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Temperature and nutrient availability interact to mediate growth and body stoichiometry in a detritivorous stream insect

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SUMMARY

1. Regimes of temperature and nutrient availability are undergoing rapid modification at global scales. Both temperature and nutrients can influence consumer physiology and growth via several mechanisms. We examined how temperature and the nutrient content of food interact to affect consumption, growth and body stoichiometry of a detritivorous consumer (the caddisfly *Pycnopsyche gentilis*).
2. In a 7-week growth study, *P. gentilis* larvae were reared at two different temperatures (5 and 10 °C) while fed conditioned red maple (*Acer rubrum*) litter at one of two stoichiometric qualities (manipulated by raising phosphorus supply in one litter conditioning treatment; Amb: mean litter P = 0.03%, mean litter N = 0.79%; Hi-P: mean litter P = 0.14%, mean litter N = 1.2%).
3. Temperature and litter quality had differential effects on bulk consumption, element-specific (N and P) consumption, growth and elemental body content of *P. gentilis* larvae. Temperature was the only factor affecting bulk feeding rates. Larvae in the Warm/Hi-P treatment had by far the highest growth rates; the negligible growth in the Cold/Amb treatment was increased by either higher temperature (Warm/Amb) or higher food quality (Cold/Hi-P).
4. Higher temperature had no effect on body P content in Hi-P treatments, but decreased body P content in the Amb treatments. Shifts in temperature and resource quality are both important components of global change and our results show that these factors can have interactive effects on detrital food webs, through which most primary production flows.

Keywords: detritus, ecological stoichiometry, homoeostasis, phosphorus, temperature

Introduction

Temperature and resource quality have fundamental influences on the physiology and life history of consumers (Sterner & Elser, 2002; Brown *et al.*, 2004; Sibly, Brown & Kodric-Brown, 2012), and both of these factors are changing rapidly across the globe. Eutrophication is altering basal resource quality (and quantity) by directly adding nutrients to ecosystems (Smith, Joye & Howarth, 2006). Increased atmospheric CO_2 is leading to higher C availability and increased C:nutrient stoichiometry in plants, as well as increasing surface air and water temperatures (van de Waal *et al.*, 2009). Although both temperature and nutrient availability affect important aspects of organismal development, they act through separate and potentially interacting mechanisms. Temperature alters the rate of biochemical reactions and changes developmental cues, affecting development time, body size, feeding, growth rate and organism metabolism (Gillooly *et al.*, 2001, 2002; Enquist *et al.*, 2003). In contrast, resource quality plays its important role by determining the absolute supply of key elements, essential fatty acids and biomolecules that serve as the building blocks for growth and reproduction (Sterner & Elser, 2002; Arts, Brett & Kainz, 2009; Kaspari, 2012).

The critical roles played by diet and temperature in controlling aquatic invertebrate growth and life histories have been explored in previous studies, which have

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revealed the potential for these two factors to interact (e.g. Sweeney & Vannote, 1986; Sweeney, Vannote & Dodds, 1986a,b; Gresens, 1997; Ferreira *et al.*, 2010). For example, temperature strongly influenced the effects of diet type on growth and development of the mayfly *Cloeon triangulifer* (Sweeney & Vannote, 1984). Studies that incorporate both temperature and diet quality typically have tested the effects of diet using different food types (e.g. different leaf species), with the role of external factors that control food resource quality receiving far less attention, especially in aquatic insects. In particular, the role of dissolved nutrients in mediating the interaction of diet quality and temperature is relatively unexplored. Recent empirical analyses of zooplankton have focussed on potentially interactive effects of diet stoichiometry and temperature, although with somewhat equivocal results. McFeeters & Frost (2011) found the greatest sensitivity of growth to low diet quality (low %P) in *Daphnia magna* at low temperature, while the effect of low-P food on body P content was larger at higher temperature. In contrast, another recent set of studies has reported the opposite effect, with P limitation of *D. magna* growth decreasing at low temperatures, presumably because of lower demands for growth (Persson *et al.*, 2011; Wojewodzic *et al.*, 2011).

To date, experimental studies investigating stoichiometric responses to the interactive effects of temperature and diet quality also have been restricted to consumers that feed on living plant tissue (McFeeters & Frost, 2011; Persson *et al.*, 2011). The majority of global primary production flows through the detrital food web (Moore *et al.*, 2004), however, despite the relatively low stoichiometric quality of detrital resources (Enríquez, Duarte & Sand-Jensen, 1993). Together, the high quantity and low quality of detritus mean that the inclusion of detritivore responses is important for any comprehensive understanding of the interacting effects of temperature and resource quality on the fate of particulate organic matter in aquatic and terrestrial food webs.

We conducted a 7-week growth study with a full factorial design in which a representative detritivorous insect (shredding larvae of the caddisfly *Pycnopsyche gentilis*) was fed one of two detrital food qualities at two rearing temperatures. We set out to determine how differences in inorganic phosphorus availability, filtered through basal detrital resources, could affect consumption, growth and whole-organism stoichiometry of *P. gentilis* at different temperatures. We predicted that increased metabolism at higher temperatures would drive increases in consumption and growth rate. Consistent with previous research on larval Trichoptera, we further predicted greater consumption on higher-quality food (Arsuffi & Suberkropp, 1986). Higher food quality increases the supply of essential or limiting nutrients, which we predicted would lead to higher growth rates in high-quality diet treatments. The effect of stoichiometric diet quality on larval growth was also predicted to be greater at higher temperatures. Finally, in accordance with the 'growth rate hypothesis' (GRH; i.e. coupling of P-rich rRNA to growth; Elser *et al.*, 1996), we predicted that body P content would track growth rate across temperature and diet treatments.

Methods

Manipulation of resource nutrient content

Autumn-shed red maple (*Acer rubrum*) leaves were picked from the ground shortly after abscission during October 2007 in Tuscaloosa County, Alabama (U.S.A.), brought back to the laboratory, air-dried for 2 weeks and stored until use. Starting on 29 November 2009, approximately 5 g of air-dried leaf material was added to each of 16–20 Nitex® (Sefar Holding Ag, Heiden, Switzerland) nylon mesh bags (0.5-mm mesh size) and incubated for 1 week in Hurricane Creek, Tuscaloosa County, Alabama (U.S.A.). Hurricane Creek is a third-order stream with mainly forested land use upstream of the incubation site. Initial incubation in Hurricane Creek allowed the natural microbial colonisation of leaf material. Periodic measurements made during the experiment showed that soluble reactive phosphorus (SRP) concentrations in Hurricane Creek ranged from 23 to 55 μg SRP L^{-1} and nitrate from 155 to 225 μg NO_3-N L^{-1}.

After removal from Hurricane Creek, litter bags were transported to one of two recirculating artificial streams [*c.* 2000 L; one low phosphorus (Amb) and one high phosphorus (Hi-P)] located in a greenhouse at the University of Alabama in Tuscaloosa, Alabama (U.S.A.). Artificial streams were initially filled with water collected from a groundwater spring (38 μg SRP L^{-1}, 445 μg NO_3-N L^{-1}; Little Schultz Creek, Bibb County, AL, U.S.A.). Microbial activity on leaf litter placed in the artificial streams depleted most of these nutrients within 1 week. The Amb treatment was then amended with NaH_2PO_4 to a target concentration of 20 μg P L^{-1} and with $NaNO_3$ to a target concentration of 200 μg N L^{-1} (dissolved molar N : P of 22). The Hi-P treatment was amended with NaH_2PO_4 to a target concentration of 100 μg P L^{-1} and with $NaNO_3$ to a target concentration of 200 μg N L^{-1} (dissolved molar N : P of 4). Nutrient

immobilisation rate was determined by the decline in nutrient concentration over a 1-week period in each of the artificial streams. Every 3–5 days, nutrients were replenished to maintain target concentrations based on measured depletion rates and to maintain an active microbial community on leaves.

Litter bags were incubated in the artificial streams (13.9 °C, SE ± 0.06) for 31–44 days before being removed and fed to *P. gentilis* located in temperature-controlled environmental chambers (see Growth trials below). Each week, one set of litter bags was removed from each artificial stream, fed to *P. gentilis* and replaced with 1-week-old litter bags incubated in Hurricane Creek.

Growth trials

Larvae of the cased caddisfly *Pycnopsyche gentilis* were collected on 30 December 2009 from unnamed spring-fed tributaries, situated within *c.* 20 m of one another, along Shope Fork at Coweeta Hydrologic Laboratory in Otto, North Carolina (U.S.A.). *Pycnopsyche gentilis* is a common and functionally important univoltine, shredding insect found in cool mountain streams across eastern North America (Flint, 1960; Wiggins, 1996). Water temperatures at Coweeta streams where *P. gentilis* can be found range from 0.7 to 20.7 °C, with *P. gentilis* generally growing from the third to fifth instar during the colder months of December to March (Eggert & Wallace, 2003). One hundred and forty-six individuals of approximately the same size (*c.* third instar) were collected for use in the study. Twenty-six of these larvae were randomly selected to provide an average initial dry mass (DM, mean 3.73 mg ± 1.3 SD) to be used in the estimation of growth rate at the end of the experiment. These 26 individuals were removed from their cases, frozen at −40 °C, freeze-dried and weighed using a Mettler digital microbalance (±0.1 μg; Mettler-Toledo, Columbus, OH, USA).

The remaining specimens (n = 120) were placed individually into 64-mm diameter, 500-μm Nitex® mesh-bottomed cylinders. Thirty cylinders, each containing one larva, were placed into each of four streamwater-filled plastic containers (54 × 40 × 14 cm) equipped with an A-130 mini-elite underwater filter (Hagan, Montreal, QC, Canada) and air pump with air stones. Two plastic containers were each placed into one of two temperature-controlled environmental chambers [Warm (10 °C) and Cold (5 °C)] and their placement within each chamber changed every 7 days to remove any effect of location within the chamber. Initially, containers were filled with water from the sites where *P. gentilis* was collected. Half of the water was changed each week, using water from Hurricane Creek. A gravel/sand mix collected from Coweeta streams was elutriated and autoclaved before being added to each individual cylinder for case making, characteristic of late fourth- and fifth-instar larvae in this species (Mackay, 1972).

The four designated treatments in the feeding trial were as follows: Cold/Amb, Cold/Hi-P, Warm/Amb and Warm/Hi-P, with Cold and Warm referring to the two temperature treatments and Amb and Hi-P specifying relative P availability to leaf litter (n = 30 per treatment; total n = 120). Larvae were fed *Acer rubrum* leaves from their representative litter quality treatment *ad libitum*. Each week, all old leaf material was removed and new leaves were added. Frass produced by larvae passed through the 500-μm mesh at the base of rearing cylinders, so was not available for consumption.

A subset of individuals from the feeding trial was also used to estimate consumption rates (CR). These individuals were treated in the same manner as other specimens, except that the leaf litter added and removed from individual cylinders was quantified to estimate consumption. To allow *P. gentilis* to acclimatise to rearing conditions and to repair any case damage that may have occurred during transport, estimates of consumption began 3 weeks after the growth trials began. Consumption was measured by feeding randomly selected *P. gentilis* larvae a known amount of leaf disc material cut using a cork borer (8 mm or 16 mm). Ten individuals from each treatment were randomly selected each week for CR estimation. After removing all visible organic matter from each individual's cylinder, a known number of leaf discs were fed to each of the assigned insects. To maintain *ad libitum* feeding conditions, enough discs were provided to ensure an excess of leaf litter available to each individual. After 1 week of feeding by *P. gentilis*, remaining leaf disc material was collected from each individual, dried at 60 °C for at least 1 week and stored in a desiccator until mass was measured. Feeding controls were included in three of the four consumption trials. These controls were incubated in the same containers that contained insects, within plastic cylinders preventing direct exposure to insects, creating an environment that differed only in exposure to feeding insects. Final masses of control leaf discs were not significantly different from initial masses (t-test, P = 0.48), so the effect of non-consumptive mass loss was ignored.

A subsample of leaf material was collected from each nutrient treatment weekly prior to feeding for stoichiom-

etric analyses (C, N and P; see Chemical analyses below). Any chemical changes to leaf discs over the course of 1 week were presumed to be minimal compared with differences between treatments; weekly measurements of resource quality were assumed to be sufficient to account for differences between treatments. Water temperature was logged every 30 min using HOBO® loggers (Onset, Pocasset, MA, U.S.A.) placed into each of the two water containers in each chamber.

Growth and consumption calculations

Growth rates were measured using the average initial mass of *P. gentilis* (W_i), as measured from the subset of individuals randomly selected at the start of the experiment. After the 49-day growth trial, *P. gentilis* larvae were frozen at −40 °C, lyophilised, weighed using a Mettler® digital microbalance (±0.1 μg) and prepared for C, N and P analysis (see Chemical analyses below). Instantaneous growth rates (IGR, % day^{-1}), assuming an exponential growth curve, were calculated as: $IGR = [\log_e(W_f/W_i)/t]*100$, where W_f = final mass, W_i is the average initial mass of individuals and time, t, is the duration of the growth trial in days.

Mass-specific leaf CR (mg DM mg^{-1} DM day^{-1}) was calculated as follows: $CR = (M_i - M_f/t)/W_t$, where M_i is initial leaf litter mass fed to *P. gentilis* and was measured by weighing 30 leaf discs from a subsample of all leaf discs cut each week from each litter treatment and calculated as the average individual leaf disc mass for that week multiplied by the number of leaf discs fed to *P. gentilis* on that day. M_f is total leaf litter mass collected after feeding had occurred for 7 days, t. W_t is the estimated mass of the individual larva at the time consumption was measured. W_t was calculated using larva-specific growth curves derived from each individual's growth rate (as determined above) to determine size of larvae on each date. Larvae that did not survive the 7-week feeding trial were excluded from all analyses except the survivorship calculations. Rates of N and P consumption were determined by multiplying litter consumption by average N and P content for each week during the 7-week trial (see Chemical analyses below).

Chemical analyses

Nitrate concentrations in Hurricane Creek, Little Schultz Creek and the artificial streams were measured using a Dionex ICS 2000 Dual RFIC ion chromatograph (Dionex Corp., Sunnyvale, CA, U.S.A.). Carbon and nitrogen analyses of leaf litter and insects were performed by micro-Dumas combustion on a NA1500 C/H/N analyser (Carlo Erba Strumentazione, Milan, Italy). P content of litter and insects was measured by persulphate digestion followed by molybdate blue colorimetric analysis (APHA, 1998). Insects were prepared for elemental analysis by grinding whole lyophilised individuals to a powder using a Wig-L-Bug® grinder (Crescent Dental, Lyons, IL, U.S.A.). A subsample of the ground insect was then weighed on a Mettler® digital microbalance (±0.1 μg) before continuing with elemental analysis as described above. Dried leaf material was ground in a Wiley Mini-Mill® (Arthur Thomas, Philadelphia, PA, U.S.A.) prior to weighing a subsample on a Mettler® digital microbalance (±0.1 μg). SRP concentrations in Hurricane Creek, Little Schultz Creek and the artificial streams were measured by molybdate blue colorimetric analysis (APHA, 1998).

Statistical analyses

Carbon, nitrogen and phosphorus contents (% DM) of litter were compared between Hi-P and Amb nutrient treatments using *t*-tests on leaf litter collected weekly prior to feeding. Mass-specific consumption and growth rates among all four treatments were compared using two-way independent ANOVAS; *t*-tests were used to test whether consumption or growth in any single treatment was different from zero. Because N and P values were not independent within diet quality treatments, statistical analyses were not performed on element-specific consumption. Body P content was compared among treatments using ANCOVA, with log body mass (mg DM) entered as the covariate. To determine significant differences among all treatment means, we conducted a one-way ANOVA followed by Tukey's *post hoc* comparison. An inadvertent loss of samples led to a low number of samples being available for body nitrogen analysis, and statistical analyses were not performed on these data. All analyses were performed in SPSS 19.0 (SPSS, 2010).

Results

Litter stoichiometry and temperature manipulations

The litter stoichiometry and temperature treatments were both successfully maintained throughout the experiment. Mean carbon content (±1 SE) of litter after incubation for 31–44 day in the artificial streams was 45.7% (±1.72, range 35–49%) and 45.2% (±0.46, range 43–47%)

in the Amb and Hi-P treatments, respectively. Mean nitrogen content was 0.79% (±0.09, range 0.67–0.87%) and 1.2% (±0.08, range 1.1–1.3%) in the Amb and Hi-P treatments, respectively, which represented a 52% increase from the Amb to the Hi-P treatment. Litter in the Amb treatment showed an average P content of 0.03% P (±0.003, range 0.02–0.03%), while litter in the Hi-P treatment averaged 0.14% P (±0.004, range 0.13–0.16%), which corresponds to a 367% increase from the Amb to the Hi-P treatment. Molar elemental ratios were 66 and 44 for C : N and 3928 and 832 for C : P in the Amb and Hi-P treatments, respectively. N and P contents of leaf litter were significantly different among treatments ($P < 0.001$ in each case); C content of leaf litter was not significantly different between treatments ($P = 0.7$).

Our temperature treatments for rearing larvae were maintained throughout the experiment. The Cold treatment had an average daily temperature of 5.1 °C (total range 3.5–7.1 °C, SD ± 0.4), and the Warm treatment had an average daily temperature of 10.0 °C (total range 9.3–10.6 °C, SD ± 0.9). There were slight differences in water temperature between rearing tanks within each temperature-controlled environmental chamber due to their placement in different sections of the chamber. As a result, there were slight differences in average temperature for Amb and Hi-P treatments within the Cold treatment (5.0 and 5.2 °C, respectively) and Warm treatment (10.0 and 10.1 °C, respectively).

Consumption rates

Only temperature had a significant effect on bulk litter consumption rate (Fig. 1, $F_{1,113} = 63.9$, $P < 0.001$), with the highest feeding rates exhibited by individuals in the Warm/Amb treatment (Fig. 1a). Consumption rate of N was highest in Warm/Hi-P treatment (Fig. 1b). Unlike bulk consumption and N consumption, P consumption was higher in both Hi-P treatments, driven by the large difference in P availability between diet treatments (Fig. 1c).

Larval growth and survival rates

There was a significant interaction between diet and temperature on growth rate of larvae (Fig. 2; $F_{1,93} = 12.3$, $P = 0.001$). Temperature and stoichiometric diet quality were also significant ($F_{1,93} = 200.9$, $P < 0.001$; $F_{1,93} = 75.0$, $P < 0.001$, respectively) but the interaction effect prevented independent interpretation of the individual effects. Growth rate increased in the order: Cold/Amb, Cold/Hi-P, Warm/Amb and Warm/Hi-P. The large difference in growth rate between Warm/Amb to Warm/Hi-P led to a significant interaction between temperature and diet quality, indicating that growth rate responded to increased diet quality more strongly at the higher

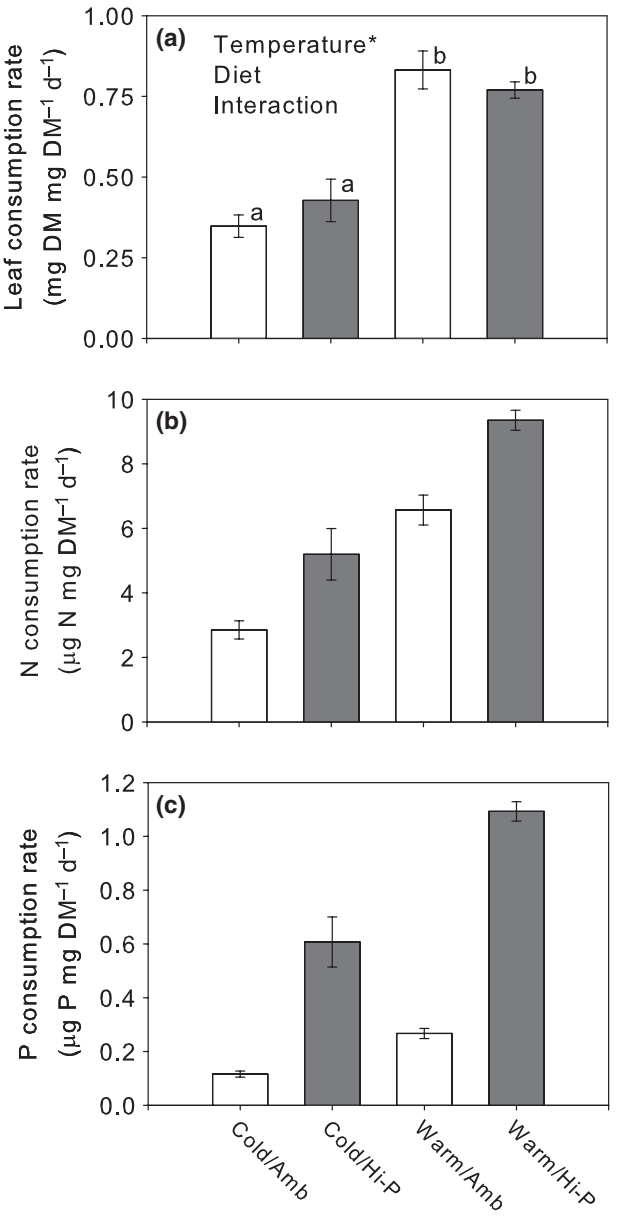

Fig. 1 Mean leaf litter consumption rates measured in random subsets of individuals ($n = 10$) within each treatment over four 1-week periods during the rearing trials. (a) Mass-specific feeding rate on bulk litter, (b) mass-specific N consumption measured as the individual mass-specific bulk feeding rate multiplied by mean %N content of the leaf litter and (c) mass-specific P consumption measured as the individual mass-specific bulk feeding rate multiplied by mean %P content of the leaf litter. *Indicates significance of factors at $\alpha = 0.05$; statistical tests were not conducted on N and P consumption data (see text). Bars with different lower-case letters represent significant differences based on a Tukey's test at $\alpha = 0.05$. Error bars indicate ± 1 SE.

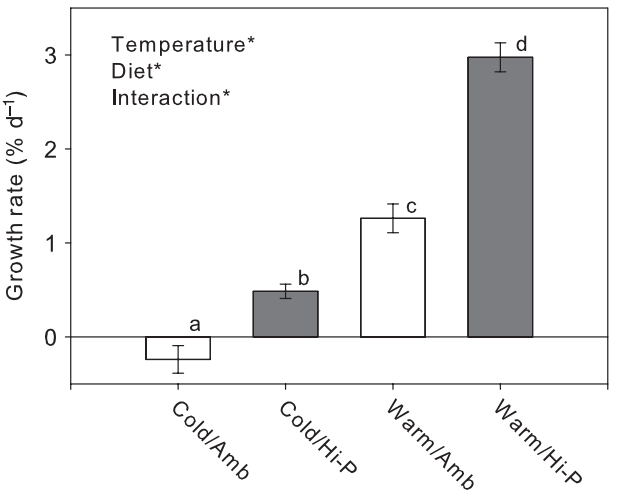

Fig. 2 Mean instantaneous growth rate (% day^{-1}) of *Pycnopsyche gentilis* larvae in each treatment. *Indicates significance of factors at $\alpha = 0.05$. Bars with different lower-case letters represent significant differences based on a Tukey's test at $\alpha = 0.05$. Error bars indicate ± 1 SE.

temperature (Fig. 2). Survival varied inversely with growth rate, with 63% in Cold/Amb, 77% in Warm/Amb, 80% in Cold/Hi-P and 93% in the Warm/Hi-P treatment.

Larval body elemental content

Body phosphorus content was negatively related to *P. gentilis* mass (Fig. 3a; $P = 0.005$). One data point in the Cold/Amb treatment had a high influence on the regression parameters for log body mass against body phosphorus content for that treatment [Cook's distance (D_i) = 1.36]. Since this value was much larger than any other (next largest $D_i = 0.14$), it was removed from the analysis (as recommended by Quinn & Keough, 2002). The resulting ANCOVA indicated a significant interaction of diet with temperature in predicting larval body P content ($F_{1,48} = 21.8$, $P < 0.001$). Stoichiometric diet quality was also significant ($F_{1,48} = 60.9$, $P < 0.001$) but the interaction effect prevented independent interpretation of this effect. When corrected for body size, Hi-P treatments had higher body P content than Amb treatments (Fig. 3b). Across all treatments, average body C : N, C : P and N : P (molar) were 6.8, 132 and 19.5, respectively.

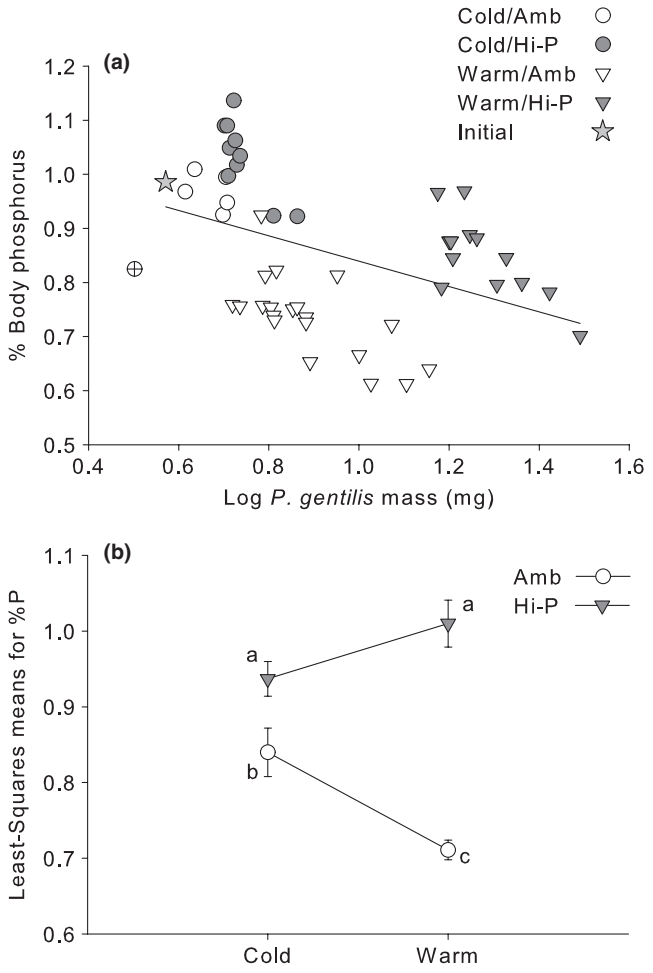

Fig. 3 (a) Relationship between body P content and log body mass in *Pycnopsyche gentilis* larvae in the four treatments. The cross-hatched circle was not included in the regression or ANCOVA analysis due to a high Cook's distance value. Fit statistics of the regression line are as follows: slope = −0.228, intercept = 1.067, $r^2 = 0.16$, $P = 0.005$. The star indicates the values for larvae at the start of the experiment. (b) Least-squares means of body P content for each treatment. The ANCOVA model indicated a significant interaction between temperature and diet. Points with different lower-case letters represent significant differences based on a Tukey's test at $\alpha = 0.05$. Error bars indicate ± 1 SE.

Discussion

Our stoichiometric diet quality manipulations differed only in the concentration of P added to the artificial streams used for litter incubation. This difference in P availability led to a predictable (*c.* fivefold) difference in the mean P content and C : P ratio of litter fed to larvae in the experiment. The N content of the litter treatments also differed, however, suggesting that P addition to the artificial streams had more general effects on litter quality. Although increases in the mass-specific N and P content of microbes colonising the litter are a possible mechanism, it seems more likely that the differences in N and P content of litter between the two treatments were driven simply by P limitation of microbial growth, resulting in varying levels of microbial biomass and

community structure among the experimental litter treatments (Cross *et al*., 2003; Güsewell & Gessner, 2009; Suberkropp *et al*., 2010). Our treatment effects on larval consumption, growth and elemental content cannot therefore be attributed to differences in P availability alone. Although the larval responses observed among treatments could have been due directly to the measured differences in N and/or P content of the litter, these elemental content differences should perhaps rather be used as a proxy for potentially broader changes in food quality (i.e. fungal species, fungal carbon, molecules such as fatty acids or other essential compounds; see Torres-Ruiz, Wehr & Perrone, 2007; Hobbie *et al*., 2012). Our study also suggests that unambiguous manipulation of detrital stoichiometry is challenging. The single-element cell content of algal cultures can often be manipulated relatively easily via short-term luxury uptake (e.g. Frost & Elser, 2002), but changing single-element cell concentrations in an equivalent way in detritus-associated heterotrophic microbes may not be feasible.

Consistent with our predictions, bulk litter consumption was approximately two times higher in the 10 °C treatments, a result probably attributed to increased metabolic rates of larvae at higher temperatures. Alternatively, microbes on leaf discs could have grown at different rates during each 7-day period of exposure to larval feeding, causing differences in consumption rate. The latter explanation seems less likely, however, given that we did not observe an interaction between diet quality and temperature in our consumption rate analysis. When diet quality has been defined by fungal species on leaf material (Arsuffi & Suberkropp, 1986) or defined as leaf species (Smock & MacGregor, 1988; Hutchens, Benfield & Webster, 1997), feeding rates of larval Trichoptera have been shown to be higher on higher-quality food. We predicted a similar increase in our study, but failed to find statistical support for any role of stoichiometric diet quality in affecting consumption rates. This is in contrast to current stoichiometric theory, which predicts that poor food quality (defined as high C:nutrient) results in increased feeding activity to compensate for low nutrient availability (Plath & Boersma, 2001; Fink & Von Elert, 2006; Suzuki-Ohno *et al*., 2012).

The fastest larval growth was observed in the Warm/Hi-P treatment (3.0% day^{-1}). The significant interaction between stoichiometric diet quality and temperature suggests that any limitation of larval growth was exacerbated at the higher temperature, a finding observed by Gresens (1997) and consistent with one of our predictions. Despite being fed *ad libitum* with litter that was of only moderately high bulk C : N and C : P ratio (Cross *et al*., 2003, 2005), growth of larvae in the Cold/Amb treatment was not statistically different from zero over the 49-day trial. However, the severe consequences of low temperature and lower diet quality were overridden by either higher diet quality (Cold/Hi-P) or higher temperatures (Warm/Amb), resulting in positive growth rates in those two treatments. This 'rescue effect' of either increased temperature or diet quality on growth was unlikely to be mediated by P consumption, which was much lower in the Warm/Amb treatment (in which growth rates were higher) than in the Cold/Hi-P treatment. It seems therefore that the dual effect of diet quality and temperature was to increase the balance of limiting factors other than P. For example, trends in N consumption among treatments largely followed the trend in growth rate across treatments. This could suggest possible N-limitation of *P. gentilis* growth or, rather, that N content of leaf litter was a more accurate proxy for growth limitation of *P. gentilis* by some other factor.

The actual mechanism for the 'rescue effect' (e.g. changes in assimilation efficiencies among treatments) cannot be isolated from our data. Given that consumption rate was clearly related to temperature (and not stoichiometric diet quality), it seems likely that higher temperature allowed higher rates of ingestion or assimilation of limiting factors in the Amb diet. These rates were presumably also increased by higher concentrations of limiting factors in the Hi-P diet, giving rise to a rescue effect in either treatment.

Pycnopsyche gentilis grows from the third to fifth instar during the cold months of January to March, when minimum water temperatures at the larval collection site can be as low as 0.7 °C. Although these periods of low temperature may be brief, our data suggest that in order for *P. gentilis* to grow during the winter, either water temperature must be higher than 5 °C or food quality must be high enough to support sufficient ingestion of growth-limiting materials. Since *P. gentilis* larvae grow during the winter, our data indicate that in natural streams, they are selecting leaf litter that is of relatively higher quality than that measured by bulk estimates (e.g. 0.3% P for bulk litter at Coweeta; Cross *et al*., 2003). These bulk estimates include various leaf species at various phases of microbial colonisation, whereas the leaf litter used in this study was taken from a single leaf species, known to be palatable to shredders, after a sufficient period of microbial colonisation (Graça *et al*., 2001).

Average growth rate in this study ranged from −0.2 to 3.0% day^{-1}. Field estimates of growth rate for *P. gentilis* have ranged from 1.5 to 3.3% day^{-1}, while laboratory-based estimates have ranged from 0.5 to 5.6% day^{-1}

for larvae fed microbially conditioned leaf litter (Mackay, 1972; Cummins *et al*., 1973; Hutchens *et al*., 1997; Chung & Suberkropp, 2009). While our Cold/Amb treatment falls outside the bounds of previous laboratory-based and field growth estimates, remaining growth rates are within the bounds of previous laboratory studies.

The positive response of consumer growth to enrichment of detrital resources that we observed is not a universal finding. Cross *et al*. (2005) observed positive effects of nutrient enrichment (N+P) on growth rates of detritivorous midges (Diptera: Chironomidae) but no growth response to the same nutrient enrichment by leaf-shredding *Tallaperla* spp. (Plecoptera: Peltoperlidae). Life history characteristics (voltinism) and feeding modes (shredder vs. collector) are factors that are likely to have led to these distinct responses. Similarly, differences in body elemental content and gross growth efficiency that vary across taxa can determine threshold elemental ratios and nutritional demand, leading to variable growth responses to resource stoichiometry (Frost & Elser, 2002; Frost *et al*., 2006).

As in other studies, we found a significant and negative relationship between body mass and % P body content of larvae (Frost & Elser, 2002; Cross *et al*., 2003; Hambäck *et al*., 2009). This relationship can be attributed to smaller individuals having higher mass-specific growth rates, with accompanying high levels of cellular phosphorus associated with rRNA (GRH; Elser *et al*., 1996, 2003). Beyond a general body-size effect on % P body content, the treatment effects observed were relatively complex. After standardising body P content across treatments, we observed a highly significant interaction between temperature and diet. The GRH has support both within species, across broad taxonomic groups (Vrede, Persson & Aronsen, 2002; Elser *et al*., 2003; Schade *et al*., 2003), and also from our data set. However, our prediction that the GRH would explain body P content across all treatments did not hold. Under warmer conditions, it was apparent that larvae increased growth and body P content when provided higher levels of dietary P, while under colder conditions, there appeared to be tighter regulation of body P content (i.e. smaller differences between the two diet quality treatments). This suggests important mediating properties of temperature on stoichiometric regulation.

Why might such differences in body P content have occurred? Relatively high P content in the Warm/Hi-P treatment may have been due in part to increases in rRNA due to increased growth (i.e. a mechanism conforming to the GRH). A trend counter to the GRH was observed in the Cold treatments, where unknown physiological processes associated with possible starvation in the Cold/Amb treatment may have been important. Consumption rates were low, and average growth rate was not significantly different from zero in the Cold/Amb treatment. Given the lack of growth in this treatment, it is likely that this treatment was under increased stress and this may have led to processing of resources in a different manner than other treatments. For instance, starving individuals may respire carbon, while excreting very little nutrients, leading to relatively high body nutrient content (as observed in the Cold/Amb treatment; e.g. see Hessen, 1990). Alternatively, storage of phosphorus as organic molecules (as seen in larvae of the tobacco hornworm moth *Manduca sexta*; Woods *et al*., 2002) is also a plausible explanation for the observed trends in P content of larvae in our study.

Survival was also lowest (63%) for the Cold/Amb treatment, but values as low as 40% have been reported for *Pycnopsyche* larvae fed white oak (*Quercus alba*) leaves in streams near to our larval collection site (Hutchens *et al*., 1997). We can also compare our survival rates with those found in cohorts of natural populations of *P. gentilis*. Survival in three streams at Coweeta ranged from 17 to 61% (mean 36%) during the equivalent stage of larval development (A.D. Huryn, unpubl. data; W.F. Cross, unpubl. data). While wild populations experience mortality from predation, these natural survival rates demonstrate that mortality in our study was not unusually high. The exact mechanisms and significance of the survival patterns that we observed are difficult to judge, but differences among our treatments suggest a strong role for diet quality, as observed in other studies of stream invertebrates (e.g. Sweeney & Vannote, 1984; Sweeney *et al*., 1986a,b).

Our results support previous studies that have documented direct positive effects of resource P availability on body P content for crustaceans (DeMott, Gulati & Siewertsen, 1998; Acharya, Kyle & Elser, 2004) and insects (Schade *et al*., 2003; Small & Pringle, 2010), including *P. gentilis* from Coweeta (Cross *et al*., 2003). The strength of homoeostatic regulation is known to vary across broad taxonomic groups (Sterner & Elser, 2002; Persson *et al*., 2010). In general, aquatic macroinvertebrates demonstrate a relatively high degree of homoeostasis (i.e. little variability in consumer nutrient content in relation to resource nutrient content) compared with terrestrial insects, but a meta-analysis has recently revealed that interspecific variation and environmental conditions play an important role in determining the degree of homoeostasis exhibited by organisms (Persson

et al., 2010). More experimental studies on single taxa may reveal further examples of stoichiometric plasticity, as well as the mechanisms driving such responses.

Temperature is understood to have a large influence on the metabolic rates of organisms (Gillooly *et al*., 2001). However, our results show that temperature-driven shifts in metabolic rate were not the sole factor determining consumption, growth and body P content of larvae among treatments. Rather the combination of resource availability and metabolic activity together determined the pattern of responses observed. By understanding the effects of temperature on physiological mechanisms, we can better understand how temperature is related to the chemical composition of organisms through its interaction with metabolism. In our study, resource availability had important mediating effects on how temperature affected larval physiology. Of these, perhaps most notable were as follows: (i) the 'rescue effect' of either higher temperature or higher food quality on larval growth and (ii) the decrease in body P content under conditions of high temperature and reduced P intake. While it is important to understand the conditions that result in such stoichiometric responses in consumers, it will also be necessary to document the consequences of these responses for broader community- and ecosystem-scale processes (Malzahn *et al*., 2007; Boersma *et al*., 2008; Small *et al*., 2011). Increased understanding may be particularly important for detritus-based food webs, through which the majority of primary production flows (Moore *et al*., 2004), particularly in the face of increases in temperature, CO_2 concentration and nutrient availability that are all components of future global change (Falkowski *et al*., 2000).

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