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Ontogenic differences in mayfly stoichiometry influence growth rates in response to phosphorus enrichment

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With 2 figures and 4 tables

Abstract: We contrasted the carbon, nitrogen, and phosphorus (C : N : P) stoichiometry of *Caenis* spp. (Ephemeroptera:Caenidae) nymphs from 2 stream reaches differing in P enrichment. We also estimated growth rates of nymphs reared on algae of different P content across four development classes in a laboratory experiment. C : N ratios of field-collected nymphs exhibited variable responses across development classes between sites whereas C : P and N : P ratios showed a clear unimodal response, increasing from classes II through IV but then declining sharply in class V (nymphs nearing eclosion) at both sites. C : P was lower at the highly enriched site for all but the last development class. Growth rates increased in response to P enrichment at the earliest development class, but this growth response diminished in later development classes resulting in a significant interaction between P treatments and development classes. Trends in field data imply that later stages of development have higher P requirements than earlier classes and nutrient enrichment may affect sequestration of P by nymphs. Laboratory data suggest that early development classes are more P limited but in light of field results, nymphs may shift P allocation from somatic growth to reproductive development as organisms mature.

Key words: C : N : P ratios, phosporus content, development classes, growth rates, Ephemeroptera, Caenidae.

Introduction

Elemental stoichiometry is gaining favor in ecology as a way to study ecosystems, especially the interactions between organisms at all trophic levels and their environment. Stoichiometry examines the ratios of key elements (nutrients), particularly carbon (C), nitrogen (N) and phosphorus (P), in organisms and their food, and how differing elemental ratios affect organisms and ecosystems (Elser et al. 1996). Nutrient ratios are an important aspect of stream ecology because they influence organism growth, reproduction and life history traits and are therefore the basis of trophic interactions. Studies on the stoichiometry of benthic invertebrates and their food sources should improve overall understanding of trophic interactions, as they are an essential part of the food web. However, current knowledge of organism stoichiometry is lacking in terms of species examined, ontogenic changes, and spatial and temporal variation in elemental composition (Frost et al. 2002).

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The importance of phosphorus in affecting growth rates has recently been demonstrated (Weider et al. 2005). Phosphorus-rich ribosomal (r)RNA is necessary for protein synthesis which directly influences growth rates. Thus, rapid growth rates are associated with increased P requirements because organisms must disproportionately increase their allocation to P-rich rRNA to meet the protein synthesis demands of rapid growth. This is the basic tenet of the Growth Rate Hypothesis (GRH) which suggests that organisms with rapid growth rates must build P-rich biomass which makes them more susceptible to P-limitation (Elser et al. 1996, 2006, Vrede et al. 2002).

Invertebrates at a given life stage are more homeostatic with respect to their body nutrient content than autotrophs despite variation in the chemical makeup of their food, although the degree of elemental homeostasis varies between species (Sterner & Elser 2002, Peck & Walton 2006). In a field study examining consumer-resource stoichiometry, Cross et al. (2003) found that P content of invertebrates exhibited much greater variability than C or N, and that some taxa might not be stoichiometrically homeostatic across life stages. Although data from this study did not find a significant correlation between body size and %P for the 40 invertebrate taxa examined, the P content of certain functional feeding groups did decrease with increasing body size. Frost & Elser (2002) found a negative linear relationship between P content and body size in *Ephemerella* spp. indicating that P content in this taxon is not necessarily fixed for its lifespan but shifts ontogenetically during development. Similar ontogenetic shifts in P content have been observed in aquatic crustaceans (DeMott 2003, Faerøvig & Hesson 2003).

Mayflies (Ephemeroptera) are a widespread and important component of stream ecosystems. Previous studies have examined the effects of C: N and C: P ratios on mayfly species growth in both field and laboratory settings. Data demonstrate that high quality food (high P and or N content) increases growth and fecundity of several species of mayfly (Soderstrom 1988, Frost & Elser 2002). Mayflies are a good organism for studying possible stoichiometric difference across their ontogeny because development classes have been described (e.g. Taylor & Kennedy 2006) or can be easily determined for most species. Whether or not the growth rate is constant across all development classes is unknown. Moreover, in organisms which undergo metamorphosis (e.g. insects), growth and the loss of juvenile structures and development of adult structures follow a time sequence which may require different nutrient levels. Because adult mayflies do not feed, all the chemical requirements of the adults must be met by the nymphs. Thus nymphal nutrition must play a large role in adult reproductive success. Since egg elemental composition is key to early nymph growth and survivorship (Tessier et al. 1983, DeMott 2003), the availability of nutrients to actively feeding nymphs is of paramount importance. Because all egg production is realized in mayfly nymphs, there should be a direct linkage between nymphal food quality, fecundity, and egg nutrient content in mayflies. This relationship has been shown in *Daphnia* (Sterner 1993, Urabe & Sterner 2001). On the other hand it is possible that this linkage is relaxed because males may contribute phosphorus in their sperm packet that is available for incorporation into eggs. This has been demonstrated in *Drosophila* spp. (Markow et al. 2001).

The objective of this study was to determine growth rates for *Caenis* spp. across a range of development classes spanning several levels of nutrient enrichment. We hypothesize that (1) nutrient stoichiometry will vary through the development cycle of *Caenis* spp., with highest levels of P at early and late development stages due to relatively high levels of somatic and reproductive growth, respectively, (2) that nutrient stoichiometry within a development class from a site will differ depending on nutrient content of food, and (3) growth rates will increase with increasing P content of food resources and decrease with increasing development class.

Methods

Study area

The North Bosque River is a 4th-order (Straehler system, 1:250,000 scale) perennial tributary of the Brazos River located in central Texas, USA. The North Bosque flows predominantly through the Cross Timbers Level III ecoregion (Griffith et al. 2004), an area characterized by semi-arid climate (annual precipitation 40–60 cm/y), shallow alkaline clay soils overlaying heavily fractured limestone bedrock, and flashy stream flow. The longitudinal profile of the North Bosque River exhibits a strong nutrient gradient caused by municipal waste water inputs and runoff associated with concentrated animal feeding operations. These inputs are highest in the upper reaches of the watershed. Consequently, concentrations of dissolved and total phosphorus and nitrogen decrease with downstream direction (Back 2003).

Two sites along the longitudinal P gradient were selected for the field study of *Caenis* spp. to contrast C : N : P ratios among nymphs of differing development classes and from habitats differing in nutrient content of food resources. We chose NBOS-03 (31.97692° N, 98.03974° W), a 4th-order reach near Hico, TX as a relatively high phosphorus site. We selected NBOS-05 (31.63760° N, 97.36640° W), a 4th-order reach

Table 1. Nutrient concentrations for the North Bosque River sample locations corresponding to the field collection of *Caenis* nymphs.

	Site	
Nutrient (µg/L)	NBOS-03	NBOS-05
TP	104	46.1
PO_4-P	10.9	9.3
TN	2058	610
NO_2-N+NO_3-N	2.1	6.6
NH_3-N	50.2	71.3

near Del Mar Ranch at Valley Mills, TX as a contrasting site of low-to-moderate levels of phosphorus enrichment (Table 1). Both stream reaches exhibited similar physical characteristics, typified by short limestone cobble riffles interspersed with long, shallow bedrock glides and pools with moderate deposits of fine sediment. Historical stream flows (United States Geological Survey, http://tx.usgs.gov/basins.html) at each site were also similar, although surface discharge was undetectable at the time of sampling and streams were reduced to a series of long glides and pools interconnected by subsurface flow.

Field study: stoichiometry of *Caenis* development classes

Caenis spp. nymphs were collected from both study reaches between 28 September and 7 October 2006. *Caenis* spp. nymphs were collected using a 250 µm Hess sampler from shallow gravel substrates at pool margins. Samples (15–25) from each site were sieved through soil sieves (2-mm, 1-mm, 0.5-mm, and 0.25-mm). The retained material in the three smallest sieves was stored on ice for transport to the laboratory. We removed in the field all large *Caenis* spp. nymphs retained in the 2 mm sieve. In the laboratory we sorted nymphs into one of five development classes following Taylor & Kennedy (2006) using a Nikon SMZ 1500 dissecting microscope equipped with a Nikon DXM 1200f digital imaging system. Development classes corresponded to external wing pad morphology and pigmentation. Development classes I through V are determined as: I = no wing-pads present, II = clear wing-pads present in thoracic region, III = wing-pads with veins present in thoracic region, IV = wing-pads with veins present in abdominal region or with veins and mottling present in thoracic region, V = wing pads enlarged, with veins and dark mottling reaching abdominal region (Taylor & Kennedy 2006). These external morphological classes are also related to internal changes associated with adult development. The use of development classes is superior to arbitrarily making groups based upon size alone because equal size among individuals does not necessarily mean they are at an equivalent development stage. Because only mature development class V nymphs can be reliably identified to species we could only contrast development classes at the genus level. We dried all development class samples to a constant mass at 50 °C, pulverized and homogenized with a mini-beadbeater-8 (Biospec Products) and stored the powder in a desiccator until determination of nutrient content was completed.

Periphyton (defined here as a composite mixture of algae, fine particulate organic matter, and sediment) was collected from each site for determination of C : N : P stoichiometry to evaluate whether food resources at the two sites differed in terms of nutrient content. Periphyton was removed from rocks by scrubbing with hard-bristle brushes. The entire slurry was dried at 50 °C for 24 hours and then pulverized and homogenized the dried periphyton with a mini-beadbeater-8 (Biospec Products) and stored the powder in a desiccator until determination of nutrient content was completed.

Surface-water samples were collected from each site for determination of dissolved (0.45 µm filter) and total (unfiltered) N and P. Samples were collected in triplicate for NH_4-N, NO_2-NO_3-N, TN, PO_4-P, and TP and stored and analyzed samples according to standard methods (APHA 1998). All surface-water nutrients were analyzed on a Lachat Quik-Chem 8500 flow-injection autoanalyzer.

We estimated %C and N content of periphyton and *Caenis* spp. nymphs from the field sites using a ThermoQuest Flash EA™ 1112 elemental analyzer. Percent P content was estimated using a Lachat QuikChem 8500 flow-injection autoanalyzer using the molybdate colorimetric method following digestion in 5-mL 32M sulfuric acid at 350 °C on a digestion block for 2-h. Soil (Thermo Finnigan 1.99 %C) and peach leaf (SRM 1547, 0.137 %P, 2.98 %N) standards were used for QA/QC to determine C, N, and P recoveries, which were all quite high (89–107 %) and consistent among replicates (n = 5 per standard).

Because of the small size of *Caenis* nymphs (development classes I–V mean mass per individual were 0.002, 0.01, 0.071, 0.289, and 0.461 mg, respectively) relative to the mass required to achieve detectable concentrations of P in our digests (10–20 mg), we analyzed composite samples of several to hundreds of nymphs from each site (Table 2). In those cases that we had less than the required mass, we decreased the acid volume and dilution volume proportionately (3–9 mg material would be digested in 2.5 ml H_2SO_4 and then diluted to 37.5 mls). We were unable to collect sufficient biomass of development class I nymphs for C : N : P determination.

Polynomial least-squares regression was used to fit continuous relationships between increasing development stages and C : P and N : P ratios of nymph composites. Regression equations were fitted to data for each site and patterns were qualitatively contrasted between sites to assess whether the higher-P site (NBOS-03) tended to have lower C : P and N : P ratios than the lower-P site (NBOS-05).

Laboratory growth experiment

Green algae (*Cladophora* and *Spirogyra* spp.) mats were collected from Neils Creek, a low-nutrient tributary of the North Bosque River, near Valley Mills, TX, to culture for food treatments in the growth experiment. Algal mats were collected on 24 September 2006 and split this material into four equal masses of approximately 20 g wet mass. We placed split fractions of algae in 1 L beakers of filtered site water enriched with Na_2HPO_4 ranging from +0 (no enrichment, background TP 10 µg/L), + 30, + 90 and + 270 µg/L P, respectively. Algal cultures were held in control and enriched stream water at 20ºC with continuous light for 48 hours to allow uptake of P into algal tissues. We did not determine the ingestion rates of *Caenis* spp. across its development classes and potentially could have not provided enough food at higher development classes. However, our lowest supply (0.15 mg C/cm^2) was 3-times higher than that used by Frost & Elser (2002) to represent low food quantity for similarly sized larvae (our development class I). Algal samples were

removed from beakers and allowed to air dry at 20 ºC for several days. Samples were ground to a fine powder using a mortar and pestle and stored in airtight containers. Nine subsamples were analyzed from each algal enrichment treatment for C, N and P content using laboratory methods previously described in the field study.

We collected *Caenis* spp. nymphs for the growth study from a perennial pool in shallow gravel/sand substrates from the lower-P site (NBOS-05) on the North Bosque River. Twenty-five Hess samples (250 µm mesh) were collected as described in the field study in order to obtain enough nymphs for the experiment. Live nymphs were separated into the five development classes and measured each individual for head capsule width (HCW) using a Nikon SMZ 1500 stereomicroscope equipped with a Nikon DXM 1200f digital imaging system. We initially considered all five development classes, but preliminary results suggested that development class V nymphs were too close to maturation and would likely emerge during the experiment. Thus, we focused only on nymphs of development classes I–IV for the growth experiment.

Nymphs were placed individually into 20 mL glass scintillation vials filled with 15 mL of filtered stream water. Ten nymphs were reared from each of the four development classes, on each of the four algal treatments in incubators set at 20 ºC with 12 hour light cycles for ten days. Nymphs were fed in order of increasing development class 0.15, 0.3, 0.6, and 1.2 mg C cm^{-2}, respectively. We replaced filtered stream water and algae every 3–4 days. Post incubation HCW measurements were made for each individual and used these measurements to calculate growth rate. Growth rate was calculated as:

$$\mu = [\ln(B_2) - \ln(B_1)] \,/\, time$$

where μ = growth rate, B_1 = estimated initial mass and B_2 = estimated final mass. We estimated initial and final masses (mg) by inserting HCW measurements (µm) into a published HCW-weight regression (dry mass = $23.09548\, e^{\,HCW + 3.19737}$, $r^2 = 0.97$) developed for *Caenis latipennis* by Taylor & Kennedy (2006).

Two-way ANOVA was performed on growth-rate data using development class (I-III) and food quality (four C : P treatments) as main effects and a development class × food quality interaction term. High mortality of nymphs in class IV treatments reduced our degrees of freedom below an acceptable level for detecting statistical interactions so we only analyzed growth-experiment data from development classes I–III . One-way ANOVA was run *a posteriori* to test the influence of food quality on growth rate within each development class, including development class IV. Tukey's studentized range test was used to assess the relationships of means between groups for ANOVA results. Because not all data was distributed normally, all analyses were conducted on ranked data. Effects were considered significant when $p \leq 0.05$. ANOVA was performed in SAS 9.1 (SAS Institute, Cary, NC, USA).

Results

Field study: stoichiometry of *Caenis* development classes

Surface-water and periphyton had higher N and P levels at NBOS-03 when compared to the downstream site (NBOS-05). This suggests that nutrient availability to periphyton indeed was higher at NBOS-03 and nutrient content of an important food resource to *Caenis* reflected these differences in its elemental composition (Table 2).

C : P ratio of *Caenis* spp. nymphs exhibited a unimodal relationship with increasing development class ($r^2 = 0.993$, $F_{2,1} = 74.7$, $p = 0.081$ and $r^2 = 0.995$, $F_{2,1} = 107.8$, $p = 0.048$ for NBOS-03 and NBOS-05, respectively; Fig. 1a). C : P ratio increased from development class II to III, but decreased slightly (NBOS-03) to markedly (NBOS-05) from class III to V. C : P ratio at NBOS-05 was greater than the more P-enriched NBOS-03 for all development classes except class V (Fig. 1a). We observed a similar hump-shaped N : P relationship across development classes and between sites ($r^2 = 0.971$, $F_{2,1} = 17.32$, $p = 0.168$ and $r^2 = 0.998$, $F_{2,1} = 207.8$, $p = 0.041$ for NBOS-03 and NBOS-05, respectively). This pattern in N : P ratio was largely due to less dramatic shifts in %N content across development classes than those observed for %P (Table 2, Fig. 1b). Neither C : N nor % C showed a consistent trend with increasing development class between sites, increasing from II–V at NBOS-03, but no clear pattern at NBOS-05 (Table 2).

Table 2. Elemental content of periphyton and *Caenis* spp. nymphs across developmental classes at each stream location.

Periphyton or Developmental Class	NBOS-03				NBOS-05			
	n[1]	% C	% N	% P	n	% C	% N	% P
Periphyton	–	10.7	1.12	0.116	–	9.70	0.96	0.085
II	504	48.2	11.7	0.93	609	47.0	11.7	0.71
III	231	48.9	12.0	0.81	352	47.2	10.4	0.59
IV	40	51.4	12.0	0.83	40	50.7	10.8	0.74
V	18	53.1	11.5	0.89	9	48.1	10.6	1.17

[1] number of individual nymphs analyzed in each composite sample

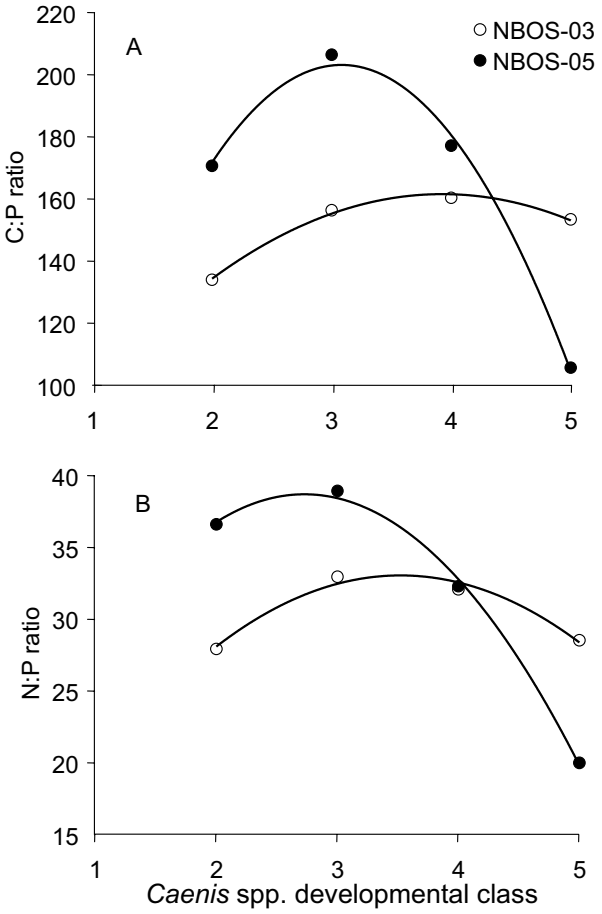

Fig. 1. Regressions of molar C : P **(A)**, and N : P **(B)** ratios across *Caenis* spp. development classes at NBOS-03 and NBOS-05 collection sites.

Laboratory growth experiment

Artificially enriched algal samples produced a strong P gradient across the four algal treatments with P increasing substantially while little change was observed in C and N. This resulted in a shift in molar C : P ratios from 960 for the control to 62 for the highest P treatment (Table 3).

Caenis spp. growth rates increased in response to P enrichment in the earliest development class but this growth response diminished in later development classes (Fig 2). Growth rate of *Caenis* spp. was significantly influenced by development class ($F_{2, 67}$ = 3.41, p = 0.0388) and the interaction between development class and P treatment ($F_{6, 67}$ = 2.36, p = 0.0397); however, P treatment ($F_{3, 67}$ = 2.51, p = 0.0661) had no effect on growth rate (two-way ANOVA on ranked data). Tukey's studentized range test (α = 0.05) could not separate the rank sums of the three development classes into statistically different groups. A post hoc analysis on data analyzed separately for development classes I through IV showed that P treatment had a highly significant effect on growth rate in development class I but not development classes II, III and IV (One-way ANOVA on ranked data; Table 4). Only the rank sums of P treatments 62 and 960 were significantly different for development class I (Tukey's studentized range test on ranked data, α = 0.05; Fig. 2).

Table 3. Elemental composition and molar ratios for the control and three P-enriched food treatments used in the growth experiments.

Treatment	%C	%N	%P	C : N	C : P	N : P
+ 0 (Control)	26.4	2.09	0.07	14.7	960	65
+ 30 µg/L	25.6	2.02	0.26	14.8	254	17
+ 90 µg/L	25.5	2.09	0.70	14.3	95	6.6
+ 270 µg/L	25.2	2.06	1.04	14.3	62	4.4

Table 4. One-way ANOVA and Tukey's Studentized Range Test results for the effects of food P content on ranked growth rates for each development class of *Caenis* spp.

Development class	df	*F*	*p*
I	3, 22	8.36	0.0007
II	3, 24	0.74	0.5411
III	3, 21	1.12	0.3650
IV	3, 15	0.18	0.9062

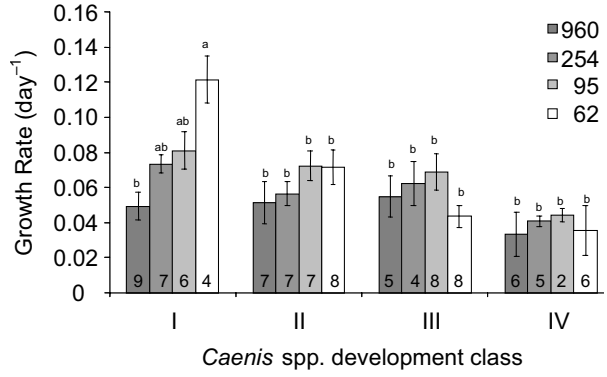

Fig. 2. Mean ± 1 SE growth rates of individually reared *Caenis* spp. nymphs in four different development classes across four different food quality treatments. All food treatments are presented as molar C : P ratios. Sample sizes are indicated within bars. Lower case letters above bars represent significant differences between rank sums of food quality treatments within each development class determined by a Tukey's Stundentized Range Test.

Discussion

Ontogenetic differences in *Caenis* stoichiometry

The C : P ratios for *Caenis* were all in the lower range of values reported 100–800 (mean 263 ± 113) for mayflies in Evans-White et al. (2005). Although we have no measurement of the amount of variation within each development class, C : P and N : P ratios showed a clear pattern of increasing and then decreasing values across development classes at both sites. Admittedly, having no measure of variation in C : P and N : P ratios decreases the certainty of a unimodal pattern. However Frost & Elser (2002) showed that the smallest mayflies had the highest P content. Extending this trend to Fig. 1 would further support a unimodal pattern. If individual species could be identified for all development classes, their stoichiometry could result in patterns different than those we observed. However, *Caenis* are collector-gathering detritivores and variability in food type and quality among species is probably low. Therefore, the level of elemental imbalance between different *Caenis* species and their food should be similar. This could lead to species with unique elemental content but not necessarily differing patterns in stoichiometry across their ontogeny.

Why would *Caenis* spp. nymphs differ in their C : N : P stoichiometry across development classes? We believe that it is a result of differing N and P demands needed for the development of somatic and reproductive structures across the ontogeny of *Caenis* spp. When eggs hatch and nymphs grow and metamorphosis progresses through development classes, the early development classes first produce somatic tissue, and later (development classes IV and especially V) produce reproductive structures and gametes. Thus the decline in C : P ratios in development class IV and V represents a high P investment in gametes, which is probably most evident in females. This idea is supported by Markow et al. (1999, 2001) who demonstrated that adult *Drosophila melanogaster* and *D. nigrospiracula* females are 3-times more phosphorus rich than males and that eggs and male ejaculate are P rich. Furthermore, males of *D. nigrospiracula*, which feed on P-poor cacti, had a longer time lag in mating after eclosion than those of *D. melanogaster*, which feed on P-rich fruit. This time lag for *D. nigrospiracula* was thought to represent a longer P acquisition time for males because of the low P content of its food (Markow et al. 2001). Follicular development in *D. nigrospiracula* was much slower than that in *D. melanogaster* at eclosion. Markow et al. (2001) suggest that *D. nigrospiracula* may have to allocate a higher proportion of P to somatic growth in the larval stages due to low P food. Thus more egg maturation takes place in the adult stage in this species. Elser et al. (2006) showed that P content of larvae in five *Drosophila* spp. decreased as larvae grew. This is not the pattern we observed for *Caenis* spp. However, mayflies have a major difference in their life history: adults do not feed. Thus, larvae must acquire all materials necessary for adult survival and reproduction. Based on the high P content of adult *Drosophila* reproductive structures (Markow et al. 2001), it makes sense that organisms with non-feeding reproductive stages would necessarily have late development stage larvae that are richer in P than earlier stages because gametes develop and mature in the larvae. The onset of morphological sexual differentiation and development of reproductive structures coincides with decreasing C : P ratios in *Caenis* spp. nymphs in our study. Examinations of mature female mayfly larvae (i.e. darkened wing pads) of *Caenis* spp. and two other mayflies at our sites, *Neochoroterpes nanita* and *Stenonema femoratum*, revealed their entire abdomens were full of eggs and no digestive tract was evident. Frost & Elser (2002) showed a steady decline in %P content in *Ephemerella* sp. mayflies. Although they did not report development classes in their study, mature larvae of *Ephemerella* sp. are 9–15 mm in total length and they complete their life cycle in 9–12 months (Edmunds et al. 1976). Since Frost & Elser (2002) collected newly hatched larvae and their experiment duration was six days, they could not have included late development class nymphs in their experiment. Thus the declining body %P demonstrated in their study supports what we demonstrated in early development class larvae of *Caenis* spp.

Trends in *Caenis* spp. nutrient stoichiometry between sites of differing nutrient status are consistent with those shown for aquatic insects by Cross et al. (2003). They showed insects from enriched sites had higher nutrient content than those from less enriched sites. Mayfly development classes showed higher percent nutrient content at the enriched (NBOS-03) site relative to the less enriched site (NBOS-05). Percent C increased across development classes. This probably represents an increase in exoskeleton and other C rich structures associated with growth (i.e. larger body size). The %N and P at NBOS-03 was equivalent or higher than NBOS-05 in every case except development class V. Again, this probably reflects the nutrient content of the dominant food source which was higher at NBOS-03 and those mayflies had the higher nutrient content.

The decrease in C : P ratio of development class V (Fig. 1a, b) at NBOS-05 when compared to NBOS-03 may be the result of the majority of individuals being female, and contributing a disproportional amount of P to the sample. The latter is supported by Markow et al. (1999, 2001) findings on the high P content of female *Drosophila* spp. Consideration of life history strategies and adult feeding status needs to be included in future studies of ontogenetic changes of elemental composition. Villar-Argaiz & Sterner (2002) demonstrated in a freshwater copepod that P deficiency in late stages of development prevented larval *Diaptomus clavipes* from developing into adults, yet younger stages grew just as well as individuals fed a P replete diet. Thus timing of increased P acquisition is probably important. Even in insects that have feeding adults, the quality of food used by adults needs to be investigated in light of larval food quality. Many holometabolous insects have larvae and adults that do not use the same food resources and each stage may face differing elemental imbalances. Future work is needed to determine the variability of C : N : P ratios within *Caenis* spp. development classes and between sexes to better understand the degree of plasticity in body chemistry among development classes and sexes.

Laboratory growth experiment

A high potential for rapid growth provides many potential advantages to species because growth and development rates can affect many life history traits (e.g. age and size at first reproduction) and ecological features (e.g. predation risk) (Elser et al. 2006). Our findings suggest that increased P content of food increases growth rates of smaller development classes of *Caenis* mayfly nymphs (Fig. 2). The GRH suggests that growth of organisms with higher potential for rapid growth will be more P limited. Our data supports this hypothesis within the context of the life history of a single taxon. As nymphs mature, growth rates and associated P requirements decrease resulting in less influence of food P content on somatic growth rate. Stream insects typically exhibit higher mortality during early stages of development (Benke & Huryn 2007). While many factors such as density affect survivorship, it is plausible that increased growth rates during early stages of development have the potential to decrease mortality and increase overall production of populations when P is not limited.

Our data showed a significant interaction between P content of food and development class on growth rates of *Caenis* spp. This interaction appears to be driven by P content of food having an effect on growth rates in development class I but not development class II through IV (Fig. 2, Table 4). However, in light of our field results it is plausible that later development classes shift P resources from somatic growth to reproductive development. Nymphs within higher P content treatments could have potentially begun developing reproductive structures earlier than nymphs in lower treatments resulting in diminishing growth rates based on HCW measurements earlier than in lower treatments. Thus linear dimensions may not increase even though biomass is accumulated via reproductive development. This supports the hypothesis that not only growth but development may be P limited. Increased development rates have the potential to limit fecundity as it is usually correlated with organism size.

Although P limitation of growth has previously been demonstrated for other mayflies including *Caenis* spp. (Frost & Elser 2002) we provide the first observation of ontogenetic shifts in P limitation of growth related to size and development class within the life cycle of a benthic consumer. Our results agree with studies on terrestrial insects done by Elser et al. (2006) that showed similar shifts in P limitation across the short life cycles of several species of *Drosophila*. However, our experimental design was limited by higher mortality rates than expected in development class IV and the short time period of the experiment. We could not determine whether these shifts were related to size specific growth potential, shifts in P allocation from somatic growth to reproductive development or a combination of the two. Nonetheless, our study demonstrates that P requirements for growth and development can vary across a species life cycle and that P availability has the potential to limit many life history factors within aquatic benthic consumers. Future stoichiometric studies of benthic consumers should consider ontogenetic shifts in P limitation of growth, the mechanisms that control these shifts and the cumulative consequences that varying P limitation across life cycles may have on populations of aquatic organisms.

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agencies, it does not necessarily reflect the views of these agencies and no official endorsement should be inferred.

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