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Use of a stable carbon isotope addition to trace bacterial carbon through a stream food web

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Abstract. The use of bacterial carbon by stream invertebrates was assessed by dripping ^{13}C as sodium acetate into a headwater spring at Coweeta Hydrologic Laboratory for three weeks during August 1992. The addition raised the $\delta^{13}C$ value of dissolved organic carbon from approximately −26‰ to approximately 100‰. Coarse particulate organic matter, fine particulate organic matter (FPOM), and 14 taxa of animals were analyzed by mass spectrometer before and after the ^{13}C addition. Pre-addition sample $\delta^{13}C$ ranged from −36‰ to −22‰; post-addition samples ranged from −35‰ to 129‰. Predators contained less ^{13}C label than collectors, shredders, and scrapers. Shredders were not uniformly labeled, suggesting low use of bacterial carbon. *Stenonema* (Heptageniidae), a biofilm scraper, was the most highly labeled taxon (up to 128‰), even though biofilm $\delta^{13}C$ was −16‰. Chironomids and copepods were clearly labeled and had a higher $\delta^{13}C$ than the FPOM, suggesting preferential assimilation of bacterial carbon relative to FPOM. Although adults and larvae of *Optioservus* (Elmidae) are believed to be scrapers, the adults were more labeled than the larvae, indicating greater dependence on bacterial carbon. Gut analyses of *Optioservus* corroborated the stable isotope results: adult guts contained mostly detritus whereas larval guts contained a high proportion of diatoms. This technique is useful for determining the relative differences in bactivory by an assemblage of stream animals.

Key words: bacterial carbon, ^{13}C, tracer, food web, DOC, *Stenonema*, *Optioservus*, spring.

Bacterial carbon is used by many benthic invertebrates (Cummins 1974); however the relative importance of this carbon to individual species is unclear (Hildrew 1992). Dissolved organic carbon (DOC), a carbon source for bacterial production, is often the largest carbon input into streams (Fisher and Likens 1973, Peterson et al. 1986); thus bacterial production from DOC could represent an important source of carbon for invertebrate production. Laboratory experiments have shown that bacteria can be important in the diets of invertebrates. For example, bacterial production was important to blackfly and mayfly growth on snags in a blackwater river (Edwards and Meyer 1987, 1990, Benke et al. 1992), and harpacticoid copepods select and consume some of the larger bacterial cells (Perlmutter and Meyer 1991). Other stream detritivores do not appear to derive much of their carbon from bacteria. Findlay et al. (1986) showed that bacterial carbon was relatively unimportant in the diet of two shredders, *Peltoperla* and *Tipula*, because assimilated carbon was derived from leaf detritus and fungi, rather than bacterial biomass.

DOC that has been enriched with ^{13}C relative to ^{12}C can be added to streams as a useful technique for examining bacterial carbon use by invertebrates. Natural abundances of $^{13}C/^{12}C$ have been used in many different ecosystems to determine carbon pathways because consumers have a $^{13}C/^{12}C$ ratio that is similar to their resource (Rounick and Winterbourn 1986, Peterson and Fry 1987). If two carbon sources, (e.g., algae vs. leaf litter) have different $^{13}C/^{12}C$ ratios (referred to as $\delta^{13}C$) then one can determine the degree these two carbon sources contribute to consumers by comparing customer $\delta^{13}C$ to the $\delta^{13}C$ of the two resources. However, natural abundances of ^{13}C are only useful if there is a difference in the $\delta^{13}C$ values between the two carbon sources of interest. Bacterial carbon is derived from stream DOC and from externally digested particulate detritus, thus bacteria should have a $\delta^{13}C$ that is similar to these two food sources. Because of this lack of a $\delta^{13}C$ difference between bacteria and other carbon sources (e.g., detritus, algae) available for invertebrate consumption in forested headwater streams, an addition of ^{13}C acetate represents a marker for bacterial carbon that can be traced into consumers. Invertebrates with high $\delta^{13}C$ should have assimilated more bacteria than those with low $\delta^{13}C$. The ^{13}C acetate can be added as a tracer at such low concentrations that only bacteria will be able to assimilate the label (Newell 1984). Unlike a laboratory experiment, a system-level stable isotope addition enables

one to determine use of bacterial carbon in the field over a longer time scale than laboratory trials, with minimal intrusion by the investigator. My study attempted to determine relative differences in bacterial consumption by several taxa of stream invertebrates using a ^{13}C DOC addition to a stream to label bacteria and trace bacterial carbon into consumers.

Study Site

^{13}C DOC was added to Cold Spring, a headwater spring seep in Dryman Fork catchment at Coweeta Hydrologic Laboratory, North Carolina. Cold Spring is surrounded by a mixed oak-hickory forest and a rhododendron understory at an elevation of 1245 m. The study reach was the first 25 m of the seep after it emerges from the ground where it has a low gradient relative to downstream sections. Substrate at the seep is primarily cobbles and organic matter (leaves and woody debris) upon fine silt. Baseflow discharge during the release ranged from 0.2 to 0.5 L/s. Water depth was 3–5 cm and the seep was approximately 2 m wide.

Cold Spring is heterotrophic; litter inputs to Coweeta streams are 400–500 g AFDM m^{-2} y^{-1} and primary productivity is 1.3 g m^{-2} y^{-1} (Webster et al. 1983, 1990). Bacterial production is much greater than primary production in Coweeta streams. Bacterial biomass is 249 mg/m^2 and productivity is 9.6 g m^{-2} y^{-1} at Cold Spring in the top cm of the sediments (Crocker and Meyer 1987), which is approximately equal to the annual invertebrate production in another Coweeta stream (Lugthart and Wallace 1992).

Methods

^{13}C release

I continuously dripped ^{13}C 1-sodium acetate into the head of Cold Spring seep from 3 to 25 August 1992 using a Marriotte bottle. Stream discharge and flow rate of the stock solution were checked twice daily and the flow rate was adjusted for discharge. The flow rate of ^{13}C was set so that $\delta^{13}C$ of the stream water DOC was approximately 100‰ relative to approximately −25‰ for unenriched DOC. From a bacterial perspective an enrichment to 100‰ is an underestimation of the $\delta^{13}C$ of available DOC because the enriched carbon is also the most labile, and thus should be preferentially assimilated by bacteria. The concentration of carbon added to the streamwater was approximately 0.002 mg C/L and amounted to only 0.55% of the total mean growing-season DOC concentration (0.39 mg/L, Crocker and Meyer 1987); thus the added ^{13}C qualified as a tracer. A total of 0.58 g of ^{13}C as sodium acetate was added during the release period. ^{13}C 1-sodium acetate was chosen because it is a labile form of carbon for bacterial uptake and because it is inexpensive. Total cost for the isotope used in this release was U.S. $130.

Sample processing and analysis

I collected samples of animals and detritus before starting and just before stopping the ^{13}C release. Sampling was distributed throughout the study area to reduce the intensity of sampling disturbance. No samples were taken within 5 m of the dripper to ensure that the labeled carbon was well mixed within the stream before reaching the sampling site. Benthic samples of coarse particulate organic matter (CPOM) were collected by hand, dried at 50°C, and finely ground in a Spex ball mill. Fine particulate organic matter (FPOM) was elutriated from the sediments, sieved through a 1-mm sieve, collected on a Gelman A/E glass fiber filter, and processes in the same way as CPOM. Biofilm was scraped from tiles into microcentrifuge tubes, which were subsequently pelletized by centrifugation and dried.

Fourteen animal taxa, spanning a range of functional groups, were used for this study (Table 1). The assemblage is dominated by shredders and collector-gatherers (Table 1). These were the most abundant taxa found during the study. Functional groups, (e.g., scraper) were determined from Lugthart and Wallace (1992), and by qualitative gut analyses of the animals. Invertebrates and salamanders were collected with a 250-μm D-net and sediment cores, and taken immediately to the laboratory. The samples were elutriated, poured onto 1-mm and 250-μm sieves, and animals were picked from the debris. I removed the guts from the animals so that assimilated carbon was not contaminated by ingested carbon in their guts (Gearing 1991). It was not possible to remove guts of copepods, chironomids, and oligochaetes because of their small size. After removing the guts, I dried all animals at 50°C. Most animals

TABLE 1. Abundances and functional groups of taxa in Cold Spring on 21 August 1992. Three sets of two 208-cm² benthic cores were used in a mixed substrate habitat. Dashes represent taxa not present in the benthic cores although present when sampled for the $\delta^{13}C$ analysis using D-nets. This discrepancy is a sampling artifact of the core sampler. Biomass was calculated using length–weight regressions (Huryn 1986).

Functional group	Taxon	Order	Mean abundance No./m²	Mean abundance mg AFDM/m²
Predators	*Desmognathus* sp.	Urodela	—	—
	Beloneuria sp.	Plecoptera	—	—
	Hexatoma spp.[a]	Diptera	40	8
	Molanna blenda[b]	Trichoptera	—	—
	Rhyacophila spp.	Trichoptera	8	17
Shredders	*Fattigia pele*	Trichoptera	72	155
	Peltoperlidae	Plecoptera	—	—
	Pycnopsyche spp.	Trichoptera	83	150
Gatherer-microbivores	Harpacticoida	Harpacticoida	11,200	34
Gatherer-miners	Chironomidae[c]	Diptera	5300	27
	Oligochaeta	Oligochaeta[d]	5100	50
Scraper-gatherers	*Optioservus immunis*-larvae	Coleoptera	1000	34
	Optioservus immunis-adults	Coleoptera	24	7.2
	Stenonema sp.	Ephemeroptera	—	—
Net-building filterer	*Parapsyche cardis*	Trichoptera	8	1.2

[a] Some shredders.
[b] Some scrapers.
[c] Tanypodinae excluded.
[d] Class.

were of appropriate size (between 200 and 5000 μg) for individual analysis on the mass spectrometer. Copepods, chironomids, elmids, and oligochaetes were pooled because they were too small (<200 μg) to analyze individually. For copepods, up to 60 individuals per sample were included.

All samples were analyzed on a Europa Tracermass mass spectrometer connected to a Carlo-Erba carbon-nitrogen analyzer. The Carlo Erba oxidized the sample at 1050°C, reduced the nitrogen oxides to N_2 and removed oxygen at 650°C over copper, and removed water using magnesium perchlorate. CO_2 and N_2 were separated chromatographically and flowed to the mass spectrometer using helium as the carrier. This system allows more inexpensive sample analysis than other mass spectrometers; however, its accuracy is not as good, and it may not be suitable for measuring natural abundances of ^{13}C. The $\delta^{13}C$ of the samples were negatively correlated with the amount of carbon in the sample. Because the size of individual animals ranged from 100 μg to 2200 μg of carbon, the $\delta^{13}C$ value ranged up to 5‰ and needed to be size corrected. This was accomplished by analyzing different amounts of leaf tissue with a known $\delta^{13}C$, and generating a regression equation that related the $\delta^{13}C$ value to the amount of carbon. Each unknown sample was corrected based on the amount of carbon it contained using the regression equation. Typically the standard deviation of size corrected replicates was 1‰. Because the samples were ^{13}C enriched, this machine error was low relative to the level of enrichment.

The data are reported as a $\delta^{13}C$ value which is the relative difference in isotopic ratio of the sample from the Pee Dee Belemnite standard (Peterson and Fry 1987). The units are in parts per thousand (‰). The $\delta^{13}C$ value for carbon is calculated by:

$$\delta^{13}C = [(R_{sample}/R_{PDB\ standard}) - 1] \times 1000$$

where R is the ratio of $^{13}C/^{12}C$. Samples enriched with ^{13}C will have higher $\delta^{13}C$ values relative to unenriched samples. I define a significantly labeled individual as one with a $\delta^{13}C$ value above the 90% one-tail confidence interval determined from samples of the taxon collected before the addition began. Because the addition of ^{13}C can only increase the $\delta^{13}C$ of

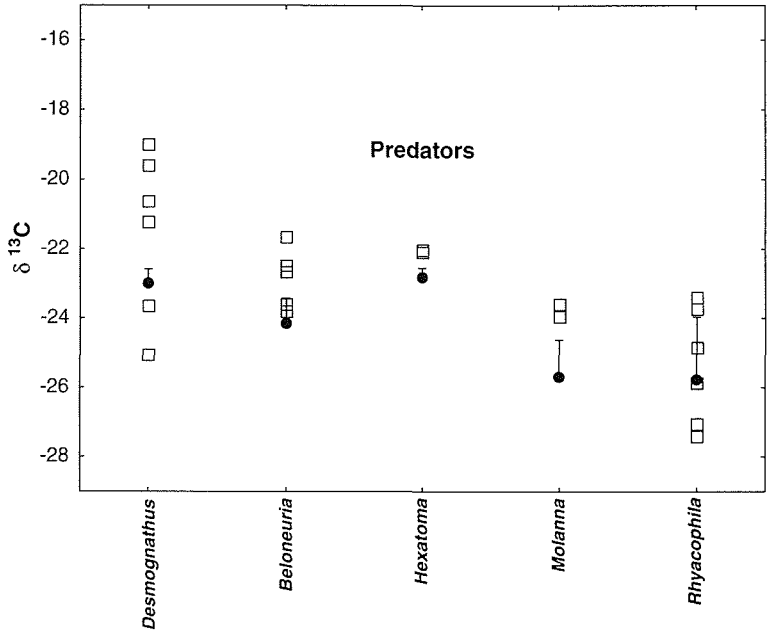

FIG. 1. $\delta^{13}C$ of predators before and after the ^{13}C addition in Cold Spring. Numbers close to 0 or positive indicate higher uptake of ^{13}C. Filled circles represent the mean $\delta^{13}C$ values pre-addition. Open squares represent $\delta^{13}C$ of individuals collected after the ^{13}C addition. Error bars are one-tailed 90% confidence intervals.

consumers, a one-tail test will give more power to detect increases in consumer $\delta^{13}C$ relative to pretreatment variation. Because I analyzed more than one sample per taxon I was able to examine the variability in the enrichment for each taxon. Increases in the variance of samples collected post-addition were compared with those collected pre-addition using a one-tail *F*-test (Sokal and Rohlf 1981).

Gut analysis

Guts of larval or adult species of the riffle beetle *Optioservus immunis* were dissected, suspended in distilled water, filtered onto a membrane filter, mounted onto a slide, and cleared with immersion oil (Cummins 1973). Three slides, each with guts of three individuals, were made for both adults and larvae. Gut contents were quantified by measuring the relative areas of diatoms and detritus on the filters using a compound microscope equipped with a video camera and image-analysis software (Benke and Wallace 1980). A two-tail *t*-test was calculated for the percent area of diatoms using an arcsine square-root transform (Sokal and Rohlf 1981).

Results

Mean predator pre-addition $\delta^{13}C$ values ranged from −25.7‰ to −23.1‰. Post-addition predator $\delta^{13}C$ values ranged from −27‰ to −18‰; only a few individuals in each taxon were significantly labeled (Fig. 1). The salamander *Desmognathus* was the only taxon to have a strong label in some individuals and have post-addition variation significantly higher than pre-addition variation (*F*-test $p < 0.005$).

The three shredders showed similar patterns of post-addition $\delta^{13}C$. Values for most individuals in each taxon did not lie above the 90% confidence interval (Fig. 2); however, 2–3 individuals per taxon lay clearly above the confidence intervals. Post-addition $\delta^{13}C$ variability was higher than pre-addition variability for both Peltoperlidae and *Fattigia* (*F*-test $p < 0.01$). There were no striking differences in the degree of labeling between the three shredders, suggesting similar use of bacterial carbon. There was no difference in $\delta^{13}C$ between pre-addition and post-addition composite CPOM samples.

Two gatherers, harpacticoid copepods and chironomids, were clearly labeled with each sample above the pretreatment $\delta^{13}C$ confidence

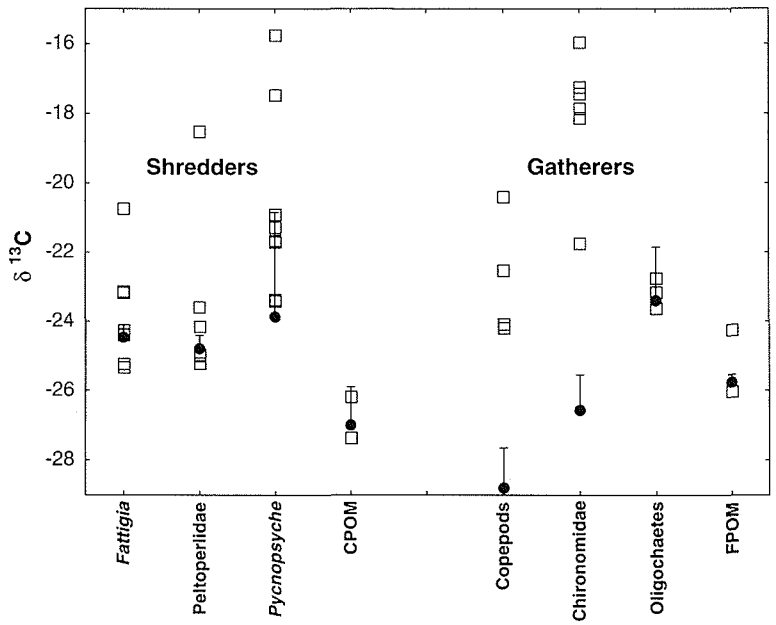

FIG. 2. $\delta^{13}C$ of shredders, gatherers, CPOM, and FPOM, before and after $\delta^{13}C$ addition in Cold Spring. Symbols are the same as Fig. 1. Open squares for gatherer taxa represent more than one individual.

interval (Fig. 2). Variability between samples was less in chironomid and copepod samples, because each sample was a composite of 20 to 60 individuals, and was not significantly greater than the pretreatment values. Post-addition oligochaetes were not labeled relative to the pretreatment oligochaetes. One FPOM sample was labeled relative to the pretreatment value; however, FPOM was less labeled than either copepods or chironomids.

Of all invertebrates sampled, *Stenonema*, a biofilm scraper, exhibited the strongest labeling (Fig. 3). All individuals were clearly above the pretreatment value, with a high $\delta^{13}C$ of 129‰. The variability of post-addition samples was significantly higher than pre-addition samples ($p < 0.001$). There were no pretreatment $\delta^{13}C$ values for biofilm, because of mass-spectrometer failure, so I cannot determine the absolute amount of label the biofilm received. However, each biofilm sample had a lower $\delta^{13}C$ than *Stenonema*, demonstrating that *Stenonema* preferentially assimilate bacteria from the biofilm. Biofilm $\delta^{13}C$ was probably near the FPOM and CPOM $\delta^{13}C$ values which ranged from −25‰ to −27‰.

Only one individual of the single filter feeder, *Parapsyche cardis*, was well labeled (Fig. 3); however, only one individual was sampled pre-addition, because filter feeders were not common in this slow-flowing low-gradient spring.

Adults and larvae of the scraper *Optioservus immunis* exhibited a different labeling pattern from each other. Both had similar pretreatment $\delta^{13}C$ near −30‰, but adults had much higher post-addition $\delta^{13}C$ values (range = −9.7 to −27.4‰), indicating greater use of bacterial carbon than larvae (Fig. 3). All post-treatment values for larvae fell within the confidence interval for the pretreatment samples. Gut analysis of *Optioservus immunis* showed that larval guts contained 43% diatoms and 57% detritus by area, and adult guts contained 2% diatoms and 98% detritus. Larvae had significantly more diatoms in their guts relative to adults (*t*-test $p < 0.001$).

Discussion

This study showed clear differences in ^{13}C labeling between taxa, showing different use of bacterial carbon. Bacterial carbon is not necessarily bacteria cells. Bacteria make exopolysaccharides which may have more mass than bacterial cells. Couch and Meyer (1992) determined that wood biofilm in artificial channels on the

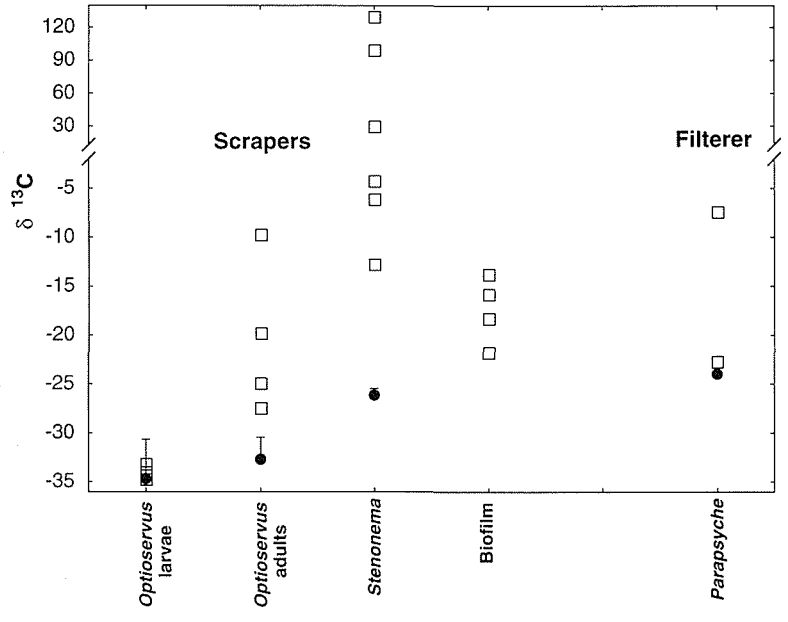

FIG. 3. $\delta^{13}C$ of scrapers, a filterer, and biofilm before and after the $\delta^{13}C$ addition in Cold Spring. Symbols are the same as Fig. 1. Note the y-axis break. There was only 1 pre-addition sample for *Parapsyche* and none for biofilm.

Ogeechee River was 7% exopolymer, which was nine times greater than bacterial cell biomass. This study cannot distinguish between bacterial cells and their exopolymers; however, exopolymers represent bacterially derived carbon.

Bacteria are most likely the only microbes taking up the labeled acetate, which is a labile carbon source for bacteria (Wright and Hobbie 1966). Algae are osmotrophic, but they exhibit diffusion kinetics for DOC; thus in order for them to be labeled, the acetate concentration would have to be high (above 0.14 mg C/L) (Wright and Hobbie 1966). Because the concentration of added acetate carbon in the stream was 0.002 mg C/L, only bacteria, which have an active transport system, would have been able to take up the label. Fungi also do not exhibit affinity for low levels of substrate. Newell (1984) reported that fungi have saturation constants of 10^{-3} to 10^{-6} M, which is higher than the concentration of acetate (1.7×10^{-7} M) during this release. Bacterial $\delta^{13}C$ values, which were not measured because of the technical difficulty of purifying bacterial carbon, could have well exceeded the calculated $\delta^{13}C$ value of 100‰ as a result of preferential uptake of acetate. Because I was interested in simply labeling bacteria rather than discerning the pattern of DOC uptake, using a labile compound ensured a strong label in bacteria, while adding only a trace amount of DOC to the stream.

It is possible for invertebrates to become labeled without having ingested bacterial carbon or a metazoan consumer. Some labeled acetate may have adsorbed onto biofilms or sediments and was subsequently ingested by invertebrates. However, many studies have demonstrated that the bulk of DOC uptake in stream sediments is due to biotic uptake, and not abiotic adsorption (Lock and Hynes 1976, Dahm 1981, Kuserk et al. 1984). Invertebrates could have consumed bactivorous protozoa and become labeled. My technique could not differentiate between consumption of bacteria or their grazers, protozoa. Because protozoans graze bacteria and have short biomass turnover times relative to metazoans, their $\delta^{13}C$ should track bacterial $\delta^{13}C$ very closely. However, in Coweeta streams, protist consumption of bacteria is low. Protists graze less than 1% of bacterial production per day, because protists are not as abundant in Coweeta sediments as in other systems (L. A. Carlough and J. L. Meyer, University of Georgia, personal communication). Metazo-

an consumption of bacteria is much higher; Perlmutter and Meyer (1991) calculated that copepods can graze up to 20% of daily bacterial production in Coweeta streams.

Differences in carbon turnover rates between the taxa I examined will affect the degree of labeling (Fry and Arnold 1983). Some animals had a population turnover time much shorter than the labeling period (3 wk); they became fully labeled. Chironomids, which can have a specific growth rate of 0.1/d (Huryn and Wallace 1986), will have a population turnover time of 10 d (Benke 1984); thus 100% of the population growth will turn over during the labeling period. A univoltine mayfly will grow and turn over less carbon during the same time period. Thus *Stenonema* may incorporate more bacterial carbon than its $\delta^{13}C$ indicates, because less than 100% of its carbon was turned over during the 3-wk ^{13}C addition.

Because predators are at least two trophic transfers away from bacteria, their uptake of label is more variable. They would have to eat enriched prey, e.g., copepods or *Stenonema*, to become labeled. Variability in the enrichment of predators is probably due to variability in prey $\delta^{13}C$. An obligate predator will not directly consume bacterial cells, but rather a diverse assortment of invertebrates, each with varying amounts of ^{13}C label. However, this technique can show patterns of bacterial carbon transfer through a food web, showing which predators ultimately derive carbon from bacterial-based pathways. Predaceous invertebrates in streams typically have varied diets; diet variability between individuals may be equal to or greater than the diet variability between taxa (Hildrew et al. 1985). Nonetheless, gut analyses are required to determine trophic intermediates between bacteria and predators.

Only a few individual shredders were labeled, indicating low consumption of bacterial carbon relative to collector and scraper taxa. Findlay et al. (1986), using a radiotracer, showed that bacterial carbon represented a small amount of the total carbon intake of two shredders, *Peltoperla* and *Tipula*. Their results showed that the bulk of the assimilated carbon was from leaf tissue and fungi, rather than associated bacteria. The low quantity of label in shredders in my study suggests that shredders assimilate more carbon from particulate, non-microbial, detritus than do collectors. However, shredders are slower growing and are probably not turning over carbon as fast as collectors with high growth rates. Shredder $\delta^{13}C$ can be compared to *Stenonema*, which has a similar growth rate to shredders, which shows that shredders incorporate less bacterial carbon than *Stenonema*.

The clear labeling of copepods and chironomids showed that they assimilate bacterial carbon, and that bacterial carbon is preferentially assimilated compared with bulk FPOM in the sediments because their $\delta^{13}C$ values are higher than $\delta^{13}C$ of FPOM. Perlmutter and Meyer (1991) showed that harpacticoid copepods select and consume individual bacteria cells on leaf surfaces. Schurr (1989) determined that assimilation efficiency for chironomids feeding on detritus was between 1 and 4%. Most of the carbon passes through the chironomid guts, with only the most labile fraction being assimilated. Any bias due to the guts not being removed on collectors would decrease their $\delta^{13}C$ values because the FPOM in their guts is less labeled than the entire individual.

Although copepods and chironomids showed a clear label, it was not nearly as high as in *Stenonema*, a scraper of biofilm from solid substrates. *Stenonema* has been shown to derive much of its carbon from bacteria. Edwards and Meyer (1990) found that bacteria compose 47% of the daily carbon needs of *Stenonema*. Benke et al. (1992) showed that *Stenonema* growth was high when fed microfine particles passed through a 0.5-μm filter, which were mostly bacteria. *Stenonema* may also be feeding upon a more highly labeled resource than other taxa. Bacteria upon rocks or wood are exposed to labeled streamflow, and probably become more labeled relative to bacteria deep in the sediments. Copepods, chironomids, and oligochaetes all live interstitially, and the label might not have reached below the first few mm of sediments. Oligochaetes occur deeper in sediments than copepods and chironomids at Coweeta (personal observation), which may explain their low uptake of ^{13}C label. The high variability of *Stenonema* $\delta^{13}C$ may be caused by patchiness in label distribution. Thus consumption of bacteria and their extracellular compounds present in biofilm may explain the high labeling of *Stenonema*.

This enrichment technique was effective in determining dietary differences between adult and larval *Optioservus immunis*. Elmids are com-

monly considered to be algivorous scrapers (Brown 1987). The low pretreatment values (−30‰ for larvae and adults) relative to other taxa correspond to other findings that algivores in a stream have lower $\delta^{13}C$ than detritivores (Rounick et al. 1982, Rosenfeld and Roff 1992). These low $\delta^{13}C$ values also suggest little dietary difference between larval and adult beetles based solely upon natural abundance of ^{13}C. The higher post-treatment $\delta^{13}C$ values suggest that adult *Optioservus* assimilated more bacterial carbon than larvae. Because adults do not grow, they will have a longer carbon turnover time than do larvae, thus adults may assimilate more bacterial carbon relative to larvae than their $\delta^{13}C$ indicates. Gut analysis showed that larvae consume more diatoms than adults, which appear to be primarily detritivores. Other studies have not shown dietary differences between larvae and adults. Seagle (1982) showed that adults and larvae of *Optioservus trivittatus* were primarily detritivores, and differed little in food habits. Tavares and Williams (1990) also reported that larvae and adults of the elmids *Promoresia elegans, Optioservus fastidius,* and *Stenelmis bicarinata* were mostly detritivorous with a low proportion of diatoms in the diet; and they found no indication of a dietary difference between larvae and adults of any of the three species. The combination of stable isotope tracer and gut analyses in this study shows that in Cold Spring, *Optioservus immunis* shows a dietary difference between larvae and adults that would not have been detected using natural abundance of ^{13}C.

The technique I have described proved to be useful in tracing bacterial carbon into a metazoan food web and showed differential use of bacterial carbon. Bacterial carbon appears to be more important to scraper and gathering taxa than to shredders and predators; however, uptake is confounded by carbon turnover rates in slow growing shredders. Unlike laboratory experiments examining bactivory by invertebrates (e.g., Montagna and Bauer 1988, Edwards and Meyer 1987, 1990, and Findlay et al. 1986) the ^{13}C addition is not able to give specific bactivory rates for individual taxa, and it is more variable than laboratory experiments. However, the technique allows the examination of patterns of bacterial use by an invertebrate assemblage in the field under natural conditions.

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