

REPORT

Growth responses of littoral mayflies to the phosphorus content of their food

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Abstract

We examined how mayfly growth rates and body stoichiometry respond to changing phosphorus (P) content in food. In two experiments, mayfly nymphs were given high or low quantities of food at different carbon:phosphorus (C:P) ratios and their growth was measured. Low food quantity resulted in negative growth rates in both experiments, regardless of food P content. However, under high food availability, mayfly growth was affected by the type of food eaten, with low C:P ratio food producing more rapid growth. In addition, mayfly growth increased somewhat when P-poor food was artificially enriched with inorganic P although this effect was not statistically significant. Mayfly body P content was inversely related to body size but increased in animals fed artificially P-enriched food. A model was constructed to simulate mass balance constraints on mayfly growth imposed by the relative supply of two elements (C and P) in food. The model shows that mayfly growth should be limited by food P content at moderately low C:P ratios (*c.* 120, by mass). Given high C:P ratios (mean *c.* 270, by mass) in periphyton from oligotrophic boreal lakes, our experimental and theoretical results indicate that stoichiometric constraints are important factors affecting benthic food webs in lakes from the Canadian Shield and perhaps in other systems with similarly high C:P ratios in periphyton.

Keywords

C:P ratio, ecological stoichiometry, growth rate, mass balance, mayfly, periphyton, phosphorus.

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INTRODUCTION

Poor nutritional quality of food can reduce growth rates of benthic consumers (Iversen 1974; Ward & Cummins 1979; Söderström 1988; Sharfstein & Steinman 2001; Stelzer & Lamberti in press). One reason that benthic consumers could experience reduced growth is that there is an insufficient content of nutrient elements in their food, which imposes mass balance constraints on biomass production (Söderström 1988; Sharfstein & Steinman 2001; Stelzer & Lamberti in press). Whereas this stoichiometric hypothesis has received considerable attention in planktonic systems (see reviews by Gulati & DeMott 1997; Sterner & Schulz 1998), the effects of high food C:nutrient ratios on growth processes in benthic consumers remain poorly understood. In this paper, we consider how growth processes of littoral mayflies relate to the phosphorus (P) content in their food.

Stoichiometric food quality is generally assessed by comparing the ratio of chemical elements found in food

to that needed for maintenance, growth, and reproduction in a particular consumer (Sterner & Schulz 1998). One implication of defining food quality with respect to the elemental requirements of each consumer is that a particular food does not have an inherent quality (Sterner 1997; Sterner & Schulz 1998). This has been studied extensively in cladoceran zooplankton where growth of the P-rich cladoceran, *Daphnia*, is strongly reduced when algal food C:P ratios are increased (Sterner 1993; Urabe *et al.* 1997; Elser *et al.* 2001; Plath & Boersma 2001). Under the same food conditions, the low-P cladoceran, *Bosmina*, experiences little, if any, growth reductions (Schulz & Sterner 1999). In addition, the quality of a food depends upon its availability to consumers (Urabe & Watanabe 1992; Sterner & Robinson 1994; Rothhaupt 1995). Negative effects of high C:nutrient ratios in food on consumer growth should be greatest at high food quantities (Sterner & Robinson 1994; Sterner 1997). Slow consumer growth caused by low food quantity lowers requirements for elements (N and P)

necessary for biomass production and increases the relative demand for carbon as respiration becomes a greater proportion of total metabolism (Urabe & Watanabe 1992; Sterner & Robinson 1994).

Thus, to examine stoichiometric food quality one must determine whether the relative supplies of elements in food material meet the requirements for growth, reproduction, and maintenance of particular consumers (Sterner & Hessen 1994). Most information available on consumer C:N:P stoichiometry is from work done on plankton, but attempts are being made to extend this approach to other groups of organisms, such as terrestrial insects (Elser *et al.* 2000b). Whereas some zooplankton taxa have considerably higher P content than others, little difference is generally found in N content (Andersen & Hessen 1991; Main *et al.* 1997). It has recently shown that C:N:P stoichiometry in terrestrial insects exhibits similar patterns of variation (Elser *et al.* 2000a). In addition, intraspecific variation in zooplankton P content is usually less than interspecific variation, which provides the basis for the assumption that planktonic consumers homeostatically maintain a relatively constant body elemental composition (Elser & Urabe 1999). Deviations in body P content observed for particular zooplankton taxa coincide with changes in zooplankton body size (Carrillo *et al.* 1996; Main *et al.* 1997; Carrillo *et al.* 2001) and with changes in their diet (DeMott *et al.* 1998). Little is known, however, about C:N:P stoichiometry of benthic consumers and hence, about their nutritional requirements for carbon and phosphorus in their food.

In this paper, we report the results of two experiments that examined how phosphorus-poor food affects the growth of littoral mayflies. Specifically, we tested whether high C:P ratios in food provided at high and low quantity reduce growth rates and alter the body stoichiometry of these benthic consumers. In addition, we used a model based on mass balance principles to explore further whether the elemental composition of natural food sources matches that needed for balanced growth in mayflies. Together, our results indicate that the stoichiometry of both consumer and food is likely to be an important aspect of trophic interactions in the lake benthos.

MATERIALS AND METHODS

Laboratory growth experiments

We initially conducted growth experiments with mayflies in the laboratory where we could carefully control C:P ratios of algal food. We tested the effects of high C:P ratios (*c.* 250, all ratios presented by mass) in a monoculture of the alga, *Scenedesmus acutus*, on growth rates of a benthic mayfly (*Caenis* sp.) given high and low food quantity. Newly hatched mayflies were collected from Kiwanis Park Pond in Tempe,

Arizona (111°55' W, 33°25' N) on February 20, 1999. All mayflies were measured for body length under a dissecting microscope and randomly chosen individuals were placed into separate 60-mL glass vials filled with 40 mL of aged tap water. No significant differences existed between initial sizes of mayflies assigned to different food treatments. Known quantities (0.15 mg C cm⁻², high quantity; 0.02 mg C cm⁻², low quantity) of phosphorus-replete (C:P *c.* 30) and phosphorus-deficient (C:P *c.* 250) *Scenedesmus acutus* were filtered onto 25 mm GF/C filters and placed on the inside of the vial caps. Caps were placed onto the vials, which were closed and inverted. Subsamples of algal material provided to animals were saved on GF/C filters and analysed for both particulate carbon and phosphorus content as described below. Animals (six replicates for each treatment combination) were transferred to new jars with fresh food every 2 days and then harvested after 6 days of incubation. Post-incubation weight measurements were taken for each animal to calculate mayfly growth rate. Growth rates were calculated as:

$$\mu = \frac{\ln(B_2) - \ln(B_1)}{\text{time}} \quad (1)$$

where μ is growth rate, B_2 is the measured post-weight and B_1 is the estimated animal pre-weight for each individual mayfly. Pre-weights were estimated for each individual mayfly by inserting measured pre-lengths into length-weight regressions developed simultaneously for these mayflies (Arizona State University experiment: $\log \text{ weight} = (3.00 \times \log \text{ length}) - 2.18$, $r^2 = 0.97$; Experimental Lakes Area (ELA) experiment: $\log \text{ weight} = (1.66 \times \log \text{ length}) - 1.23$, $r^2 = 0.85$).

Experimental Lakes Area growth experiments

With experiments at the ELA in north-western Ontario, Canada, we grew mayflies (*Ephemera* sp.) on natural organic matter collected from the littoral zones of two lakes, L239 and L302S. L302S and L239 were chosen because they normally have periphyton at the low and high ends of the C:P ratio gradient at ELA (L302S *c.* 135, L239 *c.* 290; Frost 2001). Newly hatched mayflies were collected from nearby L979 where they could be found in relatively high abundance. After measuring body lengths for all individuals, animals (six replicates for each treatment combination) were placed individually into 250 mL polyethylene jars filled with epilimnetic water from L239. No significant differences existed between initial sizes of mayflies assigned to different food treatments. Collected food was filtered onto 45 mm GF/C filters and placed on jar caps. Food was provided at high (0.32 mg C cm⁻²) and low quantity (0.05 mg C cm⁻²) by filtering different volumes of the collected slurry of periphyton. These levels (high and low) of food quantity are

somewhat low compared to periphyton biomass levels generally found in the ELA lakes (see Fig. 5). Periphyton collected from L239 was suspended in filtered lake water and that suspension was split into two halves, one of which was enriched by adding $14 \mu\text{mol P L}^{-1}$ as potassium phosphate for 4 h prior to filtering the material onto GF/C filters. P enrichment of L239 food reduced C:P ratios (by mass) from $c. 210$ to $c. 35$. Jars were randomly placed in a dark cooler at $c. 20^\circ\text{C}$. Every 2 days food was prepared as just described and replaced until the 6th day when animals were harvested, measured, dried, and then weighed on a microbalance. Growth rates were estimated as above.

Chemical analysis

All food was analysed for particulate carbon using an infrared analyser (O.I. corporation, Model 700) after digestion in an airtight bottle with potassium persulphate (Strickland & Parsons 1972; Sterner *et al.* 1993). Particulate P in food was analysed on the same digested sample using the molybdate blue–ascorbic acid reaction (American Public Health Association 1992). Carbon content (per unit dry weight) was assessed on weighed subsamples of mayflies from the ELA experiment using a mass spectrometer (PDZ Europa Corp., 20/20 IRMS) after homogenizing and then weighing small subsamples of the dried animals. Body P content (per unit dry weight) was estimated on weighed subsamples from the same mayflies as described above for food P.

Growth model and parameterization

We constructed a model to examine mass balance constraints of C and P on mayfly growth using the approach of Sterner (1997). Although our model was similar in form, we modified Sterner's model to incorporate mayfly-specific data on respiration rates, grazing rates, and body elemental

composition (Table 1). Briefly, we assumed that only one element (C or P) limited mayfly growth at a time based on Liebig's law of the minimum and that the C:P ratio of new production in mayflies remained constant. Under the second assumption, the rate of mayfly C acquisition must be proportional to the rate of P acquisition:

$$\frac{dC}{dt} = \frac{dP}{dt} \cdot \frac{Q_C}{Q_P} \quad (2)$$

where Q_C and Q_P are the C and P content of new production, respectively. In our model, we defined the rate of carbon and phosphorus acquisition as follows:

$$\frac{dC}{dt} = \frac{G_C \cdot C_F}{1 + G_C \cdot C_F \cdot T_H} \cdot A_C - R_C \quad (3)$$

$$\frac{dP}{dt} = \frac{G_C \cdot C_F}{1 + G_C \cdot C_F \cdot T_H} \cdot \frac{1}{f_{C:P}} \cdot A_P \quad (4)$$

where G_C is the grazing rate, C_F is the food quantity, T_H is the handling time, A_C and A_P are the maximum assimilation efficiencies of C and P, respectively, R_C is the rate of respiration, and $f_{C:P}$ is the food C:P ratio (see Table 1 for additional details of parameters). We modelled ingestion rates using a type II functional response fit to mayfly data (Scrimgeour *et al.* 1991). An equation for the boundary (in terms of food quantity and quality) where growth should change between C- and P-limitation is then obtained by substituting eqns 3 and 4 into eqn 2 and solving for C_F :

$$C_F = \frac{R_C \cdot Q_P}{G_C \cdot \left(A_C \cdot Q_P - \frac{A_P \cdot Q_C}{f_{C:P}} - T_H \cdot R_C \cdot Q_P \right)} \quad (5)$$

This equation represents a hyperbola in the plane of C_F vs. $f_{C:P}$. Following Sterner (1997) we derived 'thresholds' for food quantity (eqn 6) and quality (eqn 7) as the asymptotes of this hyperbola, by calculating limiting cases of infinite C_F and $f_{C:P}$:

Table 1 List of parameters and sources used in a model to examine mass balance constraints of C and P on mayfly growth. Note that grazing and respiration rates are mass-specific (mg C) values for individual mayflies derived from the listed source

Parameter	Symbol	Unit	Value(s) used in model	Derived from
Grazing rate	G_C	$\text{cm}^2 \text{ mg C}^{-1} \text{ day}^{-1}$	49, 4.9–49 ^a	Scrimgeour <i>et al.</i> (1991)
Handling time	T_H	$\text{day mg C mg C}^{-1}$	0.87	Scrimgeour <i>et al.</i> (1991)
Food C:P	$f_{C:P}$	mg C mg P^{-1}	38–580	Frost (2001)
Maximum C assimilation efficiency	A_C	None	0.50, 0.3–0.7 ^b	
Maximum P assimilation efficiency	A_P	None	0.9	
Respiration rate	R_C	$\text{mg C mg C}^{-1} \text{ day}^{-1}$	0.105, 0.01–0.20 ^c	Sweeney & Vannote (1981)
C content in <i>new</i> production	Q_C	mg C mg DW^{-1}	0.40	Frost, P.C., this study (data not shown)
P content in <i>new</i> production	Q_P	mg P mg DW^{-1}	0.0074	Fig. 3

^aRange of grazing rates used to calculate two boundaries in Fig. 4(b).

^bRange of C assimilation efficiencies used to calculate two boundaries in Fig. 4(c).

^cRange of respiration rates used to calculate two boundaries in Fig. 4(d).

$$C_F = \frac{R_C}{G_C \cdot (A_C - T_H \cdot R_C)} \quad (6)$$

$$f_{C:P} = \frac{Q_C \cdot A_P}{Q_P \cdot (A_C - T_H \cdot R_C)} \quad (7)$$

We obtained an estimate of R_C from respiration–body size relationships given in Sweeney & Vannote (1981) for *Ephemera* sp. using a respiratory quotient of 1.0 (Mathews & van Holde 1990) and the mean dry weight (1.65 mg) of pre-experimental mayflies from our ELA growth experiments. Because respiration rates can vary considerably, we explored the sensitivity of the model to a twenty-fold range in R_C (Table 1). An estimate of *Ephemera*'s grazing rate (G_C) and handling time (T_H) was obtained from Scrimgeour *et al.* (1991). We also assessed the sensitivity of our model to a lower and more conservative value of G_C (Table 1). In addition, we examined how changes to maximum carbon assimilation efficiency would alter the C- to P-limitation boundary (Table 1).

RESULTS

Growth experiments

Growth rates of laboratory mayflies (*Caenis* sp.) were affected by both the quantity and quality of food provided (Fig. 1). At low food abundance, mayfly growth rates were negative and not significantly affected by the food type offered. However at high food availability, low C:P algae resulted in significantly faster mayfly growth rates (Fig. 1). Thus, our results demonstrate a significant interaction between the effects of food quality and quantity on growth of *Caenis* sp., as has been shown for zooplankton (Urabe & Watanabe 1992).

Mayflies (*Ephemera* sp.) at the ELA also grew significantly faster at high food abundance (Fig. 2). As in the

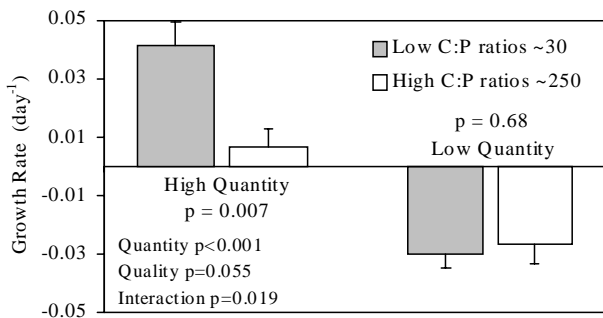


Figure 1 Effect of food quantity and C:P ratio (by mass) on the growth rates of a benthic mayfly, *Caenis* sp. fed laboratory-grown algae. Two-way ANOVA results from the cell mean's model run in SAS show an interaction between food amount and type. P -values presented next to x -axis are results of pair-wise contrasts at each food amount.

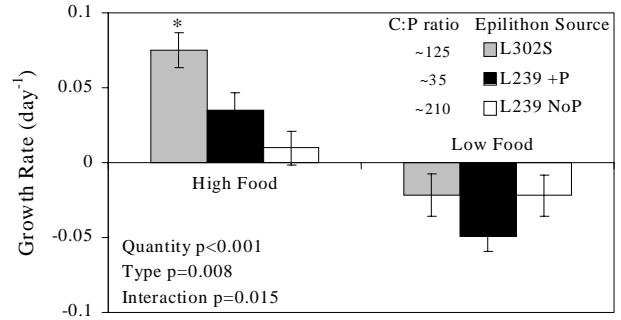


Figure 2 Effect of food quantity and C:P ratio (by mass) on growth rates of a benthic mayfly, *Ephemera* sp. fed periphyton collected from the ELA lakes. Two-way ANOVA results from the cell mean's model run in SAS show an interaction between food amount and type. The asterisk indicates that L302S was significantly ($P < 0.05$) greater than L239 +P and L239 No P at the high food quantity as determined by pair-wise contrasts.

laboratory, food type at low food quantities did not affect growth rates. However at high food quantity, food type significantly affected animal growth. L302S periphyton (naturally low C:P ratios) produced higher growth rates than L239 (+P or unenriched). Growth on P-enriched L239 food was somewhat higher than on unenriched L239 food, but this was not statistically significant (Fig. 2).

We found a negative relationship between body size and P content in mayflies from the ELA experiment (Fig. 3a). Because we assumed a fixed P content of mayfly production (Q_P) in our growth model, we fitted an inverse curve to the data to estimate this parameter from the horizontal asymptote (Fig. 3a, see discussion). We used the residuals from this non-linear regression to examine whether food type and/or quantity affected body P stoichiometry. This analysis showed that P-enriched L239 food increased mayfly body P content at both high and low food quantity relative to animals consuming unenriched L302S and L239 periphyton (Fig. 3b).

Model results

Our mass balance model delineates the combination of food conditions where different elements (C or P) are expected to limit growth in small mayflies (Fig. 4a). As has been shown in previous analyses for *Daphnia* (Sterner 1997), our model shows mayfly growth should be limited by the rate of carbon acquisition at low food quantity and low C:P ratios. However at food quantities above $0.10 \text{ mg C cm}^{-2}$, our model predicts P-limited growth in mayflies consuming food with C:P ratios (by mass) as low as 75–115 (Fig. 4a). The boundary between C- and P-limited mayfly growth shifted towards less P-limitation when we decreased grazing rates (G_C , Fig. 4b), decreased carbon assimilation efficiency

(A_C , Fig. 4c), and increased respiration rates (R_C , Fig. 4d) in our model. In each case, quantity and quality thresholds increased (Fig 4b, c, d) so that mayfly growth is more likely to be C-limited. To relate these model thresholds to field

data, we compared the model predictions to periphyton data from the Experimental Lakes Area. This comparison shows that P-limited growth in small mayfly nymphs should occur frequently in the littoral zones of these oligotrophic, boreal lakes (Fig. 5), even under a more conservative scenario of decreased grazing rate (G_C), decreased assimilation efficiency of carbon (A_C) and increased respiration (R_C).

DISCUSSION

In this study, we found that low food P content was associated with reduced growth rates of laboratory-reared

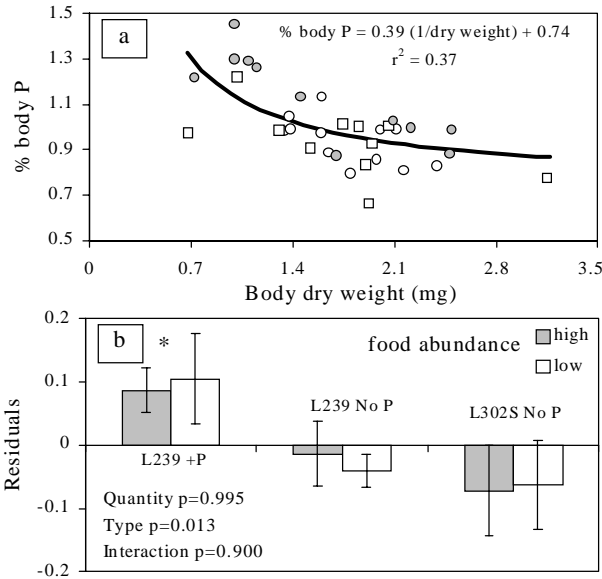
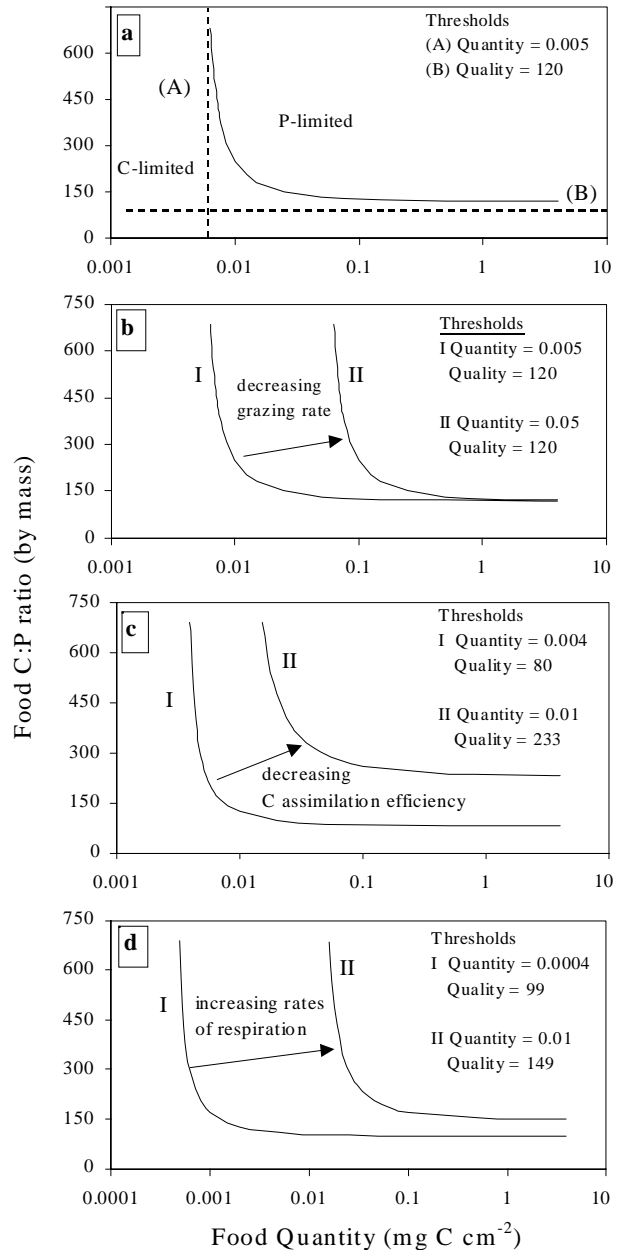


Figure 3 (a) Inverse relationship between mayfly body size and body P content (percentage of dry weight mass) in animals at the end of the ELA experiment. Symbols show mayflies eating different food types (unfilled circles (L239 No P), filled circles (L239 +P), and unfilled squares (L302S No P)). (b) Effects of food quantity and type on the residual difference between actual body P and that expected based on the regression between body size and body P content in panel a. Two-way ANOVA results, using a cell mean’s model in SAS, show food type significantly affected residuals but not food amount. The asterisk indicates that residuals for L239 +P were significantly ($P < 0.05$) greater than those for L239 No P and L302S No P food types as determined by pair-wise contrasts.

Figure 4 Boundaries (solid lines) between C- and P-limited growth in mayflies as a function of food quantity and C:P ratios (by mass). For all panels, C-limitation occurs below and to the left of the boundary whereas P-limitation occurs above and to the right. (a) Boundary produced using eqn 5 with parameter values given in Table 1. Dashed lines show the location of food quantity (A; see eqn 6, Methods) and quality thresholds (B; see eqn 7, Methods). Also provided are these thresholds calculated for the parameter values used in this model run. (b) Change in C- to P-boundary and thresholds produced by changing mayfly grazing rates (G_C ; 4.9–49 $\text{cm}^2 \text{mg C}^{-1} \text{day}^{-1}$). (c) Change in C- to P-boundary and thresholds produced by changing maximum assimilation efficiency of carbon (A_C ; 0.3–0.7) in small mayflies. (d) Change in C- to P-boundary and thresholds produced by changing mayfly respiration rates (R_C ; 0.01–0.20 $\text{mg C mg C}^{-1} \text{day}^{-1}$). Note the log scale is used in all panels for illustrative purposes but tends to exaggerate differences at low food quantities.



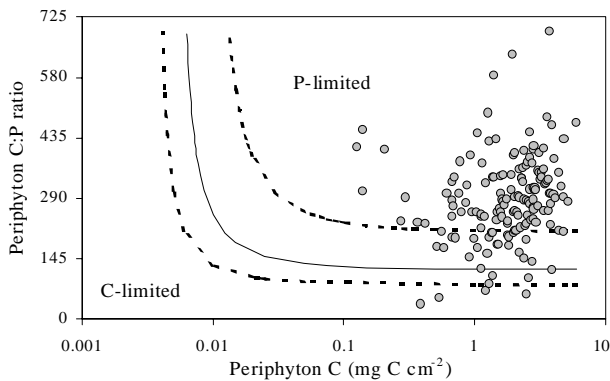


Figure 5 Periphyton C:P ratios (by mass) and C from a large survey of unmanipulated lakes at the ELA (M. Turner, Freshwater Institute, Winnipeg, Canada; unpublished data) relative to modelled predictions of C- and P-limited mayfly growth. The solid line shows the boundary between C- to P-limited growth as predicted by the mass balance model (from Fig. 4a). The dashed lines show boundaries produced by simultaneously changing R_C , G_C , and A_C . The upper dashed line resulted from a 30% increase (over values used to produce solid line) in G_C and R_C and a 30% decrease in A_C whereas the lower dashed line resulted from a 30% decrease in G_C and R_C and a 30% increase in A_C . Note that the log scale tends to exaggerate differences at low food quantities.

mayflies. However, reduced growth rates were only observed in mayflies provided with high food quantity. From a stoichiometric standpoint, this result is consistent with previous studies of pelagic consumers, particularly *Daphnia* (Urabe & Watanabe 1992; Sterner & Robinson 1994; Sterner 1997). C:P ratios (by mass) associated with reduced growth in our experiments (≈ 230) are below those typically found in natural periphyton from a wide set of ELA lakes (mean C:P ≈ 270 , Frost 2001). It thus appears that littoral mayflies in these lakes are probably limited not by the quantity but, instead, by the quality of their food resources (Fig. 5). The actual food quality situation in nature may in fact be worse for mayflies, as we have considered only one aspect of food quality (P content) and other biochemical, morphological, and elemental deficiencies could also be present in food sources at the base of benthic food webs (Ahlgrén *et al.* 1997; Vos *et al.* 2000).

On the other hand, benthic consumers may overcome growth constraints resulting from low food P content by selectively ingesting food particles of higher P content. The degree of selectiveness increases in satiated consumers (Calow 1973; Steinman 1991), who are most likely limited by food quality and not quantity. As food availability in littoral zones at the ELA is apparently high (Fig. 5), selective ingestion may be a mechanism used by mayflies to reduce potential negative effects of high C:P ratios. However, few benefits would result from this selective feeding if profitable food sources (in our case, low C:P

ratio foods) were not readily available for consumption (Begon *et al.* 1990). If food with low C:P ratios were relatively rare compared to other food types in littoral zones, benthic consumers who are selective would endure long search periods. Long search periods translate into low ingestion rates of the preferred food and increased mortality risk from predation (Begon *et al.* 1990). Ultimately, nutritional benefits must outweigh mortality and/or ingestion costs of selective feeding for benthic consumers to use food selectivity as a mechanism to avoid stoichiometric constraints on their growth.

Benthic grazers might also actively reduce their susceptibility to P-limited growth through physiological adjustments to their carbon metabolism. For example, higher respiration rates would increase the demand for C relative to P in mayflies and increase the food quantity where growth switches from C- and P-limitation (Fig. 4d). Similarly, lower grazing rates (Fig. 4b) and lower C assimilation efficiencies (Fig. 4c) reduce the likelihood of P-limited growth in mayflies. However, each of these physiological changes would result in lower mayfly growth rates as less carbon is available for biomass production. Consequently, mayflies that change carbon metabolism to reduce the extent of P constraints on growth do so only by reducing their growth potential. Nevertheless, such shifts are of considerable ecological interest, as they would directly impact on major parameters affecting overall trophic transfer efficiency through the herbivore trophic level.

We found that although C:P ratios (by mass) in the L239 +P food were ≈ 35 , mayflies consuming this food grew slower than those eating L302S food with C:P ratios of ≈ 125 . One explanation for the difference in growth rates observed between mayflies eating L302S and L239 +P food is that food quality is multifaceted. If a food lacked a sufficient amount of some essential biochemical (e.g. fatty acid, amino acid, vitamins, etc.) or was deficient in another element, then reduction of food C:P ratios by itself would not fully restore consumer growth rates to maximum values (Gulati & DeMott 1997; Urabe *et al.* 1997). It is also possible that L239 periphyton is composed of organic matter with low carbon quality (low assimilability). If so, low carbon assimilability would place additional constraints on mayfly growth beyond those already imposed by low food P content and artificial P enrichment would not always produce maximal mayfly growth rates.

An alternative explanation for lower growth with artificially low C:P ratio food was proposed by Plath & Boersma (2001). To explain anomalously low growth rates of *Daphnia* consuming algae of extremely low C:P ratios (≈ 15 , by mass), they proposed a feeding regulatory mechanism, whereby ingestion rates were governed by sensory mechanisms linked to satisfying metabolic demands for P instead of C. In their study, *Daphnia* fed this

abnormally low C:P food strongly reduced their feeding rates because they quickly met their P demands thus starving themselves amid a plenitude of food. Such a feeding response would probably occur in systems where certain consumers are constantly confronted with high quantities of low P content food (Plath & Boersma 2001), as is found at the mayfly–periphyton interface in littoral zones at the ELA (Frost 2001).

Another potential explanation for lower growth rates in the L239 +P treatment (low C:P ratios) is that P storage (and not growth) dominated metabolism of mayflies fed this P-rich food (rarely present in ELA lakes; Frost 2001). Although P storage mechanisms may allow consumers to temporally integrate growth across good and bad food (Sterner & Schwalbach 2001), it could have reduced short-term growth rates for mayflies consuming artificially P-rich periphyton in our experiments. If this explanation was true, then the duration of our experiment may have been too short to fully demonstrate the growth-enhancing potential of experimentally enriching food with phosphorus. We also found that P-enriched L239 food increased the body P content (but not growth rates) of ELA mayflies supporting the P-storage hypothesis. However, it remains to be seen whether increased growth or reproduction would eventually result in these P-enriched mayflies.

The effect of P-enriched L239 food on mayfly body P content was modest compared to the over-riding influence of body size. The negative relationship between mayfly P content and body size indicates that P content in this taxon is not fixed for its lifespan but instead shifts in a specified way during development. Strong ontogenetic and size-related changes in body elemental composition have also been shown in planktonic copepods (Carrillo *et al.* 1996, 2001). Non-constancy in body elemental content could result even if the elemental composition of *new* biomass production by consumers was fixed. A consumer that produces new tissue growth with a constant P content that is lower than its initial P content will have monotonically decreasing body P content as it grows. This negative relationship between body size and P content would thus fit a reciprocal relationship (Fig. 3) with the horizontal asymptote giving the P content of new production. One important consequence of this relationship is that it allows variations in body composition to be incorporated (as done in this study) into stoichiometric growth models, which generally assume rigid body elemental composition in particular consumer taxa (Elser & Urabe 1999).

Although ontogenetic shifts in body composition have been found in planktonic consumers (i.e. *Daphnia*, Main *et al.* 1997; copepods, Carrillo *et al.* 1996, 2001), we provide the first such observation in a benthic consumer. Size-related changes in body P content may reduce the vulnerability of smaller mayflies to P-deficient food. Lower susceptibility

results from reduced P content in *new* production and hence, lower P demands relative to that being provided in food material. However, mayflies in reproductive stages may have increased susceptibility to negative effects of low P content in food. As newly hatched mayflies appear to have body P content well above stages nearing emergence (Fig. 3), mayfly reproduction probably requires investment in P-rich, reproductive tissues, as has been shown for *Drosophila* (Markow *et al.* 2001). Future work should thus track the relative P allocation to growth and reproduction in growing mayflies under a variety of dietary conditions.

Our stoichiometric growth model captures various aspects of food–growth relationships in mayflies. For example, our model shows a greater probability of P-limited mayfly growth under conditions of high food availability. However, one main concern with this model is the accuracy of grazing, respiration, and assimilation parameters, which have received relatively little study in mayflies. We acknowledge uncertainty in these parameters (Fig. 4b–d) and suggest that future studies examine how the processes of grazing, assimilation, and respiration are affected by poor food quality. Regardless, the relative demands for C and P set by mayfly body composition and production (for which we have good data) place specific constraints on the food C:P ratios necessary to sustain growth and hence, largely dictate the dietary envelope (food quantity and C:P ratios) where mayfly growth is limited by food P content (Fig. 4b–d).

Our experimental and theoretical results provide evidence that food P content can place constraints on growth rates of mayflies in oligotrophic lakes. The question we should now ask is how often do such constraints actually act on aquatic consumers under natural conditions (Sterner 1997)? Two kinds of data are minimally required before we answer this question in the lake benthos: (1) how much food material is available in lake littoral zones and what is its elemental composition? and (2) what are the stoichiometric ratios needed by benthic consumers for growth, reproduction, and maintenance? Periphyton C and C:P data from unmanipulated ELA lakes show that P-limited growth in small mayfly nymphs should occur frequently in these lakes (Fig. 5). Unfortunately, the elemental composition of other benthic consumers remains virtually undescribed. Hence, we currently can only assume that benthic consumers have elemental needs similar to their planktonic and terrestrial counterparts, as biomass production in invertebrate Metazoa appears to require a relatively constrained balance of carbon, nitrogen and phosphorus (Sterner 1995; Elser *et al.* 1996; Elser *et al.* 2000a). If so, stoichiometric imbalances between benthic consumers and their food in littoral zones would be common and therefore, should be examined further to better understand ecological processes in benthic food webs.

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