The effect of light and nutrients on algal food quality and their consequent effect on grazer growth in subtropical streams

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Abstract: Recent investigators have emphasized that food quality has greater importance than food quantity for herbivore somatic growth. Herbivore growth is thought to be regulated by light and nutrients through changes in food elemental ratios, but our knowledge of the mechanisms driving grazer growth in stream ecosystems is scarce. We manipulated light (through shading) and nutrients (fertilizer addition) in 3 headwater streams in southeastern Queensland, Australia, and measured the responses of natural grazer communities. The growth responses of *Austrophlebioides* and *Helicopsyche* to light and nutrients differed with their body size and periphyton food quality. Large larvae were more sensitive to increased light and nutrient availability than were small larvae. Light induced a significant negative effect on *Austrophlebioides* growth, and this impact increased with increasing nutrient addition. Nutrients had a pronounced positive effect on *Helicopsyche* growth regardless of light intensity. These responses reflected changes in food quality. Periphyton food quality in terms of C:N played a more decisive role in grazer growth than algal food quantity (as chlorophyll *a*). The growth of large larvae was significantly greater under low light intensity and nutrient-enriched conditions where high-quality food was more abundant than under high-light and low-nutrient conditions where periphyton food quality was low. Our results suggest that changes in riparian vegetation or nutrient inputs can significantly influence grazer growth through changes in periphyton C:N. We recommend that future investigators of foodweb responses to riparian canopy change or nutrient enrichment on stream ecosystems pay more attention to the effects of algal food quality rather than quantity.

Key words: food quality, C:N ratio, food quantity, grazers, stoichiometry, riparian canopy

Herbivores transfer energy from basal food sources to higher trophic levels (Allan and Castillo 2007). Their somatic growth and reproduction is limited by food quantity and quality (Cross et al. 2003). Measures of food quantity, e.g., primary productivity, biomass, and chlorophyll *a* (chl *a*), have been used widely to assess herbivore growth and biomass. However, food quantity alone is insufficient for describing the resource base of consumers in many situations (Stelzer and Lamberti 2002), and the importance of food quality for herbivores is now recognized (Stern and Elser 2002). Stoichiometric theory proposes that the elemental imbalance between consumers and food sources reflects the degree of nutrient constraint on consumers and, thus, indicates food quality (Stern and Elser 2002). Aquatic consumers often have lower C:nutrient ratios than their food. If food elemental ratios resemble those of consumers, food is more easily

assimilated because the expenditure of energy to maintain consumer elemental homeostasis is reduced. Therefore, food with low C:N and C:P ratios is considered to be of high quality for the consumer (Stern and Elser 2002, Lau et al. 2008).

In benthic habitats, invertebrate grazers often are exposed to suboptimal nutritional conditions (Liess and Hillebrand 2005, Liess et al. 2009). The nutritional quality of periphyton varies with environmental conditions (Kahlert et al. 2002, Guo et al. 2016b), so the stoichiometric imbalance between algae and grazers often limits grazer growth (Fink et al. 2006, Lau et al. 2009, Ohta et al. 2011). To deal with low-quality food, grazers may alter their behavior and life history (Elser et al. 2000, Fink and Von Elert 2006, Liess 2014, Mooney et al. 2016), which may consequently affect their population dynamics, the fitness of secondary consumers, and

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nutrient cycling in benthic ecosystems (Covich et al. 1999, McManamay et al. 2011, Liess 2014). Therefore, exploration of the factors affecting algal elemental ratios and their consequences for invertebrate grazer growth is needed to untangle some of the mechanisms that underlie ecosystem processes.

Light intensity and nutrient availability are considered the 2 most important factors determining algal elemental ratios (light : nutrient hypothesis [LNH]; Sterner et al. 1997). Algae obtain C by photosynthesis and take up N and P from their surroundings. Increased light intensity can reduce algal C : nutrient ratios under nutrient-limited conditions because it can increase algal C-fixation rates (Sterner et al. 1997). Herbivore growth is expected to be highest at intermediate light-to-nutrient ratios where high-quality food is abundant. In contrast, grazer growth is expected to be nutrient limited at high light-to-nutrient ratios and C limited at low light-to-nutrient ratios (Urabe and Sterner 1996, Sterner et al. 1997). This expectation has generally been supported for lakes (Urabe and Sterner 1996, Hessen et al. 2002, Hillebrand et al. 2004, Qin et al. 2007), but results are contradictory in stream ecosystems. In one experimental study in oligotrophic streams, snail growth and reproduction were maximized at intermediate light intensity (Ohta et al. 2011), whereas in a field study, snail growth was driven mainly by periphyton production (Hill et al. 2010). Further, low light intensity can reduce mayfly and tadpole growth (Fuller et al. 1986, Mallory and Richardson 2005) but can increase the growth of some snail grazers (Liess and Lange 2011). In addition, algal elemental composition often tracks dissolved nutrients in streams (Stelzer and Lamberti 2001, Liess et al. 2012). Thus, shifts in stream nutrient status may directly affect benthic algal nutrient composition, which in turn influences grazer growth and density (Liess et al. 2012, Baggett et al. 2013). In natural streams, algal elemental ratios and grazer growth often are affected simultaneously by light and nutrients. Differing results between experiments and field surveys suggest that grazer growth in streams also may be influenced by factors other than food quality or by complex interactions between food quality and these other factors.

Snail grazers were the main model organisms in stream LNH studies, but how other invertebrate grazers respond to different light-to-nutrient conditions is still poorly understood. Further, invertebrate grazers may respond differently to light and nutrients, depending on their body size. Large larvae within a species of stream invertebrates are more easily affected by environmental changes than are small larvae (Bourassa and Morin 1995, Davis et al. 2010). Thus, field surveys including many different grazer species and explicit data on body size are required to elucidate the importance of LNH in natural streams, and improve our understanding of the combined effect of light and nutrients on grazer growth.

Our main objective was to assess the combined effect of light and nutrients on algal elemental ratios and their con-

sequent effect on invertebrate grazer growth. In southeastern Queensland, Australia, light and nutrients are the 2 most important factors affecting in-stream algal production (Mosisch et al. 2001) and nutritional quality (Guo et al. 2015). We manipulated light and nutrients in 3 headwater streams containing natural grazer communities to test the following hypotheses: 1) grazer growth is higher at low light : nutrient ratios where algal elemental ratios decrease and lower at high light : nutrient ratios where algal elemental ratios increase; 2) grazer responses to different light : nutrient conditions vary with grazer species identity and body size because large larvae are more sensitive to changes in light and nutrient regimes than are small larvae of the same species.

METHODS

Study streams

Our study was conducted in the upper Mary River catchment in southeastern Queensland (lat 152°40'E, long 26°45'S) in 3 headwater streams (Table 1) without direct human disturbance on study reaches or upstream catchments. These streams typically are at base flow from July to October, and most rainfall occurs from December to March (Guo et al. 2016a). The major land use in this region is forestry, and most agriculture involves cattle grazing on the lower slopes and in the surrounding lowland areas (Bunn et al. 1999). N (rather than P) is the primary limiting nutrient for algal growth in this area (Mosisch et al. 2001, Udy et al. 2006). The experiment was carried out for 6 wk between September and November 2013. No major rainfall events occurred during this period, and streams remained at baseflow conditions throughout the experiment.

Experimental design

Two light levels (open and shade canopy) and 2 nutrient regimes (ambient and enriched) were applied in a 2 × 2 factorial design in each of the 3 streams. Periphyton, macroinvertebrates, and water-column nutrient samples were collected on the first (pretreatment: day 0) and last (posttreatment: day 42) days of the experiment. Treatment effects were measured as changes in periphyton C : N ratio, chl *a*, grazer head width or density from pre- to posttreatment periods.

Experimental treatments

The open-canopy (O) treatment represented high light : nutrient conditions, and the shade-canopy plus added nutrients (S+N) treatment represented low light : nutrient conditions. The shade-canopy (S) and the open-canopy plus added nutrients (O+N) treatments represented intermediate light : nutrient conditions. Treatments without nutrient addition (O and S) were always situated upstream from nutrient-addition treatments (O+N and S+N) and were separated from them by ≥15 m. In each stream, 4 riffles with open canopy cover were selected. Each treatment was situated in a riffle dominated by cobble substrate, and the sampling

Table 1. Mean ($\pm$ SE) field-measured environmental characteristics and ambient nutrient concentrations obtained from the 3 study streams in southeastern Queensland, Australia. TN = total N, TP = total P, $\text{NO}_x\text{-N}$ = $\text{NO}_2\text{-N}$ + $\text{NO}_3\text{-N}$, SRP = soluble reactive P. SE = 0.00 means SE < 0.005.

Streams	Latitude	Longitude	Velocity (m/s)	Depth (cm)	Width (m)	Temperature (°C)	TN (mg/L)	TP (mg/L)	$\text{NO}_x\text{-N}$ (mg/L)	$\text{NH}_4\text{-N}$ (mg/L)	SRP (mg/L)
Bridge	26.72450°S	152.85016°E	0.25	9.8	2.0	21.0	0.23 $\pm$ 0.03	0.09 $\pm$ 0.00	0.00	0.00	0.04 $\pm$ 0.00
Scrub	26.79879°S	152.73151°E	0.24	7.8	2.0	20.5	0.14 $\pm$ 0.05	0.06 $\pm$ 0.00	0.03 $\pm$ 0.00	0.01 $\pm$ 0.00	0.01 $\pm$ 0.00
Balgwolah	26.80264°S	152.70587°E	0.36	10.0	2.8	18.8	0.16 $\pm$ 0.07	0.07 $\pm$ 0.02	0.01 $\pm$ 0.00	0.02 $\pm$ 0.00	0.01 $\pm$ 0.00

area for each treatment ranged from 4.50 to 6.08 m². Light availability was measured by photographing the riparian canopy in mid-stream 50 to 100 cm above the water surface at each potential study riffle with a Nikon D70 camera (Nikon Corporation, Tokyo, Japan) and a fish-eye lens. Images were analyzed with Gap Light Analyzer (version 2; Simon Fraser University, Burnaby, British Columbia, and the Institute of Ecosystem Studies, Millbrook, New York; Frazer et al. 1999) to extract forest canopy and gap light transmission indices. Only riffles with riparian canopy cover $\leq 20\%$ were used for this study. Shade cloth with 90% shading effects was suspended from steel posts ~ 0.5 to 1.0 m from the water surface to obtain the shaded canopy.

Nutrient enrichment was accomplished by installing stockings (made of the same shade cloth) filled with fertilizer pellets (Osmocote[®] Exact Standard 3–4 months, slow diffusing; Scotts Australia, Bella Vista, Australia) upstream of the study reaches. About 500 g/m² fertilizer was added, which can release 0.45 g N and 0.11 g P m⁻² d⁻¹ for 56 d. This value was calculated according to the Redfield ratios (Redfield 1958) needed to support gross primary production of 3000 mg C m⁻² d⁻¹ in cobble streams in southeastern Queensland (Udy and Bunn 2001).

Sample collection

Invertebrate grazers Macroinvertebrates were collected from a 30- × 30-cm quadrat. All cobbles in the quadrat were picked, and macroinvertebrates clinging to the cobbles were washed into a white tray. Three quadrats were sampled in each treatment. Cased and free-living caddisflies were separated, mayflies were identified to family, and other invertebrates were sorted to order by eye. The main invertebrate grazers in this area were mayflies and caddisflies (McKenny 2005), and specimens in these orders were preserved in liquid N in the field and placed at -80°C in the laboratory until further analysis.

Periphyton The same cobbles were used to collect periphyton samples for elemental content and biomass analysis. In each experimental unit, 4 replicate periphyton samples for elemental analysis were scraped with brushes from cobbles into distilled water. The resulting algal slurry was collected and stored in a portable freezer in the field and at -20°C in the laboratory. In each experimental unit, 3 replicate samples were collected for chl *a* analysis by placing a circular ring (area: 13.2 cm²) on the surface of a cobble and sampling the periphyton within the ring. The resulting algal slurry was filtered onto 47-mm Whatman GF/F filters and covered with Al foil. Samples were immediately placed in liquid N and later stored at -80°C in the laboratory. All samples were processed within 1 wk of collection.

Physicochemical variables Water samples were collected to assess total N (TN), NO₃ + NO₂ (NO_x-N), NH₄-N, total P

(TP), and soluble reactive P (SRP). Samples were stored in a portable freezer in the field and placed at -20°C in the laboratory. Samples were processed within 2 wk. Temperature, conductivity, and pH were measured with a portable turbidity meter (model WP-88; TPS Pty Ltd, Brisbane, Australia) (Table 1). Current velocity was measured with a current velocity meter (model 2100; Swoffer Instruments, Seattle, Washington) (Table 1). Water depth and width were measured with a measuring tape (Table 1).

Sample processing

Invertebrate grazers Mayflies and caddisflies were freeze-dried and identified to genus with the aid of a dissecting microscope. All macroinvertebrates were classified into functional feeding groups (Cummins and Klug 1979). Grazers found in the study streams included the mayfly *Austrophlebioides* and the caddisflies *Helicopsyche*, *Agapetus*, and *Tasimi*. The most abundant grazers, *Austrophlebioides* (1110 individuals) and *Helicopsyche* (462 individuals), accounted for $>75\%$ of the grazer abundance and were present in all streams. The head-capsule width of all individuals of these 2 grazers was measured with the aid of an eyepiece micrometer, and their density in each treatment was recorded. The head width of *Austrophlebioides* ranged from 0.75 to 2.50 mm and for *Helicopsyche* 0.25 to 0.90 mm.

Periphyton Periphyton samples for elemental analysis were oven-dried at 60°C for 60 h until completely dry, and all dried samples were homogenized with a mortar and pestle. Seven to eight mg of each sample was weighed into a small Sn capsule. Periphyton C and N contents were measured with a continuous flow-isotope ratio mass spectrometer (IRMS) (Sercon Hydra 20-22, Evroda EA-GSL; Crewe, Cheshire, UK). Algal chl *a* extraction and spectrophotometric analysis followed Muhid et al. (2013). Filters were immersed in ice-cold 100% ethanol, sonicated twice for 30 s at 50/50 pulse, and the extract was placed at -20°C for 24 h. Absorbances of these extracts were measured at 750, 665, 664, 647, and 630 nm (Jeffrey and Humphrey 1975). The hydrochloric acid treatment was used to adjust chl *a* values for phaeopigments (Lorenzen 1967). Chl *a* concentration was expressed as $\mu\text{g}/\text{cm}^2$.

Physicochemical variables Dissolved nutrients (NO_x-N, NH₄-N, and SRP) were analyzed with a SmartChem 200 discrete chemical analyzer (Westco Scientific Instruments, Brookfield, Connecticut) (APHA 1995). Total nutrients (TN and TP) were digested with a simultaneous persulfate digestion method (Hosomi and Sudo 1986) before analysis on the same instrument (Table 1).

Data analysis

Linear mixed-effect models were used to detect significant changes from pre- to posttreatment periods within treat-

ments. The response variables were periphyton C:N ratio, chl *a*, grazer head width or density, with the period (pre- and post-treatment) as fixed factors and the 3 streams as random factors. The protocol for linear mixed model fit and validation followed Zuur et al. (2009). The restricted maximum likelihood estimation was used to fit models, and the likelihood ratio test was used to compare and select fitted models. First, 3 models were constructed: 1) a linear model based on generalized least squares (without random effect) to investigate the fixed-factor effects, 2) a mixed-effect random intercept model (with streams as random factors), and 3) a mixed-effect random intercept and slope model (considering initial conditions in each stream). The likelihood test ratio was then used to choose the best model. If assumptions of the model were not met, quadratic terms were added, or data were rank-transformed. The optimal model obtained was refitted and validated. The estimated changes of the response factors helped assess the direction and magnitude of the treatment effect.

Assignment of larvae to size classes Effects of light and nutrients on the head width and densities of 2 grazers were first assessed graphically. Increased head width without a concomitant increase in total density would imply that the treatment increased the average individual size of at least some size groups of grazers. Next, a body-size-specific analysis was conducted to assess the effects of light and nutrients on grazer size groups. Instead of assigning a single head width (i.e., an average or maximum body size) to a taxon, each individual was assigned to an approximate size class on the basis of its $\log_{10}(x)$ -transformed head width. A linear mixed-effect model was used to compare the differences in each size group in each treatment between the pre- and posttreatment periods. This calculation was repeated for the head widths within each size group.

This body-size analysis was conducted separately for 2 grazers. Large larvae of *Austrophlebioides* (≥ 2.0 mm) and *Helicopsyche* (≥ 0.65 mm) were grouped into a size category that provided a sufficient sample size for analysis. The results from the linear mixed models indicated that the treatment effects varied among head-width groups. Divergences in the *Austrophlebioides* response were detected at a size class of 1.7 mm and in *Helicopsyche* at 0.45 mm. Each grazer was categorized into 2 representative groups: *Austrophlebioides* small (individual head width: <1.7 mm) and large larvae (individual head width: ≥ 1.7 mm), and *Helicopsyche* small (individual head width: <0.45 mm) and large larvae (individual head width: ≥ 0.45 mm). Then linear mixed models were used to estimate the changes in size of large and small larvae in *Austrophlebioides* and *Helicopsyche* from the pre- to posttreatment periods. The smallest size groups with the fewest individuals relative to other size groups (*Austrophlebioides* 0.75–1.0 mm and *Helicopsyche* 0.25–0.35 mm) were collected in only 1 stream at the pre-treatment stage, so they were excluded from further analysis.

Analysis of treatment effects Linear regression models were used to explore the relationships between grazer somatic growth, periphyton C:N, and algal biomass. The % changes of periphyton C:N, chl *a*, and grazer head width from day 0 to day 42 were calculated to standardize for initial conditions in each stream. The % change of grazer head width was the response factor, and the % change of periphyton C:N or biomass was the predictor, respectively. Data were $\log_{10}(x)$ -transformed to approximate a normal distribution. An information-theoretic approach was used for model selection (Johnson and Omland 2004). A likelihood ratio test was used to assess each model relative to a null model. The top models were selected by ranking models using the small sample unbiased Akaike information criterion (AICc) with the cut-off rule $\Delta\text{AICc} \leq 2$ (Burnham and Anderson 2001). Model averaging was used to produce 1 final model, and “0 method” was used to estimate regression coefficients (Burnham and Anderson 2002). All analyses were done in the R environment (version 3.0.3; R Project for Statistical Computing, Vienna, Austria), using the extension package *lme4* for linear mixed-effect models (Bates et al. 2012), and the package *MuMIn* for model selection and model averaging (Bartoń 2009).

RESULTS

Periphyton

Periphyton C:N and chl *a* varied with light:nutrient ratio conditions (Table S1, Table 2). In the O treatment, periphyton C:N increased significantly, whereas periphyton C:N did not change significantly in the S+N treatment. In the S and O+N treatments, periphyton C:N slightly decreased but these changes were not significant. Algal biomass (as chl *a*) did not change significantly in response to any light:nutrient treatment.

Invertebrate grazers

Head-capsule width and density in response to light:nutrient conditions differed among grazer size groups and species (Figs 1A–F, 2A–F). Density of small *Austrophlebioides* increased in all treatments (Fig. 1C), and the density of small *Helicopsyche* decreased in the O treatment (Fig. 2C). Head-capsule width of small larvae of both species was unaffected by light:nutrient conditions (Figs 1F, 2F).

Head-capsule width of large *Austrophlebioides* (Fig. 1E) decreased in the O treatment, but their density did not change (Fig. 1B), indicating that somatic growth decreased. In the S+N treatment, the average head-capsule width of large larvae increased (Fig. 1E), but their overall density did not change, indicating an increase in somatic growth. In the S treatment, overall larval density (Fig. 1A), density of large larvae (Fig. 1B), and the average head-capsule width of large larvae increased (Fig. 1E), indicating that large larvae grew even larger, and more large larvae moved into the surveyed area from other areas, whereas in the O+N treatment,

Table 2. Mean ($\pm$ SE) periphyton C:N and chlorophyll *a* (chl *a*) in 4 treatments and the change from pretreatment (day 0) to posttreatment (day 42) were tested with linear mixed-effect models. The estimated changes in periphyton C:N ratios and chl *a* were calculated based on the $\log_{10}(x)$ -transformed data. Periphyton C:N and chl *a* were used as the response factors, with period (pre- and posttreatment) as fixed factor and streams as random factors. Data were $\log_{10}(x)$ -transformed for normal distribution approximation. Model fit and validation protocols followed Zuur et al. (2009). * indicates a significant difference from pre- to posttreatment period ($p < 0.05$)

Variable	Treatment	Pretreatment	Posttreatment	Change	<i>F</i>	<i>p</i>
C:N ratio	Open (O)	7.55 $\pm$ 0.62	8.22 $\pm$ 0.45	0.03 $\pm$ 0.01	5.94	0.02*
	Open + nutrients (O+N)	9.58 $\pm$ 2.51	7.96 $\pm$ 0.49	-0.02 $\pm$ 0.09	0.06	0.76
	Shade (S)	8.27 $\pm$ 1.54	7.75 $\pm$ 0.38	-0.01 $\pm$ 0.08	0.01	0.89
	Shade + nutrients (S+N)	8.72 $\pm$ 1.21	7.71 $\pm$ 0.29	-0.00 $\pm$ 0.06	0.01	0.89
Chl <i>a</i> ($\mu\text{g}/\text{cm}^2$)	Open (O)	0.89 $\pm$ 0.54	0.90 $\pm$ 0.44	0.16 $\pm$ 0.21	0.57	0.39
	Open + nutrients (O+N)	0.61 $\pm$ 0.40	0.42 $\pm$ 0.21	-0.01 $\pm$ 0.13	0.01	0.96
	Shade (S)	1.41 $\pm$ 1.33	0.41 $\pm$ 0.19	-0.21 $\pm$ 0.39	0.29	0.54
	Shade + nutrients (S+N)	0.41 $\pm$ 0.25	0.65 $\pm$ 0.41	0.21 $\pm$ 0.01	3.56	0.06

larval density and head-capsule width of large larvae did not change (Fig 1B, E).

The density and head-capsule width of large *Helicopsyche* (Fig. 2B, E) did not change in the O treatment, whereas in

the S+N treatment, their density did not change but head-capsule width increased, which suggested they grew bigger. In the S treatment, the density and head width of large larvae did not change, whereas in the O+N treatment, their den-

Figure 1. Mean ($\pm$ SE) density (A–C) and head width (D–F) of all (A, D), large (B, E), and small (C, F) *Austrophlebioides* larvae in the pre- and posttreatment periods (Pre and Post, respectively). O = high light : nutrient conditions (open canopy), O+N = intermediate light : nutrient conditions (open canopy and added nutrients), S = intermediate light : nutrient conditions (shade canopy), S+N = low light : nutrient conditions (shade canopy and added nutrients). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Ind = individuals.

Figure 2. Mean ($\pm$ SE) density (A–C) and head width (D–F) of all (A, D), large (B, E), and small (C, F) *Helicopsyche* larvae in the pre- and posttreatment periods (Pre and Post, respectively). O = high light : nutrient conditions (open canopy), O+N = intermediate light : nutrient conditions (open canopy and added nutrients), S = intermediate light : nutrient conditions (shade canopy), S+N = low light : nutrient conditions (shade canopy and added nutrients). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Ind = individuals.

sity and head-capsule width both increased, suggesting that large larvae grew even larger, and more large larvae moved into the surveyed area from other areas.

The growth patterns of only large larvae were used for the linear regression analysis because only large larvae were affected by the light : nutrient treatments. The change in periphyton C : N was selected as the best-fit explanatory

factor predicting the somatic growth of large larvae of both species (Table 3, Fig. 3A, B). Periphyton C : N, chl *a*, and the null model explained ~77, ~20, and ~3% of the variation in head width of *Austrophlebioides*, respectively. The periphyton C : N model ($\Delta\text{AICc} = 0.00$) was selected as the best model according to the cut-off rule $\Delta\text{AICc} \leq 2$. Somatic growth of large *Austrophlebioides* decreased with

Table 3. The small sample unbiased Akaike information criterion (AICc), ΔAICc , and Akaike weights of models in the candidate linear regression models calculated for grazer growth where each model provided a statistically better fit to the data than the null model. The percentage change of grazer head width from pre- to posttreatment (day 0–day 42) was the response variable, and that of periphyton C : N ratio or biomass was the predictor form. df = degrees of freedom, logLik = log likelihood. Bold indicates the top models selected by the cut-off rule $\Delta\text{AICc} \leq 2$.

Response variable	Predictor form	df	logLik	AICc	ΔAICc	Weight
<i>Austrophlebioides</i>	C : N ratio	3	33.31	-57.6	0.00	0.77
	Chl <i>a</i>	3	31.97	-54.9	2.69	0.20
	Null	2	28.11	-50.9	6.74	0.03
<i>Helicopsyche</i>	C : N ratio	3	21.65	-34.3	0.00	0.72
	Chl <i>a</i>	3	19.94	-30.9	3.42	0.14
	Null	2	17.65	-30.0	4.34	0.09

Figure 3. Relationships between % change in head-capsule width from pre- to posttreatment (day 0–day 42) of large larvae for *Austrophlebioides* (A) and *Helicopsyche* (B) and periphyton C:N. All data were $\log_{10}(x)$ -transformed. Regression coefficients were estimated by model averaging procedure.

increasing algal C:N ($R^2 = 0.55$, $p = 0.006$), and regression coefficients were estimated by model averaging (Fig. 3A). The periphyton C:N model ($\Delta\text{AICc} = 0.00$) also was selected as the best model of somatic growth of large *Helicopsyche*. Periphyton C:N, chl *a*, and the null model explained ~72, ~14, and ~9% of the variation in head width of *Helicopsyche*, respectively. Somatic growth of large *Helicopsyche* decreased with increasing periphyton C:N ($R^2 = 0.49$, $p = 0.01$; Fig. 3B).

DISCUSSION

Our study shows that light and nutrients affected stream grazer growth by regulating periphyton C:N in subtropical streams, thereby providing field evidence in support of the LNH. The key grazers in the study streams, *Austrophlebioides* and *Helicopsyche*, were used for the first time in a LNH study. Their responses to light and nutrients depended on their body size and periphyton C:N. Large larvae were more sensitive to changes in light and nutrients than were small larvae, and the large larvae of *Austrophlebioides* and

Helicopsyche differed in their responses to light and nutrients (supporting prediction 2). Consistent with the LNH, large larvae were significantly larger on day 42 than on day 0 in the S+N treatment (low light:nutrient conditions) and significantly smaller on day 0 than on day 42 in the O treatment (high light:nutrient conditions) (supporting prediction 1).

Differences between large and small larvae

Our study provides field evidence that grazer growth response to light and nutrients was stronger in large than in small larvae. The growth patterns of small larvae of both grazers were unaffected by light and nutrients, whereas those of large larvae responded to manipulated light and nutrients. This difference in growth response of differently sized larvae was similar between mobile (*Austrophlebioides*) and sedentary grazers (*Helicopsyche*). Larger invertebrates might be able to outcompete their smaller competitors for food or space (Bourassa and Morin 1995, Davis et al. 2010). When food is limited, competition among consumers can restrict growth and fecundity, and intraspecific competition is usually stronger than interspecific competition among herbivores (Gurevitch et al. 1992). The greater ability of large larvae to compete with small larvae for food may enable large larvae to enhance their growth when food quality is high. Our data indicate that the growth of large larvae may be more strongly coupled to changes in periphyton food quality than is the growth of small larvae. This conclusion is supported by the growth pattern of small larvae in our study. The head widths of small larvae of both grazer species did not differ between treatments after 42 d of experimental manipulation. In addition, dietary plasticity resulting from omnivory could potentially contribute to the size differences. Some detritivores consume mainly detritus during small larval stages, but switch to cannibalism and intraguild predation as large larvae (Wissinger et al. 1996). However, C stable-isotope analyses from study streams show strong correlations between large larvae and periphyton (Fig. S1). Thus, large larvae were more strongly affected than small larvae by changes in periphyton food quality induced by light and nutrients.

In previous manipulative studies exploring grazer growth in response to light and nutrients, investigators mostly used grazers in certain size groups as model organisms, such as *Gyraulus chinensis* with shell-diameter 3.0 to 3.2 mm (Ohta et al. 2011), *Potamopyrgus antipodarum* with 2 to 3 mm in length and mean dry mass of 2.1 mg (Liess and Lange 2011), and *Elimia clavaeformis* ranging from 20 to 150 mg (Hill et al. 2010). Growth rates are easier to observe and compare when using similar size classes in various treatments. Nevertheless, this approach may lead to neglect of some other size classes that might have important functional roles in streams and further alter aquatic ecosystem functions (Davis et al. 2010). In our study, large and small

larvae differed in their responses to manipulated light and nutrients. Large larvae are discussed below because only large larvae were significantly affected by light and nutrients.

Grazer response to different light and nutrient conditions

Periphyton C : N rather than biomass (as chl *a*) was selected as the best-fit explanatory variable for grazer growth. Increasing algal C : N predicted decreasing grazer growth, a result suggesting that periphyton food quality played a more important role in stream grazer growth than food quantity. High light : nutrient conditions (O) induced high periphyton C : N and a pronounced decrease in the head width of large larvae of *Austrophlebioides* compared to the other experimental treatments, which suggested that grazer growth was strongly constrained by food quality (periphyton C : N). This result is in agreement with the LNH, which postulates that herbivore growth rates are more likely to be limited by food quality in environments with high light : nutrient ratios (Sterner et al. 1997).

In contrast, low light : nutrient conditions (S+N) induced an increase in head-capsule width of both grazers relative to initial conditions (i.e., growth). Fertilizer addition caused periphyton C : N to decrease slightly but not significantly. This result could have been caused by increased grazing activity. Grazers selectively remove nutrient-rich patches by direct consumption (Butler et al. 1989, Cross et al. 2005), but also spend more time in nutrient-rich patches than would be expected under random movement (Hart 1981, Kohler 1984). On the other hand, periphyton food quantity would be enhanced by added nutrients (Mosisch et al. 2001). Results of a recent field study, in which the importance of food quantity over quality for grazer growth was emphasized, suggested that grazers were C limited (Hill et al. 2010). However, in our study, all reaches were initially under open canopy ($\leq 20\%$) and received enough sunlight to support algal growth. Thus, food availability was relatively high. Moreover, algal chl *a* did not change relative to initial conditions in any treatment, a result indicating that food quantity was not limited. When food quantity is high, stream grazers should have enough C for metabolism and somatic growth, so other essential nutrients, e.g., N in our study, would constrain their growth (Stelzer and Lamberti 2002). Our results suggest that periphyton food quality (C : N) was the primary regulator of somatic growth of the grazers in our study.

The responses of *Austrophlebioides* and *Helicopsyche* to light and nutrients differed between the 2 intermediate light : nutrient conditions (S and O+N). In the S treatment, size and density of *Austrophlebioides* increased significantly, whereas those of *Helicopsyche* did not change. The *Austrophlebioides* result is in accordance with results of a previous study where grazer growth increased in response to shading (Liess and Lange 2011). However, in their study, increased

growth was not connected to higher food quality, as in our study. Liess and Lange (2011) speculated that a reduced necessity to hide from predators or high ultraviolet (UV) radiation in shaded treatments might have led to increased grazing and, thus, growth rates. Even in our study, increased growth rates of *Austrophlebioides* in S treatments might have had a similar case. However, in our study, the densities of small larvae increased in all treatments (Fig. 1C), a result suggesting that the effect of UV radiation or predation was not a structuring factor in our study streams. In addition, *Helicopsyche* was probably protected from harmful radiation by their mineral cases and, thus, did not show any growth in response to changed light intensity in the S compared to O treatment. Therefore, we conclude that the growth of *Austrophlebioides* in the S treatment was regulated by increased periphyton food quality.

On the other hand, in the O+N treatment, the head-capsule width and density of *Helicopsyche* significantly increased, but those of *Austrophlebioides* did not change. Responses of *Austrophlebioides* and *Helicopsyche* were probably affected by their species-specific traits. *Helicopsyche* are sedentary grazers and, thus, are more strongly exposed to local changes in periphyton food quality and more able to track these changes. This conclusion is supported by previous findings that cased caddisflies are more effective periphyton grazers than mobile mayflies (Lamberti et al. 1987). The increase in density of large *Helicopsyche* (Fig. 2B) indicated increased periphyton food quality in the O+N treatment, given that periphyton food quantity was not limited in study streams. Thus, in the O+N treatment, grazer growth was mainly restricted by periphyton food quality.

Previous studies suggest that grazer nutrient limitation is widespread and important in streams. As a consequence, grazers selectively choose food sources with low C : nutrient ratios and transfer this dietary energy to higher trophic levels (Stelzer and Lamberti 2002, Sperfeld et al. 2012). The link between algal C : P and grazer growth has been established in running waters (Stelzer and Lamberti 2002). In our study, only C : N was examined because N was the primary limiting nutrient for algae in the study catchment (see Mosisch et al. 2001). Light- and nutrient-induced changes in periphyton C : N were directly reflected in the growth patterns of stream grazers, with potential effects on consumers at higher trophic levels. We recommend that in future studies on the effects of riparian-canopy change or nutrient enrichment on stream ecosystems, investigators should place more attention on the effects of food quality rather than quantity.

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