

Examining primary producer–consumer interactions in a Lake Superior tributary using ^{15}N -tracer, grazer-reduction, and nutrient-bioassay experiments

KAY M. REZANKA¹

Department of Biology, University of Minnesota Duluth, Duluth, Minnesota 55812 USA

ANNE E. HERSHEY²

*Department of Biology, University of North Carolina at Greensboro,
Greensboro, North Carolina 27402 USA*

Abstract. Primary producer–consumer interactions and N dynamics were studied in a 3rd-order reach of Amity Creek, a Lake Superior tributary in northeastern Minnesota (mean discharge = 0.08 m³/s). A nutrient-limitation-bioassay experiment showed that primary production was co-limited by N and P. To evaluate the importance of grazers in controlling epilithic algal biomass, a grazer-reduction experiment was conducted using ceramic tile epilithon substrates (ambient grazing and low grazing treatments). Grazers significantly reduced epilithic biomass as measured by chlorophyll *a*, demonstrating that grazers constrain algal primary producers in this system. ^{15}N -enriched NH_4Cl was added to the stream for 6 wk, as a stable isotope tracer of N flow through producer and grazer compartments. A comparison of $\delta^{15}\text{N}$ of tile epilithon, incubated for 4 wk during the ^{15}N addition, to that of bulk epilithon collected from rock scrapings, showed that the thinner epilithon associated with tiles was much more enriched in ^{15}N than the bulk material. We propose a model of layered epilithon, where the outer, presumably newer, material is more active in N cycling than the inner, presumably older material. This more active material was used more by grazers. The caddisfly *Glossosoma* and mayflies *Paraleptophlebia* and *Ephemerella* were notably enriched in ^{15}N relative to bulk epilithon, similar to tile epilithon, suggesting that they selectively grazed and/or selectively assimilated the subcomponent of the epilithon that was most active in N cycling in this stream. The mayfly *Stenonema* was isotopically more similar to bulk epilithon than the other primary consumers. Traditional functional-feeding group classifications of these consumers did not accurately describe their food resource assimilation, as illustrated by their ^{15}N signatures. Grazers may be even more important in N cycling and retention than previously believed because they assimilate much more N than would be expected based on their apparent diets.

Key words: epilithon, grazer, nitrogen, ^{15}N , stable isotope tracer, mayfly, caddisfly, functional-feeding groups, nutrient limitation, Lake Superior tributary.

Epilithic biofilm serves as a major energy source for stream food webs and plays a critical role in retention of nutrients. Knowledge of the development and fate of this material is fundamental to advancing our interpretations of stream ecosystem structure and function (Lamberti and Feminella 1996). Grazers very commonly control epilithic algal biomass (Lamberti and Resh 1983, Power and Matthews 1983, McAuliffe 1984, Hart 1985, Lamberti et al. 1987). Such heavily grazed biofilms are typically more productive than ungrazed or lightly grazed epilithic mats, although they support lower algal

biomass (Lamberti and Resh 1983, Lamberti et al. 1987, Rosemond et al. 1993). The availability of N and/or P also constrains accrual of epilithic algal biomass (Grimm and Fisher 1986, Lohman et al. 1991), which in turn constrains consumers (Hershey et al. 1988, Hart and Robinson 1990, Rosemond et al. 1993). In the process of controlling algal biomass, grazers sequester nutrients that have been immobilized by the biofilm, and also contribute to recycling of limiting nutrients to the water. However, the degree to which these latter processes are important is less well understood than is grazer control or nutrient limitation, and will vary depending on many factors including nutrient limitation and grazer control of biomass in a particular stream.

Food preferences and feeding behavior of

¹ Present address: Fond du Lac Tribal and Community College, Cloquet, Minnesota 55720 USA.

² To whom correspondence should be addressed.
E-mail: anne_hershey@uncg.edu

stream invertebrates is fairly well studied using observational approaches, gut content analyses, controlled feeding experiments, and morphological studies of mouthparts and other feeding apparatus (see Merritt and Cummins 1996, Thorp and Covich 2001, and references cited therein). However, such methods do not provide information on the degree to which epilithic or other foods are assimilated. Stable isotopes have been used recently, in addition to traditional techniques, to further explore trophic relationships, N flow patterns, and foodweb structure of streams (Hershey and Peterson 1996, Hall et al. 1998, Mulholland et al. 2000a, Tank et al. 2000a, b, Wollheim et al. 2001). Such studies often reveal unexpected foodweb relationships. For example, in 2 streams, the ubiquitous mayfly *Baetis* assimilated a food source that was more enriched in ^{15}N than epilithon (Wollheim et al. 1999, Mulholland et al. 2000a). Another ubiquitous mayfly, *Stenonema*, assimilated only a portion of the biofilm in a heavily shaded stream, but assimilated bulk biofilm in 2 streams with higher light intensities (Tank et al. 2000a, b, Mulholland et al. 2000a). These results clearly indicate that traditional functional-feeding group assignments do not always reveal nutritional relationships among consumers and food sources, and may mask the role of consumers in nutrient processing.

^{15}N -tracer experiments differentially enrich the isotopic content of autochthonous food sources relative to allochthonous food sources. As a result of this differential allocation of ^{15}N , these foods can be isotopically distinguished from each other and, thus, traced through consumer compartments. Consumers will also exhibit a trophic enrichment of ~ 3.4 parts per thousand (ppt) or *per mil*, which reflects fractionation of N during metabolism (VanderZanden and Rasmussen 2001). Ideally, the tracer enrichment is large relative to the trophic enrichment (Hershey and Peterson 1996), and it is easy to adjust for the trophic enrichment (Kline et al. 1990, Tank et al. 2000b). Consequently, a consumer that assimilates ^{15}N -enriched autochthonous resources over time will become very enriched in ^{15}N relative to consumers that assimilate allochthonous foods. Therefore, a ^{15}N -tracer experiment is an excellent method for defining N flow from producers to consumers in lotic systems.

We describe experiments conducted in a Lake

Superior tributary to 1) evaluate nutrient limitation of algal biomass, 2) determine the effect of invertebrate grazers on algal biomass accrual, and 3) trace autochthonous N through primary producer–consumer pathways. We did a nutrient-diffusing bioassay experiment to determine nutrient limitation. We did a grazer-reduction experiment, in which grazers were discouraged from accessing plots of periphyton, to evaluate the effect of grazers on periphyton biomass accrual. We hypothesized that grazers would negatively affect algal biomass accrual. We examined incorporation of N into the epilithic biofilm and traced this N through these consumer populations by introducing ^{15}N as $^{15}\text{NH}_4\text{Cl}$ into the stream for 6 wk. We examined development of a ^{15}N signature in the bulk epilithon as well as epilithon that accrued on tiles during the experiment. We hypothesized that tile epilithon would become more heavily labeled with ^{15}N because it would lack an older or metabolically less-active component compared to bulk epilithon. Last, we hypothesized that dominant primary consumers would vary in their ^{15}N signature, depending on the degree to which they assimilated the bulk epilithon.

Study Site

Amity Creek is a woodland stream located on the North Shore of Lake Superior near Duluth, Minnesota, USA. The study area (lat $46^{\circ}51'\text{N}$, long $92^{\circ}01'\text{W}$) is a 3rd-order, low-gradient reach with a substratum of cobbles (70%), coarse gravel (15%), and boulders (15%). A canopy of predominantly black willow (*Salix nigra*), black ash (*Fraxinus nigra*), white birch (*Betula papyrifera*), quaking aspen (*Populus tremuloides*), and balsam fir (*Abies balsamea*) partially shades the stream along the study reach. Mean discharge during the experiments was $0.08 \text{ m}^3/\text{s}$, and mean wetted width and depth were 4.2 m and 0.08 m, respectively.

Dissolved nutrient levels were relatively low in the summer and autumn. NH_4^+ -N concentrations ranged from 6 to $15 \mu\text{g}/\text{L}$, $\text{NO}_3^- + \text{NO}_2^-$ -N ranged from 6 to $18 \mu\text{g}/\text{L}$, and soluble reactive P (SRP) ranged from 4 to $6 \mu\text{g}/\text{L}$ (Table 1). Leaf fall began in mid-September. Mean temperature was 14.7°C during the ^{15}N experiment.

TABLE 1. Nutrient concentrations ($\mu\text{g/L}$, mean \pm SE) during the study period. SRP = soluble reactive P. – = no data.

Date	<i>n</i>	SRP	NH_4^+	NO_3^-
18 Aug 97	3	5.2 (0.5)	7.4 (1.4)	6.6 (1.7)
25 Aug 97	8	4.5 ^a (0.2)	10.5 (0.8)	9.3 (0.4)
7 Sep 97	2	4.6, 4.7 ^b	6.6, 10.8 ^b	16.9, 13.1 ^b
14 Sep 97	8	–	15.3 (3.9)	14.8 (0.8)
24 Sep 97	5	6.1 (4)	–	13.1 (1.6)
6 Oct 97	8	–	9.5 (3.1)	10.3 (0.9)
15 Oct 97	5	5.7 (0.4)	8.0 (0.5)	12.3 (1.9)
22 Oct 97	4	4.5 (0.1)	8.4 (1.1)	10.4 (4.6)
29 Oct 97	4	5.6 (0.6)	6.3 (1.2)	17.2 (3.1)

^a *n* = 5 for SRP on this date

^b Values for 2 stations

Methods

Water chemistry

Nutrient concentrations were determined from water samples collected weekly from August to October 1997. Water samples were collected in 250 mL acid-washed polyethylene bottles. Following filtration through Whatman GF/F glass fiber filters, samples were analyzed for NH_4^+ -N, $\text{NO}_3^- + \text{NO}_2^-$ -N, and SRP using standard methods (APHA 1989).

Nutrient-diffusing substrata

To ascertain nutrient limitation of algal biomass (as chlorophyll *a*), a nutrient-diffusing bioassay experiment was conducted for 4 wk beginning 22 August 1997. A medium containing 3% DIFCO Bacto-Agar, deionized water, and either N (0.5 M NaNO_3), P (0.05 M Na_2HPO_4), N+P (0.5 M $\text{NaNO}_3 + 0.05$ M Na_2HPO_4), or no nutrient amendment (control) was prepared by autoclaving at 121°C for 15 min (Allen and Hershey 1996). The medium was poured into plastic 40 mL bioassay vials, and vials were capped with porous silica disks (26 mm diameter) (Gibeau and Miller 1989). Seven replicates of each treatment were randomly distributed between 2 plastic test tube racks. The racks were secured to the stream bottom at the head of a riffle. After 4 wk, silica disks were removed from the plastic vials. Following extraction in 90% acetone at 4°C for 24 h, phaeopigment-corrected chlorophyll *a* concentration on the disks was determined using a Perkin-Elmer Lambda UV/VIS spectro-

photometer, and concentrations were converted to mg chlorophyll *a*/m².

Bioassay chlorophyll *a* results were analyzed following a 1-way ANOVA design. Tukey's test was done on the square-root-transformed chlorophyll *a* values to determine if the addition of N, P, or N+P significantly increased chlorophyll *a* content over the control, and if chlorophyll *a* differed significantly among the 3 nutrient treatments (GLM Procedure, SAS Institute, Inc., Cary, North Carolina). Assumptions of normality and constant variance were checked using residual and normal probability plots.

Invertebrate and epilithic algal biomass

On 8 October 1997, immediately following the ¹⁵N-tracer experiment (described below), samples were taken along the study reach to assess invertebrate and algal biomass. Two random samples from each of 4 sampling stations were collected, using a Surber sampler, to assess macroinvertebrate biomass. Sites were ~50 m apart along the study reach. Invertebrates were identified to genus. Bulk epilithic biomass was determined by scraping portions of rocks collected from the stream bottom using a nonmetallic brush and a 5 × 5 cm plastic slide mount as a template (*n* = 34). Tile epilithic biomass was determined by placing 11 unglazed ceramic tiles (15.2 × 15.2 cm) in the stream, removing biofilm with a nonmetallic brush after 6 wk, and filtering onto precombusted Whatman GF/C glass fiber filters. Both invertebrate and epilithon samples were dried at 60°C for 24 h, placed in a desiccator for 24 h, weighed, and ashed at 500°C for 2 h. Following desiccation for another 24 h, samples were reweighed to determine ash-free dry mass (AFDM, g/m²).

Grazer-reduction experiment

Chlorophyll *a* content of periphyton grown in low grazing and ambient grazing conditions were compared. Pairs of 15.2 × 15.2 cm unglazed ceramic tiles were glued to bricks. All surfaces except for the tops of the tiles in the low grazing substrates were painted with 2 coats of a modified epoxy antifouling paint (Interlux Fiberglass Bottomkote) and were later coated with petroleum jelly in the field. Petroleum jelly was reapplied to low grazing treatments when the jelly layer became visibly thinner as a result of

water scouring (generally on a weekly basis). These 2 barriers discouraged crawling macro-invertebrate grazers, which were dominated by the caddisfly *Glossosoma* sp., from accessing periphyton growing on the top surfaces of low grazing tiles. These invertebrates with high specific gravities would have to crawl through the paint and jelly barriers to access the periphyton on the tops of the tiles, unlike grazing mayfly larvae that could colonize the tiles from above by drifting in the water column. McAuliffe (1984) successfully used a similar petroleum jelly technique to exclude *Glossosoma* grazers from bricks. These deterrents were not applied to the tops of the tiles, so they did not negatively affect the growth of periphyton on that surface. Ambient grazing tiles, without paint or jelly barriers, allowed all grazers to access the periphyton growing on their surfaces.

On 30 June 1997, 11 pairs of ambient grazing and low grazing treatments were positioned side by side in both riffles and runs through the 0.46 km study reach. Each pair of tiles was examined almost every day during the 51 d experiment. *Glossosoma* or other large grazers found occasionally on low grazing tiles were removed by hand. These grazers were recorded on 3 dates. Number of grazers per tile on each of these 3 dates was compared using a paired *t*-test, where mean number on low grazer tiles was subtracted from mean number on ambient grazer tiles for each date (i.e., $n = 3$ dates).

On 19 August 1997, two 8.75 cm² samples of periphyton from each pair of tiles were collected using a nonmetallic bristled brush. The 2 samples were pooled. Care was taken to avoid rinsing the sample from the tile across any jelly-coated surface. With room lights dimmed, samples were filtered onto Whatman GF/C glass fiber filters, ground with a tissue grinder, and extracted in 90% acetone for 24 h at 4°C in the dark. Following 20 min centrifugation and acidification of the supernatant with 1 N HCl, phaeopigment-corrected chlorophyll *a* concentration was determined using a Perkin-Elmer Lambda UV/VIS spectrophotometer. Concentrations were converted to mg chlorophyll *a*/m².

The effect of grazers on chlorophyll *a* standing stocks was tested using a paired *t*-test to compare ambient grazing and low grazing tiles ($n = 11$ pairs), which were paired at each station. Data were natural log transformed prior to

analysis. The assumption of normality was checked using a normal probability plot.

¹⁵N-tracer experiment

¹⁵N-tracer addition.—A ¹⁵N-tracer addition was done to study movement of N through the dominant primary consumers in the food web. The ¹⁵N-addition site (the dripper) was selected for its mixing potential, potential for hiding the dripper bottle to prevent vandalism, and for the presence of multiple riffles located downstream. Eleven sampling stations at riffles were selected along the 0.46 km reach using an approximately geometric progression of distance downstream of the dripper. A riffle located upstream of the dripper served as a reference station for ¹⁵N values in foodweb components. Samples collected at this station were used to determine background ¹⁵N levels.

A 2.35 g ¹⁵NH₄Cl/L solution was prepared using 10% ¹⁵NH₄Cl and poured into a 4 L Mariotte bottle (Hershey and Peterson 1996). Tygon capillary tubing exiting the top of the bottle functioned as a siphon to withdraw the ¹⁵NH₄Cl solution from the bottle and deliver it to the stream at a relatively constant rate (~690 mL ¹⁵NH₄Cl/d, or 0.04 g ¹⁵N/d). At this rate, the ¹⁵N:¹⁴N ratio in the dissolved NH₄⁺ pool was increased by ~400 per mil at the addition site, but there was a negligible increase in stream water NH₄⁺ concentration (<0.1 μg N/L).

Sample collection and processing.—¹⁵N addition commenced on 25 August 1997 and ceased on 8 October 1997, 44 d later. Organic matter and consumer samples were collected weekly at each of the stations as part of the Lotic Intersite Nitrogen eXperiment (LINX) sampling protocol (Tank et al. 2000a, b, Mulholland et al. 2000a, b, Peterson et al. 2001) during the 6 wk experiment. Samples were collected from as many rocks as possible within a sampling station given the geometry of the stream at the respective sites, the desire to stay within ~1 m of the site's measured distance from the dripper, and the need to minimize disturbance to the site. Each of these samples was pooled to obtain an integrated estimate of each source material and consumer at each site. Replicate samples were not collected because doing so would have caused too much disturbance to the site (depleting the site for future sampling), and cost constraints would have precluded analyses of replicate

samples. Coarse particulate organic matter (CPOM) samples were a composite of weathered and unweathered leaf litter. Fine benthic organic matter (FBOM) was collected using a turkey baster to remove material from the benthic surface and within the uppermost layers of sediment. FBOM was filtered onto 25 mm precombusted Whatman GF/C glass fiber filters. Two or 3 randomly collected rocks were scrubbed with a nonmetallic bristled brush to obtain pooled samples of bulk epilithon. Unglazed ceramic tiles that had incubated in the stream for 2 to 5 wk (day 1 to day 35 of the experiment) were scrubbed to obtain new epilithon samples. Slurries of both bulk and new epilithon were filtered onto precombusted 25 mm Whatman GF/C glass fiber filters. Young, green tips of filamentous green alga thallae (*Cladophora* sp.) were collected. Macroinvertebrate consumers were collected using both kick net and hand-picking techniques at each station. Sampling concentrated on insect taxa that represented presumed grazer and collector-gatherer functional-feeding groups and that were abundant throughout the study reach. All insects collected were in their immature (aquatic) stages. Depending on the size of the organism, 5 to 15 individuals of each taxon were collected to compose a pooled, dried sample of ≥ 1 mg.

Animals were placed in scintillation vials filled with stream water before storage in a freezer several hours later. We expected insects to clear most or all of their gut contents during this time, thereby minimizing the influence of unassimilated material on the ¹⁵N signatures. The actual gut passage time for these consumers is unknown in Amity Creek. Gut passage time varies widely depending on taxon, temperature, and food quality, but is often <30 min. (McCullough et al. 1979, Gresens 2001). A previous study in Amity Creek showed that larval black flies cleared their guts in ≤ 30 min (Miller et al. 1998), and in warm water it can be much more rapid for some taxa (Fisher and Gray 1983). Although gut passage may be longer for the consumers in the present study than it was for black flies in Miller et al. (1998), it seems likely that most or all undigested material would have passed through the guts in a few hours.

Animals were later identified to genus. Samples were dried at 60°C for 24 h, macerated with a scissors, and analyzed for ¹⁵N at The Ecosystems Center, Marine Biological Laboratory,

Woods Hole, Massachusetts, using a Finnigan Delta S isotope ratio mass spectrometer. All ¹⁵N values were expressed as δ values (ppt) calculated using the following equation:

$$\delta^{15}\text{N} = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000 \quad [1]$$

where $R = {}^{15}\text{N}:{}^{14}\text{N}$ ratio, and the N isotope standard is air (Peterson and Fry 1987). Background-corrected $\delta^{15}\text{N}$ values were obtained by subtracting the $\delta^{15}\text{N}$ of upstream control samples from the $\delta^{15}\text{N}$ of the downstream samples. ¹⁵N is typically enriched in natural food webs, such as that in the upstream portion of the river, by an average of 3.4 per mil for each trophic level because of metabolic fractionation of food sources (VanderZanden and Rasmussen 2001). For the tracer study, the background (upstream) values for each consumer were subtracted so that the tracer enrichment would not be confounded with natural trophic enrichment. Failure to do so would have a relatively larger effect on lesser-enriched components, but a negligible effect on highly enriched components.

Data analysis.—Exponential decay models were fit to these background-corrected data from day 44 of the enrichment (except tile samples, see below) to illustrate the decay rate of ¹⁵N labeling with downstream distance:

$$\delta^{15}\text{N}_D = \delta^{15}\text{N}_0 e^{-kD} \quad [2]$$

where D = distance from dripper (m) and k = slope of regression line, or decay rate of $\delta^{15}\text{N}$, with distance downstream. For each foodweb component, the natural logarithms of background-corrected $\delta^{15}\text{N}$ values at each station were regressed against the corresponding distance downstream of the dripper. The expected longitudinal pattern in $\delta^{15}\text{N}$, assuming a constant rate of $\delta^{15}\text{N}$ decay (k) downstream, is given by the solution of equation 2.

Foodweb component $\delta^{15}\text{N}$ values from day 44 were analyzed using ANCOVA with the GLM Procedure in SAS. Distance from the ¹⁵N-addition site served as the covariate regressor. Analysis focused on day 44 because these samples were more likely to be at ¹⁵N equilibrium. Samples collected earlier provided insurance in the event that high-flow conditions disrupted the experiment prematurely, but ¹⁵N determinations were done on only a few of those samples. Components included CPOM, FBOM, bulk epilithon, filamentous green algae, 2-wk to 5-wk tiles, *Ephemereilla*, *Glossosoma*, *Paraleptophlebia*, *Steno-*

nema, and *Physa*. It was important that the distance relationships between component ^{15}N values be linear and independent (i.e., the slopes of the distance vs $\delta^{15}\text{N}$ regressions needed to be parallel) for the ANCOVA analysis. Data were log transformed to meet the assumption of linearity. Prior to performing the ANCOVA, the assumption of independence was tested using the GLM Procedure in SAS. A significant interaction between component and distance would indicate nonindependence. Any component that could not satisfy both criteria was dropped from the analysis. Following ANCOVA, a priori pairwise comparisons were conducted using the least-squares means (LSMEANS) option in SAS. For these comparisons, we were most interested in determining whether autochthonous sources were dissimilar and whether consumers conformed to their expected autochthonous food sources, particularly bulk epilithon and tile epilithon. Consumers obviously do not feed on tile epilithon in nature, but we hypothesized that tiles would become more enriched than bulk epilithon because we expected them to develop a thinner coating of newly acquired material. Thus, a ^{15}N signature resembling tile would indicate that consumers either selectively feed on or selectively assimilate newer or surficial material, whereas a ^{15}N signature resembling bulk epilithon would indicate no discrimination in feeding or assimilation.

Dietary expectations and gut analysis

Four insects and one snail were expected to derive some or most of their nutrition from grazing autochthonous foods, based on their classical functional-feeding group assignments (Edmunds 1996, Wiggins 1996, Thorp and Covich 2001). *Stenonema* sp. is classified primarily as a grazer and secondarily as a collector-gatherer. *Glossosoma* sp. is reported to function as a grazer. *Paraleptophlebia* sp. and the snail *Physa* are considered primarily collector-gatherers. However, we hypothesized that *Paraleptophlebia* and *Physa* would also function as grazers because they were found on rock surfaces. *Ephemerella* sp. is primarily classified as a collector-gatherer and secondarily as a grazer. Only *Ephemerella* was frequently observed among patches of filamentous green algae in the field.

The guts of several *Stenonema*, *Paraleptophlebia*, *Ephemerella*, and *Glossosoma* were removed, and

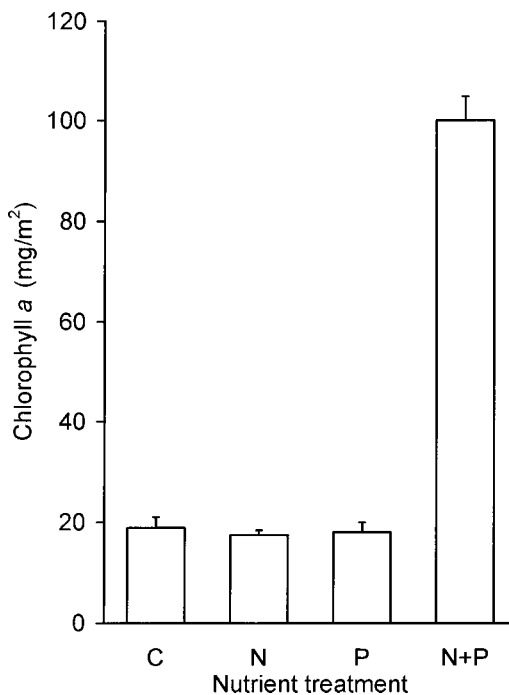


FIG. 1. Mean (+95% CI) chlorophyll *a* concentrations on nutrient-diffusing substrata. Means differed significantly (Tukey's test, $p = 0.0001$).

their contents examined under a compound microscope to determine the relative abundance of diatoms, filamentous green algae, or amorphous detritus. These potential food items were scored as either absent, present, or very abundant.

Results

Nutrient-diffusing substrata

Algal biomass accrual in Amity Creek in the late summer and early autumn was co-limited by N and P (Fig. 1). Neither N nor P treatments alone increased chlorophyll *a* response over the control at the $\alpha = 0.05$ level, but additions of both N and P increased chlorophyll *a* 5-fold over the control, N alone, or P alone treatments (Tukey's test, $p = 0.0001$).

Invertebrate and periphyton biomass

Mean biomass of tile epilithon samples after 6 wk of incubation was 0.04 ± 0.01 mg/cm² AFDM. Bulk epilithon biomass was 0.262 ± 0.019 mg/cm², more than 6-fold higher than

biomass on the tiles. Dominant grazer and collector-gatherer consumers collected at the end of the ¹⁵N-addition experiment included (in decreasing order of biomass) the snail *Physa*, the caddisfly *Glossosoma*, and the mayflies *Paraleptophlebia*, *Ephemerella*, and *Stenonema* (Table 2).

Grazer-reduction experiment

The antifouling paint and petroleum jelly barriers effectively reduced colonization by *Glossosoma* and other crawling grazers on low-grazing treatment tile surfaces. Grazer abundance on low grazer tiles was 0.68 ± 0.04 /tile (mean \pm SE) versus 3.86 ± 0.18 /tile on ambient grazer tiles ($t = 4.62$, $p = 0.022$, 1-tailed, $df = 2$). Of the total individuals observed on the tiles, 7 were *Physa* and the remainder were *Glossosoma*; other large grazers were not observed. ¹⁵N analyses (see below) demonstrated that *Physa* functioned as collectors rather than grazers. Comparison of ambient and low grazing tiles showed that grazing reduced the chlorophyll *a* content of periphyton by $\sim 42\%$ (Fig. 2). The paired *t*-test was highly significant ($t = 4.54$, $p = 0.0005$, 1-tailed, $df = 10$).

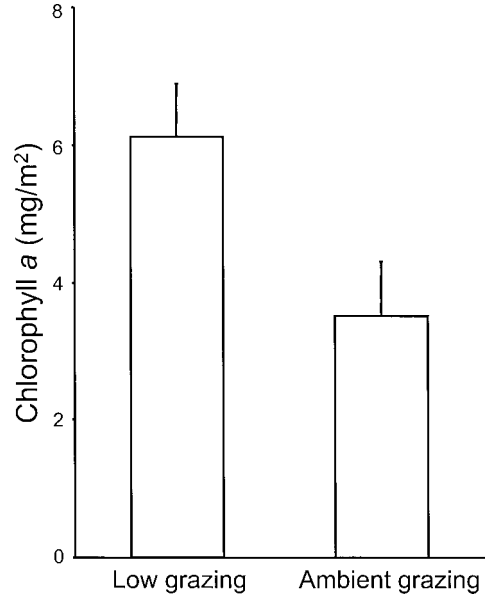


FIG. 2. Mean (+95% CI) chlorophyll *a* concentrations on low grazing and ambient grazing tiles from the grazer-reduction experiment ($n = 11$ pairs of tiles). Treatments were significantly different (paired *t*-test, $p < 0.0006$).

¹⁵N-tracer experiment

Replicate upstream analyses indicated little difference between replicate values. In addition, errors in values used for background correction would have little effect on the analyses because

variability background ¹⁵N values among consumers was also low (Table 3), and the magnitude of the enrichment above background was very high for primary consumer and autochthonous sources (Table 3). Allochthonous sources

TABLE 2. Density of common consumers in Amity Creek immediately after the ¹⁵N-tracer experiment. Biomass was also determined for taxa using significant autochthonous material. Taxa that were small and occurred rarely in samples were not identified or included. $n = 10$ for all samples. Functional-feeding group (FFG) is based on Merritt and Cummins (1996). – = no data.

Taxon	Density (no./m ²)		Biomass (g/m ²)		Presumed FFG
	Mean	SE	Mean	SE	
<i>Ephemerella</i>	67.7	45.7	0.016	0.007	Collector-gatherer/grazer
<i>Glossosoma</i>	76.3	35.4	0.128	0.070	Grazer
<i>Paraleptophlebia</i>	210.8	108.6	0.022	0.008	Collector-gatherer/shredder
<i>Physa</i>	247.3	92.7	0.135	0.060	Collector-gatherer
<i>Stenonema</i>	47.3	29.9	0.016	0.010	Grazer/collector-gatherer
<i>Prosimulium</i>	8.6	6.0	–	–	Collector-filterer
<i>Hydropsyche</i>	165.6	126.1	–	–	Collector-filterer
<i>Tipula</i>	7.5	4.3	–	–	Shredder
<i>Acroneuria</i>	32.3	12.6	–	–	Predator
<i>Atherix</i>	45.2	22.1	–	–	Predator
<i>Pteronarcys</i>	4.3	2.4	–	–	Shredder

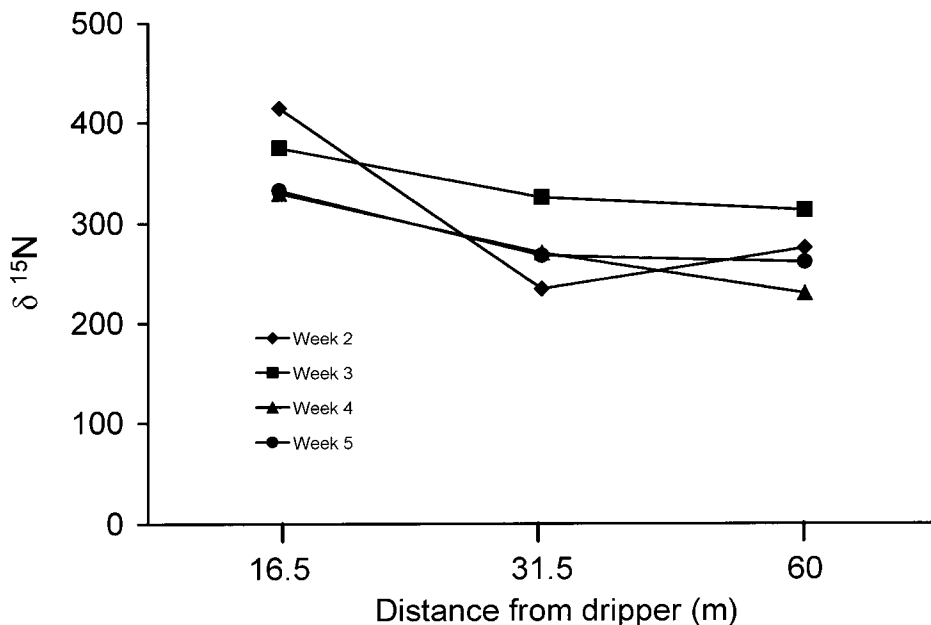


FIG. 3. $\delta^{15}\text{N}$ values on tile incubated 2, 3, 4, and 5 wk in Amity Creek during the ^{15}N -tracer enrichment study as a function of distance from the ^{15}N dripper.

were only slightly enriched above background (Table 3).

Enrichment of tile epilithon was similar for tiles incubated from 2 to 5 wk (Fig. 3). The ^{15}N -sample processing budget was focused on 4-wk

tiles (Table 3) as a compromise between epilithon biomass development between the 2-wk tiles and the bulk epilithon. $\delta^{15}\text{N}$ values of organic matter and consumer compartments were plotted against distance from the dripper (Fig.

TABLE 3. Day 44 $\delta^{15}\text{N}$ of foodweb components in Amity Creek, corrected for background $\delta^{15}\text{N}$ based on samples upstream of the enrichment site. Background values are either averages of duplicate determinations or, where indicated by an *, represent a single determination. CPOM = coarse particulate organic matter, FBOM = fine benthic organic matter, - = no data.

Downstream distance (m)	Filamentous green algae									
	Bulk epilithon	4-wk tile epilithon	CPOM	FBOM	<i>Ephemera</i>	<i>Glossosoma</i>	<i>Paraleptophlebia</i>	<i>Stenonema</i>	<i>Physa</i>	
9.7	240.25	635.60	245.50	16.10	29.20	424.35	651.80	241.45	187.60	34.70
16.5	144.85	297.10	329.60	21.90	32.30	333.55	427.80	195.55	167.10	26.40
31.5	233.15	161.30	270.30	32.50	38.70	273.85	431.90	228.95	250.30	36.00
60	164.55	30.40	229.70	24.80	39.80	298.15	305.90	240.95	214.40	17.40
74	143.35	181.00	-	14.90	34.40	291.65	306.90	214.70	182.90	25.00
142	103.55	182.30	186.10	17.30	29.20	176.85	72.00	139.45	112.50	9.50
184	61.55	83.80	-	5.10	23.40	121.15	99.50	111.85	-	11.00
202.7	46.65	78.40	118.90	5.40	18.30	115.15	38.50	104.75	89.60	118.00
282.1	43.75	35.50	75.40	2.40	6.90	69.15	52.00	-	53.30	39.30
333.6	18.95	11.10	-	1.65	10.20	40.85	31.80	32.35	39.60	16.10
463.4	7.95	7.00	-	0.80	3.30	-	12.80	14.35	13.90	10.25
Background values	6.15	3.40*	8.50*	0.70	5.00*	5.60	5.50*	5.30	5.70	6.30

4A–F). Autochthonous materials (bulk and 4-wk tile epilithon, filamentous green algae) were much more enriched in ¹⁵N than detrital compartments (CPOM and FBOM), illustrating that detrital compartments have considerably less demand for NH₄⁺ (Table 3). ¹⁵N signatures of *Ephemerella*, *Glossosoma*, *Paraleptophlebia*, and *Stenonema* generally tracked the autochthonous components, all declining exponentially with downstream distance from the dripper (Fig. 4A–E).

ANCOVA comparing food web components was highly significant ($p < 0.0001$), which partly reflected the differences among the food sources. However, we were most interested in comparing consumers with their potential foods as a means of tracing trophic transfer. FBOM and the snail *Physa* were not included in these statistical analyses because they did not meet the linearity and homogeneity assumptions, although they were included in the graphical presentations (Fig. 4). Visual inspection of these data suggested that *Physa* was functioning primarily as a collector of FBOM because it did not become heavily labeled with ¹⁵N (Fig. 4F). We did not consider *Physa* further because we were primarily interested in the grazer food web.

Four-week tile epilithon samples were more enriched in ¹⁵N than bulk (rock) epilithon samples (Fig. 4A, Table 4). $\delta^{15}\text{N}$ values of filamentous green algae were indistinguishable from bulk epilithon (Fig. 4A, Table 4).

Pairwise comparisons (Table 4) showed that $\delta^{15}\text{N}$ values for *Ephemerella* and *Glossosoma* were significantly enriched over that of bulk epilithon. However, there was no significant difference between consumer $\delta^{15}\text{N}$ values for both of these consumers and 4-wk tile epilithon, which was more enriched than the other sources (Fig. 4B, C). *Ephemerella* was consistently highly enriched along the transect (Fig. 4B). *Glossosoma* showed a somewhat uneven enrichment along the transect (Fig. 4C), although with transformation there was no significant $\delta^{15}\text{N} \times \text{distance}$ interaction. *Glossosoma* samples collected from the stations close to the dripper (9.7, 16.5, 31.5, and 60 m) were more enriched than tile epilithon, whereas samples from 3 lower stations (142, 202.7, and 282.1 m) had signatures more similar to those of bulk epilithon (Table 3).

$\delta^{15}\text{N}$ values for *Paraleptophlebia* and *Stenonema* were not significantly different from tile epilithon, but *Paraleptophlebia* was significantly en-

riched over bulk epilithon (Table 4). For *Stenonema*, this comparison was not significant, although marginal ($p < 0.1$, Table 4).

Gut analyses

Gut-content examination of *Ephemerella* revealed fragments of filamentous green algae, which was consistent with the observation that they were sometimes found associated with filamentous green algae (Table 5). *Ephemerella* guts also contained diatoms and amorphous detritus. *Stenonema*, *Paraleptophlebia*, and *Glossosoma* guts contained predominantly amorphous detritus and diatoms, but no filamentous green algae.

Discussion

Nutrient-diffusing substrata

Algal biomass accrual in Amity Creek was co-limited by N and P in the late summer and early autumn. Nutrient-limitation bioassay experiments conducted in other North Shore streams have demonstrated that nutrient limitation is temporally and spatially dynamic (Allen and Hershey 1996, Wold and Hershey 1999), but that no pattern is common to all North Shore streams. Wold and Hershey (1999) found that co-limitation was frequently observed in the early and mid-summer months in other North Shore streams, and proposed that co-limitation in these streams was likely a result of low concentrations of both inorganic N and P rather than a result of a shift from N to P limitation or vice versa. In either case, producer demand for N was high in Amity Creek during the study period, a condition which led to enhanced N uptake and subsequent trophic transfer (Mulholland et al. 2000b, Dodds et al. 2002).

Grazer-reduction experiment

The grazing activities of *Glossosoma* and potentially also *Physa*, reduced chlorophyll *a* content of epilithon growing on tiles by 42%. Many lotic studies have demonstrated that a combination of bottom-up and top-down factors can simultaneously limit primary producers (Power 1990, McCormick and Stevenson 1989, Rosemond et al. 1993, Feminella and Hawkins 1995), and both top-down and bottom-up limitation were demonstrated in Amity Creek during our

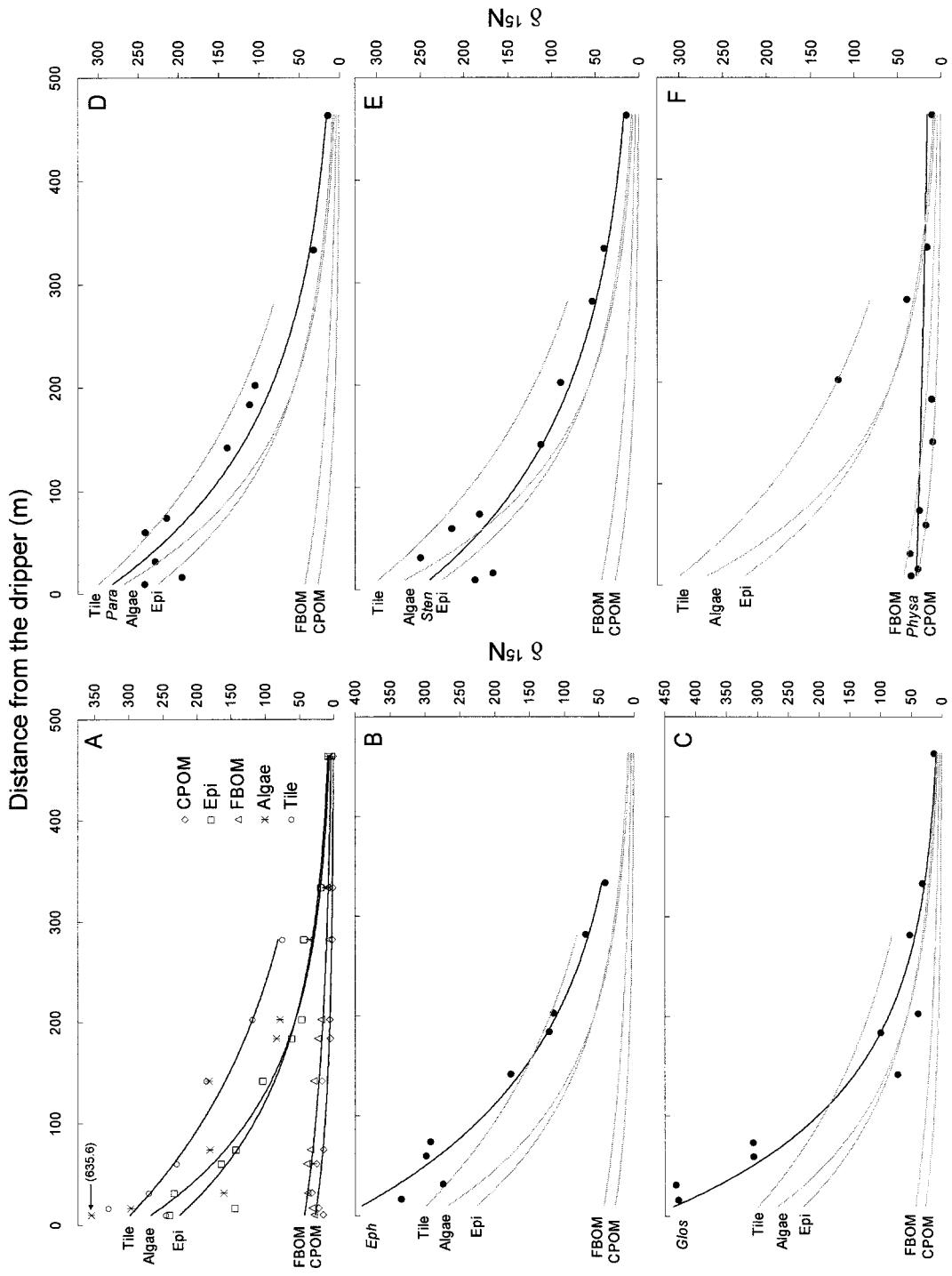


TABLE 4. Least-squares pairwise comparisons between foodweb components following ANCOVA, where distance from ¹⁵N addition was the covariate. See text for further explanation.

Contrast		<i>p</i> -value
Bulk epilithon	vs 4-wk tile epilithon	0.0048
Bulk epilithon	vs filamentous green algae	0.8229
<i>Ephemerella</i>	vs 4-wk tile epilithon	0.5541
<i>Ephemerella</i>	vs bulk epilithon	0.0002
<i>Glossosoma</i>	vs 4-wk tile epilithon	0.7760
<i>Glossosoma</i>	vs bulk epilithon	0.0038
<i>Paraleptophlebia</i>	vs 4-wk tile epilithon	0.3745
<i>Paraleptophlebia</i>	vs bulk epilithon	0.0296
<i>Stenonema</i>	vs 4-wk tile epilithon	0.1811
<i>Stenonema</i>	vs bulk epilithon	0.0934

study. Grazer control of epilithic biomass enhances trophic transfer of N. Thus, the greater the grazer control, the greater the extent to which grazers contribute to N retention in a stream reach.

We assumed that the density of swimming or drifting grazers such as mayflies was not reduced in this experiment relative to that on the ambient grazer tiles because these animals could have accessed the periphyton on the tops of the substrates by drifting in the water column rather than crawling (*sensu* Lamberti and Resh 1983). Both ambient and low grazer tiles were attached to bricks such that they were similarly elevated off the bottom and, thus, should have been equally accessible to drifters. Mayflies depress periphyton less than do larger invertebrates such as caddisflies or snails (Lamberti et al. 1987), although their impact still may be important (e.g., Kohler 1985, Marks et al. 2000). However, the experiment was a grazer reduction rather than an exclusion, and the 42% reduction caused by *Glossosoma* and potentially also *Physa* was a minimum estimate of the role of grazers

TABLE 5. Gut contents of the 4 Amity Creek insect consumers that became most heavily labeled with ¹⁵N. - = food source not observed, + = observed, +++ = observed in large quantities.

Taxon	Filamentous green algae		
	Diatoms	Amorphous detritus	
<i>Ephemerella</i>	+	+	+++
<i>Glossosoma</i>	+	-	+++
<i>Paraleptophlebia</i>	+	-	+++
<i>Stenonema</i>	+	-	+++

in Amity Creek. Thus, grazing was an important mechanism for N retention in the system.

¹⁵N-tracer experiment

Foodweb source materials.—Adding ¹⁵N to Amity Creek resulted in contrasting signatures of autochthonous and allochthonous food sources. Both CPOM and FBOM, although relatively unenriched, nonetheless acquired some ¹⁵N through the microbial colonizers of leaf litter. Caraco et al. (1998) have shown that heterotrophic microbes assimilate appreciable amounts of dissolved inorganic N, a process that likely resulted in enrichment of CPOM and FBOM in our study. However, we were primarily interested in the grazing pathway. Thus, we focused our attention on epilithon and filamentous green algae and the consumers that appeared to be feeding on them. These autochthonous sources and the primary consumers studied were much more enriched than the detrital-based food resources.

Tile epilithon was more enriched than bulk epilithon or filamentous green algae, demonstrating that N metabolism was more active in the tile community. However, bulk epilithon supported ~5-fold more AFDM biomass than

←

FIG. 4. $\delta^{15}\text{N}$ longitudinal patterns of (A) autochthonous and allochthonous sources; (B) *Ephemerella*; (C) *Glossosoma*; (D) *Paraleptophlebia*; (E) *Stenonema*; and (F) *Physa* on day 44 of the experiment (8 October 1997). Data points are background-corrected $\delta^{15}\text{N}$ values of samples collected at each station. Accompanying lines are fits of exponential decay models (equation 2) to data points. Data point indicated in parentheses on panel A represents a point that was off the scale. Abbreviations: Tile = 4-wk tile epilithon, Algae = filamentous green algae, Epi = bulk epilithon, *Eph* = *Ephemerella*, *Glos* = *Glossosoma*, *Para* = *Paraleptophlebia*, *Sten* = *Stenonema*, CPOM = coarse particulate organic matter, FBOM = fine benthic organic matter.

did tiles. Thus, bulk epilithon consisted of a thicker mat than present on tiles. It appears that the metabolic activity of bulk epilithon was uneven: the upper, enriched portion, which was readily exposed to ^{15}N in the water column, became more enriched, reflecting a greater metabolic activity than the lower, presumably older, portions beneath. When analyzed as bulk material, the overall ^{15}N signature reflected the average enrichment of these unevenly enriched layers. In contrast, the tile epilithon was all newly colonized during the experiment, and its ^{15}N signature showed that it was metabolically more active than bulk epilithon with respect to N metabolism. This result is consistent with the structural-functional model of epilithon presented by Lock et al. (1984). In this model, epilithon is described as a structurally, chemically, and metabolically heterogeneous matrix where components nearer the water interface have greater access to nutrients and other materials in the water column, and interior epilithic components may be limited by diffusion processes.

Consumer incorporation of ^{15}N .—Most consumers were enriched compared to bulk epilithon, consistent with feeding on and/or assimilating the newer, more-enriched material found on tiles. Although consumers varied somewhat in their use of this material (see below), the overall pattern of over-enrichment compared to bulk epilithon was consistent. This observation is not an artifact but an important result: consumers are very active in cycling N in the stream, more so than is apparent from the degree that they reduce algal biomass, as noted in the grazer-reduction experiment, or than is apparent from consideration of traditional functional-feeding groups. In our study, ^{15}N did not become uniformly incorporated into bulk epilithon, but was largely retained in the active, surficial compartment that was heavily utilized by grazers.

Interpretation of the ^{15}N signatures of consumers involves consideration of whether the consumers were at isotopic equilibrium with their food source(s). If a consumer naturally feeds on a single food source throughout its lifetime, its tissues will reflect that food source isotopically. Otherwise, the isotopic signature will reflect the relative assimilation of multiple food sources and, for 2 food sources, can be calculated using a simple mixing model (Kline et al. 1990, Schuldt and Hershey 1995). The ^{15}N isotopic signature of the consumer in a ^{15}N -tracer

experiment will change during the experiment as it gradually replaces unlabeled tissues with tissues enriched in ^{15}N . Previous whole-stream ^{15}N -enrichment experiments in Upper Ball Creek, North Carolina, and Walker Branch, Tennessee, have generally indicated that 6 wk is more than sufficient for insect primary consumers to reach isotopic equilibrium, and most primary consumers reached equilibrium by 3 to 4 wk (Tank et al. 2000a, b, Mulholland et al. 2000a). Water temperature was warmer during our study (14.7°C) than in either the Upper Ball Creek (7.2°C) or Walker Branch (12.4°C) studies (see Tank et al. 2000a, and above), so organisms with similar life histories should have reached equilibrium more rapidly in our stream. We focused our ^{15}N budget on samples taken after 6 wk of ^{15}N enrichment, which should have been enough time for the primary consumers studied to have achieved equilibrium with their food. However, we also discuss the consequences of nonequilibrium conditions for our conclusions.

A consumer:food ratio of unity has been used to indicate that the consumer is using the food source in question, assuming that the consumer is at equilibrium with its food (Tank et al. 2000b, Mulholland et al. 2000a). Our ANCOVA coupled with LSMEANS multiple comparisons was a way of examining this ratio statistically rather than graphically; if the consumer ^{15}N signature was the same as its food source (ratio of 1), we did not detect a difference between consumer and food source. However, a ratio different than unity could also indicate selective feeding or assimilation rather than nonequilibrium conditions.

Ephemerella's ^{15}N signature was more similar to that of tile epilithon than bulk epilithon, and the latter was indistinguishable from filamentous green algae. This greater enrichment may have occurred through selective assimilation of consumed material or through selective ingestion of more enriched surficial material. *Ephemerella* was frequently found amid tufts of filamentous green algae, and some specimens contained tufts of such algae in their gut contents. Thus, grazing of epiphytes was likely also an important mechanism for obtaining highly enriched material. We cannot resolve selective grazing from selective assimilation mechanisms with existing data, nor are they mutually exclusive.

Ephemerella species consume a variety of ma-

terials such as diatoms, detritus, moss, filamentous green algae, and animals, and the proportion of these foods in their diets shifts with seasonal changes in food quality and quantity (Shapas and Hilsenhoff 1976, Sweeney and Vannote 1981). Although the diet of *E. inermis* shifts seasonally in a Colorado stream, 80 to 90% of gut contents was detrital material (Gray and Ward 1979). In Oregon rivers, the diets of 4 *Ephemerella* species varied with habitat, size, and site, but detritus dominated the guts in all cases (Hawkins 1985). *Ephemerella infrequens* in an Indiana stream was an opportunistic collector-gatherer and selective ingestor, using cycles of maxillary brushing and mandibular biting to ingest diatoms, filamentous green algae, and detritus (McShaffrey 1988). *Ephemerella infrequens* consumed diatoms and *Cladophora* at times when its preferred food, detritus, was not as abundant, whereas *E. infrequens* in an Idaho stream were specialist detritivores (Mihuc and Minshall 1995). It is apparent that there are considerable differences in the feeding behaviors of *Ephemerella* mayflies across habitats and seasons, but that detrital material is often ingested, perhaps as a result of the relative ease of ingestion, or perhaps simply because of increased detrital availability in certain seasons. However, all of these studies used gut-content analyses to evaluate feeding behaviors and could only assess what was ingested, not what was assimilated. Our ¹⁵N-tracer results indicated that Amity Creek *Ephemerella* did not preferentially assimilate detrital foods but instead obtained virtually all of their N from highly enriched autochthonous resources in the late summer and early autumn. *Ephemerella* may be collector-gatherers in a functional-feeding sense, as other researchers have described, but in Amity Creek stream they functioned nutritionally as selective grazers.

The isotopic ratio of *Ephemerella* was statistically indistinguishable from the most-enriched food source in the samples (tile epilithon), so we assumed that *Ephemerella* was either selectively feeding or selectively assimilating the most metabolically active autochthonous material available, and that it was at isotopic equilibrium with its food. If it was not at equilibrium, then with a longer experimental period, *Ephemerella* likely would have become even more enriched in ¹⁵N. However, that finding would not change our conclusion that *Ephemerella* selectively fed and/or assimilated material that was more enriched

in ¹⁵N than either bulk epilithon or filamentous green algae.

Glossosoma caddisfly larvae are specialized to feed on the exposed surfaces of rocks, where they remove epilithon with their scraper-like, toothless mandibles and membrane-fringed labrum (Wiggins 1977). Despite these specializations, *Glossosoma*, and Trichoptera in general, are opportunistic feeders, even among members of the same species. Cummins (1973) documented that *G. nigrior* were trophic generalists, feeding on detritus in a Michigan stream but on periphyton in a Pennsylvania stream. Our transformed data showed no interaction between level of enrichment and distance from the dripper, but the raw data indicated that some upstream larvae were somewhat more enriched than tile epilithon relative to downstream larvae. It is possible that some *Glossosoma* consumed food enriched over new epilithon, whereas others consumed food resembling bulk epilithon.

The apparently (but not statistically significant) uneven enrichment might also be explained by a cohort effect for *Glossosoma*. Individuals composing these samples may have been members of different cohorts. We noted a developing cohort during the experiment, but did not record the relative composition of individuals from the younger cohort in each sample. Thus, downstream samples may have been predominantly older larvae, which fed differently or were not at isotopic equilibrium with their food, whereas a younger upstream cohort ingesting new epilithon was at equilibrium.

We do not have time-course data to sufficiently evaluate equilibrium status of *Glossosoma*. However, our statistical analysis of the entire transect revealed that, on average, *Glossosoma*, like *Ephemerella*, could not be distinguished isotopically from the most-enriched food source sampled. Thus, even if some or all individuals sampled were not at equilibrium, *Glossosoma* as a group used a food source that was more enriched than bulk epilithon. *Glossosoma* is consistently recognized as a grazer, and it was the only insect grazer found on grazer-reduction tiles, so *Glossosoma* either grazes selectively on surficial epilithon, or it selectively assimilates the highest-quality ingested food.

Paraleptophlebia is classified as either a collector-gatherer or a shredder (Merritt and Cummins 1996). Gut analyses revealed that they were ingesting diatoms and amorphous detri-

tus, similar to the other consumers studied, but isotopic data indicated that they assimilated highly enriched ^{15}N food, similar to that of tile epilithon. *Paraleptophlebia* have a relatively high rate of ingestion and the ability to extract highly nutritious material from low-quality foods (Mihuc and Minshall 1995). If their assimilation is efficient, then it is very likely that they were at isotopic equilibrium because they are smaller than *Glossosoma*.

Enrichment over tile epilithon was observed for several grazer samples at stations near the dripper. At least four possible mechanisms could explain this observation. First, there might have been analytical errors associated with the samples; however, a duplicate determination was made for one of the highly enriched samples, and it suggested there was no analytical error. Second, samples could have been contaminated during processing; however, the fact that only samples close to the dripper exhibited this over-enrichment suggests that it was not a handling problem. Third, mixing could have been incomplete at these upstream stations. We made every effort to maximize mixing potential in placement of the dripper, but this explanation remains a possibility. This problem is difficult to avoid in a small stream experiment because it is important to sample stations near the dripper to capture exponential decay of the ^{15}N signature. Consumers may be foraging in crevices or small eddies that entrain unmixed water. Fourth, grazers may have been assimilating material even more enriched than that represented by tile epilithon. Three of the 4 grazer taxa studied were consistently, on average, similarly enriched compared to tiles, so statistically half of them were enriched above tiles. Such enrichment would occur if some of them were assimilating material that was more enriched than the average tile sample. We cannot distinguish the 3rd and 4th mechanisms with our data, but they are not mutually exclusive, and it seems likely that both were operating in our study.

The ^{15}N signature of *Stenonema* was intermediate between tile and bulk epilithon and not significantly different from either, although it did appear to be slightly more enriched relative to bulk epilithon (Fig. 5E). In a previous ^{15}N experiment, *Stenonema* reached isotopic equilibrium in 4 wk (Mulholland et al. 2000a). However, we feel confident that *Stenonema* in our experiment was at equilibrium after 6 wk given the

warmer temperature in Amity Creek. Gut analyses revealed only diatoms and amorphous detritus, and we did not find *Stenonema* associated with filamentous green algae. We conclude that its diet consisted primarily of epilithon, which includes both diatoms and amorphous material.

Stenonema is a ubiquitous and often abundant mayfly and, thus, has been well studied. It appears to be opportunistic in its feeding, including microbial, algal, and detrital sources. Edwards and Meyer (1990), using a methyl- ^3H thymidine labeling technique in a 6th-order blackwater river, reported that a substantial portion (47% of daily C needs) of benthic organic matter was assimilated by *Stenonema* as microbial biomass. Hall et al. (1998) also determined, in a similar ^{15}N -tracer study in a North Carolina stream, that *Stenonema* was not more enriched than epilithon. However, Tank et al. (2000b) discovered that *Stenonema* in another stream within the same drainage basin was more highly labeled than epilithon, suggesting that in this stream, *Stenonema* was a more selective feeder or selective assimilator than in the stream studied by Hall et al. (1998). *Stenonema* in all of the systems studied is clearly ingesting some labeled material, but these tracer experiments demonstrated that the mayfly is functioning differently among streams. It is possible that different *Stenonema* species dominate in each of these systems, and the different feeding behaviors reflect these taxonomic differences. Alternatively, variations among these streams in the degree of grazing, nutrient levels, current flow, and light regimes, among other factors, could generate qualitatively different biofilm communities. Different resource opportunities may cause these taxonomically similar consumers to ingest and/or process the biofilm differently in each system. Tank et al. (2000b) concluded that *Stenonema* in a shaded stream assimilated only the high-quality portion of the epilithic biofilm, whereas *Stenonema* in an unshaded stream were not selective but instead assimilated uniformly high-quality bulk periphyton. Tank et al. (2000b) proposed that patterns of N cycling through the grazer pathway differed as a result of light availability.

In conclusion, our results have shown that grazers play an important role in controlling algal biomass in a stream that is co-limited by N and P. The ^{15}N -tracer experiment further showed that demand for N was high but also

provided insight into the heterogeneous nature of epilithon and the variability in consumer processing of autochthonous material. We compared ¹⁵N signatures of tile epilithon to those of bulk epilithon collected from rock scrapings, and showed that the thinner epilithon associated with tiles was much more enriched in ¹⁵N than the bulk material. We propose a model of layered epilithon, where the outer, newer material is more active in N-cycling than the inner, presumably older material. We also showed that the dominant consumers of allochthonous foods were assimilating material that resembled this highly enriched tile epilithon rather than the bulk material present on rocks. We did not distinguish whether assimilation of highly enriched material occurred through selective grazing on surficial material or through selective assimilation of the more enriched food that is presumably higher in quality. Further study on this topic would provide more insight into the role of consumers in processing of N through, and its retention in, stream food webs. Regardless of the mechanism, it is clear that consumers graze a very significant portion of the epilithon and assimilate the portion that is most enriched in N. Thus, consumers may be even more important in N cycling and retention than previously believed because they assimilate much more N than would be expected based on their apparent diets.

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