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Bivalve diets in a midwestern U.S. stream: A stable isotope enrichment study

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Abstract

This study examined a community of stream bivalves (unionids and fingernail clams) in a second-order woodland stream in southern Michigan using both the natural abundance of ^{15}N and a 6-week whole-stream ^{15}N enrichment experiment, as part of the Lotic Intersite Nitrogen eXperiment (LINX). Objectives included addressing what made up the diet of these bivalves and whether suspended algae consumed by bivalves were derived from pelagic phytoplankton imported from an upstream lake or attached algae sloughed from instream surfaces. Within the examination of bivalve diets, we considered whether suspension- and/or deposit-feeding modes were employed and whether bivalves selectively assimilated the algal and microbial portions of bulk material they ingested. All 12 unionid species reached a level of ^{15}N enrichment greater than the bulk suspended organic matter. *Sphaerium striatinum* (Sphaeriidae) were enriched to levels greater than all presumed food sources. Suspended algae were derived both from sloughed epilithon and pelagic phytoplankton originating from lentic waters upstream. A mixing model suggested that unionids were consuming 80% deposited and 20% suspended material. Alternatively, these bivalves were preferentially assimilating the highly enriched living component of suspended and/or benthic organic matter rather than assimilating the bulk material. These results advance our understanding of freshwater bivalve-feeding ecology, which is necessary if conservation efforts of these increasingly threatened organisms are to succeed.

Freshwater bivalves were originally ubiquitous in North American rivers and streams, but they have increasingly fallen victim to anthropogenic pressures such as overharvesting, waterway impoundment, pollution, and exotic species infestation (Ricciardi and Rasmussen 1999). Bivalve conservation efforts have included translocation, but mortality rates have averaged ~50% (Cope and Waller 1995). Captive rearing programs show highly variable mussel survival rates (Dunn and Layzer 1997). Incomplete understanding of the feeding ecology of freshwater bivalves impedes successful conservation efforts.

There are several ways to better understand the diet of organisms, including direct observation of feeding behavior, gut contents analysis, and examination of chemical constituents such as stable isotopes or nutrients within the tissues of organisms compared with their potential food sources. Direct observation of the feeding behavior of bivalves has primarily involved either measurement of filtering rates of artificial seston by adults (e.g., Kryger and Riisgård 1988; Silverman et al. 1997) or examination of filtering morphology (e.g., Ward et al. 1993). Gut content analyses of freshwater bivalves are rare and may be misleading because the method cannot distinguish ingested material that is not assimilated. Algae, for example, can survive passage through the digestive tract, and fecal material can have a high nitrogen content due to ingested material bypassing the stomach gland and moving directly to the intestinal tract (Hawkins et al. 1983; Miura and Yamashiro 1990). Also rare are energy budgets for freshwater bivalves. An energy budget for the fingernail clam *Sphaerium striatinum* was reported by Hornbach et al. (1984), who estimated that 35% of its energy was derived from suspension feeding and the rest possibly from deposit feeding.

Most stable isotope studies of food webs can be classed as either surveys of natural isotope abundance or experimental isotope enrichments. Natural abundances of stable carbon and nitrogen isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) have increasingly been used to examine the relative importance of various potential autotrophic sources in supporting food webs that include mollusks (Incze et al. 1982; Peterson et al. 1985; Thorp et al. 1998). Consumers tend to be enriched with the heavier stable isotope of nitrogen (^{15}N) relative to their diet (Minagawa and Wada 1984). Accounting for this trophic enrichment, unionids have been used to indicate the isotopic composition of the base of the food web in studies of lakes because they live long, have slow metabolism, and presumably utilize primary producers (phytoplankton) as their dominant food source. Unionids should thus integrate potential short-term fluctuations in primary producer isotope signatures over time (Cabana and Rasmussen 1996). This concept has been used to study the trophic position of fish in Canadian lakes and the effects of exotic species on food webs (Vander Zanden et al. 1997, 1999).

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Table 1. Hydraulic and geomorphological features of Eagle Creek, Michigan.

Slope*	0.3%	
Water velocity†	24 cm s^{-1}	
Water depth†	19 cm	
Channel width*	5 m	
Discharge†	202 L s^{-1}	
Substrate*	sand or FBOM	54%
	gravel	35%
	cobble	10%
Habitat*	riffle	90%
	run	10%

* Mean for study reach.
† Mean during enrichment experiment.
FBOM, fine benthic organic matter.

In an isotope enrichment experiment, the abundance of the normally rare isotope is increased in the ecosystem by the introduction of a nutrient enriched in the rare isotope, allowing a more detailed analysis of the food web. Tracer ^{15}N experiments have been conducted in the Kuparuk River, Alaska; Walker Branch, Tennessee; and Hugh White Creek, North Carolina, to elucidate food web relationships in streams (Peterson et al. 1997; Hall et al. 1998; Mulholland et al. 2000*a*,*b*). The present study and the latter two cited above were part of the Lotic Intersite Nitrogen eXperiment (LINX), a multisite examination of nitrogen cycling within stream ecosystems across North America. The study reported here is the first experimental enrichment of stable isotopes in an ecosystem containing a high abundance and diversity of freshwater bivalves.

We examined two questions of bivalve-feeding ecology: (1) What is the diet of stream bivalves? and (2) To what extent is suspended material available to filter feeders derived from benthic algae suspended within the stream or from a subsidy of pelagic phytoplankton derived from upstream? Within the context of bivalve diets, we considered whether bivalves employed deposit- and/or suspension-feeding modes and whether bivalves selectively assimilated the algal and microbial portions of bulk material ingested. To evaluate the potential food sources for bivalves in a woodland stream of the Midwestern U.S., we examined unionids, sphaeriids, and their potential food sources using both natural N isotopic abundances and a nitrogen isotope (^{15}N) enrichment experiment.

Methods

Study site—This study examined a 500-m reach of Eagle Creek, Fort Custer State Recreation Area, Michigan, near the city of Battle Creek. Originating as an outflow from an artificial impoundment 1,200 m upstream known as Eagle Lake, the stream flows through secondary forest and wetland upstream of the study reach, where there are several beaver dams. Below the study reach, the stream enters the Kalamazoo River. The study reach has little wetland and is largely shaded by deciduous forest. Hydraulic and geomorphological features are summarized in Table 1.

^{15}N tracer experiment—Experimental design and sampling methods that were standardized among LINX sites are cursorily described here; details are provided in Mulholland et al. (2000*a*,*b*) and Hamilton et al. (in press). Isotope measurements are expressed as delta values in units of parts per thousand.

$$\delta^{15}N = [(R_{sample}/R_{standard}) - 1] \times 1{,}000 \quad (1)$$

where $R = {}^{15}N:{}^{14}N$ ratio and $R_{standard}$ (N_2 in air) = 0.003663. Ammonium chloride enriched with ^{15}N was dripped into the stream for 42 d, beginning on 16 June 1998, with the intent of raising the $\delta^{15}N$ of ammonium to about +500 ‰ above natural background levels. Enrichment experiment data were corrected for background $\delta^{15}N$ by subtracting natural delta values measured either just before the experiment or during the experiment at an upstream reference site (*see* below).

Sampling—Sampling stations were established at seven locations downstream of the dripper as well as at a reference reach just above the dripper. Potential food sources for bivalves included epipsammon (EPS, or detritus and possibly algae mixed with sand) and suspended particulate organic matter (SPOM). EPS was collected using a turkey baster to suck organic matter from the surface of sand deposits, and EPS samples likely contained a substantial portion of mobile fine benthic organic matter (FBOM). SPOM was sampled by filtering water pumped from the middle of the water column. Samples of EPS and SPOM were collected on Whatman GF/F filters (nominal pore size = 0.7 μm). Additional ecosystem compartments that served as primary food sources for other organisms in the stream included coarse particulate organic matter (CPOM, collected as leaf fragments), small pieces of rotten wood, and epilithon (EPI, scraped from rocks and collected on GF/F filters). Filamentous macroalgae and macrophytes were present at very low abundances and were thus unlikely to be significant to food webs. Composite samples were made from several locations within sampling stations. Samples for natural isotope abundance were obtained from the reference station. Samples were collected weekly beginning on 15 June 1998 at all or at selected sample stations, including food sources and various macroinvertebrates. Bivalves were sampled under a different schedule.

To monitor temporal patterns of ^{15}N enrichment in bivalves, we collected three individuals of *Pleurobema sintoxia* from a single station 396 m downstream from the dripper on the seventh day of the experiment (day 7), as well as on days 14, 28, 35, and 42. We also collected three individuals of *P. sintoxia* from the same station for temporal analysis of ^{15}N turnover rate the day after the dripper was turned off (postday 1), as well as on postdays 7, 14, and 28. We studied spatial patterns of unionid isotopic enrichment by collecting three *P. sintoxia* from each station on the day of maximum enrichment (day 42) and spatial patterns of *S. striatinum* enrichment by collecting several individuals from various stations on day 42. We collected unionids of other species from two adjacent stations on day 42 for interspecific comparisons. To test for seasonal variation in natural ^{15}N abundance, we collected bivalves from the reference station on day 42 for comparison with bivalves collected the day before the dripper was turned on (day −1). We evaluated natural

isotope abundance by comparing mussels collected from the reference station on day 42 to values for food sources collected at that station averaged over the course of the enrichment experiment. We froze unionids for storage and preserved *S. striatinum* in ethanol until analysis (Peterson et al. 1993).

We surveyed unionids visible on the stream bottom to estimate abundance and biomass. A more invasive subsurface survey would have disturbed the substrate, altering seston composition and invertebrate drift. The unionid population at the surface is also that portion most directly interacting with the water column and is therefore the most relevant to food web interactions discussed in this study.

Replicate samples of suspended chlorophyll *a* (Chl *a*) were collected on GF/F filters from several points along the stream on two dates for measurement by fluorometry (Welschmeyer 1994). Suspended algae were sampled on 20 August 1998 at four locations: the Eagle Lake outlet, the outflow of wetlands 400 m upstream of the study reach, 20 m downstream of the dripper, and 460 m downstream of the dripper. Samples were concentrated by sedimentation and preserved in Lugol's solution for enumeration. Algae were identified to species and ranked as either more prevalent in benthic habitats (scored as 1), more prevalent in pelagic habitats (scored as −1), or equally prevalent in both pelagic or benthic habitats (scored as 0). The rankings were used to calculate a weighted average index from −1 to 1 (pelagic to benthic, respectively; Zelinka and Marvan 1961). These rankings were made by R. J. Stevenson of Michigan State University.

Tissue analysis—We selected individual unionid mussels for δ^{15}N analysis and did not pool any unionid samples. Due to the long nitrogen turnover times in bivalves, we analyzed three ''tissues'' rather than whole-body samples (Hawkins 1985). The stomach gland was sampled to reflect short-term assimilation of nutrients because it is a major site of nutrient absorption (Pechenik 1985). Foot muscle was sampled to integrate long-term nutrient sources. Gut contents from the intestinal tract were sampled because they contain partially undigested material. We dissected tissues from frozen specimens and dried and pulverized the samples for isotopic analysis. Shells were used for species identification. The entirety of *S. striatinum* soft tissue was dried, and up to five individuals were pooled per sample station for isotopic analysis due to the limited amounts of soft tissue per individual. The ^{15}N turnover rates for bivalve tissues and food sources were estimated from the slope of the ln(δ^{15}N) over time after the dripper was turned off (i.e., from the depletion of tracer ^{15}N).

To estimate ash-free dry mass (AFDM) and % N, we dried and ground the soft tissues (gut contents removed) of 10 whole unionids of various species. We measured dry weight and % ash gravimetrically and % N using a Carlo Erba elemental analyzer. Isotopic analysis of bivalves was done by continuous-flow isotope-ratio mass spectrometry with a Carlo Erba Elemental analyzer connected to a Finnigan Delta Plus Spectrometer at the Center for Environmental Science and Technology, University of Notre Dame, Notre Dame, Indiana. The remaining Eagle Creek LINX δ^{15}N measurements were made at that facility or by means of similar equipment at the Ecosystems Center, Marine Biological Laboratory, Woods Hole, Massachusetts.

Table 2. Biomass and nitrogen content of consumers in Eagle Creek, Michigan.

	g AFDM m^{-2}	g N m^{-2}
Unionids	1.93	0.196
Fishes	0.83	0.074
Macroinvertebrate functional feeding groups:		
Shredders	0.11	0.010
Scrapers	0.07	0.007
Collectors	0.04	0.005
Filterers	0.02	0.002
Predators	0.08	0.010

AFDM, ash-free dry mass.

Results

Unionids dominated consumer biomass in Eagle Creek (Table 2). A mean abundance of 1.9 individuals m^{-2} were present at the surface. Although the collection effort was not designed to quantify species richness systematically, we found 12 unionid species: *Anodontoides ferussacianes, Actinonais ligamentina, Elliptio dilitata, Fusconaia flava, Lampsilis cardium, Lasmigona compressa, Lasmigona costata, P. sintoxia, Pyganodon grandis, Strophitus undulatus, Venustaconcha ellipsiformis,* and *Villosa iris.* Sphaeriids were serendipitously sampled, usually from underneath rocks, and thus the biomass of sphaeriids is not known.

FBOM was abundant in the study reach, with 164 g AFDM m^{-2} in the upper 1 cm of the stream bottom and 922 g AFDM m^{-2} in the upper 5 cm (Hamilton et al. in press). The mass ratio of organic carbon to Chl *a* in surficial FBOM was 841, and the C : N mass ratio was 14. The stream carried a mean SPOM concentration of 3.3 mg AFDM L^{-1} in the study reach; the C : Chl *a* ratio of this material was 571. The results of the longitudinal survey of suspended matter above and within the study reach showed that the Chl *a* concentrations increased from the lake outflow (1,200 m upstream) to the wetland outflow (immediately upstream) then decreased through the study reach (Table 3). A similar decrease in Chl *a* concentrations through the study reach was observed on 14 July 1998, falling from 2.85 (±0.19 SD) to 2.18 (±0.08 SD) μg L^{-1} between the dripper and the 461-m station. The species composition of algae in the suspended matter collected in the longitudinal survey showed that benthic algae predominated in all samples, with an increase in the relative abundance of benthic algae along the course of the stream (Table 3).

The unionid *F. flava* was discovered during processing of *P. sintoxia* specimens; these two species are extremely similar in external appearance. Although we believe the majority of bivalves we had originally classified as *P. sintoxia* were correctly identified, we cannot rule out the possibility that a

Table 3. Chl *a* and relative abundance of planktonic and benthic algae in Eagle Creek, Michigan, on 20 Aug 1998. The wetlands occur between the lake outflow and the dripper.

	1,200 m upstream (Eagle Lake outflow)	400 m upstream of dripper	20 m downstream of dripper	460 m downstream of dripper
Chl *a* (mean μg L^{-1})	2.15	5.72	3.15	2.14
(SD)	(0.02)	(0.87)	(0.34)	(0.20)
Cell density (cell L^{-1})				
Bacillariophyta	110	136	60	20
Chlorophyta	181	122	200	170
Chrysophyta	1	144	48	20
Cryptophyta	126	141	122	168
Cyanoprokaryota	10,601	3,862	2,796	4,393
Euglenophyta	0	9	3	0
Pyrrophyta	1	1	0	0
Total	10,731	4,157	2,973	4,594
Benthic–Pelagic index	-5.4×10^{-2}	-5.3×10^{-4}	-1.9×10^{-6}	-9.3×10^{-8}

few were actually *F. flava;* we thus describe this group hereafter as *P. sintoxia* + *F. flava.*

Natural isotope abundances—Natural δ^{15}N values for the unionid community and food resources, as measured just upstream of the enriched reach, are shown in Fig. 1. EPS and FBOM had identical mean values (3.6‰) and were enriched relative to SPOM (1.3‰). Unionid muscle tissue (4.8‰) was 1.6‰ enriched with respect to stomach gland tissue (3.3‰), 1.3‰ enriched with respect to EPS and FBOM, and 3.5‰ enriched relative to SPOM. *S. striatinum* was 3.1‰ enriched relative to SPOM.

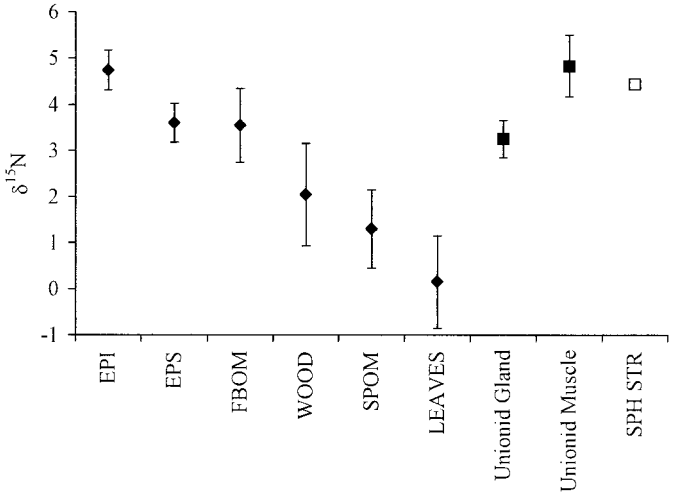

Fig. 1. Natural abundance of ^{15}N in food resources and bivalves of Eagle Creek, Michigan. SPH STR, *S. striatinum.* EPS and SPOM are presumed to be the potential food resources for bivalves. Data for food resources are means for eight collection dates at the reference station during the course of the enrichment experiment. Gland and muscle are isolated tissues from *P. sintoxia* + *F. flava.* The datum for *S. striatinum* is a composite of the whole soft tissue of several individuals. Error bars are ± 1 standard deviation.

Isotope enrichment experiment—The addition of ^{15}N-labeled NH_4^+ to the stream elevated the δ^{15}N of food resources and consumers. Unionid stomach gland and gut contents became measurably enriched with ^{15}N during the addition ($P < 0.0001$ and $P < 0.0001$, respectively) and then became depleted over time after the dripper was turned off ($P = 0.0007$ and $P = 0.0034$, respectively, Fig. 2). Estimated turnover rates for ^{15}N in the stomach gland and gut contents were similar at 78 and 85 d, respectively. Unionid stomach gland tissue was on average 0.8‰ (±0.7 SD) enriched relative to gut contents during the enrichment experiment. Muscle tissue from the foot responded very slowly to the addition of ^{15}N-enriched NH_4^+ ($P = 0.02$; Fig. 2). Although muscle tissue did become depleted after the dripper was turned off, the depletion curve was not significant ($P = 0.16$), making the estimated turnover rate of 357 d questionable. These turnover rates may be overestimated by the possible consumption of enriched FBOM after the dripper was turned

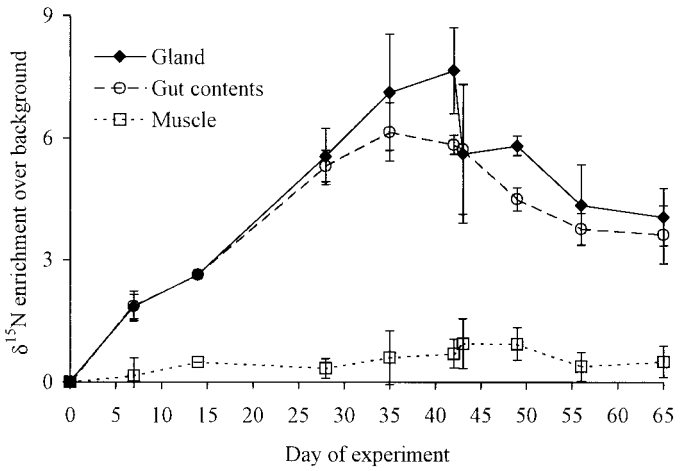

Fig. 2. Enrichment of unionid tissues over the course of the ^{15}N tracer experiment, with δ^{15}N values corrected for background δ^{15}N. Error bars are ± 1 standard deviation.

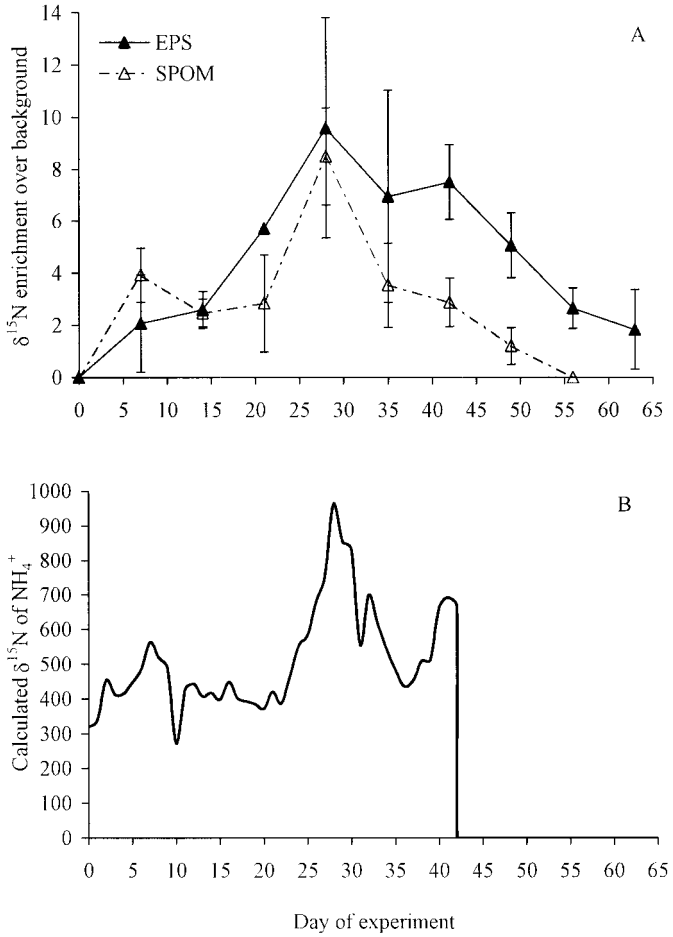

Fig. 3. (A) Enrichment of potential food resources of bivalves over the course of the ^{15}N tracer experiment, with $\delta^{15}N$ values corrected for background $\delta^{15}N$. The last SPOM datum was calculated by extrapolating the decay rate. Error bars are ± 1 standard deviation. (B) Calculated ^{15}N enrichment of dissolved NH_4^+ during the experiment, at the dripper. The enrichment decreased across the study reach as the $^{15}NH_4^+$ was assimilated or nitrified, and at the main bivalve sampling site, the $\delta^{15}N$ of NH_4^+ was 70–75% that of the dripper (Hamilton et al. in press).

off. FBOM, however, had an N turnover rate of 7 d (Hamilton et al. in press), and this rapid rate would have minimized the influence of enriched FBOM following termination of tracer addition. Based on the greater response to experimental ^{15}N enrichment of stomach gland compared with muscle, the stomach gland was used for seasonal, species, and mixing model analyses within the enrichment experiment. Muscle tissue was used for mixing model analysis of natural abundance data.

The potential food sources EPS and SPOM also became enriched during the ^{15}N addition (Fig. 3A). The temporal pattern of enrichment for EPS and SPOM followed the same general pattern as the calculated $\delta^{15}N$ enrichment of the NH_4^+in the stream water (Fig. 3B). The peak in calculated tracer $\delta^{15}N$ of NH_4^+ between days 25 and 30 was caused by a combination of lower discharge and lower ambient NH_4^+ concentrations, resulting in less dilution of the added ^{15}N (Fig. 3B; Hamilton et al. in press).

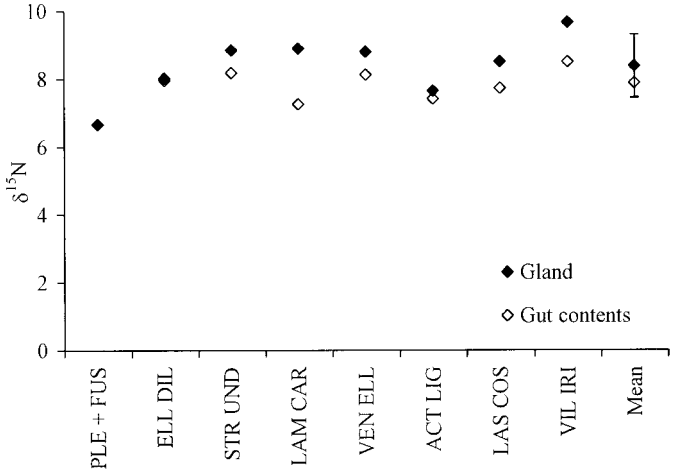

Fig. 4. Comparison of ^{15}N enrichment in unionid stomach glands and gut contents on day 42 ($\delta^{15}N$ not corrected for background levels of enrichment). PLE + FUS, *P. sintoxia* + *F. flava;* ELL DIL, *E. dilitata;* STR UND, *S. undulatus;* LAM CAR, *L. cardium;* VEN ELL, *V. ellipsiformis;* ACT LIG, *A. ligamentina;* LAS COS, *L. costata;* VIL IRI, *V. iris.*

It was not clear whether unionid tissues approached isotopic equilibrium during the ^{15}N enrichment period. Gut contents may have reached a plateau by the end of the enrichment experiment (Fig. 2). Enrichment of stomach gland tissue appeared still to be rising on day 42, although variability in $\delta^{15}N$ values of this tissue increased near the end of the experiment.

The stomach gland $\delta^{15}N$ values from various unionid species were similar between days −1 and 42 at the station above the dripper (mean for community = 3.4‰ ± 0.6 SD and 3.3‰ ± 0.4 SD, respectively). The $\delta^{15}N$ values of *S. striatinum* were also similar between days −1 and 42 at the station above the dripper (4.4 and 4.3‰, respectively). Thus, no seasonal isotopic change occurred in either the unionid community or *S. striatinum.* The various unionid species reached similar levels of ^{15}N enrichment (Fig. 4).

The $\delta^{15}N$ for EPS, SPOM, and unionid stomach glands varied over the length of the stream on day 42 (Fig. 5). Enrichment of EPS appeared to increase in the downstream part of the reach, as did the $\delta^{15}N$ of unionid stomach glands. *S. striatinum* varied widely between locations with an average enrichment of 13.9‰, which exceeds that of EPS and SPOM, the presumed potential food sources (Fig. 5).

Discussion

The simultaneous consideration of natural abundance of stable isotopes with the results of our isotope enrichment experiment yields several insights into the feeding ecology of stream bivalves that are not available from other kinds of studies, but it also raises some interesting new questions that challenge our traditional view of freshwater bivalves as primarily suspension feeders. In the following discussion, we address the evidence regarding dietary sources of nitrogen for the bivalves, the role of bivalves in the overall cycle of nitrogen in the stream, and the implications for conservation.

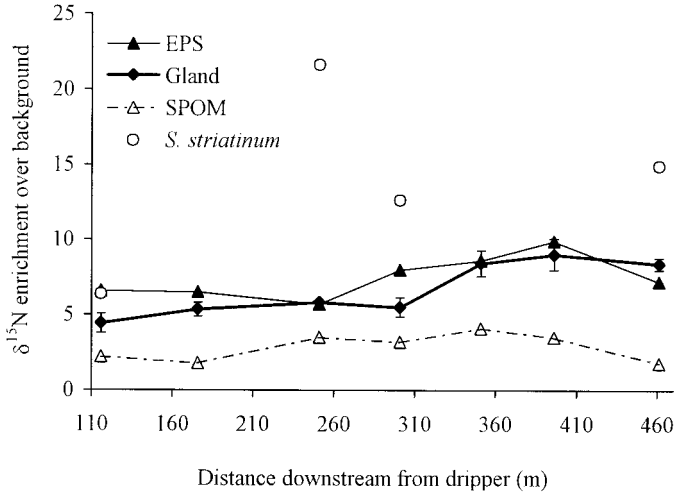

Fig. 5. Longitudinal patterns of ^{15}N enrichment for unionid stomach gland samples, *S. striatinum* whole-body samples, and potential food resources across the study reach on day 42 of the ^{15}N enrichment experiment (values corrected for background $\delta^{15}N$). Error bars are ± 1 standard deviation.

Patterns of ^{15}N enrichment in bivalves—The observed enrichment of ^{15}N in bivalve gut contents and tissues was due to the experiment rather than seasonal changes in natural isotope abundance. The community was remarkable in its similar response, suggesting that all unionid species were utilizing similar food resources. The small response of unionid muscle compared with stomach gland during the enrichment experiment indicated that muscle tissue N integrates food resources over a much longer time period than does the stomach gland.

Turnover times for muscle calculated in this study agree well with a 333-d whole-body N turnover time calculated for *Mytilus edulis* by Hawkins (1985). Studies utilizing unionids as a baseline for comparison of food webs between ecosystems would benefit from analysis of isolated muscle tissue rather than the whole body because of longer term integration of dietary sources within muscle, whereas studies needing shorter term dietary information should use the stomach gland.

Food resources reached isotopic equilibrium or nearly so during the experiment (Hamilton et al. in press), as illustrated by the similar patterns of changing $\delta^{15}N$ values between the tracer, SPOM, and EPS (Fig. 3). This equilibrium reflected the rapid turnover rates and direct utilization of suspended ammonium by algae and heterotrophic microbes within SPOM and EPS. Unionids did not clearly reach isotopic equilibrium during the course of the enrichment experiment. Two explanations of unionid food resource utilization are thus possible: (1) a diet of both EPS and SPOM (their presumed potential food resources), and/or (2) differential assimilation of food resource components within the SPOM rather than uniform assimilation of the bulk organic material.

Sources of suspended algae—The total amount of algae decreased along the study reach as indicated by Chl *a*, while the relative abundance of benthic algae increased. This pattern can be explained by settling and active removal of suspended pelagic algae by bivalves and macroinvertebrate filtering collectors, coupled with sloughing of benthic algae from the stream bottom. Lake outlet streams have long been recognized to support a high density of filter feeders including unionids (Brönmark and Malmqvist 1982; Richardson and MacKay 1991). While the study reach was not strictly a lake outlet because there are also wetlands below the lake, the importation of high-quality food from lentic environments might explain the observed bivalve abundance and diversity. Thus, within the suspension mode of feeding, the bivalves of Eagle Creek could be supported both by constant resuspension of material from deposited pools and an ecological subsidy of pelagic phytoplankton supplied by the upstream lake and wetland. The concept of an ecological subsidy could also be extended to organic detritus exported from the wetland and entering the stream (Polis et al. 1996).

Food resource mixing model—The relative abundance of multiple food sources in the diet of organisms can be quantified using an isotope mixing model.

$$\delta^{15}N_{\text{consumer}} = \sum f_i \delta^{15}N_i \quad (2)$$

where f_i and $\delta^{15}N_i$ are the fractional contributions and $\delta^{15}N$ values of each food resource i, and $\Sigma f_i = 1$. Use of a mixing model assumes both the organism and potential food resources to be at isotopic equilibrium (Gannes et al. 1997).

Bulk SPOM could not be the predominant food resource for bivalves in the stream because we observed a consistent enrichment of stomach gland tissue to levels greater than that of SPOM (Fig. 5). The simplest explanation of food resources for unionids then becomes a mixture of SPOM and EPS. In the enrichment experiment, each sampling station was treated as a different test of unionid food resource partitioning. The ^{15}N enrichment for stomach gland slightly exceeded that of EPS at the 251-m station (5.8 and 5.7‰, respectively) and at the 461-m station (8.4 and 7.2‰, respectively). Unionids were assumed to have consumed EPS exclusively at these stations for the purposes of mixing model analysis. The mixing model using stomach gland data from the enrichment experiment suggested unionids consumed 80% EPS and 20% SPOM on average (Table 4).

Application of the mixing model to natural muscle isotope abundance data required correction of consumer $\delta^{15}N$ values due to the fractionation that occurs in consumers relative to their food resources. In the enrichment experiment, trophic fractionation was accounted for by the subtraction of background (natural) $\delta^{15}N$ values from the measured $\delta^{15}N$ values. A 3.4‰ enrichment per trophic level is commonly used in food web studies, but this is an average value for a wide variety of animals (Minagawa and Wada 1984). Data on marine bivalves from the literature suggest that bivalves tend to be ~1.7‰ enriched with ^{15}N relative to suspended food resources (calculated from Minagawa and Wada 1984; Fry 1988). Application of the mixing model to natural abundance muscle $\delta^{15}N$ values, assuming a trophic enrichment of 1.7‰, indicated that unionids consumed 81% EPS and 19% SPOM. Using the same trophic correction of 1.7‰ with whole-body *S. striatinum* $\delta^{15}N$ values, the mixing model indicated that

Table 4. Food resource partitioning for bivalves in Eagle Creek, Michigan, based on observed ^{15}N enrichment on day 42 of the experiment. Stomach gland $\delta^{15}N$ was used for bivalves unless otherwise noted in text.

Location (m)	% EPS	% SPOM
116	50	50
176	77	23
251	100*	
301	48	25
351	96	4
396	86	14
461	100*	
Mean	80	20
Unionid muscle†	81	19
S. striatinum†	64	36

* $\delta^{15}N$ of tissue slightly exceeded $\delta^{15}N$ of EPS at this location.
† Natural abundance $\delta^{15}N$ with 1.7‰ correction for trophic level enrichment.
EPS, epipsammon; SPOM, suspended particulate organic matter.

S. striatinum consumed 64% EPS and 36% SPOM. Application of the mixing model to natural isotope abundance data for *S. striatinum* agreed well with Hornbach et al.'s (1984) energy budget for this species, which indicated greater reliance on deposit feeding than on suspension feeding.

Suspension versus deposit feeding—Suspension feeding is the removal of suspended particles including phytoplankton from the water column. Deposit feeding is the consumption of particles from the sediment. Descriptions of unionid behavior and the role of unionids in aquatic ecosystems emphasize the suspension mode of feeding (e.g., McMahon 1991; Strayer et al. 1994, 1999; Box and Mossa 1999).

Use of the deposit-feeding mode by freshwater bivalves has generally been described within specific contexts in which direct feeding upon sediment by adults is not usually considered to be typical. Examples include (1) illustrations of exceptional deposit-feeding behaviors (Way 1989 cited in McMahon 1991; *see* also Reid et al. 1992); (2) burial of sphaeriids in the substrate (e.g., Pennak 1989; McMahon 1991); and (3) pedal transport of sediment by juvenile unionids (Reid et al. 1992; Yeager et al. 1994). Resuspended sediment has been examined as a factor affecting suspension-feeding behavior and clearance rates, but this is not an example of deposit feeding (e.g., Kiørboe et al. 1980; Bricelj et al. 1984).

In the scientific literature, descriptions of adult unionids as both suspension and deposit feeders are uncommon (e.g., Singh et al. 1991), while the belief that adult unionids are exclusively suspension feeders is pervasive yet not supported by empirical evidence. Ecological studies often examine unionids within the context of suspension feeding only (Kramer 1979; Brönmark and Malmqvist 1982; McCall et al. 1995; Parker et al. 1998). If citations are given in reference to stating that unionids are suspension feeders, the citations are usually not of studies that quantify freshwater bivalve-feeding ecology (Brönmark and Malmqvist 1982; Yeager et al. 1994). To state that adult unionids are exclusively suspension feeders is therefore an inductive conclusion.

The mixing model used in this study suggested a predominance of the deposit mode of feeding in unionids, with good agreement between model results obtained with natural abundances and results obtained from the enrichment experiment. Indeed, application of a mixing model might be considered a standard procedure in a study of this kind. Use of the model, however, requires satisfaction of several conditions, including isotopic equilibrium and assimilation of bulk material. The ^{15}N enrichment of gut contents appeared to reach a plateau over the 42-d addition, but the stomach gland became more variable at the end of the experiment. These results obscure evaluation of whether unionids reached isotopic equilibrium. If unionids were near equilibrium by the end of the experiment, then the mixing model is still reliable. If not, the model yields a maximal estimate of the importance of the relatively ^{15}N-depleted SPOM because the $\delta^{15}N$ of unionid tissues would have continued to rise had the experiment been longer.

The duration of the present study was insufficient to evaluate feeding mode preference definitively. Future in situ isotope enrichment studies of bivalves will need longer periods to accommodate slow N turnover rates. Laboratory experiments, however, should prove more valuable in determining the extent to which adult freshwater bivalves employ the deposit mode of feeding (Gannes et al. 1997). Laboratory isotope enrichment experiments could entail use of smaller amounts of ^{15}N, thereby costing less, and different levels of isotopic enrichment between suspended and deposited organic matter could be produced in the laboratory.

Preferential assimilation of microbes and algae— Pennak (1989) describes the diet of freshwater bivalves as consisting chiefly of fine organic detritus dislodged from the substrate, with phytoplankton of minor importance. By contrast, McMahon (1991) lists phytoplankton, bacteria, and fine detritus as the chief components of the freshwater bivalve diet, with deposit feeding as a potential, and perhaps underestimated, supplement. This dichotomy of opinion should not be surprising given the common assertion that the ecology of freshwater bivalves is poorly understood (e.g., Bogan 1993; Neves 1993, 1997; Box and Mossa 1999).

In LINX experiments in other streams, macroinvertebrate consumers of EPI often became more highly labeled than their food resources (e.g., Tank et al. in press). This could only have occurred if the organisms were assimilating highly labeled algal components of EPI, rather than the bulk material that included algae, mucilage, and entrained detritus. Unionids could also have preferentially assimilated the microbial and algal components of the bulk material they ingested. Nichols and Garling (2000) found that unionids in a Michigan river and lake fed primarily on bacteria and concluded that the unionids were selectively assimilating bacteria from ingested detritus. The enrichment of *S. striatinum* to levels greater than that of bulk potential food sources supports the preferential assimilation explanation (Fig. 5). Due to small body size relative to unionids, *S. striatinum* likely had higher N turnover rates, resulting in greater enrichment during the course of the experiment.

If bivalves differentially assimilate living components of bulk fine organic material, we cannot use the $\delta^{15}N$ data of bulk samples to distinguish between suspension and deposit

feeding. This is because SPOM represented suspended material of multiple origins, including lentic phytoplankton exported to the stream, attached algae sloughed from surfaces, and FBOM in constant exchange between suspension and deposition. Lake phytoplankton, however, passed through the study reach very quickly (~32 min) due to high current velocities, and this short residence time may have prevented much uptake of labeled ammonium by plankton that entered the study reach already in suspension. Labeled microbial portions of SPOM were more likely derived from resuspended stream sediments and benthic algae. In light of the probability of preferential assimilation of microbial and algal components of food, the mixing model becomes questionable. The possibility of preferential assimilation does not, however, eliminate the potential for deposit feeding to exist in this community. The bivalves of Eagle Creek may to a substantial extent feed directly on sediments and likely derive their nutrition from the living microbial and algal components of fine organic material on the sediment surface.

Role of bivalves in stream N cycling—Due to their large size and abundance, unionids represented the largest pool of consumer N in the stream ecosystem. The present study shows that the slow N turnover rate of unionids, however, effectively prevented N bound in biomass from cycling in the short term. The importance of this pool of N may appear upon the death of a unionid by releasing large amounts of N to the sediment in a localized area. Predation upon bivalves by terrestrial mammals such as raccoons represents a loss of this N from the stream.

Implications for conservation—Both the mixed diet of EPS + SPOM and differential assimilation explanations of patterns seen in this study could improve our understanding of the relationship between stream bivalves and sediment. For example, while improvements in the success of freshwater bivalve translocation efforts have been made by refining handling techniques, limited understanding of unionid ecology still impedes optimal site selection (Dunn and Sietman 1997). Poor success of translocation efforts might be explained in part by differences between locations in the microbial and algal components of sediment and seston. A radical change in sediment characteristics can be encountered by unionids during a translocation project, and living components of sediment may differ in abundance or palatability in the new location. Such differences in the sediment would affect deposit feeding directly and suspension feeding indirectly by altering one source of suspended organic matter. Changes in the microbial community of sediment and seston may also explain why greater translocation success has been seen in projects that minimize the distance mussels are moved, especially to new locations in the same river (Valovirta 1998). Such alterations might not need to occur in the stream itself to have an impact if an upstream wetland or lake were providing an ecological subsidy of suspended organic material. Clearly, we should consider how alteration of the sediment affects the food sources of unionids within the context of the general ecology and conservation of these increasingly threatened organisms.

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