting that it may be a useful surrogate to assess dietary sufficiency of food resources in natural settings.

Hydrodynamic environments range from relatively still (wind-driven mixing) in lentic waters to turbulent (fluvial- and tidal-driven mixing) in streams, rivers, and estuaries. Cross-system differences and seasonal variation in physical forces are important determinants of the quantity and composition of suspended particulate matter available for consumers. Food quality (edibility, digestibility, nutritional sufficiency) is determined in large part by the relative contributions from allochthonous sources (terrestrial in-

puts) and autochthonous production (Findlay et al. 2001; Sobczak et al. 2005). The former is detrital material of low nutritive value, whereas the latter (algal fraction) is enriched in mineral nutrients and important biochemicals (fatty acids, amino acids, etc.) whose concentrations vary with algal species composition and nutritional status (Brett and Muller-Navarra 1997; DeMott et al. 1998; Von Elert and Wolffrom 2001). Consumers in advective systems (rivers, estuaries) are thought to benefit from allochthonous subsidies to a greater extent than their lentic counterparts as a result of the physical forces that transport and maintain particulate matter in suspension. More recent work, however, has shown that the importance of allochthonous inputs to lakes may be underappreciated (Pace et al. 2004), and concurrent studies in rivers and estuaries suggest that autochthonous sources may be nutritionally important even in systems dominated by allochthonous inputs (Thorp and DeLong 2002; Guelda et al. 2005; Sobczak et al. 2005).

Zooplankton frequently experience reduced growth from low food quantity or poor food quality (Boersma et al. 2001; Acharya et al. 2004; DeMott et al. 2004). Recent

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studies have taken a stoichiometric approach focusing on the nutrient content of consumers relative to that of their food (Gulati and DeMott 1997; Sterner and Elser 2002). Zooplankton biomass is more nutrient rich than seston by one or two orders of magnitude (Hessen 1992; Elser and Hassett 1994; Sterner et al. 1997); therefore content, especially N and P, is an important aspect of food quality. Most of the recent work on N and P stoichiometry has focused on *Daphnia* because of their ecological importance (high grazing rates in the plankton) and their amenability to experimental studies. In laboratory and field studies, *Daphnia*'s growth was correlated with the P content of its food when dietary intake was below threshold levels (Urabe and Watanabe 1992; Sterner et al. 1993; Gulati and DeMott 1997). Experiments that used P supplements have shown that at least some of this growth suppression is a direct result of dietary P deficiency (DeMott et al. 1998; Elser et al. 2001). The effects of low dietary P intake on growth rate appear to be associated with altered allocation of P-rich ribosomal RNA (the "growth rate hypothesis"; Sterner and Elser 2002; Elser et al. 2003). An implication of this chain of mechanisms is that growth rate, body P content, and body RNA content should all be tightly correlated when growth is limited by dietary P intake. When growth rate varies as a result of some other factor (nitrogen or protein intake, or overall energy limitation), growth and body P content may no longer be correlated (Elser et al. 2003).

Lotic environments support diverse and at times abundant zooplankton communities (Basu and Pick 1997; Thorp and Mantovani 2005), although these have received comparatively little attention in stoichiometric food-web studies (Woodward and Hildrew 2002). Previous studies on riverine zooplankton have typically emphasized the direct effects of physical processes (river discharge) in regulating abundance (e.g., Pace et al. 1992). Within the constraints imposed by hydrologic conditions, ecological factors (food resources, predation) may dictate whether plankton abundance will increase or decrease as parcels of water are transported downriver (Jack and Thorp 2000; Jack and Thorp 2002; Guelda et al. 2005). The relative importance of hydrologic and ecological constraints on plankton development is also subject to anthropogenic influences arising from the presence of water regulation structures. Water storage in impounded rivers increases transit times and the importance of in situ ecological processes. Impoundments also alter the proportions of autochthonous and allochthonous sources of particulate matter by fostering phytoplankton production (Sellers and Bukaveckas 2003) and promoting sedimentation of watershed-derived materials (Syvitski et al. 2005). Because few large rivers remain unregulated, there is a critical need to link spatial and temporal variation in hydrologic conditions to the quantity and quality of food resources for riverine consumers.

*Bosmina* is a common component of freshwater plankton communities (Kerfoot 1975; DeMelo and Hebert 1994) and typically the dominant cladoceran occurring in many rivers (Thorp et al. 1994; Thorp and Casper 2003). Unlike *Daphnia*, *Bosmina* are thought to be selective feeders (DeMott 1982) and this selective ability might give them an advantage in riverine environments where mineral turbidity might interfere with grazing. Though a number of studies have examined dietary effects on *Bosmina* (Kerfoot 1975; DeMott 1985; Urabe 1991), studies linking *Bosmina* growth rates to discharge-related changes in food resource conditions are lacking. The algal fraction of river seston is highly variable depending on discharge conditions (Sellers and Bukaveckas 2003; Guelda et al. 2005), and therefore we anticipated that seston P content would vary in response. Previous studies indicate that *Bosmina* have low body P content (0.7% dry wt; Hessen and Lyche 1991) relative to *Daphnia* (1.5%; DeMott et al. 1998; Acharya et al. 2004b). Based on homeostatic assumptions, *Bosmina* should exhibit high growth efficiency (per unit P) when growth is constrained by P availability and a high threshold for P limitation (high food C : P; Schulz and Sterner 1999). We hypothesized that variation in *Bosmina* growth rates would correspond to shifts from P-rich (low discharge) to P-poor (high discharge) food quality. During high discharge when low-P allochthonous food sources dominate, we expected strong associations between growth rates and the body content of P and RNA (here, content refers to the percent of dry weight contributed by P or RNA) if increases in seston C : P give rise to P limitation. Alternatively, if riverine *Bosmina* did not experience growth limitation arising from P deficiency, then we predicted a poor association between growth rate and body P content. To test these predictions, we measured the effects of changing food resource conditions on *Bosmina* growth rates by using natural seston obtained over a range of hydrologic conditions.

## Methods

*Study site*—The Ohio River is formed by the confluence of the Allegheny and Monongahela Rivers at Pittsburgh, Pennsylvania, and flows 1,579 km in a generally south-western direction to enter the Mississippi River at Cairo, Illinois. The basin is densely populated, with approximately 48% of land area appropriated for human use (USGS 2000). Wastewater inputs from urban centers and runoff from agricultural areas result in nutrient-rich conditions throughout the year and high sediment transport during periods of elevated discharge (typically February–April; Bukaveckas et al. 2005). The annual hydrograph of the river is characterized by peak discharge in winter months (December–May; White et al. 2005). Although low discharge is typical in summer, rain events driven by moisture from the Gulf of Mexico frequently result in high discharge and sediment transport even during periods when the river is normally at base flow (July–October). The river

is regulated by a series of low dams and lock structures (height, <10 m) which act to maintain a minimum water depth for navigation. At low flow (water elevation < dam height), the river is subdivided into a series of pools characterized by shallow depths in the upper reaches and progressively deeper waters in lower reaches proximal to dams. The combined effect of climatic events and water management practices gives rise to a wide range of hydrodynamic conditions which were used in this study to explore linkages between seston properties (C : N : P) and consumer growth potential. Previous work has shown that growth rates of Ohio River zooplankton are susceptible to manipulations of food quantity and its algal carbon fraction during periods when discharge and seston concentrations are low (Guelda et al. 2005).

*Field sampling*—*Bosmina* were collected near the midpoint of a navigational pool formed by the McAlpine Dam (located at Louisville, Kentucky). Water transit times through the McAlpine Pool range from 2 to 3 d at high flow to >25 d at low flow. During periods of low flow, peak algal biomass is observed near the midpoint of the pool (our sampling location) owing to positive algal carbon balances in the upper, shallower portion of the pool (Sellers and Bukaveckas 2003). Zooplankton communities of the Ohio River are dominated by *Bosmina*, small cyclopoid copepods (e.g., *Diacyclops* sp.) and the calanoid copepod *Eurytemora affinis* (Thorp et al. 1994; Jack and Thorp 2000, 2002). *Bosmina*, seston, and physical-chemical data were collected monthly from May to October 2004. *Bosmina* densities were estimated from 10-liter samples filtered through 60- $\mu\text{m}$  mesh and preserved in sugar formalin (Haney and Hall 1973). *Bosmina* used in feeding experiments were collected with a 60- $\mu\text{m}$  plankton net. Water samples for analyses of seston (particulate organic carbon [POC], C : N : P), chlorophyll *a* (Chl *a*), and dissolved constituents were collected in 1-liter acid-washed Nalgene bottles. Live animals and water samples were transported to the lab within an hour of collection.

*Laboratory feeding experiments*—To test the effects of variable food quantity, we compared individual growth rates at ambient concentrations of river seston (hereafter, “100%AMS”, where AMS = ambient seston concentration) to growth rates at reduced seston concentrations (33% and 66% of ambient; hereafter, 33%AMS and 66%AMS). Reductions of food concentrations were obtained by mixing river water (zooplankton removed) with filtered river water (particles >0.45  $\mu\text{m}$  removed with a GF/F filter; 0%AMS) at 2 : 1 and 1 : 2 ratios. A no-food treatment (0%AMS) was included in the design to assess interexperiment variation in maternal effects on juvenile growth rate. To test the effects of variable food quality, river seston was replaced by or supplemented with 1.5 mg C L<sup>-1</sup> of laboratory-cultured green algae (*Scenedesmus acutus*; hereafter, “Alg” and “Alg+S,” where Alg+S denotes algae-seston mix). Previous studies by DeMott (1982, 1986), Urabe (1991), and Schulz and Sterner (1999) have shown that green algae such as *Scenedesmus* and *Chlamydomonas* provide a suitable diet for *Bosmina*. The *S. acutus* was

grown in a chemostat in batch cultures on filtered (0.45- $\mu\text{m}$  GF/F) and autoclaved river water modified to contain at least 1,000  $\mu\text{mol N L}^{-1}$  and 50  $\mu\text{mol P L}^{-1}$  (molar N : P ratio 20). C : N : P ratios for *Scenedesmus* cultures exhibited little variation across months/experiments (C : P = 150–175; C : N = 8.5–10).

Neonates born within a 24-h period from gravid *Bosmina* collected in the field were used during each of the six experiments (May–October). Experiments were conducted in 25-mL vials (one individual per vial); a volume that exceeds by 4–5 times the expected daily clearance rate of *Bosmina*. Food and media were replenished every 24 h to ensure that food concentrations were constant throughout the 3-d growth experiment and comparable (for 100%AMS) to those observed in the river. Vials were maintained at 14 : 10 light : dark cycle (25°C) and gently rotated twice a day to resuspend settled particulates. A random subset of neonates was used to estimate initial body size ( $N = 30\text{--}40$ ), P% (three replicates of 12 animals each) and RNA% (five replicates of 2 animals each). Eighteen animals were used for each of the six diet treatments. After 3 d, all animals were photographed and body area was estimated by image analysis. For growth rate estimation, initial and final body area measurements were converted to body dry mass by a previously derived dry weight–body area regression ( $p < 0.0001$ ,  $R^2 > 0.90$ ; Acharya et al. 2005). Growth rate,  $\mu$  (d<sup>-1</sup>), was calculated as

$$\mu = \ln(\text{final weight}/\text{initial weight})/\text{days}$$

Two replicates of six animals each were placed in 25-mL glass tubes (for P analysis), and five or six replicates of one animal each were placed in nuclease-free Eppendorf tubes (for RNA analysis) with ultrafine tweezers (Dumont) and immediately frozen at  $-80^\circ\text{C}$  for later analysis.

*Sample analysis*—*Bosmina* were enumerated under a compound microscope (Olympus SZX-ILLD100, Olympus Optical) and separated into three approximate size classes (neonates, juveniles, and adults) based on body lengths. Adults were identified as males or females and females were further classified as egg-bearing and non-egg-bearing females. River *Bosmina* consisted of a mixed population of *B. (sinobosmina) freyi* and *B. leideri* (formerly *Bosmina longirostris* “complex”). These two species are similar in size and morphology and are discriminated from each other by the number of spines on the proximal pecten and curvature of the antennule (DeMelo and Hebert 1994). From the pool of live *Bosmina* collected in the field, subsamples were taken for subsequent determination of body C, N, P, and RNA content. *Bosmina* collected from the field were classified as adults, juveniles, and neonates (based on body size) before measuring their RNA. For P, there were three replicates of each age class (12, 8, and 4 animals per replicate for neonates, juveniles, and adults, respectively) and five replicates of each for RNA (two animals per replicate for neonates and one each for juveniles and adults). Body RNA content did not differ



significantly among age groups in any month (analysis of variance [ANOVA],  $p > 0.10$ ), and therefore subsequent data on field-collected animals are for the mixed population. For C and N determinations, a mixed population (all age groups) was used because a larger number of animals were required for analysis. There were two replicates of at least 20 animals each for C and N determinations (Perkin-Elmer model 2400 elemental analyzer).

All animals for C, N, P, and RNA analyses were photographed for body size measurements (egg-bearing animals were avoided). Samples for P were oven-dried at  $60^{\circ}\text{C}$  and stored in a desiccator, and RNA samples were stored at  $-80^{\circ}\text{C}$ . RNA content of *Bosmina* (field populations and experimental animals) was determined by the microfluorometric method of Kyle et al. (2003) and converted to percentage of dry mass by means of the body area–dry mass regression. P content was determined by persulfate oxidation followed by colorimetric analysis using the acid molybdate technique (APHA 1998).

Measurements of turbidity, Chl *a*, POC, and C : N : P stoichiometry were used to characterize food quantity and quality. Turbidity was measured with a HACH 2100P turbidity meter. Water samples for Chl *a* determination (three replicates) were filtered through  $0.45\text{-}\mu\text{m}$  filters (47-mm-diameter GF/F) and extracted in acetone for 24 h. Chl *a* was measured fluorometrically (Turner 10-AU fluorometer) with acid correction (Arar and Collins 1997). POC (three replicates) was determined by filtering a known volume of water through a precombusted  $1\text{-}\mu\text{m}$  (47-mm-diameter GF/F) pore filter, removing the volatile organic matter at high temperature ( $450^{\circ}\text{C}$ ) and determining POC by difference. Seston and *S. acutus* were collected on precombusted  $0.45\text{-}\mu\text{m}$  (GF/F) pore filters and analyzed for C and N with a Perkin-Elmer model 2400 elemental analyzer. P content was determined by persulfate oxidation by the acid molybdate technique (APHA 1998).

**Statistical analysis**—We used repeated-measures ANOVA with “month” as a time factor to assess the effects of food quality and quantity on *Bosmina* growth rates. We also ran Tukey honestly significant difference (HSD) multiple-comparison tests to identify months and treatments where significant differences in growth rates occurred. Stoichiometric data obtained during feeding experiments (P%, N%, RNA%) were analyzed by a two-way ANOVA with diet treatments (six levels) and discharge conditions (months pooled according to high vs. low discharge; two levels) used as explanatory factors. Effects of discharge variation on the stoichiometry (P%, RNA%) of field-collected *Bosmina* were analyzed by one-way ANOVA and the Tukey HSD multiple-comparison tests. Regression analyses were used to test for associations among P%, RNA%, and growth rates on pooled field and experimental data (all dates and treatments).

## Results

Variations in turbidity, POC, Chl *a*, and seston C : N : P ratios suggest that the quantity and quality of food resources in the river varied as a function of discharge

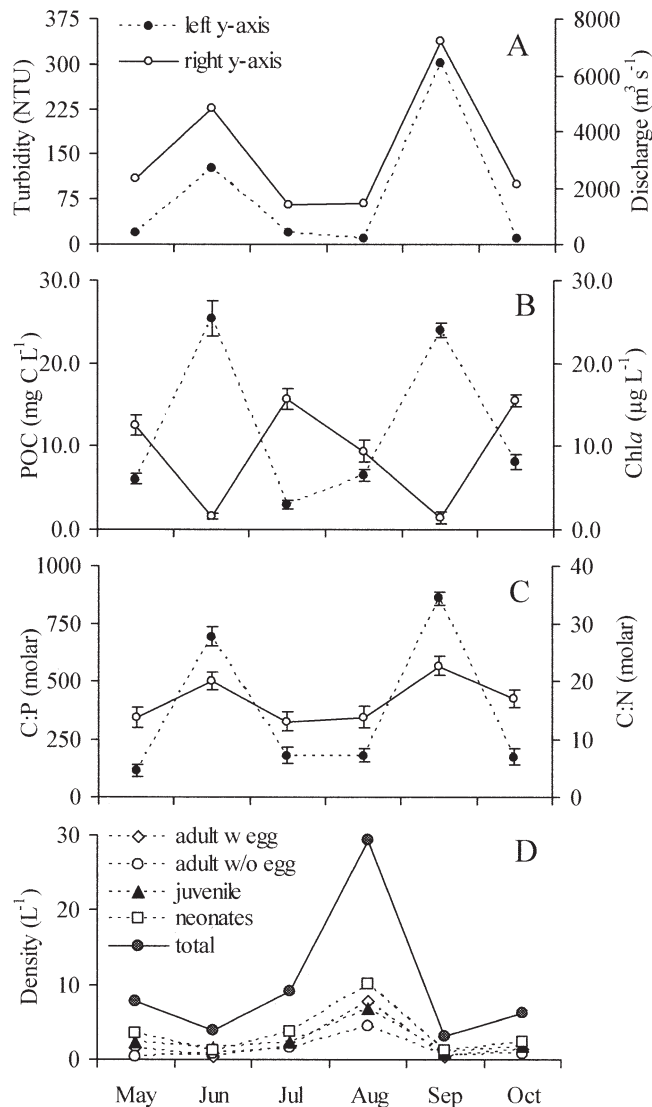


Fig. 1. (A) Turbidity (NTU) and discharge ( $\text{m}^3 \text{s}^{-1}$ ), (B) POC ( $\text{mg C L}^{-1}$ ), and Chl *a* ( $\mu\text{g L}^{-1}$ ), (C) seston C : P (molar) and C : N (molar) ratios, and (D) *Bosmina* population density of the Ohio River (at Westport, Kentucky) during May to October 2004. Error bars are standard errors.

(Fig. 1). Highest discharge occurred in June and September in association with hurricane-driven rain events. Turbidity, POC, and seston C : P, C : N, and N : P were positively related to discharge, whereas Chl *a* was inversely related. At low flow, turbidity was less than 25 nephelometric turbidity units (NTU) but exceeded 300 NTU during the June and September high-flow events. POC concentrations in the river were also highest ( $>25 \text{ mg C L}^{-1}$ ) during elevated discharge but did not exceed  $10 \text{ mg C L}^{-1}$  when the river was at typical summer flow (May, July, August, October). In contrast, Chl *a* was lowest ( $<1 \mu\text{g L}^{-1}$ ) during high flow and highest ( $>10 \mu\text{g L}^{-1}$ ) at low flow. We observed a strong negative relationship between Chl *a* and turbidity ( $R^2 = 0.87$ ,  $p < 0.05$ ). The N and P content of seston varied widely in response to changes in discharge. June and September (high flow) were characterized by low

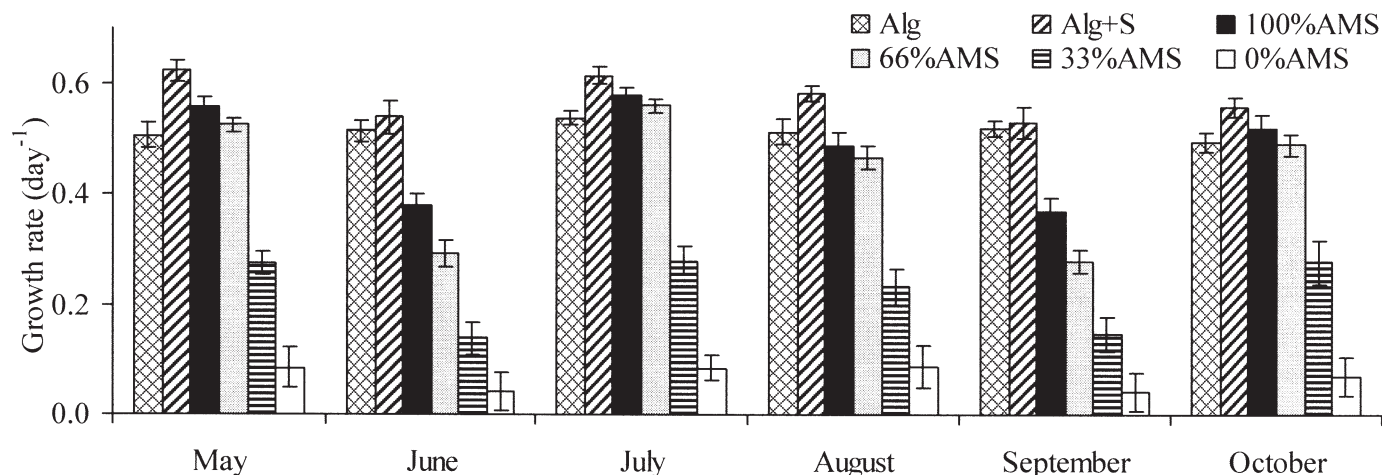


Fig. 2. Average growth rates,  $\mu$  ( $\text{d}^{-1}$ ), of *Bosmina* fed algae (Alg), river seston supplemented with algae (Alg+S), or varying concentrations of river seston alone (0–100% ambient: 0%AMS, 33%AMS, 66%AMS, and 100%AMS) during experiments conducted between May and October. June and July represent low-flow and May, July, August, and October represent high-flow periods. Error bars are standard errors.

N and P content ( $C:P > 600$ ;  $C:N > 20$ ) whereas seston was relatively nutrient rich in other months ( $C:P = 115$ – $180$ ;  $C:N = 13$ – $17$ ). During low discharge, the P content of seston was similar to that of *Scenedesmus* ( $C:P = 150$ – $175$ ) while the N content was somewhat lower (*Scenedesmus*  $C:N = 8.5$ – $10$ ). The abundance of *Bosmina* in the Ohio River varied as a function of discharge with lowest densities ( $< 5$  individuals [ $\text{ind}$ ]  $\text{L}^{-1}$ ) occurring in June and September (Fig. 1D). Peak abundance of neonates, juveniles, and adults occurred in August when total density reached  $\sim 30$   $\text{ind L}^{-1}$ . During September, a significant fraction ( $\sim 12\%$ ) of the total population consisted of males ( $0.4$   $\text{ind L}^{-1}$ ); the male population declined in the following month (October) as the river discharge decreased.

Experiments varying food quality and quantity had significant effects (overall treatment  $F_{5,46} = 31.09$ ,  $p < 0.001$ ) on *Bosmina* growth rates with group means for various treatments ranging from  $0.1$  to  $0.6$   $\text{d}^{-1}$  (Fig. 2). Consistently high growth rates ( $\mu > 0.5$ ) were observed among *Bosmina* feeding on green algae and there were no significant differences between treatments where river seston was replaced by or supplemented with algae (Alg, Alg+S). *Bosmina* feeding on seston at ambient concentrations (100%AMS) exhibited growth rates comparable to those receiving algal-amended diets except during months associated with high discharge (June, September). In these months, growth rates at ambient seston concentrations were significantly lower ( $\mu < 0.4$ ) relative to algal-amended diets ( $p < 0.01$ , Tukey HSD). Reductions of food quantity (dilution of seston) had a significant effect on growth rates during each of the six experiments. Growth rates declined significantly at 0%AMS and 33%AMS in all months whereas effects at the 66% level of dilution were apparent only during months with high discharge. Some of the *Bosmina* died at 0%AMS, but the remaining animals exhibited positive growth rates, possibly the result of a maternal effect. During low-flow conditions, *Bosmina* did not experience food limitation effects at ambient or

moderately reduced (66%) seston concentrations. Changes in seston quantity and quality associated with high flow resulted in reduced growth rates (relative to algal-amended diets) at all seston concentrations, including ambient.

Because growth responses to diet manipulations varied depending on discharge-related changes in seston, subsequent analyses (*Bosmina* P%, N%, RNA%) were based on pooling data according to high- versus low-discharge conditions (Fig. 3). *Bosmina* RNA content ranged from 1.5% to 4.5% with significant differences associated with flow conditions ( $F_{5,31} = 5.77$ ,  $p < 0.001$ ). Highest RNA content ( $> 4\%$ ) was measured for animals receiving algal-amended diets (Tukey HSD). Similar values were obtained from animals feeding on seston at ambient concentrations during low-flow months. Reductions in seston quantity had a significant effect on RNA% at all food levels during low-flow months. During high-flow months, RNA content was significantly lower at 100%, 66%, and 33% of ambient seston concentrations relative to corresponding treatments during low-flow months. Treatment effects produced a smaller range of variation (low vs. high discharge  $F_{5,9} = 21.34$ ,  $p < 0.001$ ) in *Bosmina* P content (0.5–0.8%) relative to that observed for RNA content (twofold) and growth rate (sixfold). The highest P content was measured in animals receiving algal-amended diets. Similar values were obtained during low-flow months over a broad range of seston concentrations (33–100% ambient). Lower body P content was observed in corresponding treatments during high flow and at 0%AMS (all months). The C and N content of *Bosmina* was not significantly affected by the treatments with C ranging between 42% and 45% and N ranging between 7% and 8.5% across treatments and months.

Analyses of pooled data (all dates and treatments) revealed significant covariation of P and RNA content with growth rates ( $R^2 = 0.58$  and  $0.67$ , respectively; Fig. 4). The P and RNA contents of *Bosmina* were also found to be significantly correlated, although the predictive power of this relationship was weaker ( $R^2 = 0.49$ ). Relationships

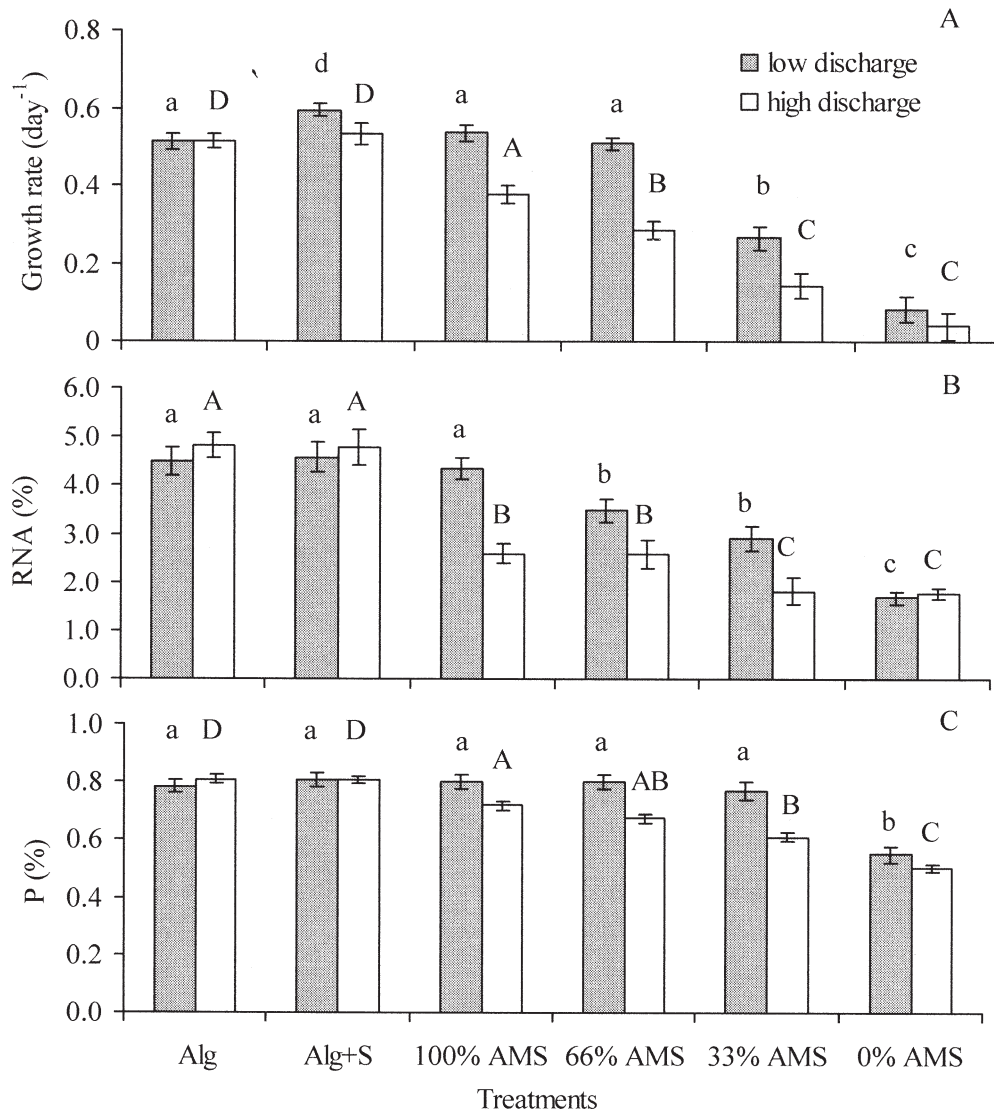


Fig. 3. (A) Average growth rates,  $\mu$  ( $\text{d}^{-1}$ ), (B) RNA content (% dry mass), and (C) P content (% dry mass) of *Bosmina* fed algae (Alg), river seston supplemented with algae (Alg+S), or varying concentrations of river seston alone (0–100% ambient: 0%AMS, 33%AMS, 66%AMS, and 100%AMS). Results from six experiments conducted between May and October are pooled according to low-discharge (May, July, August, and October; shaded bars with lowercase letters) and high-discharge (June and September; unshaded bars with uppercase letters) conditions. Letters above bars represent results of pairwise (Tukey honestly significant difference [HSD]) comparison test. Error bars are standard error.

among the three parameters were particularly strong ( $R^2 = 0.86\text{--}0.96$ ) in the subset of data obtained during high-flow conditions (June and September experiments). During low discharge, only the correlation between RNA% and growth rate was significant ( $R^2 = 0.46$ ,  $p < 0.01$ ), whereas P% was not significantly correlated with RNA% or growth rate. Overall, these findings show that diet-induced changes in *Bosmina* growth rates were consistently associated with variation in RNA content. *Bosmina* P% was also found to covary with growth rates but only during periods when the P content of seston was low (high discharge).

The RNA content of field-collected *Bosmina* exhibited significant monthly variation (Fig. 5;  $p < 0.001$ , Tukey

HSD) with lowest values (2.5%) occurring during high discharge and highest values (4%) measured in low-flow months. These values were comparable to those observed in the 66% and 100% ambient seston treatments (respectively) during corresponding months. The P content of field-collected *Bosmina* exhibited a somewhat narrower range of variation (0.7–0.8%; Fig. 5B) in comparison to animals subjected to diet experiments (0.5–0.8%). The P content of animals collected in high-flow months (June and September) was significantly lower than that in animals collected in May ( $p < 0.02$ , Tukey HSD) but did not differ significantly from animals collected in other low-flow months. Pooled data (all months) also revealed a significant

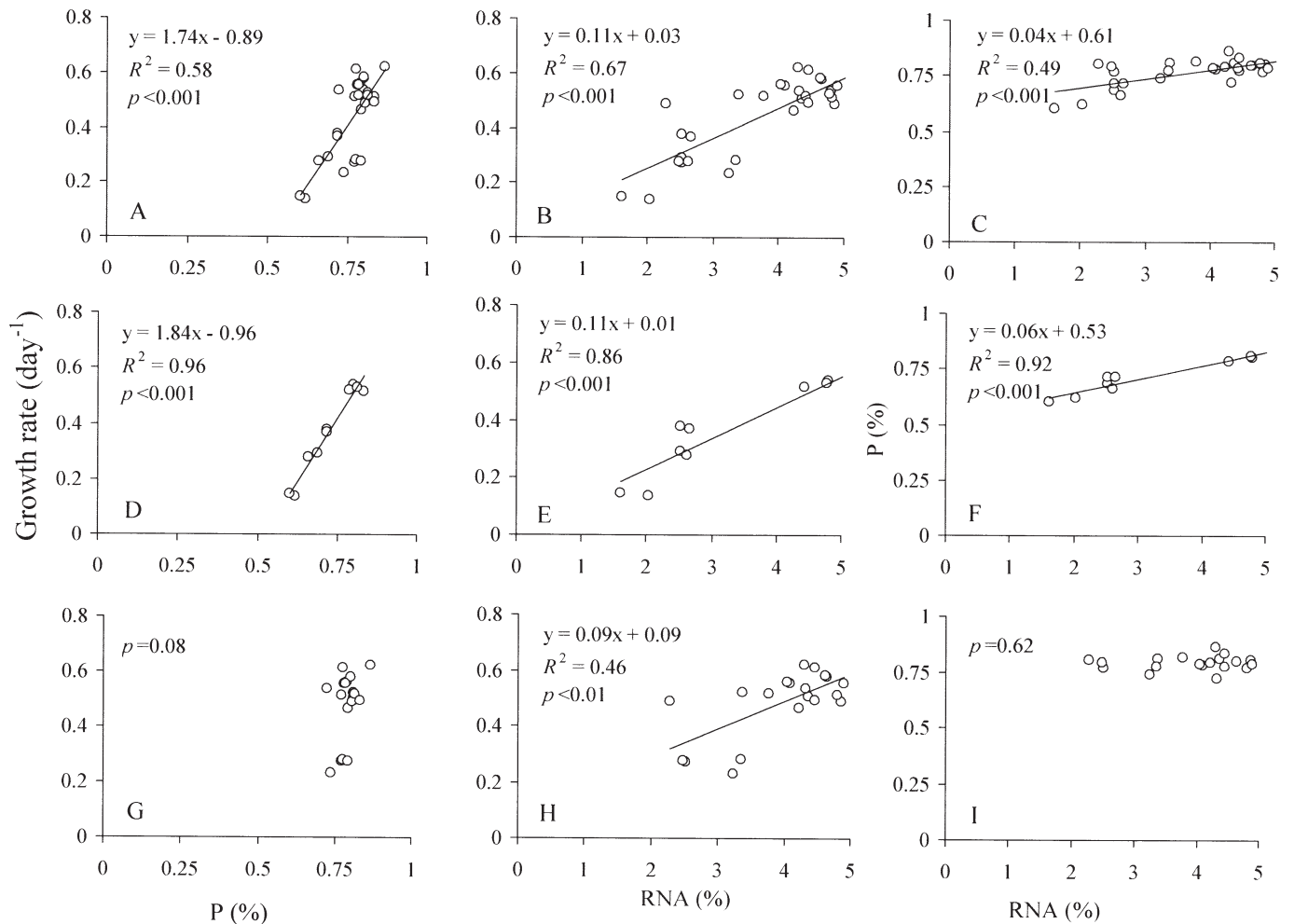


Fig. 4. Relationships between growth rate,  $\mu$  (d<sup>-1</sup>), and body P content (P%), growth rate,  $\mu$  (d<sup>-1</sup>), and RNA content (RNA%), and P content and RNA content of Ohio River *Bosmina*. Regressions shown for (A, B, C) all data (May to October), (D, E, F) high-discharge conditions (June and September), and (G, H, I) low-discharge conditions (May, July, August, and October).

association between P% and RNA% for the field-collected *Bosmina* (Fig. 6) with regression parameters (slope = 0.035, intercept = 0.62) similar to those obtained during laboratory feeding experiments (slope = 0.042, intercept = 0.61), although the strength of the relationship was somewhat lower ( $R^2 = 0.40$  and  $0.49$  for field and lab, respectively). The C and N content of field-collected *Bosmina* did not differ among months.

## Discussion

*Bosmina* growth rates were found to be highly sensitive to changes in food resource conditions arising from monthly variation in river discharge. Previous work on riverine zooplankton has suggested that high concentrations of particulate organic matter associated with elevated discharge may reduce feeding efficiency through mechanical interference (Saunders and Lewis 1988; Pace et al. 1992). Although *Bosmina* feeding on river seston exhibited lower growth rates during months with elevated turbidity, our experimental data suggest that this was not a result of high particulate concentrations. If high seston concentra-

tions diminished feeding rates by interference, we would expect individuals feeding on seston supplemented with green algae to exhibit lower growth rates than those feeding on green algae alone (Alg, Alg+S treatments). However, similar growth rates were observed for both diets in all flow conditions, suggesting that the presence of high seston concentrations did not adversely affect *Bosmina*. This finding is consistent with previous studies suggesting that *Bosmina* is a highly selective feeder (DeMott 1982, 1985) and may in part account for its success in riverine environments.

Elevated discharge was associated with increases in food quantity (as measured by POC) but poor food quality (low N and P content). Changes in seston characteristics associated with elevated discharge had a deleterious effect on growth rates despite high concentrations of potential food resources. We attribute reductions in seston quality during high discharge to dilution of the nutrient-rich, algal fraction by nutrient-poor materials of presumably terrestrial origin. The algal carbon fraction of seston (estimated from Chl *a* and POC concentrations and assuming C:Chl *a* = 20; Sellers and Bukaveckas 2003) declined from 5%



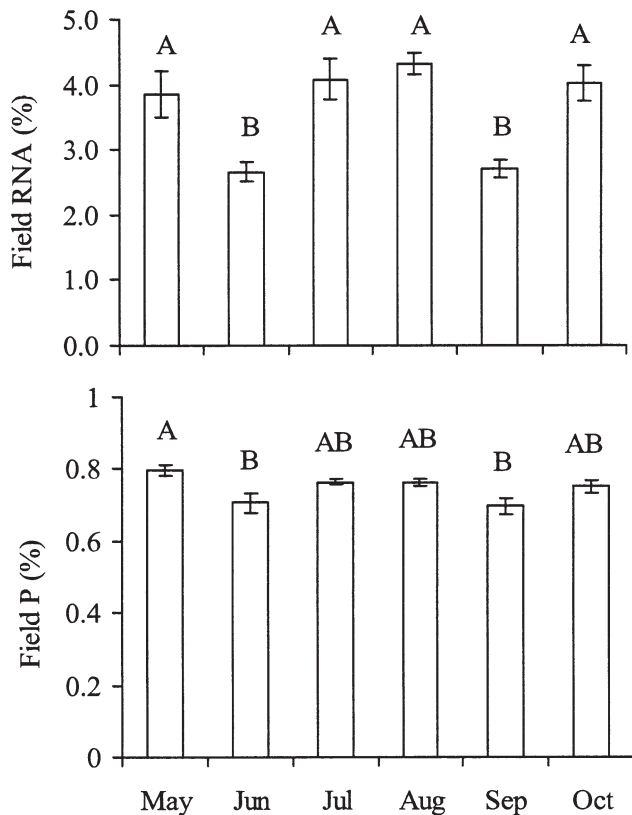


Fig. 5. Average P content (%) and RNA content (%) of *Bosmina* collected from the Ohio River during May to October. Letters above bars denote significant differences based on pairwise (Tukey HSD) comparisons. Error bars are standard errors.

during low-flow months to 0.2% in high-flow months. At elevated discharge, growth rates were lower throughout the range of food treatments (33–100% of ambient) despite twofold to threefold higher POC concentrations. Increases in food quantity apparently did not offset declines in food quality and resulted in lower intake of key nutritional factors. Our findings are consistent with previous studies suggesting that phytoplankton constitute the nutritionally important fraction of seston in systems dominated by detrital carbon inputs (Thorp and Delong 2002; Sobczak et al. 2005).

The coupling between zooplankton growth and autochthonous food resources suggests that algae contribute a disproportionate fraction of one or more key dietary components. Identification of the specific dietary factors restricting growth is complicated by uncertainty regarding nutritional needs relative to environmental availability for factors such as mineral nutrients (P) and various biochemicals (e.g., essential fatty acids). Elser et al. (2003) argued that P plays a central role in the diet of consumers because high growth rates require increased allocation to P-rich ribosomal RNA. They showed that growth, RNA%, and P% were tightly coupled for diverse biota when conditions favored maximal growth or if P deficiency restricted growth. We also observed covariation in growth, RNA%, and P% for both field and laboratory animals that was generally consistent with this hypothesis. Our data suggest

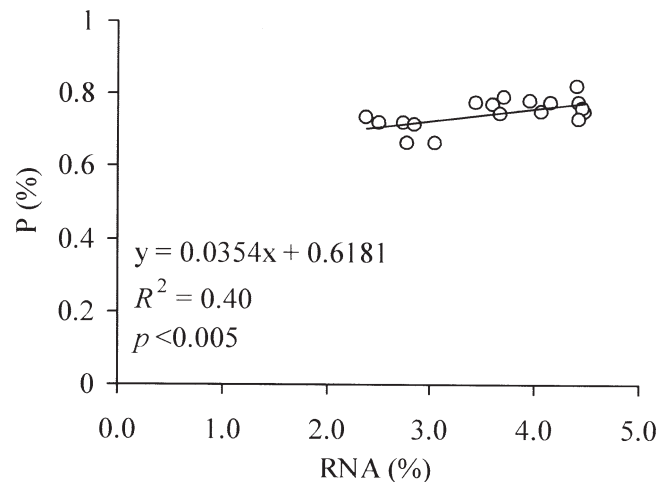


Fig. 6. Relationship between RNA content (%) and P content (%) of *Bosmina* collected from the Ohio River during May to October 2004. Coefficients are from the results of linear regression.

that riverine *Bosmina* may suffer from P limitation during periods of elevated discharge when the P content of their food is low (seston C : P > 600). Under these conditions, P% and RNA% were closely coupled to growth ( $R^2 = 0.96$  and  $0.86$ , respectively) and to each other ( $R^2 = 0.92$ ). P limitation may arise in riverine consumers when hydrologic conditions favor terrestrial inputs, sediment resuspension, and low algal productivity, thereby resulting in seston fractions dominated by low-P materials.

During low-flow conditions, riverine seston was found to be P rich relative to *Bosmina* requirements and growth rates were decoupled from body P content, suggesting that P limitation was not operating during these periods. Animals fed low C : P seston grew faster and exhibited higher RNA% but body P% did not change appreciably. Similar decoupling of growth, RNA%, and P% has been noted for bacteria when substrate C:P ratios are low (Makino et al. 2003), for P-sufficient cyanobacteria and eukaryotic algae (Rhee 1978; Healey 1985), and for *Daphnia* when food is N deficient (Acharya et al. 2004b). It was previously hypothesized that when dietary intake of P is in excess, a greater fraction of P will be contained in pools other than RNA (Elser et al. 2003; Acharya et al. 2004b). This hypothesis is supported by our data for *Bosmina*. When river seston was low in P (high flow), RNA-P in *Bosmina* was ~50% of total body P (assuming RNA-P = 10% RNA). This fraction declined to 35% when seston was P rich (low flow). Maternal effects were not found to be important, as growth rates, P%, and RNA% in the no-food treatments (0%AMS) did not differ between months with high or low discharge.

Body RNA% was found to be a strong predictor of juvenile growth during laboratory feeding experiments. RNA content was strongly and linearly related to growth over a broad range of resource and growth conditions ( $\mu = 0.1$  to  $0.6$ ) and regression parameters were similar to previously reported values for *Daphnia* (Elser et al. 2003). The RNA% of field-collected *Bosmina* responded to



natural variation in food resources with shifts in RNA% that were comparable to those observed during laboratory experiments. However, our findings show that *Bosmina* differs from *Daphnia* in that RNA was correlated with growth rate under both P-sufficient and P-deficient dietary conditions. Also, *Bosmina* exhibited lower RNA content (~4.0% of body mass) compared to *Daphnia* (~8.0%; Vrede et al. 2002; Acharya et al. 2004b) but their maximum growth rates were similar ( $>0.5 \text{ d}^{-1}$ ). Thus, *Bosmina* achieves higher growth rates per unit RNA. This demonstration of coupling between RNA content and growth using natural food sources and field-collected animals extends the validity of the use of RNA as an indicator of individual performance for this widespread and ecologically significant taxon.

Lacking additional data on the biochemical properties of river seston, we can not exclude the possibility that other dietary factors may have contributed to growth limitation. In a related study, we compared life history traits of *Bosmina* raised on seston (collected during low discharge; C:P = 115) with those fed P-sufficient green algae (Acharya et al. 2005). Although the P content of both diets was similar, *Bosmina* raised on green algae grew larger, produced more offspring, and exhibited larger lipid reserves than those fed river seston. Biochemical analyses revealed that seston contained only a third of the essential fatty acid content found in the green algae diet. Taken together, these studies suggest that discharge-related changes in seston characteristics may be associated with shifts in dietary limitation from P (high discharge) to fatty acids (low discharge). This hypothesis assumes a precedence for P limitation (over biochemical limitation) during elevated discharge based on the strong coupling between growth, P%, and RNA% when seston C:P is high. However, we can not discount the potential for colimitation by P and biochemical factors because hydrodynamic conditions favoring low seston P% would also favor low concentrations of other algal-derived dietary factors (essential fatty acids, proteins, etc.).

Results from our feeding experiments suggest that consumer growth potential in riverine environments is sensitive to shifts in food resources associated with changing discharge conditions. The timing and frequency of these shifts is unpredictable (arising from climatic events) particularly in regulated rivers. Because water regulation structures are common worldwide (Nilsson et al. 2005), our findings may be generally applicable in rivers where seasonal flood-pulse cycles have been replaced by irregular hydrographs (Sparks et al. 1998). Although differences in growth rates between high and low discharge conditions (ambient seston concentrations) were relatively small ( $\mu = 0.40$  vs.  $0.55 \text{ d}^{-1}$ , respectively), our related study showed that small differences in juvenile growth rates were later followed by differences in adult body size, age at maturation, brood size and frequency, and size of neonates (Acharya et al. 2005). The cumulative impact arising from the various life history responses (twofold difference in total fecundity) may be of sufficient magnitude to account in part for the sixfold range of variation in population size between low- and high-flow periods (Fig. 1D).

Incorporating knowledge of consumer response to variation in food quality is an important component to building spatially and temporally explicit models of trophic interactions. Of central importance to the application of stoichiometry approaches in rivers is the issue of whether dietary restrictions are relevant to the population ecology of pelagic consumers during high discharge conditions. We argue that this is largely an issue of scale. In the McAlpine Pool (length = 122 km), transit times during high discharge are short (~2 d). In a reach-scale assessment of growth conditions, deleterious effects arising from poor food quality would be confined to a single clutch interval. However, at the scale of the Ohio River, the effects would be manifested over the length of the river (1,600 km) such that food quality effects may ultimately determine whether there is a net gain or loss of individuals in parcels of water traveling downriver.

Our findings support the utility of ecostoichiometric approaches to studies of riverine food webs by demonstrating that variation in consumer growth rates can be related to changes in seston elemental composition even for a selective feeder such as *Bosmina*. Previous demonstrations of this approach have focused largely on *Daphnia* in lakes where its role as dominant grazer strengthens the importance of species-specific stoichiometry to trophic dynamics. Despite its numerical dominance in the plankton, it is unlikely that *Bosmina* could fulfill the same role of dominant grazer given its low individual filtration rates. However, other benthic or pelagic consumers have been shown to individually or collectively exert a strong influence on organic matter transformations in riverine systems (Caraco et al. 1997) such that investigations of dietary imbalances arising from environmental dynamics may yield useful insights. In this context, measurements of consumer RNA% may be a useful surrogate for laboratory growth experiments to assess nutritional sufficiency of food resources within a stoichiometric framework. Rivers, like estuaries, provide good model systems for testing stoichiometric effects on consumers because temporal variation in physical processes gives rise to a wide range of food quality conditions.

## References

- ACHARYA, K., J. D. JACK, AND P. A. BUKAVECKAS. 2005. Dietary effects on life history traits of riverine *Bosmina*. *Freshwater Biol.* **50**: 965–975.
- , M. KYLE, AND J. J. ELSER. 2004a. Effects of stoichiometric dietary mixing on *Daphnia* growth and reproduction. *Oecologia* **138**: 333–340.
- , ———, AND ———. 2004b. Biological stoichiometry of *Daphnia* growth: An ecophysiological test of the growth rate hypothesis. *Limnol. Oceanogr.* **49**: 656–665.
- APHA. 1998. Standard methods for the examination of water and wastewater. American Public Health Association/Water Environment Federation.
- ARAR, E. J., AND G. B. COLLINS. 1997. In vitro determination of chlorophyll a and phaeophytin a in marine and freshwater algae by fluorescence. National Exposure Research Laboratory, U.S. Environmental Protection Agency.
- BASU, B. K., AND E. R. PICK. 1997. Phytoplankton and zooplankton development in a lowland, temperate river. *J. Plankton Res.* **19**: 237–253.

- BOERSMA, M., C. SCHÖPS, AND E. McCAULEY. 2001. Nutritional quality of seston for the freshwater herbivore *Daphnia galeata hyalina*: Biochemical versus mineral limitations. *Oecologia* **129**: 342–348.
- BRETT, M. T., AND D. C. MULLER-NAVARRA. 1997. The role of highly unsaturated fatty acids in aquatic food-web processes. *Freshwater Biol.* **38**: 483–499.
- BUKAVECKAS, P. A., D. L. GUELDA, J. D. JACK, R. W. KOCH, T. SELLERS, AND J. SHOSTELL. 2005. Effects of point source inputs, sub-basin delivery and longitudinal variation in material retention on C, N and P fluxes within the Ohio River basin. *Ecosystems* **8**: 825–840.
- CARACO, N. F., J. J. COLE, P. A. RAYMOND, D. L. STRAYER, M. L. PACE, S. E. G. FINDLAY, AND D. T. FISCHER. 1997. Zebra mussel invasion in a large, turbid river: Phytoplankton response to increased grazing. *Ecology* **78**: 588–602.
- DEMELO, R., AND P. D. N. HEBERT. 1994. A taxonomic reevaluation of North American Bosminidae. *Can. J. Zool.* **72**: 1808–1825.
- DEMOTT, W. R. 1982. Feeding selectivity and relative digestion rates of *Daphnia* and *Bosmina*. *Limnol. Oceanogr.* **27**: 518–527.
- . 1985. Relations between filter mesh size, feeding mode, and capture efficiency for cladocerans feeding on ultrafine particles. *Ergeb. Limnol.* **21**: 125–134.
- . 1986. The role of taste in food selection by freshwater zooplankton. *Oecologia* **69**: 334–340.
- , J. R. EDINGTON, AND A. J. TESSIER. 2004. Testing zooplankton food limitation across gradients of depth and productivity in small stratified lakes. *Limnol. Oceanogr.* **49**: 1408–1416.
- , R. D. GULATI, AND K. SIEWERTSEN. 1998. Effects of phosphorus-deficient diets on the carbon and phosphorus balance of *Daphnia magna*. *Limnol. Oceanogr.* **43**: 1147–1161.
- ELSER, J. J., AND R. P. HASSETT. 1994. A stoichiometric analysis of the zooplankton–phytoplankton interaction in marine and freshwater ecosystems. *Nature* **370**: 211–213.
- , H. HAYAKAWA, AND J. URABE. 2001. Nutrient limitation reduces food quality for zooplankton: *Daphnia* response to seston phosphorus enrichment. *Ecology* **82**: 898–903.
- , AND OTHERS. 2003. Growth rates—stoichiometric couplings in diverse biota. *Ecol. Lett.* **6**: 936–943.
- FINDLAY, S., J. QUINN, C. HICKEY, G. BURRELL, AND M. DOWNES. 2001. Land-use effects on supply and metabolism of stream dissolved organic carbon. *Limnol. Oceanogr.* **46**: 345–355.
- GUELDA, D. L., R. W. KOCH, J. D. JACK, AND P. A. BUKAVECKAS. 2005. Experimental evidence for density-dependent effects and the importance of algal production in determining population growth rates of riverine zooplankton. *River Res. Appl.* **21**: 1–14.
- GULATI, R. D., AND W. R. DEMOTT. 1997. The role of food quality for zooplankton: Remarks on the state-of-the-art, perspectives and priorities. *Freshwater Biol.* **38**: 753–768.
- HANEY, J. F., AND D. J. HALL. 1973. Sugar-coated *Daphnia*: A preservation technique for Cladocera. *Limnol. Oceanogr.* **18**: 331–333.
- HEALEY, F. P. 1985. Interacting effects of light and nutrient limitation on the growth rate of *Synechococcus linearis* (Cyanophyceae). *J. Phycol.* **21**: 134–146.
- HESSEN, D. O. 1992. Nutrient element limitation of zooplankton production. *Am. Nat.* **140**: 799–814.
- , AND A. LYCHE. 1991. Inter- and intraspecific variations in zooplankton elemental composition. *Arch. Hydrobiol.* **114**: 321–347.
- JACK, D. J., AND J. H. THORP. 2000. Effects of the benthic suspension feeder *Dreissena polymorpha* on zooplankton in a large river. *Freshwater Biol.* **44**: 569–579.
- , AND ———. 2002. Impacts of fish predation on an Ohio River zooplankton community. *J. Plankton Res.* **24**: 119–127.
- KERFOOT, W. C. 1975. Seasonal changes of *Bosmina* (Crustacea, Cladocera) in Frains Lake, Michigan: Laboratory observations of phenotypic changes induced by inorganic factors. *Freshwater Biol.* **5**: 227–243.
- KYLE, M., T. WATTS, J. D. SCHADE, AND J. J. ELSER. 2003. A microfluorometric method for quantifying RNA and DNA in terrestrial insects. *J. Insect Sci.* **3**: 1–7.
- MAKINO, W., J. B. COTNER, R. W. STERNER, AND J. J. ELSER. 2003. Are bacteria more like animals than plants? Growth rate and resource dependence of bacterial C:N:P stoichiometry. *Funct. Ecol.* **17**: 121–130.
- NILSSON, C., C. A. REIDY, M. DYNESIUS, AND C. REVENGA. 2005. Fragmentation and flow regulation of the world's large river systems. *Science* **308**: 405–408.
- PACE, M. L., S. E. G. FINDLAY, AND D. LINTS. 1992. Zooplankton in advective environments: The Hudson River community and a comparative analysis. *Can. J. Fish. Aquat. Sci.* **49**: 1060–1069.
- , AND OTHERS. 2004. Whole-lake carbon-13 additions reveal terrestrial support of aquatic food webs. *Nature* **427**: 240–243.
- RHEE, G. 1978. Effects of N:P atomic ratios and nitrate limitation on algal growth, cell composition, and nitrate uptake. *Limnol. Oceanogr.* **23**: 10–25.
- SAUNDERS, J. F., III, AND W. M. LEWIS, JR. 1988. Zooplankton abundance and transport in a tropical white-water river. *Hydrobiologia* **162**: 147–156.
- SCHULZ, K. L., AND R. W. STERNER. 1999. Phytoplankton phosphorus limitation and food quality for *Bosmina*. *Limnol. Oceanogr.* **44**: 1549–1556.
- SELLERS, T., AND P. A. BUKAVECKAS. 2003. Phytoplankton production in a large, regulated river: A modeling and mass balance assessment. *Limnol. Oceanogr.* **48**: 1476–1487.
- SOBCZAK, W. V., J. E. CLOERN, A. D. JASSBY, B. E. COLE, T. S. SCHRAGA, AND A. ARNSBERG. 2005. Detritus fuels ecosystem metabolism but not metazoan food webs in San Francisco Estuary's freshwater delta. *Estuaries* **28**: 122–135.
- SPARKS, R. E., J. C. NELSON, AND Y. YIN. 1998. Naturalization of the flood regime in regulated rivers. *Bioscience* **48**: 706–720.
- STERNER, R. W., J. J. ELSER, E. J. FEE, S. J. GUILDFORD, AND T. H. CHRZANOWSKI. 1997. The light:nutrient ratio in lakes: The balance of energy and materials affects ecosystem structure and process. *Am. Nat.* **150**: 663–684.
- , AND ———. 2002. *Ecological stoichiometry: The biology of elements from molecules to the biosphere*. Princeton Univ. Press.
- , D. D. HAGEMEIER, W. L. SMITH, AND R. F. SMITH. 1993. Phytoplankton nutrient limitation and food quality for *Daphnia*. *Limnol. Oceanogr.* **38**: 857–871.
- SYVITSKI, J. P. M., C. J. VOROSMARTY, A. J. KETTNER, AND P. GREEN. 2005. Impact of humans on the flux of terrestrial sediment to the global coastal ocean. *Science* **308**: 376–380.
- THORP, J. H., A. R. BLACK, K. H. HAAG, AND J. D. WEHR. 1994. Zooplankton assemblages in the Ohio River: Seasonal, tributary and navigation dam effects. *Can. J. Fish. Aquat. Sci.* **51**: 1634–1643.
- , AND A. F. CASPER. 2003. Importance of biotic interactions in large rivers: An experiment with planktivorous fish, dreissenid mussels, and zooplankton in the St. Lawrence. *River Res. Appl.* **19**: 265–279.

- , AND M. D. DELONG. 2002. Dominance of autochthonous autotrophic carbon in food webs of heterotrophic rivers? *Oikos* **96**: 543–550.
- , AND S. MANTOVANI. 2005. Zooplankton of turbid and hydrologically dynamic prairie rivers. *Freshwater Biol.* **50**: 1474–1491.
- URABE, J. 1991. Effect of food concentration on growth, reproduction and survivorship of *Bosmina longirostris* (Cladocera): An experimental study. *Freshwater Biol.* **25**: 1–8.
- , AND Y. WATANABE. 1992. Possibility of N-limitation or P-limitation for planktonic cladocerans—an experimental test. *Limnol. Oceanogr.* **37**: 244–251.
- VON ELERT, E., AND T. WOLFFROM. 2001. Supplementation of cyanobacterial food with polyunsaturated fatty acids does not improve growth of *Daphnia*. *Limnol. Oceanogr.* **46**: 1552–1558.
- VREDE, T., J. PERSSON, AND G. ARONSEN. 2002. The influence of food quality (P:C ratio) on RNA:DNA ratio and somatic growth rate of *Daphnia*. *Limnol. Oceanogr.* **47**: 487–494.
- WHITE, D. S., K. JOHNSTON, AND M. MILLER. 2005. Ohio River Basin., *In* A. C. Benke and C. E. Cushing [eds.], *Rivers of North America*. Elsevier.
- WOODWARD, G., AND A. G. HILDREW. 2002. Food web structure in riverine landscapes. *Freshwater Biol.* **47**: 777–798.

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