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Differences in phosphorus demand among detritivorous chironomid larvae reflect intraspecific adaptations to differences in food resource stoichiometry across lowland tropical streams

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Abstract

We tested whether assemblages of detritivorous chironomid larvae (Chironomidae: Diptera) varied in phosphorus (P) demand based on local nutrient conditions using a series of streams in lowland Costa Rica that exhibit a natural range in ambient dissolved P and detrital P due to inputs of solute-rich groundwater. Chironomids collected from three high-P streams had growth rates and P-excretion rates that were similar to, or lower than, those of chironomids collected from four low-P streams. Chironomids from a naturally low-P stream that was experimentally amended with P over 8 yr showed an increase in P-excretion rates but not growth rates, indicating an inability of these chironomids to use additional dietary P. Chironomids from a high-P stream showed greater evidence of P limitation (lower growth rates, P-excretion rates, and ribonucleic acid content) when fed low-P detritus compared to chironomids from a low-P stream. Our findings support the hypothesis that chironomid assemblages are adapted to local food quality in this heterogeneous landscape, thereby circumventing P limitation. Genetic analysis indicates that chironomid assemblages across the study streams are similar in species composition, suggesting that differences in P demand may be due to microevolution. The lower P demand among chironomids from low-P streams appears to limit their ability to respond to nutrient-enriched food, effectively stabilizing the food web in response to changes in nutrient availability.

Variable rates of phosphorus (P) loading across landscapes can lead to differences in food resource P content among streams in a watershed (Stelzer and Lamberti 2001; Cross et al. 2003; Bowman et al. 2005), creating a heterogeneous template for resource–consumer stoichiometric relationships. In low-P environments, species with lower P requirements should be favored in competitive interactions (Tilman 1982). Simultaneously, within species, genotypes that lead to increased P-use efficiency should be favored (Jeyasingh and Weider 2007). As a result of either of these mechanisms, the optimal dietary ratio of carbon (C) and P, or threshold elemental ratio ($TER_{C:P}$; Frost et al. 2006), of stream consumer assemblages could reflect interstream differences in food resource P content. However, despite an increasing number of studies measuring the short-term responses of individual consumer species to changes in the P content of food resources (Frost and Elser 2002; Kyle et al. 2006; He and Wang 2007), we know relatively little about how the P demand of entire consumer assemblages may be altered due to chronic P loading in stream ecosystems.

Detritivores face a unique nutritional challenge because detritus is typically a nutrient-poor food resource, with carbon : phosphorus (C : P) ratios among the highest experienced by all consumers (Enriquez et at. 1993; Cross et al. 2003). Many invertebrate detritivores use a combination of physiological strategies to decrease their P demand, including having high body C : P ratios (decreasing the imbalance between their food and their own biomass; Frost et al. 2006) and slow growth rates (resulting in a greater fraction of ingested carbon being lost through respiration; Anderson et al. 2005). Stoichiometric theory predicts that taxa with slower growth rates should have a low P demand (and therefore be less susceptible to P limitation) because of the smaller required investment in P-rich ribonucleic acid (RNA) required for protein synthesis (Sterner and Hessen 1994; Elser et al. 1996).

Chironomid larvae, which can be important detritivores in many freshwater ecosystems (Armitage et al. 1995), may be especially prone to P limitation due to their fast growth rates (Huryn 1990; Hauer and Benke 1991; Benke 1998) and low body C : P ratios (Cross et al. 2003). As a result, selection pressure due to dietary P availability should be high for chironomids. Although detritus is a nutrient-poor food resource in general, the P content of detritus varies in response to stream P availability (Cross et al. 2003; Small and Pringle 2010). Consistent with this prediction of general P limitation, chironomid growth rates have been shown to increase in response to an experimental whole-stream nutrient enrichment (Cross et al. 2005) and along a natural P gradient (Rosemond et al. 2001; Ramírez and Pringle 2006). However, no study to date has explicitly tested for potential differences in P demands for larval chironomid assemblages from high- and low-P streams. Moreover, because chironomid larvae are notoriously difficult to identify to species using morphological characters, relatively little is known about the way in which sustained P loading may alter chironomid species composition.

To test the hypothesis that the nutrient demands of a consumer assemblage may be adapted to local food

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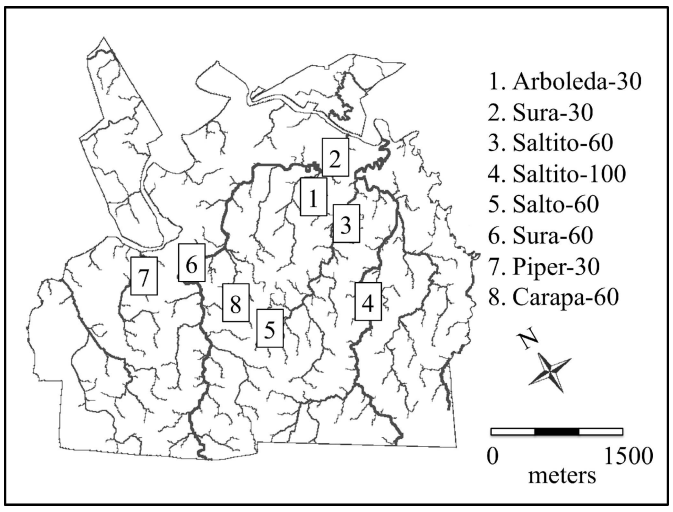

Fig. 1. Map of La Selva Biological Station, Costa Rica, showing locations of study sites.

resource stoichiometry, we assessed P limitation for detritivorous chironomid assemblages from naturally high- and low-P streams when fed leaf litter ranging in P content. To test whether observed differences were due to changes in species composition among streams, we analyzed species composition in two focal streams using mitochondrial deoxyribonucleic acid (DNA). We predicted that chironomid P demand would increase with stream P levels due to a combination of species shifts and intraspecific genetic variation.

Methods

Site description—La Selva Biological Station (10°26′N, 84°01′W) is situated on the Caribbean slope of Costa Rica and receives almost 4000 mm of rainfall annually (Sanford et al. 1994). Geomorphological features of this landscape result in natural interbasin transfers of solute-rich groundwater entering some La Selva streams (Pringle et al. 1993). Groundwater is modified by volcanic activity at high elevations, cools as it moves downhill, and emerges at the base of ancient lava flows. These groundwater inputs are characterized by high solute concentrations (e.g., P, Na^+, Cl^-, HCO^{3-}; Pringle et al. 1993).

We chose seven streams that varied widely in average soluble reactive phosphorus (SRP) concentration (2–135 μg L^{-1}) due to differential inputs of groundwater (Table 1). These streams are second- and third-order, are all within close proximity (< 2 km), and they are surrounded by dense forest (Fig. 1). Channel widths range from 1 to 3 m, and the dominant substrata are detritus, silt, and clay, with boulders present at some sites. These seven streams are a subset of sites used in a long-term study of the physicochemistry of La Selva streams (Pringle and Triska 1991; Triska et al. 2006), for which continuous monthly data are available since 1997.

Experiment 1: Growth and excretion rates of chironomid assemblages across a natural P gradient—From February to

Table 1. Physical and chemical properties of streams used in this study. Values are means (and ranges) from monthly data collected in 2006–2007. Dissolved phosphorus in streams is reported as soluble reactive phosphorus (SRP). The Carapa-60 was experimentally enriched in P from 1998 to 2006. Background SRP is reported for this site; SRP concentrations for the P-enriched reach varied with distance from the injection site, with a maximum concentration of 91 μg L^{-1} during the current study.

Stream	SRP (μg L^{-1})	NO_3-N (μg L^{-1})	NH_4-N (μg L^{-1})	Temp. (°C)	Discharge (m^3 s^{-1})	Conductivity (μS cm^{-1} at 25°C)	pH
Arboleda-30	135 (27–397)	126 (63–162)	20 (7–42)	25.6 (24.7–26.4)	0.17 (0.09–0.21)	257 (173–310)	6.2 (5.9–6.5)
Sura-30	83 (39–150)	163 (60–277)	18 (0–87)	25.4 (24.7–26.7)	0.61 (0.43–0.86)	157 (73–188)	6.1 (5.8–6.8)
Saltito-60	33 (1.6–87)	98 (35–170)	19 (0–64)	25.0 (24.0–25.9)	0.11 (0.04–0.2)	110 (37–170)	6.1 (5.9–6.9)
Salto-60	10 (4.3–21)	180 (101–261)	20 (0–59)	24.7 (23.7–25.6)	0.45 (0.07–0.93)	32 (28–44)	5.9 (5.6–6.6)
Saltito-100	3 (0–7.0)	163 (78–460)	37 (18–60)	24.3 (23.6–25.6)	0.03 (0.02–0.06)	19 (17–24)	5.7 (5.3–6.7)
Sura-60	3 (0–9.0)	199 (58–353)	21 (0–51)	24.8 (24.0–26.2)	0.19 (0.05–0.55)	20 (16–26)	5.6 (4.7–6.5)
Piper-30	2 (0–6.8)	188 (99–404)	40 (6–166)	25.0 (24.3–25.9)	0.03 (0.01–0.10)	22 (19–26)	5.5 (4.8–6.2)
Carapa-60	6 (0–17)	176 (131–220)	23 (2.5–132)	25.0 (23.0–26.0)	0.02 (0.01–0.073)	19 (13–56)	5.6 (5.1–6.5)

June 2006, we measured growth rates and P-excretion rates for chironomid assemblages collected from the study sites, feeding on detritus conditioned in those respective sites. Methods for measuring growth rate were modified from Ramírez and Pringle (2006). Chironomid growth rates were measured for each stream site in the laboratory using four chambers (8 × 10 cm) with 90-μm mesh windows that were placed in a 12-liter plastic tub filled with stream water. Stream water was collected from each source stream on the day each experiment was initiated, and it was filtered through 1-mm mesh to remove large particles. Water was continuously aerated and maintained at ambient temperature throughout the experiment. We collected a water sample from each site for SRP analysis and measured pH, conductivity, and water temperature in each plastic tub during each growth trial.

Larval chironomids for growth experiments were obtained by placing *Ficus insipida* leaf packs in each of the study streams. Leaf packs were removed after 6–7 d, and chironomids (excluding the predator subfamily Tanypodinae) were removed. Length was measured to the nearest 0.1 mm under a dissecting microscope, using a reference grid, to obtain initial biomass based on a length–weight regression for chironomid larvae from these streams (r^2 = 0.68). A group of ~ 20 larvae with an initial length of 2–4 mm was placed in a growth chamber. Thirty conditioned *Ficus insipida* leaf disks (1.5 cm) were cut from leaves incubated in the source stream for 15 d before the experiment to use as food in each chamber. After 3 d, chironomid larvae were recovered from the chambers and measured. Instantaneous growth rates (IGR) for each experimental unit (chamber) were estimated using the equation

$$\mathrm{IGR} = (\ln Wf - \ln Wi)/t \quad (1)$$

where *Wi* and *Wf* are the mean initial biomass and mean final biomass for all chironomids in each chamber, respectively, and t is the incubation time (*see* Web Appendix, www.aslo.org/lo/toc/vol_56/issue_1/0268a.html).

Immediately following final length measurements, we measured P-excretion rates of chironomids from each growth chamber. Chironomids recovered from each growth chamber were rinsed in distilled water and placed into an acid-washed beaker containing 30 mL of distilled water (one beaker for each growth chamber, each containing ~ 20 chironomids; *see* Web Appendix). Two controls (distilled water with no chironomids added) were used with every four beakers containing chironomids. SRP values from the two controls were averaged for each set of excretion experiments. After a 3-h incubation period, a 20-mL water sample was removed, filtered through a 0.45-μm Millipore filter into a scintillation vial, and frozen until later SRP analysis. SRP was measured spectrophotometrically using the ascorbic acid method (APHA 1998) at the Analytical Chemistry Laboratory, University of Georgia. Excretion rate was calculated as the increase in SRP per unit chironomid biomass divided by the incubation time. While these rates likely do not accurately represent ambient chironomid excretion rates due to the potentially confounding effects of fasting and stress (Whiles et al. 2009), the excretion values should be comparable among treatments within each experiment as an indicator of relative P-excretion rates.

After leaf disks were removed, the remainder of leaf material was dried at 50°C and analyzed for C : P ratios. For C analysis, samples were analyzed on a Carlo Erba NA 1500 elemental analyzer (Carlo Erba). For P analysis, samples were acid-digested (Aqua Regia double acid) and analyzed spectrophotometrically. All C : P values are reported as molar ratios.

Growth rate experiments were performed using chironomid and leaf disks from the seven sites along the natural P gradient in both March and July 2006. Four replicate chambers were used at each site on two separate sampling dates. For statistical analysis, each sampling date at each site was treated independently. We used multiple regression analysis to examine relationships between the mean growth rate measured for each chamber and detritus C : P, with average initial chironomid mass within each chamber as a covariate. Similarly, P-excretion rate was also regressed against detritus C : P, with average chironomid mass (the mean final chironomid mass within each growth chamber) as a covariate. Because growth rates and excretion rates were measured for groups of chironomids rather than individuals, variance in individual body size could affect our measurement of these rates, potentially leading to slight underestimates of these allometric rates (e.g., the growth rate of the average-sized individual could be lower than the average growth rate if growth rates decline exponentially with body size). However, variance was low and consistent among treatments within each experiment (*see* Web Appendix) and therefore should not influence trends observed across treatments.

Experiment 2: Growth and excretion of a chironomid assemblage across an experimental P gradient—Using the methodology described already, we measured growth rates and excretion rates for chironomid assemblages from six sites along a naturally low-P stream that was experimentally P enriched over 8 yr. A first-order stream, the Carapa, was experimentally enriched in P from July 1997 to February 2006. Dissolved phosphate was added continuously from a Mariotte bottle to increase phosphorus concentrations from background levels of < 5 μg L^{-1} to ~ 300 μg L^{-1} SRP over the study reach. The whole-stream P enrichment is described in more detail in Ramírez and Pringle (2006) and Small et al. (2008). Growth rate experiments were conducted using chironomids, conditioned *Ficus* leaves, and water from six sites along this stream (10 m upstream of the injection site, and 10 m, 50 m, 100 m, 200 m, and 500 m below the injection site) in February 2006 (during P enrichment) and June 2006 (4 months after P enrichment ended), creating an experimental P gradient. As described previously, the four growth rate and excretion rate measurements from each site at each sampling date were regressed against detritus C : P, with mean chironomid body mass as a covariate.

Experiment 3: Response of chironomid assemblages from a naturally high- and low-P stream to range of litter C : P—

In April–June 2007, we measured the growth rates of chironomids from the high-P site (Arboleda) and a low-P site (Sura-60) when feeding on detritus conditioned in streams across the natural P gradient. These two streams are similar in discharge, substrate, and invertebrate assemblage (Ramírez et al. 2006), but detritus P content is > 4-fold higher in the high-P stream (Small and Pringle 2010). Growth rate experiments were conducted as described already, except that instead of using chironomids from each site along the P gradient, four chambers of chironomids collected from the high-P stream and four chambers of chironomids from the low-P stream were incubated in water from each site, feeding on *Ficus* leaf disks conditioned in those sites. Fifteen different leaf C : P values were used, achieved by incubating *Ficus* leaf packs for 15 d in stream water ranging in dissolved P. Incubation times were decreased from 3 d to 2 d in experiment 3, which still resulted in an average 3-fold increase in biomass but prevented pupation during the study. Leaf C and P analyses were conducted as described previously.

P-excretion rates were measured for eight of the treatments from experiment 3 using methods slightly modified from experiments 1 and 2. Instead of distilled water, filtered water collected from the low-solute Sura-60 was used for all excretion trials, and the length of incubation time in excretion trials was decreased from 3 h to 1 h. Following the excretion measurements, most chironomids were analyzed for body C : P ratios. Due to the small size of individuals, chironomids from each treatment were combined in a single composite sample.

RNA content was measured as % RNA by dry weight for eight individual chironomids ranging in size for each of eight different food C : P treatments. Nucleic acids were measured on frozen individual insects using protocols modified from methods used for zooplankton (Wagner et al. 1998; Gorokhova and Kyle 2002). DNA and RNA were quantified by extraction with N-laurylsarcosine, followed by sonication and staining with Ribogreen® (Molecular Probes). Nucleases were used to determine DNA and RNA separately.

Multiple regression was used to test for the effects of detritus C : P, average body size, and chironomid identity (i.e., collected from high-P stream or low-P stream) on growth rates, P-excretion rate, chironomid C : P, and RNA content. All statistical analyses were conducted using the general linear model procedure (PROC GLM) in SAS (version 9.2).

Genetic characterization of chironomid assemblage—In June 2007, chironomid larvae from *Ficus* leaf packs in each of the two focal streams (14 from Sura-60 and 5 from Arboleda) were reared to adults and preserved in ethanol along with pupal cases for taxonomic identification. In March 2009, approximately 80 chironomids from each of the two focal streams were collected from *Ficus* leaf packs and were preserved in molecular-grade ethanol for DNA sequencing. DNA was extracted from these larvae, as well as from adult specimens that had been previously identified to species, following the methods used to sequence chironomid DNA by Sinclair and Gresens (2008). A 650 base-pair (bp) fragment of the mitochondrial cytochrome c oxidase subunit I (COI) gene was amplified using the primers 911 (5′-TTTCTACAAATCATAAAGATATTGG-3′) and 912 (5′-TAAACTTCAGGGTGACCAAAAAATCA-3′) (Guryev et al. 2001). DNA was amplified in the following 25-μL reaction: 5 μL of polymerase chain reaction (PCR) buffer, 5 μL of $MgCl_2$, 2 μL of deoxyribonucleotide triphosphate (DNTP), 1 μL of each primer, 0.15 μL of *Taq* polymerase, and 1 μL of template DNA. The PCR thermocycling program used an annealing temperature of 40°C. PCR products were verified by electrophoresis and were sequenced in one direction (using the 911 primer) on an ABI 3730xl automated sequencer at the Georgia Genomics Facility. Quality-trimmed nucleotide sequences of ~ 550 bp were aligned using CodonCode ALIGNER 2.0.4 (CodonCode). Sites with a Phred quality score (Ewing and Green 1998) < 20 were coded as ambiguous.

Genetic distances were calculated using the Kimura-2-parameter (K2P) distance model (Kimura 1980). Neighbor-joining and maximum parsimony trees of K2P distances were created using Phylogenetic Analysis Using Parsimony software (PAUP*) 4.0b10 (Swofford 2002). Nonparametric bootstrap analysis was performed with 100 replicates for maximum parsimony. We identified species as clades with K2P distances < 0.06, a criterion previously used for other chironomid taxa (Sinclair and Gressens 2008). We then used the χ^2 statistic to test for differences in species distributions between the two focal streams.

Intraspecific genetic variance for each of the common species was analyzed within and between the two study sites using the Snn "nearest neighbor" statistic of Hudson (2000). Snn is a sequence-based statistic that measures how often nearest-neighbor sequences (haplotypes that are most similar to one another) are found in the same geographic location. Significance of the nearest-neighbor statistic was determined via permutational test (1000 replicates) using DNA Sequence Polymorphism (DNASP) version 5.0 (Librado and Rozas 2009). We also calculated summary statistics such as haplotype diversity (Hd) and nucleotide diversity (π).

Results

Experiment 1: Growth and excretion rates of chironomid assemblages across a natural P gradient—Across the natural P-gradient in the 2006 experiments, *Ficus* leaf litter C : P ranged from 268 to 1397 (Fig. 2). Chironomid growth rates were high, with a mean of 0.39 mg mg^{-1} d^{-1} (range: 0.18–0.42 mg mg^{-1} d^{-1}). Average initial chironomid mass in each chamber explained 16% of variance in growth rates. Chironomids from low-P streams feeding on low-P food grew at a marginally faster rate ($F_{1,43} = 3.75$, $p = 0.059$, $r^2 = 0.23$; Fig. 3A) and excreted P at a higher rate ($F_{1,43} = 6.40$, $p = 0.0153$, $r^2 = 0.14$; Fig. 3B) compared to chironomids from high-P streams feeding on high-P detritus. Pupae and adult chironomids were observed in some of the growth chambers at the end of the 3-d growth trials (mean < 1, but a maximum of 7 out of 20 in a chamber). The number of pupae and adult chironomids observed in growth chambers increased with increasing

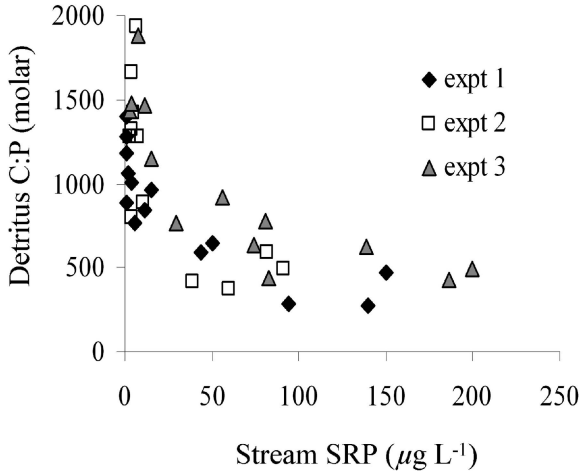

Fig. 2. Relationship between stream soluble reactive phosphorus (SRP) levels and detritus C : P used in the three experiments.

litter P content ($F_{1,43} = 30.76$, $p < 0.001$, $r^2 = 0.42$), while average initial larval size was not a significant factor ($F_{1,43} = 0.28$, $p = 0.6023$).

Experiment 2: Growth and excretion of chironomid assemblage across an experimental P gradient—Across the experimental P gradient, *Ficus* leaf litter C : P ranged from 417 to 1933 (Fig. 2). Chironomids had a mean growth rate of 0.27 mg mg^{-1} d^{-1} (range: 0.19–0.37 mg mg^{-1} d^{-1}). Initial size explained 11% of variance in measured growth rates. Litter P content had no effect on growth rates ($F_{1,44} = 1.18$, $p = 0.28$, $r^2 = 0.18$; Fig. 4A) after accounting for average initial size ($F_{1,44} = 7.93$, $p = 0.0073$). However, chironomid P excretion increased significantly with increasing leaf litter P content ($F_{1,42} = 48.67$, $p < 0.0001$, $r^2 = 0.54$) after accounting for variation in average larval biomass ($F_{1,42} = 1.21$, $p = 0.28$; Fig. 4B). The number of pupae and adults at the end of the 3-d growth trials was negatively related to leaf litter P content ($F_{1,44} = 3.87$, $p = 0.035$, $r^2 = 0.18$) after accounting for initial average biomass ($F_{1,44} = 3.87$, $p = 0.056$).

Experiment 3: Response of chironomid assemblages from a naturally high- and low-P stream to range of litter C : P—*Ficus* leaf litter C : P ranged from 426 to 1884 across all treatments in the 2007 experiment (Fig. 2). Chironomid growth rates in this experiment had an overall mean of 0.21 mg mg^{-1} d^{-1} (range: 0.05–0.35 mg mg^{-1} d^{-1}). Initial size of larvae in each chamber explained 44% of variance in growth rates ($F_{1,112} = 98.13$, $p < 0.0001$).

The chironomid assemblage from the high-P stream showed increasing growth rates with increasing litter P content, while the chironomid assemblage from the low-P stream showed no response (chironomid identity × leaf litter C : P: $F_{1,112} = 4.15$, $p = 0.044$; Fig. 5A). Measured P-excretion rates in experiment 3 were highly variable and showed no statistical relationship to leaf litter C : P ($p > 0.05$; $r^2 = 0.15$). However, for all leaf litter C : P values > 700, chironomids from the high-P stream had lower rates of

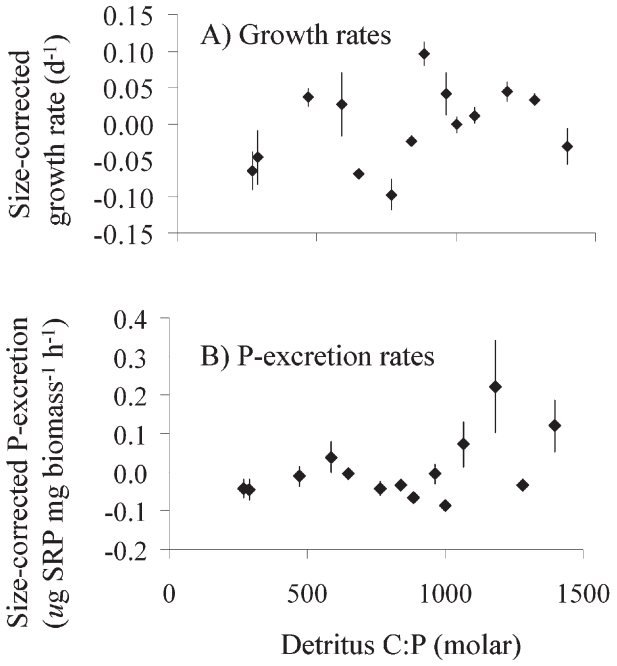

Fig. 3. (A) Size-corrected chironomid growth rates (residual of instantaneous growth rate vs. average initial larval size for each treatment) vs. detritus C : P for in situ chironomid assemblages along the natural-P gradient in experiment 1. (B) Size-corrected P-excretion rates (residual of P-excretion rate vs. average size of larvae in each replicate) vs. detritus C : P along the natural P gradient in experiment 1. Error bars represent 1 SE.

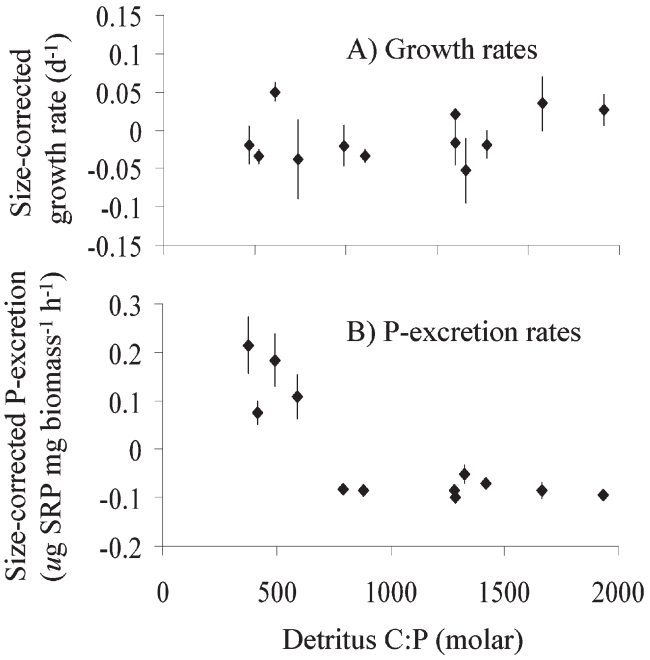

Fig. 4. (A) Size-corrected chironomid growth rates (residual of instantaneous growth rate vs. average initial larval size for each treatment) vs. detritus C : P along the experimental P gradient in experiment 2. (B) Size-corrected P-excretion rates (residual of P-excretion rate vs. average size of larvae in each replicate) vs. detritus C : P along the experimental P gradient in experiment 2. Error bars represent 1 SE.

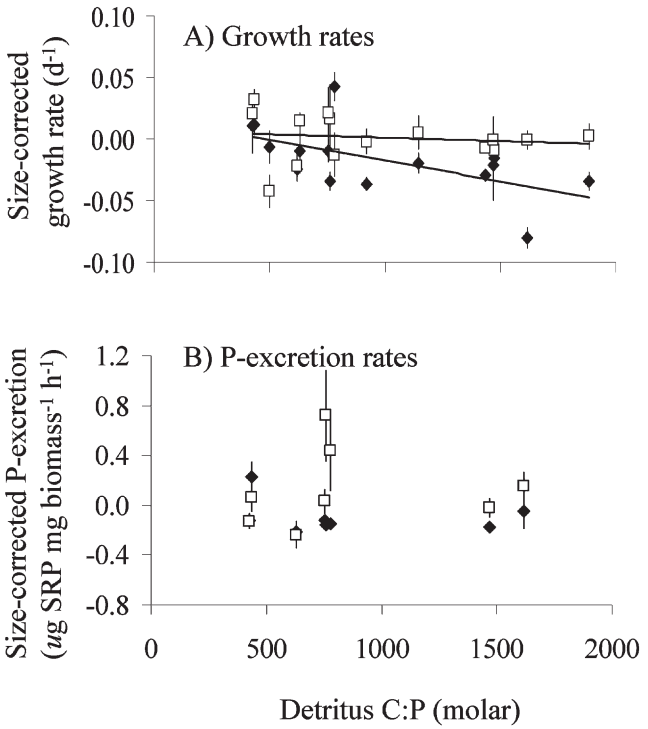

Fig. 5. (A) Size-corrected chironomid growth rates (residual of instantaneous growth rate vs. average initial larval size for each treatment) vs. detritus C : P for chironomids collected from a high-P stream (shaded diamonds) and a low-P stream (open squares) in experiment 3. (B) Size-corrected P-excretion rates (residual of P-excretion rate vs. average size of larvae in each replicate) vs. detritus C : P for chironomids collected from a high-P and low-P stream. Error bars represent 1 SE.

P excretion compared to chironomids from the low-P stream (Fig. 5B).

Chironomid biomass C : P increased with increasing detritus C : P ($F_{1,22} = 6.24$, $p = 0.02$, $r^2 = 0.23$; Fig. 6A), but there was no difference in this relationship between chironomids from the high-P stream and low-P stream (chironomid identity $p = 0.87$; identity × leaf C : P, $p = 0.91$). However, RNA content was higher for chironomids from the low-P stream across the range of detritus C : P in this study ($F_{1,120} = 22.24$, $p < 0.0001$, $r^2 = 0.48$; Fig. 6B). Chironomid RNA content was negatively related to size of the individual ($F_{1,120} = 32.77$, $p < 0.001$) and leaf C : P ($F_{1,120} = 15.18$, $p = 0.0002$), but the chironomid source × leaf C : P interaction term was not significant ($F_{1,120} = 1.77$, $p = 0.1857$). When data from both chironomid assemblages were considered together, chironomid RNA content was a significant predictor of size-corrected growth rates ($F_{1,14} = 6.4014$, $p = 0.0021$, $r^2 = 0.31$; Fig. 7).

Genetic characterization of chironomid assemblage—From chironomids reared to adults, three species were identified from the high-P Arboleda and low-P Sura-60 streams: *Polypedilum pterospilus* Townes (1 in Arboleda, 2 in Sura-60), *Endotribelos* cf. *hesperium* (Sublette) (1 in Arboleda, 7 in Sura-60), and an undescribed species of *Endotribelos* (3 in Arboleda, 5 in Sura-60; J. H. Epler pers. comm.).

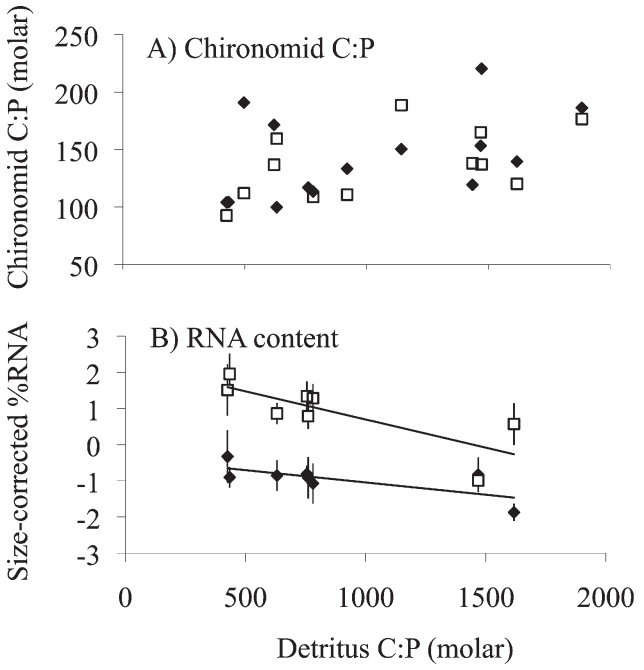

Fig. 6. Chironomid (A) body C : P and (B) size-corrected % RNA vs. detritus C : P for chironomids collected from a high-P stream (shaded diamonds) and low-P stream (open squares) in experiment 3. Size-corrected RNA content is calculated as residual of % RNA by dry mass vs. mass of individual larvae. Error bars represent 1 SE.

We successfully sequenced mtCOI DNA from 146 chironomid larvae from the two focal streams, as well as 5 adult chironomids that were identified to species (Genbank GU565707–GU565917). In total, 752 characters were evaluated in PAUP from the 650-bp fragment (some sites had multiple substitutions). Of these, 511 characters were constant, 41 characters were parsimony-uninformative, and 200 characters were parsimony-informative.

Across the 146 chironomid larvae that were sequenced, 129 (88%) fell into four species (defined by monophyletic

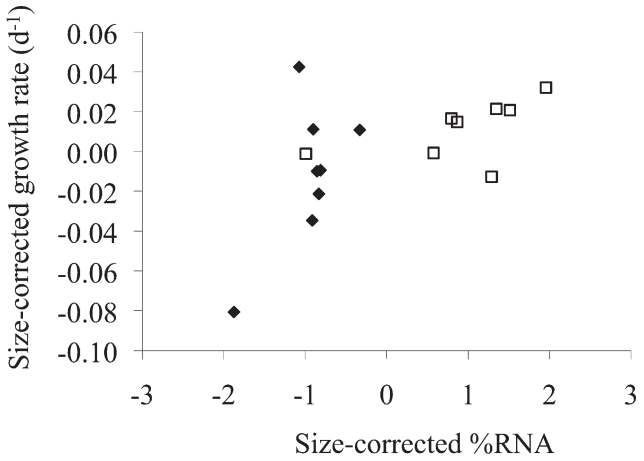

Fig. 7. Size-corrected growth rate vs. size-corrected %RNA for chironomids collected from high-P stream (shaded diamonds) and low-P stream (open squares) in experiment 3. Error bars are not shown for clarity.

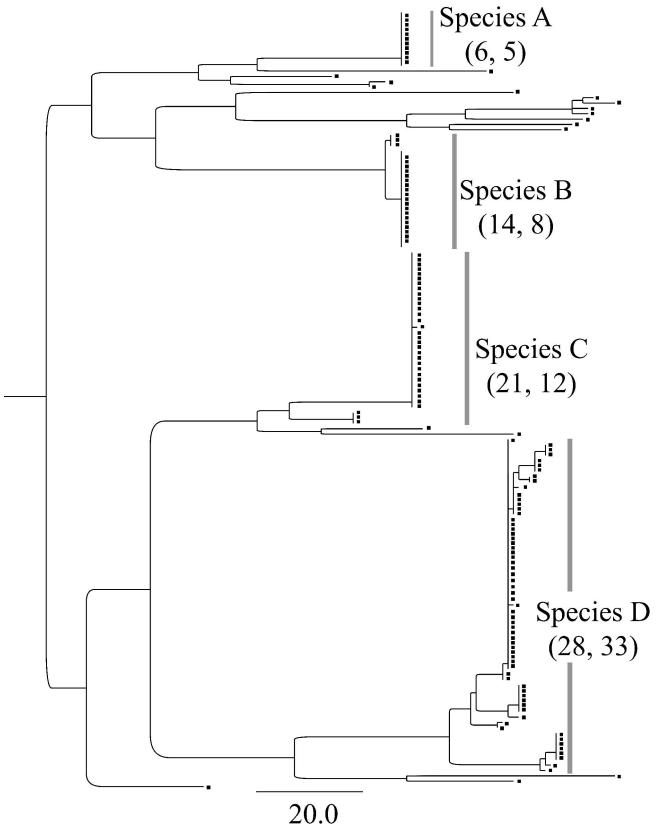

Fig. 8. Phylogram of 146 chironomid larvae collected from the two focal streams from experiment 3, based on mitochondrial COI DNA sequences. Species are defined by K2P divergence of $< 6\%$. Numbers in parentheses indicate the number of individuals of each species identified from the high-P Arboleda-30 and low-P Sura-60, respectively.

groups of divergence $< 6\%$; Fig. 8). We were unable to provide unambiguous associations between sequenced adults that had been identified based on morphological characteristics and the phylogeny-defined species. For this reason, we only refer to the phylogeny-defined species by letters rather than by species names.

Based on these sequencing data, community composition is similar between these two focal streams ($\chi^2 = 3.77$, df = 2, $p > 0.25$). Of the 69 chironomid larvae sequenced from the low-P Sura-60, 5 (7%) were identified as species A, 8 (12%) as species B, 12 (17%) as species C, and 34 (49%) as species D. Of the 77 larvae sequenced from the high-P Arboleda, 6 (8%) were identified as species A, 14 (18%) as species B, 21 (27%) as species C, and 29 (38%) as species D. Within each of these dominant species, we found no evidence of phylogenetic separation between the two focal streams. For species B, C, and D, the Snn statistic has p-values > 0.25 (Table 2). A single haplotype was found for species A, so Snn cannot be calculated for this group.

Discussion

Our results support the hypothesis that chironomid P demand reflects local food quality across a landscape characterized by nutrient heterogeneity. Chironomids from a naturally high-P stream showed evidence of P limitation when fed low-P detritus, while chironomids from naturally low-P streams showed no evidence of P limitation. Previous studies of effects of P on chironomid growth rates at La Selva used chironomid larvae collected from a single stream (intermediate in dissolved P levels) and did not measure the P content of food resources. These prior studies (Rosemond et al. 2001; Ramírez and Pringle 2006) reported increases in chironomid growth rates with increasing stream P levels. In our study, we explicitly considered the potential for variation in chironomid P demand across a stoichiometrically heterogeneous landscape.

Table 2. Number of chironomid larvae sequenced (n), haplotype diversity (Hd), and nucleotide diversity (π) for each of the four common species detected in the low-P Sura-60 stream and the high-P Arboleda stream. The nonparametric Snn statistic, and accompanying p-value, is shown for species B–D. Because we found a single haplotype of species A, Snn cannot be calculated for this species.

	n	Hd	π	Snn	p
Species A					
Sura-60	5	0.000	0.000		
Arboleda	6	0.000	0.000		
Species B					
Sura-60	8	0.429	0.001	0.515	0.543
Arboleda	14	0.143	0.000		
Species C					
Sura-60	12	0.000	0.000	0.546	0.204
Arboleda	21	0.338	0.016		
Species D					
Sura-60	34	0.731	0.017	0.52	0.263
Arboleda	29	0.564	0.014		

Several lines of evidence from our study indicate that chironomids from naturally low-P streams are not P limited when feeding on high-C : P detritus. First, chironomid assemblages from low-P streams, feeding on high-C : P detritus, showed marginally *higher* growth rates and P-excretion rates compared to chironomids from high-P streams that fed on low-C : P detritus (Fig. 3). These results suggest that the chironomids in our low-P study streams that feed on high-C : P detritus ingested more P than is required for biomass production. When feeding on low-C : P detritus, chironomid larvae in the experimentally enriched stream showed no change in growth rates but a sharp increase in P-excretion rates (Fig. 4), suggesting that the chironomids in this historically low-P stream were not capable of converting the extra P into new biomass. Finally, the chironomid assemblage from the low-P focal stream showed no changes in growth rates over a wide range of detritus C : P values (Fig. 5). The fact that chironomid growth rates in experiment 3 were consistent across the 4-fold range in detritus C : P also indicates that this chironomid assemblage, which typically feeds on low-P detritus, can tolerate high-P detritus with no apparent negative effects (Boersma and Elser 2006).

Our results also indicate that P demand is higher among chironomids in naturally high-P streams. The relatively low

growth rates and P-excretion rates observed for chironomids from high-P streams feeding on high-P detritus (Fig. 3) suggest that their P intake may have been insufficient for the demands of biomass production, even while feeding on high-P food resources. Additionally, compared to chironomids from the low-P focal stream, chironomids from the high-P focal stream showed decreases in growth rates and lower P-excretion rates when fed detritus with C : P $>$ 800 (Fig. 5).

The differences in P demands among chironomids from high- and low-P streams appear to be due to altered P allocation. Within each assemblage, RNA content declines with increasing food C : P, consistent with other studies on invertebrate consumers (Schade et al. 2003; Elser et al. 2005), and, for chironomids of a given size, RNA content is a good predictor of growth rate, consistent with the "growth rate hypothesis" (Elser et al. 1996). However, across the range of detritus C : P examined in this study, chironomids from the low-P stream had consistently higher levels of RNA (Fig. 6).

To illustrate the way in which P is differentially allocated with changes in dietary P content for chironomids from high- and low-P streams, we modeled the contribution of RNA and DNA to the total P content of a chironomid with a mass of 0.05 mg, typical for our study (Fig. 9), based on regressions of total body P, % RNA, and % DNA vs. detritus C : P (with body size as covariate) for both chironomid assemblages. % RNA and % DNA were converted to RNA-P and DNA-P by assuming that nucleic acids are 8.7% P by mass (Sterner and Elser 2002). For a chironomid from the high-P stream feeding on high-P detritus, RNA accounts for 30% of its body P, compared to 35% when feeding on low-P food. For chironomids from the low-P focal stream, RNA-P increases proportionately with body P, accounting for approximately 46% P across all food P levels. In all cases, DNA accounts for approximately 3% of body P.

The differences in P allocation between chironomid assemblages suggests that chironomids from the low-P stream have a lower non-RNA P demand, so that for a given food C : P value, these chironomids would have more P remaining to allocate to RNA. Kyle et al. (2006) found that different zooplankton taxa have different maintenance costs of RNA and P; similarly, chironomids in low-P La Selva streams may have higher P-use efficiency due to lower baseline P demands. In addition to altered P allocation, it is also possible that chironomids in low-P streams have adopted a combination of behavioral and physiological strategies to mitigate the elemental imbalance in their food, such as selectively feeding on patches of leaves colonized by nutrient-rich microbes (Arsuffi and Suberkropp 1985), compensatory feeding to increase total P intake, higher assimilation of P (He and Wang 2007), or compensatory respiration to burn off excess C (He and Wang 2008).

The apparent higher P demand for chironomids from high-P streams could be due to differences in P storage. For example, elevated levels of non–nucleic acid P have been documented in other invertebrates feeding on P-enriched food as a result of P storage in hemolymph (Woods et al. 2002) or in metal-containing granules along the digestive

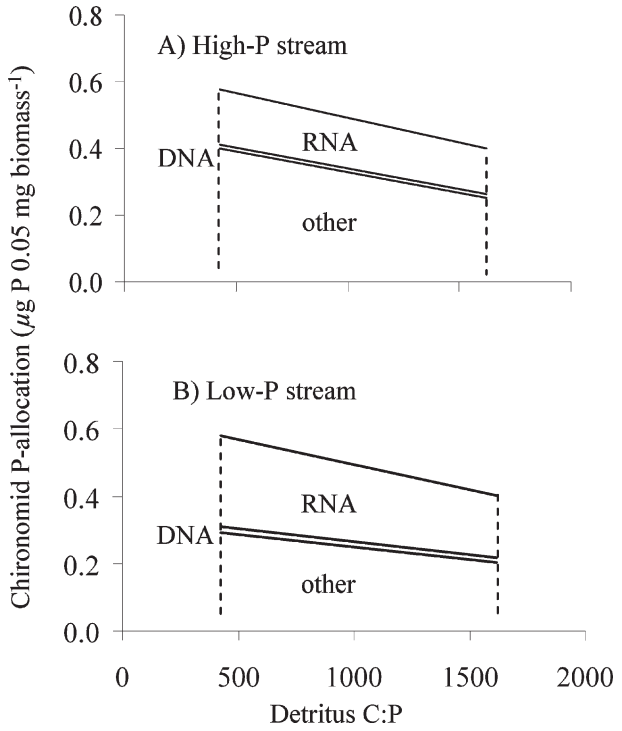

Fig. 9. Estimated contribution of RNA and DNA to total P content of a chironomid with mass 0.05 mg from (A) high-P stream and (B) low-P stream, across range of detritus C : P values, based on modeled relationships between % RNA, % DNA, and total % P vs. detritus C : P for each assemblage.

tract (Hopkin 1989). The higher levels of non-RNA P storage in chironomids from high-P streams could lead to increased fitness in that environment. For example, even though larval growth rates were marginally lower for chironomids in high-P streams (Fig. 3A), we found indirect evidence of more rapid larval development (higher numbers of pupae and adults found at the end of the 3-d growth trials), as has been observed in other insect taxa feeding on P-enriched food resources (Perkins et al. 2004).

Differences in P demand among chironomid assemblages could potentially be attributable to differences in species composition, intraspecific genetic differences, or phenotypic plasticity, but our results allow us to eliminate several possible mechanisms. The similar species composition between the two focal streams leads us to rule out species shifts as the primary driver of the different physiological responses to food P content. At the individual level, another possible mechanism is differences in gene expression during early developmental stages (i.e., before larvae were collected for use in our experiments) determining P demand in later instars (Jeyasingh and Weider 2007), but this explanation is inconsistent with the lack of response by chironomids in the experimentally P enriched stream. Transgenerational phenotypic effects of food quality (through maternal nutrition) have been documented in some invertebrates (Frost et al. 2010), but this explanation is also inconsistent with our results, because it would imply that chironomid larvae from high-P streams would generally be less prone to P limitation.

By ruling out alternative explanations, our results suggest that genetic adaptations within populations may be responsible for the observed differences. Many populations have considerable standing genetic variation for P physiology, and microevolutionary responses to spatial or temporal P supply have been observed in other systems (reviewed in Jeyasingh and Weider 2007). For example, a similar genotype × environment interaction creates a competitive tradeoff in *Daphnia*, based on genetic variation at the phosphoglucose isomerase locus (Jeyasingh et al. 2009). Although we found no phylogenetic divergence between populations based on the (presumably neutral) mitochondrial DNA sequences, a small amount of gene flow between populations would be sufficient to maintain this homogeneity, and is not unlikely given that the sample sites are separated by only 1500 m. Local genetic adaptation can occur despite gene flow between populations, for example, as a result of genotype-specific mortality (Schmidt and Rand 2001). If microevolution explains the differences among chironomid assemblages in the naturally high- and low-P streams, the fact that no differences in P demand were observed after 8 yr of experimental P enrichment in the Carapa (corresponding to hundreds of generations) would seem to suggest a low heritability of this trait. However, the lack of genetic response by chironomids in the Carapa could also be explained by the fact that, due to high rates of uptake of dissolved P (Small et al. 2008), only ~ 100 m of the stream experienced consistently high-P conditions, effectively decreasing the selection pressure on this population. In order to test the hypothesis that microevolution is responsible for the differences in P demand among populations, future research should examine polymorphic loci in these populations, and test whether genotypes within each species from high- and low-P streams are competitively dominant within their respective environments.

Our finding that chironomid larvae in the low-P streams were not P limited has important ecological implications. The correspondence between chironomid P demand and local food quality across this heterogeneous landscape could serve to stabilize the food web against perturbations caused by nutrient loading. In streams where primary consumers are P limited, nutrient loading can have important effects at multiple trophic levels. For example, in a detritus-based temperate stream, a 2-yr nutrient (N + P) addition resulted in a 1.5-fold increase in growth rates and a 3-fold increase in production of larval chironomids (Cross et al. 2005). This increased production of chironomids, along with other short-lived primary consumers, led to a decrease in leaf-litter standing stock, and increased abundance and biomass of predators during the initial years of the experiment (Cross et al. 2006). By contrast, our study found that chironomids from low-P streams did not respond to increased P availability. Chironomids from naturally low-P streams showed no increase in growth when feeding on low C : P detritus (Figs. 4, 5). Furthermore, no increases in chironomid biomass were observed in the experimentally P-enriched stream over 8 yr (Ramírez and Pringle 2006). The lower P demand of chironomids in low-P streams appears to limit the ability of these consumers to respond to nutrient-enriched food. This limited physiological response may act to stabilize the detritus-based food web against changes in nutrient availability, given that chironomid larvae are the dominant group of detritivores in these lowland tropical streams.

In summary, assemblages of larval chironomids in low-P streams showed no evidence of P limitation under ambient conditions as a result of their lower P demand relative to chironomid assemblages in nearby high-P streams. While ecological stoichiometry theory has considered a consumer's nutrient demand to be a species-level property (Frost et al. 2006), our results, showing differences in P demand among similar assemblages, suggest that microevolution may cause differences in nutrient demand among populations. Anthropogenic nutrient pollution, through land-use change, fertilizer runoff, and wastewater discharge, can all lead to landscape-scale heterogeneity in stream basal food resource nutrient content, and, over time, may lead to similar responses in other stream consumers.

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