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## Examination of the carbon base in southern Ontario streams using stable isotopes

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**Abstract.** Dependence of the invertebrate and fish community on terrestrial versus aquatic carbon sources in southern Ontario streams was studied using stable carbon isotope analysis. Algal carbon was significantly more <sup>13</sup>C depleted (-35.5‰) than terrestrial carbon (-27‰), but high variability in algal δ<sup>13</sup>C precluded use of a quantitative mixing model. δ<sup>13</sup>C values at the unforested sites show a dependence of invertebrates on primarily autochthonous carbon. δ<sup>13</sup>C values of many taxa at the forested sites also show a substantial dependence of invertebrates on algal carbon during the spring. Fish appear to derive more carbon from terrestrial sources than do aquatic invertebrates at both the forested and unforested sites. Carbon isotope analysis has the potential to elucidate carbon pathways in streams only when local conditions cause algae to be <sup>13</sup>C depleted relative to terrestrial carbon.

**Key words:** autochthonous, allochthonous, invertebrates, fish, stable carbon isotope, streams.

The origins of the carbon driving stream ecosystems are of longstanding interest. Organic carbon in streams can come from two primary sources; it may be terrestrial in origin (allochthonous carbon), or it may be synthesized by primary production of autotrophs within the stream itself (autochthonous carbon). Although terrestrial inputs predominate in most forested streams, and algal inputs dominate at unshaded sites, many streams have substantial inputs of both autochthonous and allochthonous carbon. Even in streams with obvious disparities in carbon inputs, it is often unclear which source is most important in supporting the ecosystem, largely because differences in "food quality" (Cummins 1974, Bird and Kaushik 1984) make the availability of carbon to higher trophic levels disproportionate to gross inputs.

Contributions of different carbon sources to the food base of stream communities is usually determined by stomach content analysis of individual animals. Although a direct and useful method, stomach content analysis has several shortcomings which reduce its practicality and resolution. It is labour intensive, different food items vary widely in their assimilability, and the gut contents at any given instant may not be representative of the animal's diet over time. Thus the actual dependence of an organism on

a food resource may not be accurately reflected by its relative abundance in the gut. In addition, the detrital nature of carbon in streams makes it difficult to identify the origin of gut contents in many invertebrates.

Although several studies have examined the diets of stream invertebrate communities in great detail (e.g., Nelson and Scott 1962, Coffman et al. 1971, Koslucher and Minshall 1973, Gray and Ward 1979), few were able to distinguish clearly between autochthonous and allochthonous carbon in the diet, largely because of the presence of substantial quantities of detritus of unknown origin. Even for stream fishes, the origin of as much as 30% of the stomach contents may be unidentifiable (e.g., Chapman and Demory 1963); the true carbon base of fish production will be further obscured by the indeterminate carbon base of the identifiable invertebrates. To date, the real importance to the stream community of autochthonous and allochthonous carbon sources under comparable conditions of abundance remains, at best, rather speculative.

Stable carbon isotope analysis is an alternative technique for discriminating between carbon sources, used extensively in marine and estuarine systems (Fry and Sherr 1984) and freshwater lakes (e.g., Rau 1978, Araujo-Lima et al. 1986), but only recently applied to streams (Rounick et al. 1982, Winterbourn et al. 1986, Bunn et al. 1989). Carbon 13 (<sup>13</sup>C) is a stable isotope of carbon that is naturally present in air and water in approximately fixed proportion

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(O'Leary 1988). Terrestrial and aquatic organic matter often differ significantly in their  $^{13}\text{C}/^{12}\text{C}$  ratios, because of different metabolic pathways associated with the uptake of  $\text{CO}_2$  during organic carbon synthesis (Rounick and Winterbourn 1986). Since consumption alters  $^{13}\text{C}/^{12}\text{C}$  ratios very little, or in a predictable fashion up a food chain (DeNiro and Epstein 1978, Rau et al. 1983), the  $^{13}\text{C}/^{12}\text{C}$  ratio of an organism should reflect that of its food source. Carbon isotope analysis has the advantage over stomach contents analysis in that the isotope ratio reflects the carbon that is *assimilated* by the animal, rather than that which is simply present in the gut. In addition, because turnover of body carbon is not instantaneous, the carbon isotope ratio of an animal integrates its diet over time, and is not dependent on the most recent feeding event.

If autochthonous and allochthonous carbon can be shown to have significantly different  $^{13}\text{C}/^{12}\text{C}$  ratios, then a simple two-source mixing model can be applied (Fry and Sherr 1984). An invertebrate with a  $^{13}\text{C}/^{12}\text{C}$  ratio close to either source indicates a dependence primarily on that source; an invertebrate with an isotope ratio intermediate between both sources indicates a dependence on both that can be quantitatively determined by the intermediacy of its isotope ratio. Carbon isotope analysis has been successfully applied to streams in New Zealand to determine the relative contributions of autochthonous and allochthonous carbon to stream invertebrates (Rounick et al. 1982) and it has the potential for doing so wherever the  $^{13}\text{C}/^{12}\text{C}$  ratios of autochthonous and allochthonous carbon are significantly different.

Our study involved stable carbon isotope analysis of carbon sources, invertebrates, and fishes in southern Ontario streams, in an attempt to determine the dependence of consumers on autochthonous versus allochthonous carbon. Samples were collected from forested and unforested headwater streams in order to have maximum contrast in the levels of availability of autochthonous carbon, and to test the assumption that the carbon base of forested headwater streams is primarily terrestrial. Selected fishes and an invertebrate species common in local streams were also collected and raised in the laboratory on known diets to verify that the carbon isotope ratio of consumers reflects that of their carbon source.

## Methods

### *Feeding experiments in the laboratory*

Larval white suckers (*Catostomus commersoni* [Lacepede]; mean wet weight 0.024 g) and common shiners (*Notropis cornutus* [Kirtland]; mean wet weight 0.046 g) were collected from the unforested streams and reared in the laboratory on a diet of tropical fish flakes for a period of 40 d.  $\delta^{13}\text{C}$  (see below) was determined for fish flakes, a subsample of the original fish collected for each species, and fish of both species reared in the laboratory. Early instar caddisfly larvae (Hydropsychidae) were collected and raised in the laboratory in a recirculating plexiglass chamber on a diet of trout chow for a period of 20–25 d. Trout chow, a subsample of the original larvae (mean dry weight 0.8 mg), and larvae raised in the lab (final mean dry weight 4.0 mg) were analyzed for their stable carbon isotope ratios.

### *Collection and preparation of field samples*

Samples for carbon isotope analysis were collected during the spring (April–early June) of 1988 and 1989 from two forested streams (Limestone Creek, App's Mill Creek) and two unforested streams (Carrol Creek and Swan Creek); incidental samples were also collected from two additional forested streams, Hanlon Creek and the West Credit River. All sites were part of a larger study investigating energy flow in southern Ontario streams. The unforested sites were characterized by high epilithic primary production, whereas the forested sites had greater terrestrial inputs characteristic of shaded headwater streams and had primary production rates an order of magnitude lower (Rosenfeld 1989).

Dead (leached) leaves of representative riparian tree species were collected from the forest floor and the stream itself; samples of epilithon (the organic biofilm containing autotrophs, heterotrophs, and detritus) were scraped from randomly selected rocks using a chisel. Samples of algae, both unicellular and filamentous, were scraped from rocks, and purified in the laboratory by removing non-algal contaminants under a dissecting microscope. Three to five of the most visually abundant macroinvertebrate species were collected and analyzed from each site, as well as less abundant

macroinvertebrates of potential interest as indicators of carbon dependence (e.g., crayfish, predatory invertebrates). Invertebrate samples were not replicated, but consisted of 1–22 individuals of each species (mean  $n = 10$ , Fig. 3) pooled for analysis from a given site. Analyses were performed on pooled samples rather than individual organisms because (1) a minimum 10 mg of carbon was required for analysis, (2) the expense of isotope analysis restricted the number of analyses we could perform, and (3) the objective of this study was to examine the average carbon dependence of animals under different conditions of resource availability. Although pooling the carbon of animals collected gives no information about the variance of isotope ratio between individuals, it does show the average isotopic composition of animals collected at a given time and place, and therefore their average carbon dependence.

To prevent contamination by inorganic carbon, samples of allochthonous and autochthonous carbon were acidified using 10% v/v HCl in clean scintillation vials, and placed in a water bath at 50°C for 8–24 h. This process was repeated at least twice, or until no further carbon dioxide evolution was observed. Invertebrates were rinsed with 10% v/v HCl to remove inorganic carbonates from their cuticle, and guts were removed by dissection to prevent contamination of samples by stomach contents. All samples were oven dried at 55°C before analysis.

Analysis was done at the Environmental Isotope Laboratory, Department of Earth Sciences, University of Waterloo, Ontario, using a Leco furnace and a Micromass 903 triple collector ratio mass spectrometer (Drimmie 1976), with an analytic precision of  $\pm 0.5\%$ . In accordance with convention, carbon isotope ratios are expressed as parts per thousand (per mil, ‰) difference between the sample and the PDB standard (Craig 1957, Peterson and Fry 1987):

$$\delta^{13}\text{C} = \frac{^{13}\text{C}/^{12}\text{C}_{\text{sample}} - ^{13}\text{C}/^{12}\text{C}_{\text{standard}}}{^{13}\text{C}/^{12}\text{C}_{\text{standard}}} \times 1000$$

The  $\delta^{13}\text{C}$  of organic matter is typically negative relative to the PDB standard (O'Leary 1988); samples that are depleted in  $^{13}\text{C}$  tend to have more negative  $\delta^{13}\text{C}$  values, and samples that are enriched tend to be less negative.

## Results

### Feeding experiments

The  $\delta^{13}\text{C}$  of hydropsychids raised on trout chow closely approximated that of their food source after 20–25 d (Fig. 1a). The  $\delta^{13}\text{C}$  of young-of-the-year white suckers fed tropical fish food was also close to that of their food source after 46 d (Fig. 1b), although the  $\delta^{13}\text{C}$  of common shiners was still significantly more negative than tropical fish food after 40 d of feeding (Fig. 1c).

### $\delta^{13}\text{C}$ of carbon sources and consumers

The  $\delta^{13}\text{C}$  of terrestrial leaves ( $-29.0 \pm 0.8\%$ ) differed significantly from that of aquatic algae ( $-37.0 \pm 4.6\%$ ) across all sites ( $p < 0.01$  for log transformed data), although the variability of algal  $\delta^{13}\text{C}$  was very high (Fig. 2, Table 1). The  $\delta^{13}\text{C}$  of algae was generally more  $^{13}\text{C}$ -depleted (negative) at the forested sites (Table 1). In contrast, the isotopic composition of terrestrial leaves showed very low variability between species and sites (Table 2).

In addition to algae, a liverwort and a moss were also present at the Limestone Creek site; the isotopic label of the liverwort ( $-38.9\%$ ) was similar to that of algae (Fig. 3a), whereas the moss ( $-26.9\%$ ) bore a signature close to that of terrestrial carbon. The  $\delta^{13}\text{C}$  of epilithon was variable, but generally intermediate between that of terrestrial leaves and algae (Fig. 2).

Invertebrates from forested sites showed a wide range in  $\delta^{13}\text{C}$  (Figs. 3a, b). Although shredders such as *Pycnopsyche* spp. and *Leuctra* spp. (Figs. 3a, b) at the forested sites had  $\delta^{13}\text{C}$  values close to that of their presumed carbon source (terrestrial leaves), the caddisfly *Hesperophylax* sp. had a  $\delta^{13}\text{C}$  intermediate between those of terrestrial and algal carbon, and nemourids from App's Mill Creek had an even more distinct algal label (Fig. 3b).

The  $\delta^{13}\text{C}$  of *Pycnopsyche* from Limestone Creek showed little variation between years (Fig. 3a). *Pycnopsyche* collected from another forested site, the West Credit River, also showed a terrestrial label ( $-27.8\%$ ), as did the shredder *Lepidostoma* sp. from forested Hanlon Creek ( $-27.5\%$ ).

Generally speaking, filtering collectors (hydropsychids) and gathering collectors (*Ameletus* sp.) from the forested sites showed  $\delta^{13}\text{C}$  values intermediate between those of algal and ter-

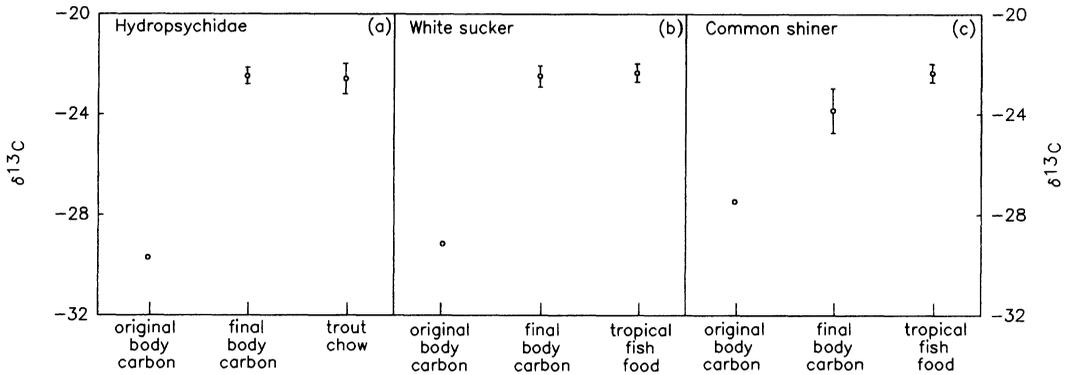


FIG. 1. Stable carbon isotope ratios ( $\delta^{13}\text{C}$ ; mean  $\pm$  1 SD) of carbon sources and body carbon from animals raised in the laboratory;  $n = 1$  for original body carbon,  $n = 2$  for final body carbon and carbon sources. Rearing periods: *Hydropsyche* 20–25 d, white sucker 46 d, common shiner 40 d.

restrial sources, although the  $\delta^{13}\text{C}$  of the single crayfish collected at Limestone Creek was much closer to that of leaves and moss. The only predator analyzed from the forested sites, the stonefly *Acroneuria* sp., had a  $\delta^{13}\text{C}$  slightly more negative than the non-shredding invertebrates from Limestone Creek (Fig. 3a).

The  $\delta^{13}\text{C}$  values of invertebrates from Carrol Creek (unforested) were generally close to that of algal carbon collected at the same site, with the exception of the crayfish, which showed a distinct terrestrial label (Fig. 3c). Invertebrates from the other unforested site, Swan Creek, were more negative than the single sample of algal carbon, which was very close to terrestrial carbon (Fig. 3d). The  $\delta^{13}\text{C}$  of *Pycnopsoche* from Carrol Creek appeared to show some seasonal variation, and was considerably more negative than *Pycnopsoche* collected from either Limestone Creek or the West Credit River.

The  $\delta^{13}\text{C}$  of fishes was quite variable. The range in  $\delta^{13}\text{C}$  values for brook trout (*Salvelinus fontinalis* [Mitchell]) from forested App's Mill Creek was almost as large as the range in invertebrate  $\delta^{13}\text{C}$ , and virtually spanned the difference between terrestrial and aquatic carbon (Fig. 3b). Fishes from forested Limestone Creek had a slightly more terrestrial label, but the variation in  $\delta^{13}\text{C}$  between individual brook trout was still high (Fig. 3a). Fishes from the unforested sites had isotopic labels much closer to terrestrial than to aquatic carbon (Figs. 3c, d), although blacknose dace (*Rhinichthys atratulus* [Hermann]) from Carrol Creek were substantially more negative than blacknose dace collected at forested Limestone Creek.

**Discussion**

*Feeding experiments*

Previous studies have demonstrated that  $\delta^{13}\text{C}$  values of consumers are usually within  $\pm 1\%$  of their carbon sources (DeNiro and Epstein 1978, Haines and Montague 1979, Gearing et al. 1984). The feeding experiments in this study showed a close isotopic resemblance of consumers to their diet for hydropsychids and common suckers (Fig. 1), less so for common shiners (1.5‰ difference). This suggests a certain interspecific variability in the fidelity of an animal's isotope ratio to its diet. Alternatively, the isotope ratio of common shiners may not have equilibrated with that of their food source by the time they were killed for analysis. Since common shiner biomass increased only 250–550% during the

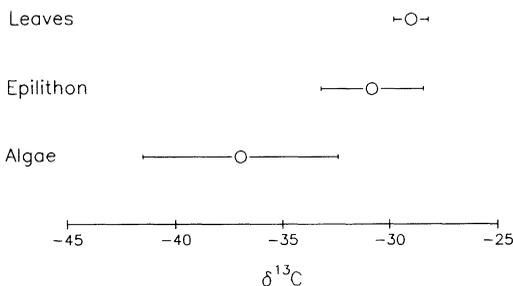


FIG. 2. Stable carbon isotope ratios ( $\delta^{13}\text{C}$ ; mean  $\pm$  1 SD) of terrestrial leaves ( $n = 9$ ), epilithon (the epilithic biofilm including autotrophs and heterotrophs,  $n = 9$ ), and epilithic algae ( $n = 6$ ) from all sites.

TABLE 1. Stable carbon isotope ratios ( $\delta^{13}\text{C}$ ) of algae from study sites in southern Ontario.

Forested		
Hanlon Ck.	<i>Ulothrix</i>	-42.73
Limestone Ck.	<i>Ulothrix</i>	-41.96
	Diatoms (mostly <i>Meridion</i> and <i>Synedra</i> )	-36.65
App's Mill	Diatoms (mostly <i>Gomphonema</i> )	-35.12
Unforested		
Carrol Ck.	Diatoms (mostly <i>Meridion</i> and <i>Gomphonema</i> )	-34.27
Swan Ck.	Diatoms (mostly <i>Meridion</i> and <i>Gomphonema</i> )	-31.05

feeding experiment, compared with over 1000% for white sucker, incomplete carbon turnover following a dietary switch (Fry and Arnold 1982) may partially explain the more pronounced disparity between consumer and food resource. Although only three taxa were used during the feeding experiments, the data appear to confirm the generalization that the difference between consumers and their carbon resource is on average less than 1‰, although certain taxa seem to conform to the generalization better than others.

#### $\delta^{13}\text{C}$ of carbon sources

The observed  $\delta^{13}\text{C}$  of -29‰ for terrestrial leaves is close to the average of -28 expected for terrestrial C3 plants (O'Leary 1988). Since the leaves were leached, having been collected from the stream or forest floor, the slight  $^{13}\text{C}$  depletion may be due to a higher content of refractory lignin, which is generally isotopi-

cally lighter than whole tissue (Benner et al. 1987). Regardless, the variability in terrestrial  $\delta^{13}\text{C}$  across all sites is very low, particularly when compared with algal  $\delta^{13}\text{C}$ .

Algal samples had to be free of all terrestrial detritus to produce an accurate algal isotopic signature. Collection of pure algal samples is difficult, particularly for non-filamentous algae. Biasing of the algal signature is evident in the epilithon samples, whose intermediate  $\delta^{13}\text{C}$  values (Fig. 2) suggest contamination by allochthonous carbon, presumably by direct deposition of fine detritus within the epilithon, or by uptake of allochthonous dissolved organic carbon by microheterotrophs.

Although variability of algal  $\delta^{13}\text{C}$  within a site may be high (Fig. 3a), variability between sites is greater still (Table 1), suggesting that the algal isotope ratio is site (or reach) specific and dependent on local conditions. Algal  $\delta^{13}\text{C}$  will depend on the physiology of the plant (i.e., the isotopic discrimination of the particular pathway used during carbon fixation), the isotopic composition of dissolved inorganic carbon in the stream water, and diffusion barriers related to current velocity (O'Leary 1981, Fry and Sherr 1984). Although the small sample size makes generalizations difficult,  $\delta^{13}\text{C}$  values for algal carbon from the forested sites appear to be more negative than those from the unforested sites (Table 1). Dissolved inorganic carbon in groundwater may become  $^{13}\text{C}$ -depleted owing to respiration of terrestrial carbon during percolation through the soil community, resulting in algae with a more negative  $\delta^{13}\text{C}$  label in spring-fed streams (Rounick and James 1984). The more negative  $\delta^{13}\text{C}$  values for algae in the forested streams is consistent with higher inputs of potentially  $^{13}\text{C}$ -depleted groundwater in these watersheds.

TABLE 2. Stable carbon isotope ratios ( $\delta^{13}\text{C}$ ) of dead riparian tree leaves from the forest floor at study sites in southern Ontario.

Location	Source	$\delta^{13}\text{C}$
Limestone Ck.	Beech <sup>a</sup>	-28.67
	Beech <sup>a</sup>	-30.75
	Maple	-28.71
App's Mill	Oak	-28.61
	Oak and ash <sup>b</sup>	-28.93
	Oak and poplar <sup>b</sup>	-29.92
Hanlon Ck.	Eastern white cedar	-28.43
	Maple	-28.19
	Alder	-29.40

<sup>a</sup> Independent samples collected on the same day.

<sup>b</sup> Combined sample.

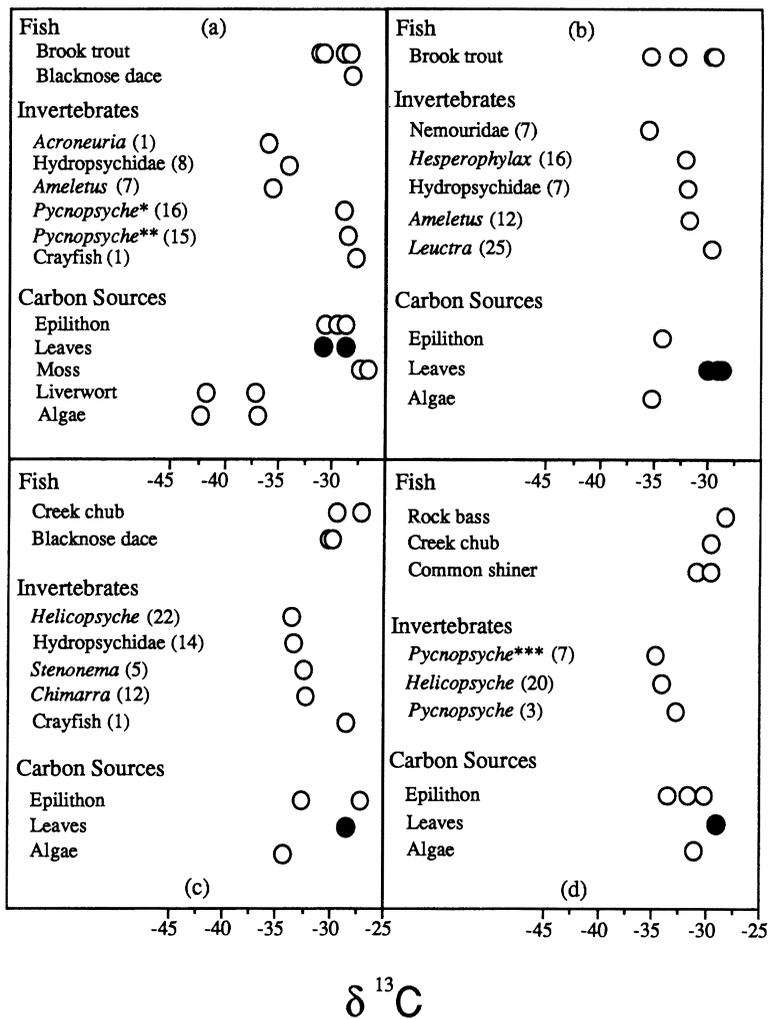


FIG. 3. Stable carbon isotope ratios ( $\delta^{13}\text{C}$ ) of fishes, invertebrates, and carbon sources collected during the spring from (a) forested Limestone Creek, (b) forested App's Mill Creek, (c) unforested Carrol Creek and (d) unforested Swan Creek. Filled circles represent terrestrial carbon. *Pycnopsyche* collected: \* during 1989, \*\* during 1988, and \*\*\* in July. Terrestrial carbon in (d) is the average from all sites, since terrestrial leaves were not collected at Swan Creek.

Because of the variability in algal  $\delta^{13}\text{C}$ , conclusions concerning carbon dependence of animals at a given site should be based on the algal isotope ratio characteristic of the particular stream (or reach) in question. Unfortunately, the variability in algal  $\delta^{13}\text{C}$  in this study is high enough to preclude using a quantitative mixing model (e.g., Fry and Sherr 1984) with any confidence to infer dependence on autochthonous versus allochthonous carbon sources. Nevertheless, the differences in isotope ratios (Fig. 2) are still consistent enough to make qual-

itative inferences concerning carbon dependence of consumers at the different sites.

Although there is a strong temptation to compare absolute  $\delta^{13}\text{C}$  of similar taxa between sites, this should be done with caution to avoid erroneous interpretations; the similarity in  $\delta^{13}\text{C}$  between hydropsychids from forested Limestone Creek and unforested Carrol Creek, for example, does not mean that they have similar carbon dependences, because the label of the algal carbon source differs between sites (Fig. 3).

*$\delta^{13}\text{C}$  of invertebrates*

$\delta^{13}\text{C}$  values of many invertebrates from the forested sites are negative ( $^{13}\text{C}$ -depleted) relative to terrestrial carbon, suggesting either isotopic fractionation during consumption (DeNiro and Epstein 1978), or a partial dependence on autochthonous carbon. As discussed above, fractionation during consumption is probably insignificant, certainly not enough to account for the 3–5‰ depletion observed relative to terrestrial carbon (Fig. 3). In addition, fractionation during consumption along a food web typically leads to enrichment (DeNiro and Epstein 1978, Haines and Montague 1979, McConnaughey and McRoy 1979, Rau et al. 1983) rather than depletion. Thus it seems reasonable to interpret depletion at the forested sites as a real indicator of partial dependence on autochthonous carbon.

On the whole, the stable carbon isotope data from the forested sites (Figs. 3a, b) suggest a substantial dependence on autochthonous carbon by many of the taxa analyzed. Despite the low visual abundance of algae at the forested sites, Rosenfeld (1989) estimated that algal production rates were high enough to provide 30% of the assimilatable carbon available to invertebrates in riffles, assuming indiscriminate consumption of available resources. The intermediate  $\delta^{13}\text{C}$  values of those taxa analyzed that were functionally capable of feeding on algal carbon (i.e., filtering and gathering collectors, e.g., hydropterygids and *Ameletus*) suggest that they are assimilating algal carbon to at least this degree. The carbon isotope data, however, may not be representative of carbon dependence throughout the year at the forested sites, since invertebrates were collected during the spring when algal production was at a peak (Rosenfeld 1989). Dependence of the invertebrate community on autochthonous carbon could be considerably less in other seasons when algae are less abundant.

Although shredders such as *Pycnopsyche* and *Leuctra* (Figs. 3a, b) had  $\delta^{13}\text{C}$  values close to that of their presumed carbon source (terrestrial leaves), some of the invertebrate isotopic labels were unexpected. Nemourids collected at App's Mill Creek, for instance, had a surprisingly distinct algal label. This apparent anomaly may be partly explained by the fact that nemourid stoneflies also feed as collector-gatherers (Harp-

er and Stewart 1984), increasing their potential consumption of senescent algal cells deposited in lentic habitats. Future studies would be wise to include limited stomach content analysis of invertebrates to complement isotope data, and to verify this kind of post hoc hypothesis.

As expected, most of the invertebrates at the unforested sites have  $\delta^{13}\text{C}$  values indicating a primary dependence on autochthonous carbon. The one exception is the crayfish from Carrol Creek, which shows a distinct terrestrial label. It is also interesting to note that late instar *Pycnopsyche* were more enriched during the summer than the spring (Fig. 3d), suggesting an increasing dependence on autochthonous carbon, or an ontogenetic shift in feeding habits, as seen in *P. lepida* by Cummins (1964) in a Michigan stream. The former possibility is consistent with the summer peak in availability of algal carbon at the unforested sites, which have a reversed pattern of algal growth relative to the seasonally shaded forested streams (Rosenfeld 1989).

There are also certain inconsistencies in the data, particularly at unforested Swan Creek, where separation between terrestrial and aquatic carbon sources is less distinct.  $\delta^{13}\text{C}$  values of invertebrates from Swan Creek are far more negative than algae (–31.0‰), whose label is close to that of terrestrial carbon. The presence of more negative  $\delta^{13}\text{C}$  values in the epilithon suggests that the  $\delta^{13}\text{C}$  of the single sample of algae collected was not representative of the algal pool available to aquatic invertebrates.

 *$\delta^{13}\text{C}$  of fish*

Blacknose dace, the only species collected at both a forested and unforested site, was 3‰ more negative at the unforested site, implying a greater dependence on autochthonous carbon. Despite this, there is very little overall difference between sites in absolute fish  $\delta^{13}\text{C}$ . The other notable feature in the data is the high intraspecific variability in  $\delta^{13}\text{C}$ , up to 6‰ for brook trout at App's Mill Creek alone (Fig. 3d), possibly reflecting the isotopic variability of the invertebrates on which they feed. Brook trout in particular are known to be idiosyncratic feeders (Bryan and Larkin 1972, Allan 1981), and the degree of terrestrial enrichment can be expected to vary with the foraging habits of the fish; it is quite conceivable that an individual

feeding selectively on benthic *Ameletus*, for example, could have a different ratio from an individual with a preference for infalling terrestrial invertebrates (Main and Lyon 1988). Trout also tend to be mobile (Smith 1958), and it is possible that trout collected at a given location may previously have been resident in an area with different carbon sources.

Despite the lack of a consistent difference in fish  $\delta^{13}\text{C}$  between forested and unforested sites, certain relationships in the data are still evident. Generally speaking, previous studies have observed a 1–2‰ enrichment associated with consumption at each trophic level (McConnaughey and McRoy 1979, Rau et al. 1983, Fry and Sherr 1984); although the average  $\delta^{13}\text{C}$  for fish from the App's Mill Creek site is in the expected 1–2‰ enrichment range, fish from Limestone Creek are on average 4.2‰ enriched relative to the mean  $\delta^{13}\text{C}$  of their potential invertebrate prey. Fish from Limestone Creek would therefore appear to derive more of their carbon base from terrestrial sources than do aquatic invertebrates from the same site. This suggests that terrestrial inputs in the form of infalling invertebrates (secondary producers) may contribute as much to vertebrate production in a forested stream as do primary terrestrial carbon inputs (leaves, detritus) via the aquatic food chain.

Although a strong dependence on terrestrial invertebrates is well documented elsewhere (e.g., Chapman and Demory 1963), it is interesting to note that the situation appears to persist even at the unforested sites, despite the increased availability of autochthonous carbon; at Carrol and Swan creeks, fish are still on average 3.9‰ enriched relative to the average invertebrate  $\delta^{13}\text{C}$  (Figs. 3c, d). This apparent dependence, however, may be exaggerated by infalling terrestrial invertebrates grazing on grasses, and bearing an enriched label characteristic of C4 plants ( $\sim -14\%$ , O'Leary 1988). Rounick and Hicks (1985) also found little difference between  $\delta^{13}\text{C}$  values of fish at forested and unforested sites in New Zealand, despite a clear difference between autochthonous and allochthonous carbon sources.

Conventional stream theory predicts a primary dependence on terrestrial carbon at forested headwater sites (e.g., Fisher and Likens 1973, Cummins 1974). While the invertebrate data repudiate this to a degree by showing a

partial dependence on autochthonous carbon during the spring, the isotope data for fishes, albeit limited, tend to support the conventional assumption that forested headwater streams are allochthonous-based. As discussed above, this is not necessarily because carbon dependence changes during transmission up the aquatic food chain, but rather because fish receive additional allochthonous inputs in the form of infalling terrestrial invertebrates. The generality of this observation, however, may be limited to smaller streams, because inputs of terrestrial invertebrates probably decrease on an areal basis as rivers get wider, or become less important as in situ primary production increases.

#### Overview

It appears that carbon dependence tends to become obscured with increasing trophic level in streams, in contrast with less complex marine pelagic systems with only a single potential carbon source. In streams, there are at least two potential carbon sources, and the variability in the feeding habits of individual organisms superimposed on possible fractionation effects between trophic levels makes the results difficult to interpret. This situation is also compounded by the fact that the difference between autochthonous and allochthonous carbon sources is relatively small ( $\sim 5\text{--}7\%$ ).

Although attempts to quantify carbon dependence using the available data would be unrealistic because of variability in algal  $\delta^{13}\text{C}$  and small sample sizes, this study clearly shows a substantial dependence of invertebrates on autochthonous carbon even at the forested headwater sites (Figs. 3a, b). Nevertheless, the utility of carbon isotope analysis as a survey tool to quickly determine the carbon base of different stream communities is limited (Winterbourn et al. 1986, this study), primarily because of spatial variability in algal  $\delta^{13}\text{C}$ .

Despite these limitations, carbon isotope analysis can still be used to elucidate carbon pathways in simple systems (such as headwater streams), where there are two distinct carbon sources well separated in their isotope ratios. Limestone Creek, for example, has a good separation between terrestrial and algal  $\delta^{13}\text{C}$ , but the presence of bryophytes complicates the situation; isotopic labels of terrestrial leaves and moss overlap, so that carbon dependence of *Pyc-*

*nopsyche* or crayfish could be attributable to either source or a combination of both (Fig. 3a). Careful measurement of variability in algal  $\delta^{13}\text{C}$  through space and time at an appropriate site would validate application of a two-source mixing model, and permit tracing of carbon dependence throughout the year. In conjunction with complete sampling of the major invertebrate taxa over different seasons and life cycle stages, it should be possible to derive a quantitative measure of carbon dependence for the invertebrate community. Nevertheless, researchers wishing to use isotope analysis to study carbon flow in streams should take care to find a system where carbon sources are more distinctly separated than in this study, and would be wise to include some gut content analysis to corroborate their results.

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