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Differential Utilization of Allochthonous and Autochthonous Carbon By Aquatic Insects of Two Shrub-Steppe Desert Spring-Streams: A Stable Carbon Isotope Analysis and Critique of the Method

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DIFFERENTIAL UTILIZATION OF ALLOCHTHONOUS AND AUTOCHTHONOUS CARBON BY AQUATIC INSECTS OF TWO SHRUB-STEPPE DESERT SPRING-STREAMS: A STABLE CARBON ISOTOPE ANALYSIS AND CRITIQUE OF THE METHOD

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ABSTRACT

DIFFERENTIAL UTILIZATION OF ALLOCHTHONOUS AND AUTOCHTHONOUS CARBON BY AQUATIC INSECTS OF TWO SHRUB-STEPPE DESERT SPRING-STREAMS: A STABLE CARBON ISOTOPE ANALYSIS AND CRITIQUE OF THE METHOD

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Old Dominion University, 1992
Director: Dr. Frank P. Day

Stable carbon isotope analysis, a technique that measures the ratio of stable carbon-12 to stable carbon-13 in abiotic and biotic components of ecosystems, was used to assess the differential utilization of autochthonous and allochthonous carbon by aquatic insects of two cold desert spring-streams in the Columbia Basin of Washington State. The aquatic insects of Snively Springs, a heavily overgrown and densely shaded stream, had isotopic values close to that of stream aufwuchs (substrata scrubate) indicating substantial autochthonous carbon dependence. Aquatic insects of Douglas Creek, the more open and less shaded of the two streams, were equally dependent upon autochthonous and allochthonous carbon. In addition to insects, isotopic values of several other consumers were determined at both streams. The stable carbon isotopes of insects, combined with the isotopic values of the additional consumers, strongly suggest that the food webs of both streams are principally based on autochthonous energy.

Allochthonous carbon isotope values at both streams were consistently in the expected -27.0 °/oo -28.0 °/oo range. Autochthonous carbon isotopic values varied widely within each stream among the various types of producers: epilithic aufwuchs, filamentous green algae, emergent macrophytes, and submerged macrophytes. The wide isotopic variance among autochthonous carbon sources, some of which were consistently in the expected allochthonous isotopic range, made it impossible to *quantify* differential utilization of autochthonous and allochthonous carbon at either stream. However, within the study assumptions, stable carbon isotope technology did enable a limited *qualitative* indication of relative dependence on in-stream and out-of-stream carbon.

The application of stable carbon isotope technology to cold desert spring-stream carbon utilization has substantial problems. Many of the assumptions upon which this study and similar studies in the literature have been based lead to incomplete and/or erroneous data interpretations. I conclude that stable carbon isotope technology is not helpful in unambiguously assessing the differential utilization of allochthonous and autochthonous carbon in the study streams and question its value in similar studies of all freshwater streams.

Key Words: stable carbon isotope, autochthonous, allochthonous, aquatic insects, stream energetics, cold desert, spring-stream

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FOREWORD

I started this study with a conviction that stable carbon isotope technology provided a long sought after new approach to making sense out of stream carbon utilization. As the data developed and as I got deeper into the literature, I became increasingly convinced that many stable carbon isotope investigators have taken insupportable liberties with incomplete and often questionably valid data. Of special concern is the manner in which investigators select one autochthonous carbon component, often epilithon (which will be shown to be as much allochthonous as autochthonous), and proceed to use the questionable epilithon data to represent all autochthonous carbon in the stream. Allochthonous carbon will also be shown to be a much more complex stable carbon isotope mixture than is reported by most investigators. In this paper, the Results based on my initial study plan set forth in the Introduction and Materials and Methods sections are reported. In the Discussion section, several critical fallacies in the assumptions that were used to produce the Results are identified and I attempt to show why most stable carbon isotope studies that purport to successfully compare stream allochthonous/autochthonous carbon utilization in freshwater streams should be considered questionable at best, and probably, invalid.

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1. INTRODUCTION

Odum (1971) showed that understanding energy sources and energy flow through an ecosystem is essential to understanding how that ecosystem is structured and how it functions. Cummins (1974) and Cummins et al. (1984) contended that all streams are in a constant state of change in response to man-engendered perturbations and that any successful stream management strategy must rely on fundamental knowledge of the ecosystem's structure and function which, in turn, demands intimate knowledge of energy flow and material cycling. Minshall (1978) and Minshall et al. (1985) concluded that knowledge of stream energy sources is essential in developing a robust theory of stream ecosystem dynamics and stream structure and function.

The earliest autecological investigators of stream energetics (1930-1960) emphasized the importance of autochthonous carbon (principally algae) as the dominant source of stream energy (Minshall et al. 1983). From 1963 to 1978 the Hynes (1963, 1975) synecological concept of allochthonous carbon as the principal energy source in all streams that were not anthropogenically enriched was essentially unquestioned. Minshall (1967), Fisher and Likens (1973), and Cummins (1974), working in eastern deciduous forest landscapes, published papers strongly supporting Hynes' (1963) generalization that most streams were allochthonously based. Cummins (1974) went so far as to say that maintenance of stream ecosystem quality requires that autochthonous production be subservient to allochthonous inputs.

Minshall (1978) conducted a review of published stream energy studies and discovered that at least half of the studies found predominant autochthonous energy bases and that anthropogenic enrichment was not necessarily a factor. Minshall presented evidence that autochthonous energy drives the larger streams of forested areas and streams of all sizes in arid and semi-arid regions.

In support of Minshall, Busch and Fisher (1981) studied the energetics of an Arizona desert stream and found that their study stream was autochthonously fueled. Naiman (1981) presented evidence that most desert stream energy is produced within the stream. Cushing and Wolf (1982) determined that Rattlesnake Springs, a cold desert spring-stream of the Columbia Basin (near Snively Springs) was autochthonously driven. Interestingly, Busch and Fisher (1981) further suggested that many desert streams not only produce adequate energy for their own sustenance but that they transport organic matter to terrestrial consumers -- an intriguing reversal of the original Hynes (1963) terrestrial to aquatic energy exchange generalization.

The River Continuum Concept (RCC) (Vannote et al. 1980) predicts that as temperate, forested streams increase in size and the forest canopy opens, instream photosynthesis increases, grazing on algae increases, and a gradual shift from allochthonous to autochthonous carbon resources takes place. In addition to this natural stream continuum shift in energy resource dependence, disturbances such as logging, burning, flooding, earthquakes, pesticide or other chemical contamination, and added nutrient sources can affect the relative quantities of allochthonous and autochthonous energy available to the stream (Cummins et al. 1983). How do these changes in energy sources affect the stream? Are some niches destroyed and others created? Is stream stability affected? Are some stream consumers restricted to certain streams because of the availability or lack of autochthonous and allochthonous carbon? Is stream biodiversity affected? Or because most stream primary consumers are indiscriminate, opportunistic detritus feeders (Koslucher and Minshall 1973), does it matter whether the stream energy is allochthonous or autochthonous or some mixture thereof?

It is generally agreed that stream ecosystems are primarily fueled by the metabolism of organic detritus (Wetzel 1975). Stream detritus comes from two major sources. Allochthonous detritus consists of the residue of carbon that is fixed outside the stream boundaries and transported into the stream. Examples of allochthonous detritus are: leaves, twigs, flowers, nuts, limbs, roots of terrestrial vegetation, feces and carcasses of terrestrial animals, respired CO_2, and dissolved organic matter from terrestrial sources. Autochthonous detritus is the residue of plants and animals that are produced within the stream wetted perimeter, e.g., all floating and sessile algae, carbon-fixing and heterotrophic bacteria, aquatic fungi, submerged aquatic macrophytes, emergent macrophytes, and the feces and carcasses of zooplankton, aquatic insects, crustaceans, fish, and other stream fauna.

Determining the relative importance of allochthonous and autochthonous carbon sources to aquatic consumers has always been difficult (Chapman 1966). Traditionally, the methods used to study autochthonous/allochthonous carbon resource utilization have been field and/or laboratory observation (Pimental 1961), antigen/antibody analyses (Rothschild 1966), biological pigment tracers (Putnam 1965), radioactive tracers (Odum and Golley 1963, McCullough et al. 1979 a,b) and gut content analysis (Isely and Alexander, 1949). Gut content analysis seems to be the method most often used (Petelle et al. 1979).

Chapman and Demory (1963) used gut content analysis in an attempt to determine whether aquatic insects of an Oregon stream fed principally on aquatic algae or terrestrial detritus. They found the gut content method unsatisfactory and the results unreliable, largely because of the inevitable large quantity of amorphous organic matter that could not be identified to source. Chapman (1966) attempted again to use gut content analysis in assessing the relative contribution of allochthonous and autochthonous energy to Deer Creek, Oregon. He again concluded that gut content analysis is a poor tool for determining stream energy sources and suggested the need for vastly improved methods such as chemical analysis, polarized light separation, carbon/nitrogen ratio analysis, chromatography, and immunoelectrophoresis. Stable carbon isotopes were not considered (D. Chapman, personal communication).

Aside from the unidentifiable amorphous mass always present in gut contents, investigators cannot be sure which identified stomach contents will actually be assimilated by the animal. Stable carbon isotopes ostensibly minimize both of these problems. Isotope ratios are taken directly from animal tissue and reflect only assimilated carbon (Fry and Sherr 1984, Estep and Vigg 1985). If desired, a ratio of stomach contents can be compared to the tissue ratio and thereby examine assimilation (McConnaughey and McRoy 1979).

A major advantage of the stable carbon isotope approach is that field study measurements of existing isotopic distributions in some ecosystems can show how ecosystem components are energetically connected. Stable carbon isotopes can then be used to track alterations in energy flow following perturbations and to show which processes and components are most sensitive (Peterson and Fry 1987). With these data, stream ecosystem managers can more accurately predict the probable effect of decisions that might alter the relative availability of terrestrial and aquatic carbon resources to a stream's trophic economy.

AUTOCHTHONOUS DEFINED FOR THIS STUDY

Some investigators, e.g., Mook and Tan (1991) and Rosenfeld and Roff (1992) equate autochthonous production to algal production. In stable carbon isotope analysis it is attractive to adopt this equation because algal isotopic values are usually distinct from allochthonous carbon; whereas, other autochthonous production isotopic values (submerged macrophytes, emergent macrophytes) are less distinct or not distinct at all from terrestrial carbon (O'Leary 1981, Osmond et al. 1981, Raven et al. 1982). However, it is not biologically correct to say that stream autochthonous production consists only of algae or any other single autochthonous component. Autochthonous production, as defined by Wetzel (1975), and for purposes of this study, includes any producer indigenous to the stream. That includes filamentous algae, suspended algae, epilithic and epiphytic aufwuchs, sessile algae, autotrophic bacteria, heterotrophic bacteria and fungi, submerged macrophytes, emergent macrophytes and carcasses and feces of stream-dwelling animals. Among these, this study specifically addresses the emergent macrophytes, (watercress and cattails), epilithic aufwuchs, filamentous algae, and the submerged macrophyte (Potamogeton pectinatus).

Of particular concern in comparing autochthonous and allochthonous carbon utilization is that in stable carbon isotope analysis, there is a critical physiological distinction between emergent macrophytes and other autochthonous primary producers. Emergent macrophytes convert atmospheric CO_2 into plant tissue during photosynthesis. As a result, emergent macrophytes consistently have essentially the same $\S^{13}C$ signature as terrestrial C-3 plants, i.e. about -27.0 ^{o}/oo to -28.0 ^{o}/oo. In sharp contrast, other autochthonous primary producers use some component of dissolved inorganic carbon (DIC) in the stream as a carbon source during photosynthesis. These DIC-converting autochthonous producer isotopic values vary spatially, temporally, among and within species, and in response to environmental factors such as stream flow velocity, photosynthetic rate, pH, and insolation (O'Leary, 1981). In the present study's streams, DIC-converting autochthonous producer §^{13}C values ranged from -18.3 °/oo to -26.2 °/oo. In the literature, these autochthonous values range from -10.0 °/oo ((Rounick and James 1984) to -43.0 °/oo (Rau 1980).

For the present study, terms "autochthonous," "endogenous," and "in-stream" are used synonymously. In like manner, "allochthonous," "exogenous," "terrestrial," and "out-of-stream" are used interchangeably.

STABLE CARBON ISOTOPE TECHNOLOGY

The stable isotopes of carbon are carbon-12 (^{12}C) and carbon-13 (^{13}C); these isotopes occur in inorganic carbon in an approximate ratio of 98.9:1.1, (O'Leary 1988). As this inorganic carbon is taken up by the biosphere and fixed into organic carbon, physical and chemical fractionation processes cause the ^{13}C/^{12}C ratio to change. These isotopic fractionations cause the ^{13}C/^{12}C ratio to be distinctive in various types of primary producers and to a lesser degree in consumers in different trophic levels (McConnaughey and McRoy 1979, Rau et al. 1983, Rounick and Hicks 1985, Winterbourn et al. 1986, Peterson and Fry 1987).

To illustrate how stable carbon isotopes can be used to track energy flow through an ecosystem the following example is provided. The world-wide §^{13}C value of atmospheric CO_2 is -7.0 °/oo (O'Leary 1988), indicating that atmospheric CO_2 is 7.0 °/oo more negative (less ^{13}C) than the PDB standard. When atmospheric CO_2 is fixed by C-3 photosynthesizing plants, ^{13}C is discriminated against (Park and Epstein 1960, Deniro and Epstein 1978) and the diffusion/carboxylation fractionation causes further depletion of the §^{13}C value of C-3 plants to an average of about -27.0 °/oo globally (O'Leary 1981). In other words, terrestrial C-3 plants have an average fractionation or delta (Δ) §^{13}C value of about -20.0 °/oo [-27.0 °/oo - (-7.0 °/oo) = -20.0 °/oo].

When animals consume these C-3 plants, ^{12}C is sometimes discriminated against very slightly and there can be a small enrichment (about 0.0 °/oo to 1.0 °/oo) at the primary consumer level (Rau et al. 1983). Rau et al. (1983) and Peterson and Fry (1987) suggested that each successive consumer trophic level has a 0.0 °/oo to 1.0 °/oo enrichment, so a tertiary consumer in a pure C-3-based terrestrial energetics scheme might have a §^{13}C value of -24.0 °/oo. However, it should be pointed out that many investigators find the stable carbon isotope level of resolution too coarse to delineate consumer trophic levels (Gearing et al. 1984, Winterbourn et al. 1986). The possibility of errors in sample processing and in isotope analysis often exceeds the very slight enrichment between consumer trophic levels (Gearing et al. 1984). Although the stable carbon isotope technology is somewhat coarse in resolving power, Cowie (1980), Rounick et al (1982), Rounick and Hicks (1985) and Rosenfeld and Roff (1992) contended that the method is exceptionally well-suited for assessing the proportional utilization of autochthonous and allochthonous carbon by stream consumers.

PURPOSE OF THE STUDY

The purpose of this study was to utilize stable carbon isotope technology to compare the carbon base of a small cold desert spring-stream that is heavily shaded (Snively Springs) to that of a similar nearby stream that is unshaded and open to insolation (Douglas Creek). Based on the literature (see Previous Studies below), the more shaded Snively Springs was expected to have a proportionally greater dependence on allochthonous carbon than the more insolated Douglas Creek.

In addition to the purpose stated above, the study was designed to evaluate the suitability of stable carbon isotope technology for establishing differential carbon resource utilization in cold desert spring-streams. On a broader scale, the study was intended to contribute to a better understanding of the energy linkage between aquatic and terrestrial ecosystems.

The null hypothesis was:

> *"Using stable carbon isotope values of composite samples of aquatic insects as indicators, there is no difference in the proportions of autochthonous and allochthonous carbon resources used by the aquatic insects of Snively Springs and Douglas Creek."*

ASSUMPTIONS OF THE STUDY

The derived/stated and implied assumptions under which this study was conducted are listed below. Most of the stated assumptions were derived from Rounick et al. (1982), Rounick and Winterbourn (1986), Peterson and Fry (1987), and personal discussions with Brian Fry and Robert H. Garrit (Woods Hole Ecosystems Center), and Greg H. Rau (University of California, Santa Barbara).

DERIVED/STATED ASSUMPTIONS

1. Consumers have the same stable carbon isotope ratio as their principal food source.

2. The stable isotopes of carbon move with little or predictable alteration through food chains.

3. Stable carbon isotopes provide an integrated history of a consumer's recent feeding habits.

4. Autochthonous carbon is isotopically distinct from allochthonous carbon.

5. Terrestrial C-3 plants have a globally consistent isotopic value and detritus from these plants has the same isotopic ratio as the living plants.

6. Stream microheterotrophs, bacteria and fungi, have the same isotopic value as their substrate.

7. The isotopic value of total dissolved inorganic carbon (DIC) determines the isotopic value of DIC-photosynthesizing autochthonous producers.

IMPLIED ASSUMPTIONS

The implied assumptions listed below were not actively considered in developing the study constraints. However, as the study progressed, it became increasingly apparent that these passively-derived, built-in, implied assumptions had a substantial effect on the study-especially the interpretation of data.

1. Chemoautotrophic bacteria are negligible carbon sources in streams and need not be considered in assessing allochthonous/autochthonous carbon utilization.

2. Terrestrially-derived respired CO_2 (used as a photosynthesizing carbon source) is useful in explaining why some stream algae have an exceptionally depleted isotopic ratio, but is not considered an allochthonous carbon source.

3. If anthropogenic activity is remote from a stream stretch and there are no surface streams entering the study stretch for several km upstream, there is little likelihood of nutrient enrichment which, in

turn, might affect the stable carbon isotope analysis.

PREVIOUS STUDIES

Fry and Sherr (1984), Rounick and Winterbourn (1986), and Peterson and Fry (1987) provided reviews of stable isotope literature. Stable carbon isotopes have been used to identify major carbon pathways in rivers and lakes (Rau 1980, Rounick et al. 1982, Peterson et al. 1986, Bunn et al. 1989, Kling et at. 1992) the ocean, (McConnaughey and McRoy 1979, Fry 1981, Fry et al. 1982), and estuaries and salt marshes (Peterson et al. 1985, Cifuentes et al. 1988, Matson and Brinson 1990). Stable carbon isotopes have also been used to track terrestrial carbon flow (Craig 1953, Fry et al. 1978, Petelle et al. 1979, Smedley et al. 1991).

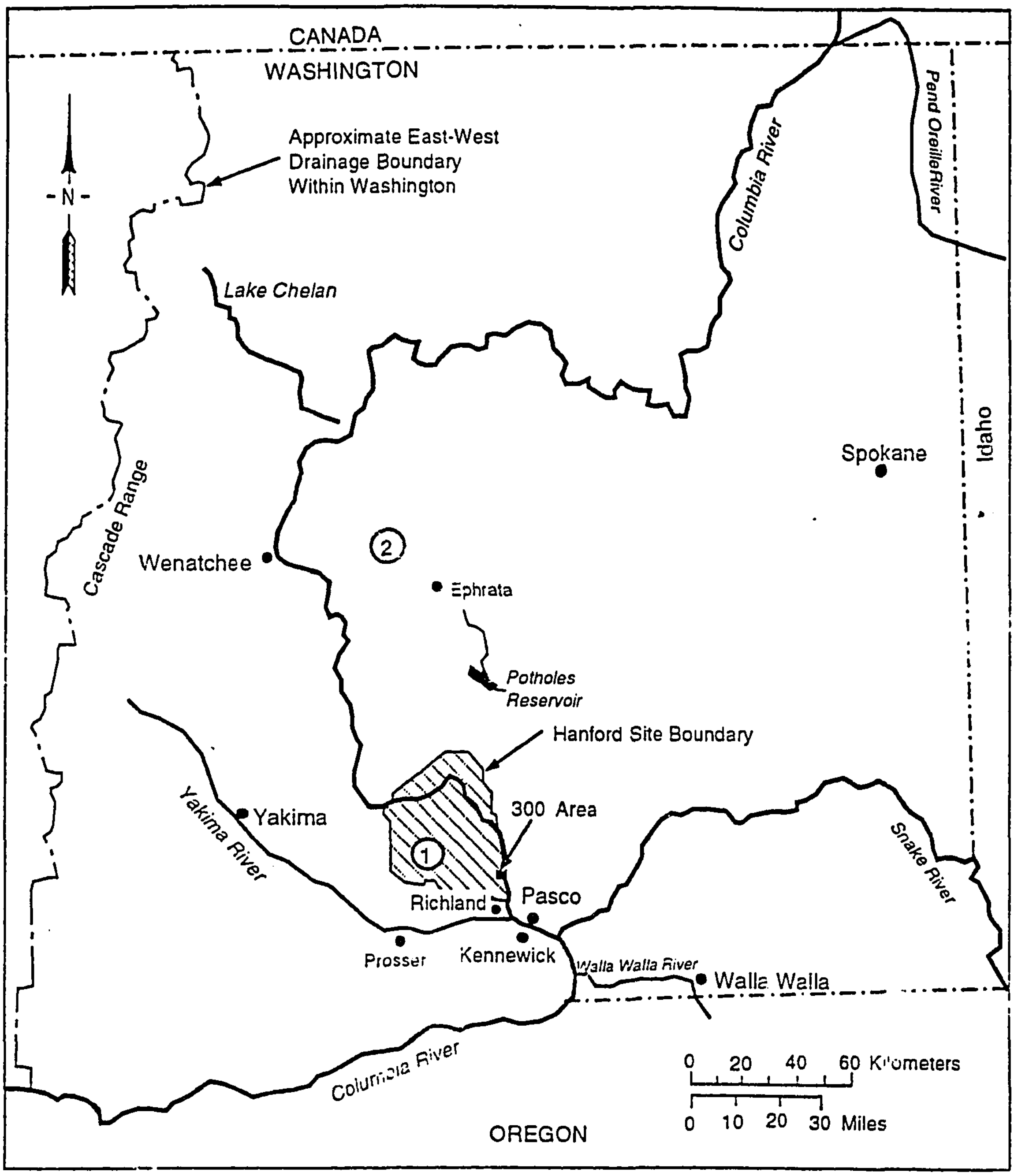
Several investigators have used stable carbon isotopes specifically to address autochthonous/allochthonous carbon resource partitioning in streams. Rounick et al. (1982) found that fauna of small forested streams in New Zealand depended primarily on allochthonous sources of carbon; whereas, the fauna of grassland streams demonstrated variable utilization of endogenous and exogenous energy sources. Peterson et al. (1986) found that stable carbon isotope technology was not suitable for assessing autochthonous and allochthonous carbon utilization in a tundra river in Alaska because carbon sources were not isotopically distinct. However, Bunn et al. (1989) claimed to have successfully used stable carbon isotopes to determine that terrestrial carbon was the principal energy source for aquatic invertebrates in a tundra river of Quebec. Araujo-Lima (1986) examined the relative in-stream and out-of-stream carbon resources used by detritivorous fishes of the Amazon River and concluded that these critically important human food fishes depend principally on autochthonous carbon. Rounick and Winterbourn (1986) investigated carbon resource utilization by benthic invertebrates in two British river systems, and concluded that small woodland stream invertebrates depended principally on allochthonous carbon while the benthos at more open stream sites had a greater dependence on autochthonous carbon. Rosenfeld and Roff (1992) discovered that invertebrates of southern Ontario's unforested streams fed primarily on autochthonous carbon and that invertebrates from forested streams also had a substantial seasonal dependence on in-stream carbon sources.

STUDY AREA

Snively Springs is a small endorheic stream located within the U.S. Department of Energy's Hanford Reservation near Richland, Washington (Figure 1). It arises from seeps in a canyon and then flows through open country for about 3.6 kilometers before disappearing as a result of seepage and evapotranspiration (Note: the exact stream terminus varies with environmental conditions).

The following descriptive data are from Gaines (1987). The drainage basin is about 40 km^2. The Stream average width is 1 m and average depth is 10 cm. Stream base flow is 0.04 m^3/s, and the gradient is 2 %. Mean annual

Figure 1. Stream Locations Within the Columbia Basin: 1) Snively Springs; 2) Douglas Creek.


CANADA
WASHINGTON
N
Approximate East-West Drainage Boundary Within Washington
Lake Chelan
Columbia River
Pend OreilleRiver
Idaho
Spokane
Cascade Range
Wenatchee
②
Ephrata
Potholes Reservoir
Hanford Site Boundary
300 Area
①
Yakima
Yakima River
Richland
Pasco
Snake River
Prosser
Kennewick
Walla Walla River
Walla Walla
Columbia River
0 20 40 60 Kilometers
0 10 20 30 Miles
OREGON

precipitation in the area is about 14 cm. Maximum and minimum water temperatures were 22° (July) and 2° (January), respectively, during 1985-1986. The surrounding terrestrial community is a shrub-steppe desert dominated by big sage (Artemesia tridentata). Peachleaf willow (Salix amygdaloides) and wild rose (Rosa sp.) are the dominant riparian plants. The in-stream macrophyte community is dominated by watercress (Nasturtium officinal) and cattail (Typha latifolia). The combined riparian vegetation and dense emergent macrophyte growth provide a conspicuous canopy to the stream. Because of shading, the algal community is not well developed. Stream macrofauna consist of a variety of aquatic insects (see Gaines et al. 1989 for complete list), one species of amphipod (Gammarus sp.), and a small population of crayfish (Pacifastacus leniscul us). There are no snails or fish in Snively Springs. See Cushing (1988) for a more detailed description of the ecosystem.

Three sites at Snively Springs were sampled (Figure 2). These sites were spaced at roughly equal intervals along the permanently flowing stream bed as described by Cushing (1988). Site 1 was at the spring source of the stream near the abandoned Snively homestead. Site 2 was near a ford on the down-stream side of a moderately steep-walled canyon. Site 3 was about 800 m downstream of Site 2 in an open, flat meadow and several hundred meters upstream of where the stream ends.

Douglas Creek is a cold desert spring-stream located about 300 km north of Snively Springs in the desert rain shadow of the Cascade Mountains (Figure 1).

Figure 2. Photographs of Snively Springs Sampling Sites (from top to bottom, sites 1-3).

It is in Douglas County near the small village of Palisades. The following descriptive data are from Gaines (1987). The average width of the stream is 4.0 meters and the average stream depth is 31 cm. The flow is 0.6 m^3s. The drainage basin is 530 km^2. Stream temperature is highest in July at 19°C and lowest in February at 5°C.

Riparian vegetation is dominated by rice cutgrass (Leersia oryzoides L.) and peachleaf willow (Salix amygdaloides). Aquatic macrophyte growth is dominated by watercress (Nasturtium officinal) and there are seasonally dense growths of pondweed (Potamogeton pectinatus) in the stream pools. Periphytic growth is abundant and there are seasonal blooms of filamentous green algae (Cladophora sp.). Stream macrofauna consist of a wide variety of aquatic insects (see Gaines et al. 1992 for complete list), snails, crayfish (Pacifastacus lenisculus), mountain suckers (Catostomus platyrynchus), and rainbow trout (Onchorhynchus mykiis).

Douglas Creek is a much longer stream than Snively Springs; however, access is limited on the upstream end of the study area by an irreparable road washout. About 300 m downstream of the stream study stretch, the entire stream water flow is removed for irrigating adjacent farmland. The accessible, undisturbed length of Douglas Creek is about the same as Snively Springs (3.6 km) and again three sampling sites were used. Site 1 was just downstream (50 m) from the road washout in a steep-walled, narrow canyon. Site 2 was about 150 m downstream from the mouth of the canyon. Site 3 was in an open desert plain.

Site 1 received noticeably less insolation than Sites 2 and 3 because of the shading effect of the steep canyon walls (Figure 3).

Figure 3. Photographs of Douglas Creek Sampling Sites (from top to bottom, sites 1-3).

2. MATERIALS AND METHODS

PILOT STUDY

Prior to the main study, during the period July through September 1990, a pilot study was conducted with the following objectives:

1. Determine if the allochthonous and autochthonous carbon isotopic values were distinct at the study streams.

2. Determine the total number of isolable and isotopically distinct carbon sources available to the food web of each study stream.

3. Determine if there were any C-4 photosynthesizing plants or crassulacean acid metabolizing (CAM) plants in the riparian zone of either study stream. The presence of substantial numbers of either C-4 or CAM plants would probably have caused the study plan to be abandoned (see explanation below).

4. Determine if Snively Springs had enough aquatic insect biomass to sustain monthly sampling without adversely affecting the ecosystem.

Gaines et al. (1989) commented on the paucity of the aquatic insect population of Snively Springs.

5. Determine the availability of additional food web components of various trophic levels whose isotopic values might lend added perspective to the trophic economy of both streams.

6. Determine if living and dead allochthonous and autochthonous plants had different isotopic signatures.

The pilot study samples were collected and processed in essentially the same manner as the main study samples as described below. Woods Hole Oceanographic Institute expedited turn-around time on the ratio mass spectrometry of the pilot study samples so I could adapt my materials and methods in response to the pilot study findings.

At least one sample of each genus of riparian vegetation at all sites was identified and analyzed for isotopic value. The literature shows that C-4 plants are consistently and substantially more enriched than C-3 plants and that CAM plants have a wide range of isotopic values that overlap both C-3 and C-4 plants (O'Leary 1981). There were no C-4 or CAM plants at either stream. This finding simplified the allochthonous sampling procedures because all terrestrial C-3 plants are reported to have a consistent and narrow range of values (Peterson and Fry 1987) which enabled the collection of composite allochthonous samples that were not necessarily collected according to relative abundance.

Allochthonous composites that were taken directly from the living tree, shrub, herb, or grass were tested against recognizable allochthonous detritus taken from the stream (leaves, twigs, branches, roots). Based on three samples each of live and detrital material, there was no significant difference in the isotopic values of these composites. Allochthonous composites taken from live plants were used for samples because they were easier to gather and there was less chance of leaching and contamination by in-stream micro-decomposers.

At least one sample of each of the following autochthonous producer groups; filamentous algae, epilithic aufwuchs (scrubate), submerged macrophytes, and emergent macrophytes was collected. Isotopic analysis indicated that emergent macrophytes (watercress and cattail) had the same isotopic value at both streams and that these isotopic values were not significantly different from the isotopic value of riparian plants at both streams. Aufwuchs, filamentous algae, and submerged macrophytes were found to be isotopically distinct from each other and from terrestrial carbon. As a result of the isotopic differences among the various autochthonous producers, the autochthonous carbon samples were not composited. Because of the difficulty in recognizing autochthonous detritus in the stream and because the small samples indicated that live and detrital tissue had the same isotopic value, live autochthonous samples for filamentous algae, emergent, and submerged macrophytes were collected. The aufwuchs samples, of course, necessarily contained both live and detrital material (Lock and Williams 1981, Hutchinson 1981).

The primary concern of this study was the assessment of proportional utilization of energy sources in the two study streams using aquatic insects as the principal indicator. At the outset, there was some doubt that there would be adequate insect biomass (10 mg dry weight) for all monthly samples. The pilot study indicated that there would be adequate insect biomass to obtain the 10 mg dry weight required at each site if insect composites were used. There was not adequate biomass to support sampling insects by species or functional feeding group as described by Cummins (1973). At this point, it was determined to use composite insect samples and to test these composites against a single functional feeding group at some point during the study when seasonal population increases provided enough biomass for such a test. Insect samples were held in submerged net baskets for 12 hours to permit gut purging prior to drying and powdering.

Sampling fortuitously revealed an abundance of crayfish at all sites. It was reasoned that the omnivorous general scavenger feeding habits of crayfish should yield a good cross check against the composite insect samples and should provide a broader picture of carbon resource partitioning in both streams. Consequently, crayfish were sampled along with insects at every site and at every monthly sampling period. Pilot study sampling revealed additional aquatic food web components at each stream, e.g., amphipods, snails, trout, and suckers. These additional food web components were sampled quarterly in order to gain a broader understanding of the total food web carbon dependence.

During the pilot study, several attempts were made to isolate suspended algae (phytoplankton) from both streams; however, all samples were heavily contaminated with detrital seston of unknown origin. Possible alternative sampling procedures for suspended algae were discussed with two established stable carbon isotope investigators: Bruce Peterson (personal communication) and Greg Rau (personal communication) indicated that there is no adequate procedure for isolating stream phytoplankton for isotopic analysis. Bruce Peterson (personal communication) is working on a procedure to extract chlorophyll from seston-contaminated algae samples and analyze the chlorophyll for isotopic value; however, the procedure has not yet been tried. The end result was that isotopic values for suspended algae and sessile algae were not obtained.

Autochthonous carbon has many different isotopically distinct components (Tables 1 and 3). A pivotal decision in this study concerned how to derive one autochthonous value to represent all autochthonous carbon. Watercress and cattails were not good choices, because they are atmospheric CO_2 converting plants and are, therefore, not isotopically distinct from terrestrial carbon. Filamentous green algae is grazed by zooplankton, amphipods, and crayfish (Pennak 1978), and is used by most aquatic insects in detrital form (Cummins 1974); however, the filamentous form, which is the only form that can be consistently collected, is only available in the summer. The difficulty in sampling phytoplankton and sessile algae has already been explained. Averaging isotopic values of the various autochthonous components was not deemed acceptable because of the wide variance in isotopic values, seasonality of some components and because the respective component biomasses varied widely. Epilithic aufwuchs seemed to offer the best available representative autochthonous isotopic value because it was isotopically distinct from terrestrial carbon, consistently available year-round, and universally recognized as a major food source for aquatic insects (Lock and Williams 1981). Epilithic aufwuchs (epilithon, rock scrubate) had also frequently been used by previous investigators as the autochthonous basis of comparison with allochthonous carbon (Rounick et al. 1982, Rounick and Hicks 1985, Peterson et al. 1986, Bunn et al. 1989)

MAIN STUDY - FIELD COLLECTION PROCEDURES

Three replicates of allochthonous carbon, epilithic aufwuchs, insect, crayfish, and water samples were collected monthly at each stream. Single amphipod, sucker, and trout samples were collected quarterly. Snail, submerged macrophyte, emergent macrophyte, and filamentous algae were sampled without replication as frequently as laboratory analysis funding permitted but with no predetermined schedule. Sample size for all tissue samples was approximately 250 mg wwt. Water sample size was 500 ml.

Leaves and twigs of live riparian plants were collected into composite replicates, generally according to relative abundance at each site, but with no rigid protocol in that regard. Autochthonous primary production samples were not composited because of the wide isotopic variance among these producers. Aufwuchs-bearing substrata were taken from the stream by hand and kept submerged in a bucket of stream water until returned to the lab. Submerged macrophytes (leaves and stems) were collected by hand and rinsed free of sediment in the stream. Filamentous green algae was collected by hand or with a large (100 ml) syringe used as a slurp gun. Emergent macrophyte (watercress and cattail) leaves and stems were collected by hand.

Aquatic insects were collected using D-ring dip nets and a Surber sampler. All aquatic insects collected at a site, regardless of species or functional feeding group, were combined into one composite sample (see exception below). During June and July, 1991, insects were abundant at both streams and caddisflies (*Hydropsyche* sp.) were analyzed separately from aquatic insect composites for each site. The purpose of this effort was to see if the isotopic signature of this particular insect genus from the filter/collector functional feeding group varied significantly from the multiple feeding-group composites. The caddisflies were collected with dip nets and/or removed by hand from substrata.

Crayfish were collected using seines, dip nets, and a bait cast net. Amphipods (*Gammarus* sp.), another crustacean, were found only at Site #2 at Snively Springs and were collected as a cross check against the isotopic value of the aquatic insects at that one site. The amphipods were collected with D-ring dip nets.

Mountain suckers (Catostomus platyrhynchus) were present at two of the three Douglas Creek sites (a large waterfall has served as a barrier to sucker migration at the farthest upstream site). These fish are not found in Snively Springs. This particular sucker feeds by scraping stream substrata and was collected as a cross check against the epilithic aufwuchs collection method. It was thought that if the aufwuchs collection method was reliable, and the stable carbon isotope technology was predictable, the sucker tissue isotopic signature should match the aufwuchs isotopic value closely (Rounick et al. 1982). Suckers were collected quarterly using a bait cast net or electroshocker.

Rainbow trout (Oncorhyncus mykiis) were found at all Douglas Creek sites, but not found at Snively Springs. These fish were collected because they constitute the top aquatic predator in the Douglas Creek ecosystem and it was thought, based on Rounick et al. (1982), that their isotopic value should represent an isotopic integration of carbon resources of the total Douglas Creek food web. Trout were collected quarterly using a bait cast net or electroshocker.

Snails (Physa sp.) were found only at Douglas Creek. These gastropods feed principally by grazing aufwuchs from stream substrata and were collected for isotopic comparison with suckers and aufwuchs. The "predictability" aspect of the technology would indicate that these three organisms should have essentially the same §^{13}C. Snails were collected with dip nets and picked by hand from the stream.

WATER SAMPLES

Three replicates of water samples for total dissolved inorganic carbon (DIC) analysis were collected monthly at each stream. Approximately 500 ml of water were taken from each site in an acid washed, 1-liter Nalgene screw cap bottle. The water samples were fixed immediately with 1 ml saturated mercuric chloride per 250 ml of water. The water sample bottles were then sealed with black electrical tape and refrigerated constantly until analyzed at Woods Hole Oceanographic Institute (see laboratory analysis procedures below for instrumentation).

MAIN STUDY - LABORATORY PREPARATION OF SAMPLES

Allochthonous composite samples were placed in brown paper bags and oven-dried at 60°C for 72 hours. Dried samples were ground into a fine powder using a Wiley Mill (40 mesh) and at least 10 mg dry weight was placed in a screw cap glass vial for shipment. (Note: All following tissue samples were dried at 60°C for 72 hours, powdered with mortar and pestle or Wiley Mill, and stored in screw cap glass vials for batch shipment.)

Epilithic aufwuchs was scraped from stream substrata using a single edge razor blade or scalpel. The substrata was then scrubbed with a stiff bristle brush and rinsed with distilled water. The scrapings and rinse water were poured in a large wide-mouth beaker and placed in a drying oven at 60°C until all water evaporated. The residue was treated with 1N-HCl to remove carbonates prior to isotopic analysis. Submerged macrophytes, filamentous green algae, and emergent macrophytes were rinsed thoroughly with distilled water and dried separately in paper bags; they were then prepared for shipment in the manner described above for allochthonous composites.

Insects and amphipods were held in separate submerged small mesh nylon baskets for 24 hours to permit gut purging. They were then oven-dried in glass beakers. The separate caddisfly samples of June and July were prepared in the same manner.

Snail samples were dried and powdered whole. The powdered sample was bathed in 1N-HCl to remove all inorganic carbon from the shell and then redried at 60°C for 72 hours.

Pure crayfish tail muscle and sucker and trout hypaxial muscle tissue were extracted with scalpel and tweezers. Special care was taken to avoid contaminating fish muscle samples with bone, skin, or scales because these tissues seem to fractionate carbon in an inexplicable fashion (Estep and Vigg 1985).

STABLE CARBON ISOTOPE LABORATORY ANALYSIS PROCEDURES

Tissue samples were flash combusted at 1800°C and water samples were acidified; the resulting gases were reduced in a Heraeus elemental analyzer. Combustion and acidification gases were cryogenically separated and pure CO_2

was measured for stable carbon isotope values using a Finnigan Delta S stable isotope ratio mass spectrometer. High purity tank carbon dioxide was used as a working standard during sample analysis. This working standard was calibrated against Pee Dee Belemnite (PDB) limestone which has an arbitrary §^{13}C value of zero. This PDB standard is developed from the fossil skeleton of a Cretaceous belemnite, Belemnitella americana, an extinct cuttlefish found in South Carolina's Pee Dee formation (Craig 1957). The §^{13}C of samples is expressed in parts per thousand (^{o}/oo) either enriched (more positive) or depleted (more negative) relative to the PDB standard. The formula for calculating the §^{13}C value of any sample is:

$$\S^{13}\text{C} = \frac{{}^{13}\text{C}/{}^{12}\text{C sample} - {}^{13}\text{C}/{}^{12}\text{C PDB standard}}{{}^{13}\text{C}/{}^{12}\text{C PDB standard}} \times 1000 = {}^{o}/\text{oo}$$

STATISTICAL ANALYSIS

Statistical analyses were done with the STATVIEW II software package on a Macintosh MAC-2 computer. The STATVIEW II software has copyrighted T-Test and analysis of variance (ANOVA) and regression procedures which were used without modification. For comparison of population means, I used the Mann-Whitney T-Test and for analysis of variance, I used the Kruskal-Wallis Test. Unless otherwise specifically stated, all statistical significance findings are based on a 95% confidence level.

3. RESULTS

SNIVELY SPRINGS

Snively Springs insects had an annual mean §^{13}C value of -26.2 ^{o}/oo (Table 1). A Kruskal-Wallis analysis of variance showed no significant difference among the four seasons. Because the study sampling methods were biased toward the capture of larger insects, most of the insect biomass of the composite samples was from carnivorous insects. Damselflies (Odonata), water boatmen and water striders (Hemiptera), and carnivorous water beetles (Coleoptera) comprised approximately 95% of the sample biomass. Caddisflies, black flies, horseflies, and midges accounted for the remaining sample biomass.

During June and July 1991, a caddisfly (Trichoptera) was separated from the insect composites. The caddisfly sampled (Hydropsyche sp.) is a net filter feeder that strains particulate organic matter from the water (Pennak 1978). Table 2 shows a mean §^{13}C value for the Snively Springs caddisflies of -26.2 ^{o}/oo which is not significantly different from the insect composites (Table 1). Therefore, the caddisfly was included in the discussions with aquatic insect composites and not addressed separately.

Table 1. Annual Mean Stable Carbon Isotope Values of Allochthonous and Autochthonous Carbon, Insects, and Crayfish in Snively Springs and Douglas Creek (October 1990 through September 1991), n=36 for all Parameters. Isotope values are shown with one standard deviation. Units are $^{o}/oo$.

	Alloch	Autoch	Insects	Crayfish
Snively Springs	-27.5±1.1	-26.0±2.8	-26.2±1.1	-24.7±0.6
Douglas Creek	-27.2±0.8	-21.5±4.0	-24.3±2.3	-22.0±1.8

Table 2. Mean Isotopic Values of Selected Additional Fauna at Snively Springs and Douglas Creek Between October 1990 and September 1991. Fauna Sampled Were Not Necessarily the Same at Both Streams and the Numbers of Samples Varied - (n) Is Shown With the Mean Value. Isotopic values are shown with one standard deviation. Units are ^{o}/oo.

	Caddisfly	Amphipod	Snail	Sucker	Trout
Snively Springs	-26.2± 0.8 n=4	-26.3± 0.3 n=7	None -	None -	None -
Douglas Creek	-24.9± 1.4 n=6	None -	-21.0± 1.1 n=3	-18.5± 1.3 n=12	-23.5± 1.8 n=15

Allochthonous carbon at Snively Springs is all from C-3 plants and the annual mean isotopic value of -27.5 °/oo (Table 1) is within the expected isotopic range of terrestrial C-3 plants (Boutton et al. 1980, O'Leary 1981, Petersen and Fry 1987). A Kruskal-Wallis test showed no significant variance among seasons. A Mann-Whitney T-test showed a significant difference ($p=0.0001$) between the population means of aquatic insects and allochthonous carbon. The underlying premise of the $§^{13}C$ approach to assessing differential carbon utilization is that consumers will have the same isotopic value as their dominant or integrated food sources (Rounick and Winterbourn, 1982). Because the aquatic insects of Snively Springs are significantly different ($p=0.0001$) from allochthonous carbon, allochthonous carbon is probably not the principal energy source for this trophic group.

Contrary to expectation (see Introduction), the insects of Snively Springs appear to depend principally on autochthonous carbon. A Mann-Whitney T-test showed that the population mean isotopic value of the insect composites was not significantly different from the epilithic aufwuchs isotopic mean (Table 1). It should be noted that the filamentous green algae (Table 3) is even more isotopically removed from terrestrial carbon. It is particularly important to note that the identified DIC-converting autochthonous components (epilithic aufwuchs and filamentous green algae) are influential enough in the insect diet to overcome the combined influence of terrestrial carbon and the very considerable influence

Table 3. Individual Components of Autochthonous Production With Mean Stable Carbon Isotope Values. The Number of Samples Varied - (n) Is Shown With Each Mean. Isotopic values are shown with one standard deviation. Units are ^{o}/oo.

	Aufwuchs (Scrubate)	Watercress	Cattail	Filamentous Green Algae	Sago Pond-Weed
Snively Springs	-26.0± 2.8 n=36	-28.3± 1.2 n=4	-26.7± 0.6 n=5	-24.2± 2.5 n=2	None -
Douglas Creek	-21.5± 4.0 n=36	-27.7± 0.8 n=8	-26.4± 0.1 n=2	-22.6± 5.1 n=13	-23.4± 1.7 n=9

of atmospheric CO_2-converting autochthonous producers, such as watercress and cattails.

The crayfish at Snively Springs were significantly more enriched than terrestrial carbon (p=0.0001) and were also significantly more enriched (p=0.0001) than the aufwuchs component of autochthonous carbon. The crayfish mean §^{13}C value of -24.7 ^{o}/oo (Table 1) was closest to the mean isotopic value for filamentous green algae at -24.2 ^{o}/oo (Table 3). The crayfish is an omnivorous, general scavenger that indiscriminately ingests living plants and animals and detritus from both terrestrial and aquatic sources. Perhaps the crayfish enrichment can be explained by the preferential assimilation of filamentous algae. Enrichment because of inorganic carbon contamination in the samples from the carapace or digestive tract is ruled out, because only pure, carefully extracted tail muscle was used in the samples. However, there is a possibility that crayfish assimilate inorganic carbon, e.g., marl encrusted on living or detrital foods and carbonaceous mineral particles. Inorganic carbon has a very enriched isotopic value (about 0.0 ^{o}/oo to -2.0 ^{o}/oo) and, if assimilated, would certainly cause enrichment.

A Kruskal-Wallis analysis of variance showed that the crayfish isotopic values did not differ significantly among the four seasons. Crayfish results indicate that this top aquatic predator of Snively Springs is principally dependent on autochthonous carbon and to a significantly greater extent (p=0.0001) than aquatic insects. Crayfish data reinforce the insect composite data (both sample sizes being equal and both sampling periods the same) and provide additional evidence that the carbon base of Snively Springs is autochthonous.

Amphipods are omnivorous general scavengers with a penchant for browsing epilithic aufwuchs (Pennak 1978). Table 2 shows that amphipods had a mean isotopic value of -26.3 ^{o}/oo which was not significantly different from the isotopic value of insect composites. This result reinforces the reliability of the insect composite findings and adds additional evidence to support a conclusion that the entire Snively Springs food web has a predominantly autochthonous carbon base.

The food web of Snively Springs is not complex (Figure 4). There are four grazer chain trophic levels and isotopic samples were taken from three of the four. Zooplankton, e.g., rotifers, cladocerans, ostracods, and copepoda were not specifically sampled. Zooplankton, of course, are included to some degree in epilithic aufwuchs. All fauna sampled at Snively Springs (insects, caddisflies, crayfish, and amphipods) reflect a clear and consistent primary dependence on DIC-photosynthesizing autochthonous carbon resources. This endogenous carbon dependence did not vary by season. Despite the dense canopy of riparian vegetation and the exceptionally high biomass of atmospheric CO_2 converting watercress (Figure 5) which has the same isotopic value as allochthonous carbon, the DIC-converting autochthonous producers apparently provide the dominant carbon base for Snively Springs. Because of the variety of autochthonous

Figure 4. Snively Springs Trophic Levels. Representative Biota For Each Trophic Level Are Provided.

SNIVELY SPRINGS

GRAZER CHAIN Crayfish

Crayfish Carnivorous Insects

Herbivorous Insects Amphipods Zooplankton Crayfish

CARBON SOURCES Detritus Live Autochthonous
(Autochthonous/Allochthonous)

Bacteria - Fungi

Amphipods Zooplankton Filterer - Collector Insects

Crayfish Carnivorous Insects

DECOMPOSER CHAIN Crayfish

Figure 5. Photograph of Watercress in Snively Springs Illustrating Exceptionally High Biomass in this Plant.

production components and their different isotopic values, the proportional utilization of allochthonous and autochthonous resources could not be quantified; however, all evidence supports a qualitative conclusion that the Snively Springs food web depends principally on autochthonous carbon.

DOUGLAS CREEK

Allochthonous carbon at Douglas Creek is derived from C-3 plants (see Pilot Study) and the mean annual isotopic value for allochthonous carbon shown in Table 1 is typical for the range of values established for such plants by Boutton et al. (1980), O'Leary (1981) and Peterson and Fry (1987). A Kruskal-Wallis test indicated no significant difference among seasons for allochthonous carbon.

Autochthonous primary production at Douglas Creek has greater variety and is generally more isotopically enriched than Snively Springs (Tables 1 and 3). As expected, the atmospheric CO_2-converting autochthonous producers (watercress and cattails) had statistically the same isotopic value as allochthonous carbon. The DIC-converting autochthonous producers, on the otherhand, showed a wide variety of isotopic values (Table 3). Using the rationale set forth in the Materials and Methods section, epilithic aufwuchs was chosen to represent autochthonous carbon for comparison with allochthonous carbon. A Kruskal-Wallis test showed no significant seasonal variance for epilithic aufwuchs.

The aquatic insect composite annual mean isotopic value for the study stretch of Douglas Creek was -24.3 °/oo (Table 1). There was no seasonal variance in insect $^{13}C/^{12}C$ ratio. The isotopic value of insect composites was significantly different ($p=0.001$) from both allochthonous carbon and aufwuchs (Table 1). The insect isotopic ratio was approximately mid-way between the isotopic ratios of autochthonous and allochthonous carbon and suggests that the insects use endogenous and exogenous carbon in approximately equal proportions.

Douglas Creek crayfish had a mean annual $\S^{13}C$ value of -21.5 °/oo which is not significantly different from the mean annual $\S^{13}C$ of aufwuchs (Table 1). There was no significant seasonal variance in crayfish isotopic value. The very enriched crayfish $\S^{13}C$ value suggests a principal dietary dependence on aufwuchs which may be ingested with detritus. Crayfish probably also graze filamentous green algae and the sago pondweed (Pennak 1978) which are also isotopically enriched. The possibility that the crayfish assimilate inorganic carbon, which could also cause enrichment of muscle tissue cannot, be ruled out. Although the exact carbon source or sources cannot be determined, it appears that the crayfish population of Douglas Creek depends principally on autochthonous carbon.

Snails (Physa sp.) are aufwuchs grazers (Pennak 1978) and should, therefore, have a $\S^{13}C$ value very close to that of aufwuchs. Table 2 shows that snails have a $\S^{13}C$ value of -21.0 °/oo which is not significantly different from aufwuchs (Table 1). The enriched snail isotopic ratio leaves little doubt that snails are principally dependent on autochthonous carbon and that aufwuchs is probably the specific principal source. The small sample size for snails precluded any analysis of seasonal variance and, to some degree, limits the reliability of inferences from the samples; however, there seems little reason to doubt the snail-aufwuchs carbon relationship.

Suckers (Catostomus platyrynchus) were found only at Site 2 and Site 3. This is a relatively rare fish in Washington that feeds almost entirely on algae which it scrapes from the stream substrata (Wydoski and Whitney 1979). Douglas Creek suckers have an extremely enriched isotopic value (Table 2) -- the most enriched of any identified producer or consumer at either stream. Other investigators have found similar enriched isotopic ratios for suckers (Estep and Vigg 1985, Bunn et al, 1989). Perhaps the sucker enrichment results from preferential assimilation of the pure algal component of aufwuchs as suggested by Estep and Vigg (1985). Hargrave (1973), Calow (1975) and Greig (1976) also found that many aquatic fauna have as high as 65% assimilation efficiency for algae compared to 10% assimilation efficiency for detritus and living macrophytes. Because the isotopic ratio of sucker hypaxial muscle only reflects assimilated foods and since these fish are known to be algae eaters, there is reasonable evidence to indicate that a pure algal sample (probably diatoms) of Douglas Creek might have an isotopic value closely matching that of suckers. It also suggests an indirect sampling method for getting at hard to sample autochthonous carbon components. By sampling a consumer that is known to feed principally on a difficult to isolate carbon source, such as diatoms, one should be able to derive a somewhat reliable isotopic value for the carbon source, since the consumer should have very nearly the same isotopic value as its principal food (Rounick and Winterbourn 1982).

For suckers there is another possible explanation for the enriched §^{13}C. Suckers are substrata scrapers and must incidentally ingest substantial quantities of inorganic carbon such as marl encrustations or particles of mineral carbon. It would seem possible that, like crayfish, these inorganic carbon ingestions could have some effect on tissue carbon. If so, the effect would be enrichment since limestone has an isotopic value of about 0.0 ^{o}/oo. Although the exact carbon sources and/or assimilation mechanisms are not clear, suckers demonstrate a particularly strong principal dependence on autochthonous carbon. It is interesting that although both suckers and snails graze epilithic aufwuchs, suckers are significantly more enriched than snails ($p=0.0001$). The reasons for this isotopic difference are not clear.

Rainbow trout (Onchorhyncus mykiis) were present at all three sites. The trout is the top aquatic predator in Douglas Creek and should, therefore, reflect the total food web's integration of carbon sources (Rounick et al. 1982). The trout mean annual isotopic value for the entire study stretch was -23.5 ^{o}/oo (Table 2). There were no seasonal isotopic differences for trout. The trout §^{13}C was slightly more enriched but not significantly different from insect composites and caddisflies at the stream. Not surprisingly, trout appear to feed primarily on aquatic insects (Hynes 1970).

There are four grazer chain trophic levels in the aquatic food web of Douglas Creek (Figure 6), all of which were sampled during this study. Suckers and snails are primary consumers and both show a decisive, principal dependence on autochthonous carbon. Crayfish span two trophic levels as primary consumer and predator; they also demonstrate a strong principal dependence on endogenous carbon. The insect composites and caddisflies are both primary consumers and predators and both these food web components indicate approximately equal dependence on in-stream and out-of-stream carbon. Trout are the top aquatic predators and their stable carbon isotope ratio shows a slight bias toward autochthonous carbon.

It is reiterated that "autochthonous" in the above discussion refers only to DIC-converting autochthonous producers (aufwuchs, sago pondweed, filamentous green algae). The atmospheric CO_2-converting autochthonous producers (watercress and cattails) and their effect on the food web cannot be separated from allochthonous carbon. Therefore, in order for food web components to register a predominantly DIC-converting autochthonous carbon dependence, they must overcome the combined effects of allochthonous stream inputs and autochthonous emergent macrophytes which contribute a substantial carbon input to Douglas Creek.

Rounick and Winterbourn (1986) suggested that an investigator should be able to quickly determine a stream's carbon base by ascertaining the stable carbon isotope ratio of its top aquatic predator. The basis for this proposal is that the

Figure 6. Douglas Creek Trophic Levels. Representative Biota For Each Trophic Level Are Provided.

Douglas Creek

Trout

Grazer Chain Crayfish

Carnivorous Insects Crayfish

Collector/Filterer Insects Amphipods Suckers Crayfish

Carbon Base Detritus (Alloch/Autoch)/live Autoch/DOM

Bacteria - Fungi

Zooplankton Filterer/Collector Insects

Carnivorous Insects

Crayfish

Decomposer Chain Trout

top predator ostensibly integrates the total carbon resources of the stream and that its tissue isotopic value should reflect the autochthonous/allochthonous carbon dependence of the entire stream. Although this proposal is strongly repudiated by Rounick and Hicks (1985) and Rosenfeld and Roff (1992), trout alone were examined to see how the isotopic ratio of this one trophic level compared with the combined analyses of multiple tropic levels. The trout isotopic ratio suggests the Douglas Creek food web uses allochthonous and autochthonous carbon resources in generally equal proportions but with a slight principal dependence on in-stream carbon. In this case, one would reach the same conclusion about stream carbon dependence by looking at isotopic values of multiple components of the food web as by looking at the top aquatic predator's isotopic value. This carbon integrator/top predator concept is examined further in the Discussion section.

DISSOLVED INORGANIC CARBON

In certain stream ecosystems, some autochthonous carbon (e.g., algae) is isotopically depleted relative to terrestrial carbon (Rau 1978, Rounick et al. 1982). In other streams, algae is enriched relative to out-of-stream carbon (Rau personal communication, Rosenfeld and Roff 1992, this study). Dissolved inorganic carbon (DIC) isotopic values are sometimes thought to be the principal factor that causes endogenous carbon to be enriched or depleted relative to exogenous carbon (Osmond et al. 1981, Lazerte and Szalados 1982, Hynes 1983, Mook and Tan

1991).

Table 4 shows matched pairs of isotopic values for total DIC and epilithic aufwuchs. These samples were taken at the same site within minutes of each other. Using aufwuchs as the dependent variable and DIC as the independent variable, contrary to expectation, a simple linear regression analysis showed no significant regression relationship between aufwuchs and DIC. There are several possible explanations:

1. It could mean that aufwuchs is made up of a low percentage of autochthonous primary producers; therefore, any uptake of DIC is minimal and of no consequence in the isotopic constituency of aufwuchs.

2. It could mean that other factors, besides DIC, determine the §^{13} ration of stream primary producers: perhaps plant diffusion resistance as suggested by Lazerte (1983).

3. It could mean, as Mook and Tan (1991) contended, that the §^{13}C of total DIC is meaningless. They contended that each DIC component ($C0_2$, HCO_3^-, and CO_3^{--}) has a distinct isotopic value and that the specific DIC component, used by each plant, must be known in order to develop a DIC/primary production isotopic relationship.

Table 4. DIC and Aufwuchs (Auf) §^{13}C Paired Values Taken From the Same Site on the Same Day. Units are °/oo.

	SS1		SS2		SS3		DC1		DC2		DC3	
Month	DIC	AUF	DIC	AUF	DIC	AUF	DIC	AUF	DIC	AUF	DIC	AUF
Oct.	-2.5	-26.4	N/S	-26.4	N/S	-25.0	-8.0	-23.9	-1.0	-24.8	-5.4	-24.6
Nov.	-6.0	-26.5	-4.3	-23.0	-4.7	-26.7	-6.6	-25.3	-5.2	-18.6	-6.3	-16.3
Dec.	-0.3	-25.3	-4.4	-24.1	-1.1	-26.5	-8.0	-25.9	-9.8	-13.5	-8.9	-16.6
Jan.	0.0	-26.6	-6.5	-25.7	-4.3	-26.8	-9.6	-24.8	-9.5	-16.6	-9.2	-16.5
Feb.	-1.9	-26.8	-4.6	-25.8	-1.1	-26.9	-6.1	-19.9	-5.3	-19.9	-5.9	-24.2
Mar.	-3.3	-26.4	-1.9	-25.7	-0.8	-24.5	-4.7	-23.9	-6.5	-25.4	-4.4	-24.8
Apr.	-4.8	-26.3	-3.0	-22.8	-2.4	-23.6	-5.3	-22.0	-5.4	-23.5	-4.4	-22.3
May	-2.2	-39.7	-6.7	-22.3	-2.3	-24.3	-6.3	-21.6	-6.8	-26.4	-6.4	-18.6
Jun.	-3.4	-27.1	-0.5	-26.0	-7.0	-26.7	-8.5	-24.3	-6.4	-24.3	-7.0	-20.0
Jul.	-8.8	-26.4	-7.8	-25.3	-7.9	-27.6	-8.5	-27.0	-7.7	-28.1	-7.8	-17.5
Aug.	-6.7	-26.0	-8.2	-22.4	-5.9	-27.2	-8.3	-24.7	-6.9	-14.3	-6.7	-16.9
Sep.	-6.0	-24.1	-3.8	-25.5	-2.9	-27.1	-8.3	-21.5	-8.2	-16.6	-7.5	-17.7

N/S = no sample

The mean annual $\S^{13}C$ of DIC at Douglas Creek was -6.8 $^{o}/oo$; at Snively Springs it was -4.1 $^{o}/oo$. According to Rounick et al. (1982), Rosenfeld and Roff (1992), and others, the more isotopically depleted Douglas Creek DIC should have resulted in more isotopically depletion in primary producers and in the food web as a whole. In fact, the reverse was true (Table 1, Table 2, and Table 3); Douglas Creek autochthonous producers were significantly more enriched ($p=0.0001$).

Simplistically, at the outset of this study, it was expected that the allochthonous and autochthonous values would be isotopically similar at both streams and that the difference between the streams would be that the isotopic ratio of insects from the more insolated Douglas Creek would reflect a greater use of autochthonous carbon. What was found instead was that autochthonous carbon was significantly different between the two streams and that there were several isotopically distinct autochthonous components at each stream. Aquatic insects at both streams had isotopic values that were intermediate between allochthonous and autochthonous carbon values which immediately led to a very complex analysis. In this situation, $\S^{13}C$ of total DIC was expected to explain these differences between streams. Had there been a strong regression relationship between DIC and aufwuchs, it might have been possible to develop a mathematical equation that would have enabled a somewhat quantitative comparison of biota in the two streams.

The DIC results did indicate that both streams have high levels of dissolved mineral carbon and relatively lower levels of respired CO_2. The average river, worldwide, has a DIC §^{13}C of -11 °/oo (Mook and Tan 1991) or -12 °/oo (Peterson and Fry 1987). Most of these rivers are more isotopically depleted than the study streams because they contain higher levels of respired terrestrial CO_2 (-24.0 °/oo) in the total DIC (Deines et al. 1974). Perhaps the relatively enriched DIC at Snively springs and Douglas Creek explains why all autochthonous biota at both streams are more enriched than is generally reported in the literature, but the data were of little utility for any other purpose.

4. DISCUSSION

The primary purpose of this study was to utilize stable carbon isotope technology to compare the differential utilization of allochthonous and autochthonous carbon by aquatic insects of two cold desert spring-streams. The aquatic insects of Snively Springs indicated a principal dependence on epilithic aufwuchs (the autochthonous carbon component chosen to isotopically represent all autochthonous carbon). Douglas Creek aquatic insects demonstrated almost equal utilization of allochthonous carbon and epilithic aufwuchs.

In essence, these results showed that shading (lack of insolation) has little effect on the differential utilization of allochthonous and autochthonous carbon in cold desert spring-streams. This finding conflicts with the River Continuum Concept (Vannote et al. 1980) and disputes most stream ecologists who consistently demonstrate that allochthonous carbon consumption/assimilation by aquatic insects increases with shading (Rounick et al. 1982, Rounick and James 1984, Rounick and Hicks 1985, Peterson and Fry 1986, Rounick and Winterbourn 1986, Bunn et al. 1989, Rosenfeld and Roff 1992). Naiman (1976), Minshall (1978), and Fisher (1986) suggested that most desert streams are principally dependent on autochthonous carbon and the results supported these suggestions. However, given that both study streams were principally authochthonously based, the substantially more shaded Snively Springs should have demonstrated relatively more allochthonous carbon dependence than the sparsely shaded Douglas Creek. Results indicated the reverse.

The results of this study are based on the stated assumptions and the implied assumptions set forth in the Introduction. As previously explained, the stated assumptions used in this study were derived largely from Rounick et al. (1982) who claimed to have successfully determined allochthonous/autochthonous carbon differential utilization in several New Zealand streams. There have been approximately ten stable carbon isotope investigations of stream carbon utilization since the technology was developed in 1958; half of these reports have been published since this study began. All of these studies have used essentially the same stated assumptions and the same procedures as Rounick et al. (1982) (see reviews by Rounick and Winterbourn 1986, Peterson and Fry 1987). The last three assumptions, which are listed as Implied Assumptions, were inadvertently built-in to the study by the methods and procedures, and their effect on data interpretation was not discovered until the study was under way.

As this study evolved, and the extant literature was critically evaluated from a stronger personal base of knowledge and as new studies were published, all of the assumptions (stated and implied) in the Introduction were reexamined. In the following discussion, an attempt is made to show how these assumptions often lead to misinterpretation of collected data and to the exclusion of other critically important data. This discussion will attempt to show that this study and other similar studies use stable carbon isotopes to draw conclusions about carbon relationships in freshwater streams that do not stand up well under critical scrutiny. A case will be presented that stable carbon isotope technology offers little or no meaningful improvement over traditional study methods such as gut content analysis, feeding observations, feces analysis, and radioactive tracers.

CONSUMER/FOOD SOURCE ISOTOPIC RELATIONSHIPS

The results of this study are principally based on the assumption that consumers have the same isotopic ratio as their food. Rounick et al. (1982) contended there is only a small increase in the ^{13}C/^{12}C ratio of animals relative to their food and that this fact provides a basis for identification of food utilization of aquatic animals. Rau et al. (1983) claimed that consumer ^{13}C/^{12}C ratios accurately reveal their food sources. Rounick and Hicks (1984) stated that "because only a small ^{13}C/^{12}C alteration (fractionation) has been found in animals relative to their food, carbon isotope ratios can be used to identify their source of food." Winterbourn and Rounick (1985) wrote, "...as the ^{13}C/^{12}C ratio of an animal reflects that of its food, it can be inferred that terrestrial plant material was their main source of carbon." Rosenfeld and Roff (1992) stated "since consumption alters ^{13}C/^{12}C ratios very little, or in a predictable fashion up a food chain, the ^{13}C/^{12}C ratio of an organism should reflect that of its food source."

Using this assumption, all elements of all trophic levels of a food web should have the same §^{13}C value as the carbon base of the food web. For example if herbivorous aquatic insects show an isotopic value of -28.0 ^{o}/oo then carnivorous insects, crayfish, and trout of the same food web would be expected to have the same isotopic value, i.e., -28.0 ^{o}/oo. Rounick et al. (1982) went so far as to postulate that isotopic analysis of stream top aquatic predators alone (usually fish) provides a useful shortcut method for determining proportional carbon utilization in streams. They said that isotopically analyzing trout, for example, would reveal the sources of carbon supporting benthic communities since the ^{13}C/^{12}C of predators accurately reflects all the trophic linkages beneath them.

Stable carbon isotope technology is new and not all investigators agree with this assumption. Deniro and Epstein (1978), Haines and Montague (1979) and Gearing et al. (1984) demonstrated that consumers can be isotopically the same as a controlled food source or they can be significantly enriched or depleted. Macko et al. (1982) found that amphipods were consistently depleted relative to their food. Generally these investigators found that consumers were within plus or minus 2.0^{o}/oo of the isotopic value of their food.

The selection of a consumer-food source isotopic relationship paradigm affects the interpretation of data. To illustrate, autochthonous and allochthonous carbon isotopic values at Snively Springs were -27.5 ^{o}/oo and -26.0 ^{o}/oo respectively; aquatic insects were -26.2 ^{o}/oo (Table 1). Using the assumption that consumers have the same isotopic value as their principal food and finding no significant difference between insects and autochthonous carbon, it was concluded that the insects were feeding on autochthonous carbon. If one of the other equally well-supported consumer food source isotopic relationships had been selected (see Table 5) the data would have been interpreted differently. For example, McConnaughey and McRoy (1979) and Rau (1983) contended there is a 1.5 °/oo and 1.0 °/oo enrichment respectively at every tropic level. If either of these enrichment assumptions had been used, it would have been concluded that the insects (-26.2 °/oo) were feeding on allochthonous carbon (-27.5 °/oo) and enriching this carbon by 1.4 °/oo -- a very defensible finding and quite different from the results reported.

At this stage in the evolution of stable carbon isotope methods there is no consensus as to the isotopic relationship of consumers and food. Deniro and Epstein (1978) concluded that the degree of accuracy in stable carbon isotope technology is poor and that applications should be restricted to comparison of diet sources where large isotopic differences are found. Gearing et al. (1984) warned that the range of isotopic differences between consumers and food sources is too great to generalize with any certainty about how consumers fractionate stable carbon isotopes. Peterson et al. (1985) pointed out that many consumers can have the same isotopic value, although their respective diets are starkly different.

The results reported in this dissertation are based on the selection of a commonly used consumer/food source isotopic relationship. There is no proof that this choice was correct. The evidence indicates that there is no tried and

Table 5. Different Trophic Levels Isotopic Values That Could Be Expected Using Snively Springs Carbon Base Data and Using Different Investigators' Concepts of How Stable Carbon Isotopes Are Altered as They Move Through Food Webs. The Values Shown Under Gearing et al. (1984) Assumes 2 °/oo Depletion, While Those in the Deniro and Epstein Column are Based on a 2 °/oo Enrichment. Units are °/oo.

Trophic Levels	Rounick et al. 1982		McConnaughey & McRoy 1979 (+1.5 °/oo)		Gearing et al. 1984 (-2%)		Deniro & Epstein 1978 (+2%)	
1	-26.0	-27.5	-26.0	-27.5	-26.0	-27.5	-26.0	-27.5
2	-26.0	-27.5	-27.5	-29.0	-24.0	-25.5	-28.0	-29.5
3	-26.0	-27.5	-29.0	-30.5	-31.5	-22.0	-30.0	-31.5
4	-26.0	-27.5	-30.5	-32.0	-33.5	-20.0	-32.0	-33.5

proven choice, i.e., there is no established, agreed-upon consumer-food source isotopic fidelity paradigm. Depending on which assumption or theory one chooses, the same data can be interpreted in different ways.

PREDICTABILITY OF STABLE CARBON ISOTOPE FRACTIONATION

Peterson and Fry (1987) postulated that "......isotopic compositions change in predictable ways as elements cycle through ecosystems." They added that this characteristic enables stable carbon isotopes to be used to track energy flow through ecosystems. Rau et al. (1983) developed a marine pelagic food web model based on the idea that stable carbon isotopes move with predictable alteration through a food chain.

Predictability is not a complicated concept. In the case of stable carbon isotopes, it simply means that an investigator can realistically expect the stable carbon isotope ratio of various food web components to conform to a predictable pattern of alteration. The following findings are offered from the literature and from the results of this study, that show that stable carbon isotopes do not move with predictable alteration in food chains:

1. Winterbourn et al. (1986) said that estimates of allochthonous/autochthonous carbon dependence of stream invertebrates are not easy to make, because the stable carbon isotope value of aquatic plants can vary among individuals of the

same species and among species within and between sites, and may overlap the isotopic ratio of terrestrial plants. Winterbourn et al. (1986) further warned that, "ecologists must be cautious in their interpretation of $§^{13}C$ values, because of this variability, and be especially careful in extrapolating (predicting) from one situation to another".

2. Rounick and Hicks (1985), Peterson et al. (1986), Bunn et al. (1989), and Rosenfeld and Roff (1992) found great isotopic variability among fish in their respective study streams and were unable to show any consumer-carbon source isotopic relationship for many fish species and for individual fish. It should be pointed out that, if stable carbon isotopes did move predictably through a food chain, the stream top aquatic predator's isotopic ratio would provide a short-cut method for deducing the carbon base of a stream -- an idea first presented by Rounick et al. (1982). The fact is that stream fish isotopic ratios are often highly variable and are very difficult to relate to a specific food source or a carbon base. From the opposite perspective, an investigator who has determined the isotopic ratio of aquatic insects in a certain stream stretch should be able to predict, with reasonable accuracy, the isotopic value of predaceous fish such as trout since trout are known to feed

principally on aquatic insects (Hynes 1970). The literature shows that this predictability does not exist (Rounick and Hicks 1984, Peterson et al. 1986, Hesselin et al. 1992, Rosenfeld and Roff 1992). For example, Hesselin et al. (1992) showed that predaceous fish of the MacKenzie River in Canada were inexplicably 7 °/oo depleted relative to the stream's carbon base.

3. Because stable carbon isotopes do not move predictably through food webs (and because investigators have been led to believe they should), writers often make unsupportable speculations about feeding relationships. For example, Cowie (1980) and Rounick et al. (1982) showed that a mayfly that was known to feed exclusively on epilithon differed significantly (>10 °/oo) in isotopic value from that of the epilithon. They then <u>speculated</u> that this obvious lack of predictability was explained by the insect's preferential assimilation of the algal component of the epilithon -- although simultaneous gut content analysis showed no algae in the gut. Rosenfeld and Roff (1992) could find no plausible carbon relationship between brown trout and any endogenous carbon base of a southern Ontario stream. As a result, they <u>speculated</u> that the trout were feeding principally on terrestrial insects, although no terrestrial insect isotopic values were determined. In both examples, it is suggested

that there were many other possible explanations, including the simplest of all, i.e., various consumers fractionate different foods in different ways and an investigator cannot predict how individual consumers, feeding groups or trophic levels will alter the isotopes of their various foods. The assumed predictability of isotopic movement through food chains often causes investigators to speculate carbon relationships rather than investigating the many possible combinations of factors that can result in a consumer's isotopic ratio.

4. Lazerte and Szalados (1982) and Lazerte (1983) showed that submerged aquatic plants have a wide range of isotopic values among individuals of a species and among species at any given site. They also showed that submerged aquatic plants' isotopic values can be enriched, depleted, or the same as terrestrial plant values. These isotopic values were not predictable, but varied by DIC component, water velocity, diffusion resistance, photosynthetic rate, pH, and individual plant physiology. Rounick et al. (1982) showed that a single submerged aquatic plant species, Potamogeton pectinatus, had a stable carbon isotope ratio of -5.0 ^{o}/oo in slow water and -30.0 ^{o}/oo in fast water. One can imagine the difficulty in predicting a consumer-food source relationship with these variances among

individuals and species of one component of autochthonous carbon. How can an investigator predict the isotopic value of any consumer that feeds extensively on submerged aquatic vegetation when the food source itself has such a documented broad range of isotopic values?

5. Many investigators use stream algae as the autochthonous carbon isotopic value when comparing autochthonous/allochthonous carbon utilization in streams (Rounick and Winterbourn 1982, Rosenfeld and Roff 1992). Algal isotopic values have been shown to be highly variable and unpredictable. Oana and Deevey (1960) found that algal samples from the same site varied as much as 10.0 °/oo; Stiller and Nissenbaum (1980) reported variances as high as 15.0 °/oo. Rosenfeld and Roff (1992) concluded that the variability of algal isotopic values precluded any supportable conclusions about autochthonous/allochthonous carbon utilization in their analysis of southern Ontario streams. How can an investigator track carbon transfers in a food web when a single component of one category (algae of autochthonous) has a range of isotopic values that brackets both consumers and allochthonous carbon isotopic values?

To further illustrate the lack of predictability concerning algae (which it must be remembered is only one of several equally complex and variable autochthonous components), I offer the following paradox. Rau et al. (1978) and Rounick et al. (1982) claimed that aquatic algae are always depleted relative to terrestrial carbon. Rosenfeld and Roff (1992) added that stream algae must be depleted relative to terrestrial carbon in order for §^{13}C technology to be used effectively. However, this study and Bunn et al. (1989) showed that stream algae can be consistently and significantly enriched relative to terrestrial carbon. These conflicting findings are not fully explained by DIC or any other combination of factors, which must cause one to question the predictability aspect of stable carbon isotope fractionation.

6. Coffin et al. (1989) suggested that aquatic heterotrophic bacteria should have the same carbon isotope ratio as their substrate. If an investigator predicts (as was done in this study) that this principal food source for aquatic insects can be isotopically discounted because its isotopic ratios are subsumed in the isotopic ratios of stream detritus, he or she is probably making a major mistake that will lead to erroneous interpretations of data. Macko and Estep (1984) showed that aquatic heterotrophic bacteria often differ significantly from their substrate in isotopic value. Again we have two diametrically different views as to the isotopic predictability of a

major carbon source in streams and no proof as to which view is accurate. In fact, both Coffin et al. (1989) and Macko and Estep (1984) point to the need for more in situ investigations in order to clarify the isotopic relationship of microheterotrophs and substrate.

7. In the light of stable carbon isotope predictability, it must be pointed out that we know essentially nothing about carbon turnover time and associated isotopic adjustment in adult consumers. It is not difficult to construct a scenario wherein an individual consumer or an insect cohort could develop its tissue isotopic ratio during growth with a certain foraging strategy, then change diets dramatically as an adult, and die before isotopic adjustment to the changed diet takes place. How can there be a "predictable alteration" of stable carbon isotopes through a food chain until carbon turnover time and isotopic adjustment is better understood? Carbon turnover time is discussed further in the following sections.

In summary, this very key "predictable alteration" assumption in all stable carbon isotope studies must be considered invalid. Some writers claim that a consumer has the same isotopic value as its food source (Rounick and Winterbourn 1986). Others say there is an approximate 1.0% enrichment at every trophic level (Rau 1983). Others say consumers can be enriched or depleted relative to their food (Gearing et al. 1984). Field studies show that there is no pattern, no predictability, for how any consumer's isotopic value may relate to a probable food source or carbon base at any given time (Cowie 1980, Rounick et al. 1982, Hesselin et al. 1982, Rosenfeld and Roff 1992).

To illustrate the lack of predictability of stable carbon isotope alteration and the uncertainty of consumer-food source isotopic relationship consider the following report. Peterson et al. (1986) studied the Kuparuk River of Alaska and discovered that all carbon sources (allochthonous and autochthonous) available to stream consumers had the same $^{13}C/^{12}C$ ratio of -27.0 °/oo. Given this situation, one would predict that all stream consumers would have an isotopic value within a few parts per thousand of the carbon sources of the stream. Aquatic insect isotopic values ranged from -25.7 °/oo to -32.3 °/oo. Grayling fish, the stream's top aquatic predator, ranged from -23.4 °/oo to -28.5 °/oo. Peterson et al. (1986) could offer no explanation for these consumer isotopic values. It seems clear that investigators cannot assume any predictable alteration of stable carbon isotopes-not in inorganic-organic carbon transfers and not at any individual consumer or trophic level.

DO STABLE CARBON ISOTOPES PROVIDE AN INTEGRATED HISTORY OF A CONSUMER'S RECENT FEEDING HABITS?

Rounick et al. (1982), Winterbourn et al. (1986), Peterson and Fry (1987) and Rosenfeld and Roff (1992), claimed that a major advantage of stable carbon isotope technology is that a consumer's $\S^{13}$C value reflects an integrated history of its recent feeding habits. This claim implies that the $\S^{13}$C value of consumers is continually reflective of the current diet. For example, if an adult detritivorous fish feeds on terrestrial plant leaf detritus from October through June and feeds on aquatic macrophyte detritus from July through September, a fish sample taken in August should show the autochthonous macrophyte carbon dependence. On the other hand a sample from this fish in December should show an allochthonous carbon dependence. In reality, however, the December fish sample will probably still show an isotopic value close to that of aquatic macrophytes because tissue isotopic adjustment does not occur rapidly. Lack of knowledge about carbon turnover time in consumers, and lack of knowledge about the time required for consumers to establish isotopic fidelity with food sources makes it very difficult to determine what time frame of any consumer's feeding history is represented. Rounick and Winterbourn (1986) pointed out that a "major shortcoming of stable carbon isotope technology is that very little is known about consumer carbon turnover time."

Fry and Arnold (1982) estimated that an organism, during the rapid growth life stage, requires a 400 percent biomass increase on a laboratory food diet in order to isotopically reflect a change in diet. Although Rosenfeld and Roff (1992) found no isotopic fidelity between common shiners and laboratory food after a 400 percent biomass increase, the biomass quadrupling hypothesis is the only postulation available, and seems reasonably supportable in the rapid growth phase of an organism's life. If we accept the four-fold biomass increase hypothesis, there are still major unanswered questions regarding carbon turnover time and consumer isotopic response to a change in diet. For example, at various life stages, how long does it take a consumer in the field to increase biomass by 400 percent? Does the biomass quadrupling hypothesis hold for all consumers? Is it realistic to expect that a consumer will fractionate the large variety of natural foods at the same delta level as carefully prepared laboratory foods?

Adult consumers present an even more complex isotopic constituency. In adults, consumer-food isotopic fidelity is developed by cell maintenance -- not by new growth. The literature contains no studies and no hypotheses of isotopic adjustment and carbon turnover time in adult consumers. How long does it take an adult consumer to replace enough cells to accurately reflect a diet change? Is this process affected by seasonally low metabolic rates, e.g., fish in winter?

It is well recognized that some aquatic insects can have significantly different diets at various life stages (Pennak 1978). An early instar of an insect could feed on bacteria and very fine particulate detritus and, a few months later, the adult might feed carnivorously. Fish, such as the largemouth bass, feed on algae as fry and are strict carnivores as fingerlings and adults. The critical question is, "How long does it take for these life-stage dietary shifts to be reflected in an organism's stable carbon isotope ratio?" Is it possible that the stable carbon isotope ratio adjustment of an adult consumer could be months or years behind a major dietary shift? The answers to these questions are not available. Until they become available an adult consumer's §^{13}C can not be considered to accurately reflect recent feeding history.

To illustrate how the carbon turnover time issue could lead to misinterpretation, consider the following: Largemouth bass are often the top aquatic predator in streams, and they are known to migrate for long distances (Hall 1971). The carbon base of streams changes from low-order to high-order streams (Vannote et al. 1980) and often differs dramatically by site and stretch (Rosenfeld and Roff 1992) over distances that are well within the migratory travels of the largemouth bass. It is quite conceivable that an individual bass or school of bass could establish stable carbon isotope ratios in one stream stretch with an autochthonous carbon base and, subsequently, before any isotopic adjustment is made, be captured as a study sample in a stretch of stream that is allochthonously based. This scenario could easily lead an investigator to erroneously conclude that the stream stretch from which the bass was taken has an autochthonous carbon base.

Fish, in particular, frequently show very little isotopic relationship to documented food sources (Rounick et al. 1982, Rounick and James 1985, Peterson et at. 1986, Rosenfeld and Roff 1992). Occasionally an investigator explains this finding by stating that the fish sample does not isotopically agree with its documented food source because other, different food sources are "held in isotopic memory" (Rounick and Hicks 1985). How long does "isotopic memory" last? Given the unknown tenure of "isotopic memory", an investigator often cannot determine what carbon sources combined to produce the §^{13}C value of any fish sample or tissue sample of other stream consumers.

The claim that a consumer's §^{13}C represents recent feeding history needs to be viewed with great caution. Given the uncertainty of carbon turnover time and the uncertainty and variability of isotopic adjustment to dietary changes, an investigator does not know what time frame of assimilated feeding history is reflected. Moreover, there may be foods that are essential to an organism that are oxidized immediately and not assimilated, which will never show up in a consumer's carbon isotope ratio (Rounick and Winterbourn 1986).

AUTOCHTHONOUS CARBON IS NOT ALWAYS ISOTOPICALLY DISTINCT FROM ALLOCHTHONOUS CARBON!

Rounick et al. (1982) wrote, "Since $^{13}C/^{12}C$ ratios of terrestrial plants are distinct from aquatic algae as a result of the ^{13}C-depleted HCO_3-source utilized in photosynthesis by aquatic plants, this provides a basis for identification of food utilization by aquatic animals." Rounick and Hicks (1985) stated: "Stable carbon isotope analysis has been used successfully in New Zealand streams to document the differential utilization of allochthonous and autochthonous carbon by invertebrates. This has been possible because $^{13}C/^{12}C$ ratios of aquatic algae (autochthonous inputs) are generally isotopically distinct from those of terrestrial plants (allochthonous inputs)." Rounick et al. (1982), Winterbourn et al. (1984), and Winterbourn and Rounick (1985) all reported successful utilization of stable carbon isotope technology to determine differential utilization of allochthonous and autochthonous carbon resources in New Zealand streams, because the isotopic values of these two carbon categories were consistently distinct.

"Autochthonous" is not defined in the reports cited above. The implication in "autochthonous carbon" is that the investigator is addressing all of the carbon that is produced within the wetted perimeter of the stream (Wetzel 1975). "Autochthonous" carbon consists of single cell algae (planktonic and sessile), filamentous and colonial algae, rooted algae, bryophytes, submerged macrophytes, emergent macrophytes, chemoautotrophic and photautotrophic aquatic bacteria, aquatic fungi, aquatic heterotrophic bacteria, carcasses of stream dwelling animals, feces of stream dwelling animals, and exudates of aquatic primary and secondary producers. Each of these individual autochthonous carbon components often has an isotopically distinct stable carbon isotope ratio, and there is frequently significant isotopic variance among individual components (Oana and Deevey 1960, Lazerte and Szalados 1982).

Peterson and Fry (1987) postulated that the stable carbon isotope technology requires that there be no more than two carbon sources available to a given consumer; otherwise, the true carbon source cannot be determined. Realizing that all stream consumers have access to many isotopically distinct autochthonous carbon components and several isotopically distinct allochthonous components, investigators often attempt to meet the two carbon source restriction by combining all individual autochthonous and allochthonous carbon components into two isotopically distinct grouping categories, i.e., autochthonous and allochthonous. This grouping process creates artificial isotopic values for both carbon categories, especially for autochthonous carbon.

Emergent macrophytes (e.g., watercress and cattails) are autochthonous carbon components. These plants are C-3 photosynthesizers that use atmospheric CO_2 as a carbon source during photosynthesis. As a result, these plants are not isotopically distinct from allochthonous carbon. At Snively Springs and, to a lesser extent at Douglas Creek, watercress was unquestionably the dominant source of plant carbon. The watercress biomass was so extensive (Figure 5) that it is suggested that the combined live and detrital carbon produced by watercress alone was enough to provide all of the carbon required by the Snively Springs food web. Yet in evaluating the results, watercress (and cattails) were reluctantly ignored in assessing the differential utilization of allochthonous and autochthonous carbon simply because they were not isotopically distinct from terrestrial carbon. Other investigators also have ignored this autochthonous carbon component for the same reason, although it is often present in substantial density in their study areas (Rau personal communication, Rosenfeld personal communication).

Emergent macrophyte exclusion by itself causes a distorted autochthonous carbon value derivation and an inaccurate comparison of endogenous and exogenous carbon utilization. However, when we put emergent macrophytes aside and only examine the DIC-converting autochthonous primary producers, there are still many isotopically distinct autochthonous components which can be isotopically the same, or depleted, or enriched relative to allochthonous carbon. Faced with the many distinct carbon components and faced with the paradigm that stable carbon isotope technology is only effective when there are just two carbon sources available to consumers, how does an investigator derive a single autochthonous carbon isotopic value? Most investigators select one DIC-converting autochthonous carbon component that happens to be isotopically distinct from allochthonous carbon and use this single component to represent all autochthonous carbon in the stream (Rounick et al. 1982, Rosenfeld and Roff 1992, Peterson et al. 1986, this study).

Frequently, the single autochthonous component of choice is epilithon -- often called by other names, such as rock scrubate, stone layer, Lock Layer, and epilithic aufwuchs. Peterson et al. (1986), Bunn et al. (1989), and Rosenfeld et al. (1992) used epilithon as the "autochthonous" carbon value for comparing stream allochthonous/autochthonous carbon dependence. In this study, I also used epilithic aufwuchs as the "autochthonous" comparator. Epilithic aufwuchs consists of a complicated array of carbon components, many of which are of allochthonous origin (Hynes 1963, Lock and Williams 1981). Table 6 provides a general breakdown of the autochthonous, allochthonous, and inorganic carbon components of epilithic aufwuchs. Inorganic carbon components are listed but

Table 6. Autochthonous, Allochthonous, and Inorganic Carbon Components of Epilithic Aufwuchs.

Autochthonous	Allochthonous	Inorganic Carbon
Single cell algae	DOM < .5 micron	DIC
Filamentous algae	POM	Mineral particles
Colonial algae	Plant exudates	Marl encrustations
Photoautotrophic bacteria	Land animal carcasses	
Chemoautotrophic bacteria	Land animal feces	
Heterotrophic bacteria	Enzymes	
Fungi		
Micrometazoans < 2 mm		
Zooplankton		
Bryophytes		
POM		
Dissolved organic matter		
Plant exudates		
Stream animal carcasses		
Stream animal feces		
Enzymes		

can safely be ignored because thorough acidification (see Materials and Methods) converts these components to CO_2 gas that moves out of the sample (Rounick et al. 1982).

Looking at Table 6, one must ask, "Isotopically speaking, what is epilithic aufwuchs?" Is it one-third pure autochthonous carbon at a component average isotopic value of -18.0 °/oo and two-thirds allochthonous carbon at a component average isotopic value of -28.0 °/oo? Or is it some other virtually infinite combination of isotopic values? At this time there is no technique to accurately determine the relative allochthonous/autochthonous isotopic constituency of epilithic aufwuchs. As a result it is difficult to have any confidence that epilithic aufwuchs accurately reflects the autochthonous stable carbon isotope ratio in any stream (certainly not in the study streams of this report).

Many stream ecologists contended that terrestrially-derived DOM is the single major contributor to stream energy budgets (Lock and Williams 1981, Hynes 1983, Masser and McDiffit 1986, Peterson et al. 1986). Hynes (1983) said that terrestrially-derived DOM finds its way via ground water to the polysaccharide slime of the epilithon where it is immediately taken up by bacteria and fungi of the epilithic aufwuchs. It seems, then, that epilithic aufwuchs is not only a questionable "autochthonous" comparator, but that a case could be made that epilithic aufwuchs is principally based on allochthonous carbon (DOM specifically). When we combine the influence of terrestrially-derived respired CO_2 on epilithic algae (discussed below) and terrestrially-derived DOM on bacteria and fungi of the epilithon, there is clearly a substantial allochthonous carbon influence in the isotopic value of epilithic aufwuchs. Sometimes this influence is so prevalent that there is no isotopic distinction between allochthonous carbon and epilithic aufwuchs (Peterson et al. 1986).

Investigators choose stream filamentous algae at about the same frequency as epilithon for an autochthonous carbon isotopic comparator (Rounick et al. 1982). Filamentous algae is purely autochthonous carbon which gives it an accuracy advantage over epilithon; however, algal samples vary widely in isotopic value. Rosenfeld and Roff (1992) attempted to use stream algae as the autochthonous comparator in §^{13}C studies of some Canadian streams, but found that algal isotopic values varied so widely that no supportable conclusions about stream carbon bases could be drawn. Moreover, algae may or may not be a significant carbon contributor to streams, depending primarily on the degree of insolation (Vannote et al. 1980, Colbert Cushing, personal communication).

Submerged aquatic macrophytes (SAM) are often major autochthonous carbon contributors to stream energy budgets (Westlake 1965). SAM vary significantly in isotopic value among individuals of the same species at the same site, among species at the same site, and among individuals and species at different sites along the stream and at different times of the year (Osmond et al. 1981) These plants respond isotopically to changes in DIC, pH, temperature, diffusion resistance at the boundary layer, insolation and photosynthetic rate (Osmond et al. 1981, Lazerte and Szalados 1982, and Raven et al. 1982).

Most stable carbon isotope investigators ignore the autochthonous carbon contribution of SAM to consumer isotopic constituencies, perhaps because Hynes (1963), Cummins and Klug (1979), and Minshall (1978) suggested that submerged macrophytes are not grazed and that carbon from these plants does not enter a stream's trophic economy until it becomes detritus. Pennak (1978), however, argued that SAM plants are indeed grazed by a variety of stream consumers and that SAM detritus is readily ingested by all stream consumers. Regardless of form, live or detrital, submerged macrophyte carbon is a substantial source of carbon in many streams and was a major autochthonous carbon contributor to the energy budget of Douglas Creek. Site 2 and Site 3 at Douglas Creek had massive growths of Potamogeton pectinatus from June through August, and there can be little doubt that this submerged macrophyte contributed substantially, both as a living plant and as detritus, to the trophic economy of Douglas Creek. Unfortunately, the stable carbon isotope method does not provide for including this carbon source in determining the isotopic constituency of consumers and their related carbon sources.

Epilithic aufwuchs, filamentous algae, and submerged macrophytes are autochthonous carbon components in streams that can usually be isolated and isotopically evaluated. However, individually or collectively, they do not present a complete picture of autochthonous carbon. In addition to these autochthonous components that can be isotopically evaluated, there are very important autochthonous carbon components in all freshwater streams that are difficult or impossible to separate and isotopically analyze. As a result, they are excluded from the §^{13}C analyses of carbon resource partitioning. Autochthonous DOM, phytoplankton, autotrophic and heterotrophic bacteria, aquatic fungi, aquatic animal carcass and feces and autochthonous respired CO_2 are seldom included in stream stable carbon isotope studies and were not included in this one. Autochthonous DOM and autotrophic bacteria, especially, are known to be major carbon contributors in all streams and, particularly, in desert streams (Naiman 1981, Estep and Vigg 1985). Excluding these major carbon components leaves doubts about the reliability of any single autochthonous carbon comparator.

The point of this discussion is that autochthonous carbon is not always distinct from terrestrial carbon and autochthonous carbon is too complex to derive any meaningful single autochthonous value. There are many autochthonous carbon components of a stream. These components may be individually or collectively isotopically the same as allochthonous carbon, or they may be enriched or depleted. Taking one autochthonous component such as epilithic aufwuchs or algae that happens to be isotopically distinct from terrestrial carbon and using this one component as a basis for comparing differential utilization of allochthonous and autochthonous carbon forces the investigator into using faulty and incomplete data. Future studies would be more realistic if they abandoned the autochthonous/allochthonous comparison format and compared the single autochthonous component of choice to allochthonous carbon. For example, one could study the differential utilization of aquatic phytoplankton and allochthonous carbon by aquatic insects. There would still be major problems in such a study, but at least the study objective would not be misrepresented.

ALLOCHTHONOUS CARBON (LIVE AND DETRITAL)

Peterson and Fry (1987) reviewed stable isotope literature and stated that terrestrial C-3 plants have a globally consistent and very narrow range of carbon isotope values with an average isotopic ratio of -28.0 °/oo. They further suggested that C-3 plant detritus does not differ in §^{13}C from the living plants. In a study of a Canadian tundra river, Bunn et al. (1989) seemed to confirm the Peterson and Fry statements by finding that live riparian C-3 plants had an isotopic value of -27.8°/oo and that detritus from these plants had the same isotopic value as the living plants. The pilot study for this dissertation tested the isotopic ratios of living riparian plant leaves and twigs against the isotopic ratios of assorted in-stream terrestrial detritus and found no significant difference between them. Fry and Sherr (1984) have indicated that living plants and their detritus should have the same isotopic ratio.

Schidlowski et al. (1983) studied terrestrial C-3 plant species around the world and found a range of stable carbon isotope values from -23.0 °/oo to -32.0 °/oo and a mean value of -27.0 °/oo. Broadmeadow et al. (1992) reported wide spatial and temporal variances in the §^{13}C of C-3 trees, and Lowden and Dyck (1974) reported significant seasonal isotopic variations. Leavitt and Long (1986) showed that individual terrestrial C-3 plants of the same species at the same site can vary as much as 3.0 °/oo in isotopic ratio. They also demonstrated that different tissues from the same plant can vary by 3.0 °/oo, e.g., leaves might be -28.0 °/oo while stems and roots might be -25.0 °/oo. O'Leary (1988) pointed out that C-3 plant lipids can be as much as 6.0 °/oo depleted relative to cellulose and lignins in the same plant. Rounick et al. (1982) found major isotopic differences between live and detrital beech tree tissue:

	°/oo
Leaves from a living tree	-30.7
Leaves on forest floor	-28.5
Leaves after stream conditioning	-27.3
Beech wood conditioned in stream for 3 years	-25.0
Beech leachate	-25.3

These data reveal an isotopic difference of nearly 6.0 °/oo between live and stream-conditioned detrital tissue from the same tree species. Peterson et al. (1986) also suggested that aquatic detritus differed isotopically from the living plant. They explained these differences as the result of anaerobic decomposition and associated chemotrophy.

The isotopic range of live and detrital tissue described above by Rounick et al. (1982) is greater than the range of isotopic values that separated allochthonous and autochthonous carbon at both Snively Springs and Douglas Creek (Table 1). Because allochthonous samples for this study were composed of leaves and twigs from living plants, the possibility that stream-conditioned detritus from other plant tissues (roots, boles, branches) would have had a significantly different value cannot be ruled out. There is increasing evidence that stream conditioning causes isotopic enrichment of terrestrial organic material (J.V. McArthur, personal communication). With detrital enrichment, it is possible that the isotopic value of stream-conditioned allochthonous detritus in this study could have had the same isotopic value as epilithic aufwuchs which was used as the autochthonous comparative value. If so, the results might have shown equal utilization of allochthonous and autochthonous carbon at Snively Springs and allochthonous carbon dependence at Douglas Creek -- a marked difference from the results that were reported.

In addition to the problems already discussed, perhaps a greater concern is that all stable carbon isotope studies of stream carbon utilization use allochthonous carbon samples from living plants or detrital particulate organic matter (from tree trunks to seston). These studies fail to consider the isotopic ratio of terrestrially derived DOM and its impact on stream consumer isotopic ratios. Although it is generally agreed that DOM is a far greater contributor to stream carbon budgets than POM (Hynes 1983, Masser and McDiffit 1986, Peterson et al. 1986), this carbon source is never included in stable carbon isotope analyses of stream consumer carbon utilization. Deines (1980) demonstrated that respired terrestrial CO^2 has an isotopic value of -24.0 °/oo; i.e., 4.0 °/oo enriched relative to living plant samples. Rounick and Hicks (1985) and Rosenfeld and Roff (1992) suggested that terrestrially derived DOM might have about the same isotopic value, i.e., -24.0 °/oo. This is a significantly different isotopic value from allochthonous samples that are customarily used, such as leaves and twigs from the living plant and assorted detritus from the stream and the shore. Rau and Anderson (1981) demonstrated that stream invertebrates can readily assimilate DOM. It is suggested that an investigator can not draw defensible conclusions about how much endogenous and exogenous carbon are used respectively by stream consumers, when the single principal allochthonous carbon contributor, DOM, is left out of the equation.

As was the case with autochthonous carbon, investigators generally oversimplify allochthonous carbon -- probably in an effort to adhere to the two carbon source rule of Peterson and Fry (1987). This study has shown how C-3 plants can differ among species, individuals, tissues, and cells and has called attention to evidence of significant differences between living plants and detritus. It has also been pointed out that excluding terrestrial DOM results in an incomplete analysis of carbon utilization in streams. There are also terrestrial animal feces and carcasses (e.g., terrestrial insects and frass) that provide substantial allochthonous carbon to a stream food web and that can have a major impact on stream consumer isotopic ratios (Chapman 1963, Rounick and James 1984, Rosenfeld and Roff 1992). Because ground water provides all of the basal flow of streams and can transport terrestrial plant exudates over great distances and store them for decades or longer (Hynes 1983), there is the possibility that groundwater DOM contains allochthonous carbon from C-4 plants and from CAM plants which could significantly affect the isotopic ratio of stream consumers at all tropic levels.

In summary, the allochthonous carbon isotopic value in most reported studies, including this one, is essentially meaningless. It leaves out DOM, which is consistently a greater carbon contributor to streams than POM. It leaves out the carbon contribution of terrestrial animal feces and carcasses. The data that are used fail to consider isotopic differences among species, among individual plants and in tissues of the same plant and fail to consider the probable enrichment of terrestrial carbon as it is conditioned in the stream.

MICROHETEROTROPHS AND STREAM CONSUMER ISOTOPIC VALUES

Cummins et al. (1984) stated, "for stream energy considerations clearly most energy and material fluxes are accounted for by microorganisms - algal primary producers and bacterial and fungal consumers." Goldman and Kimmel (1978) showed that the association of microorganisms with suspended particles constitutes a major route of energy flow in aquatic ecosystems. Minshall (1978) said that stream bacteria and fungi are the "peanut butter on indigestible detrital crackers." Clearly, microheterotrophs are a major carbon source for stream consumers. The important question is, "Do microheterotrophs have the same isotopic value as their substrate?" If so, it may be safe to ignore them as isotopic entities and trust that the allochthonous and autochthonous carbon values that we compare will accurately subsume the microheterothroph isotopic values. On the other hand, if microheterotrophs differ significantly in isotopic value from their substrate, investigators will undoubtedly draw false conclusions about consumer-carbon source relationships in a stream.

At the outset of this study, the lack of effective sampling procedures for determining the §^{13}C of stream heterotrophic bacteria and fungi was a source of concern. Brian Fry (personal communication) suggested that stream microheterotrophs need not be sampled separately because the isotopic values of bacteria and fungi are the same as their substrate (this advice was based on Coffin et al. [1989] in which they performed some laboratory experiments with the isotopic fidelity of estuarine bacteria and their substrate). In the literature, stable carbon isotope investigators of stream carbon utilization do not mention microheterotrophs as a contributor to the isotopic constituency of stream consumers except as unisolable components of the organic matter stew that makes up epilithic aufwuchs.

There is evidence suggesting that aquatic bacteria do not have the same stable carbon isotope ratio as their substrate. Macko and Estep (1984) demonstrated that aquatic bacteria differ consistently and significantly in isotopic value from their substrate. Tieszin et al. (1979) showed that terrestrial fungi differ substantially in isotopic value from their substrate. Macko and Estep (1984) and Coffin et al. (1989) both suggested that the isotopic fidelity of microheterotrophs and substrate is still uncertain and that extensive in situ study is required in order to accurately determine how these microbes fractionate the stable carbon isotopes of naturally occurring substrates.

Heterotrophic bacteria and fungi are probably the major food source for stream aquatic insects (Barlocher and Kendrick 1973, Cummins 1973, Lock and Williams 1981, Hynes 1983). There is evidence that these organisms have significantly different isotopic values from their substrate. Moreover there is little knowledge concerning microheterotroph substrate feeding preferences. It is quite possible that microheterotrophs could prefer one or the other category of carbon (allochthonous or autochthonous) which could cause a distorted interpretation of the carbon base in a stream if investigators assume that $^{13}C/^{12}C$ ratio's of microheterotrophs and substrate are the same and that microheterophs consume substrate in proportion to availability.

It is unrealistic to ascribe consumer/food source or consumer/carbon base relationships in freshwater streams without deliberate consideration of the isotopic contribution of both heterotrophic bacteria and fungi. Failure to include these organisms which constitute a major food source for aquatic insects results in an incomplete understanding of stream carbon utilization and food web relationships. If microheterotrophs cannot be isolated and isotopically evaluated, the results must indicate that this major carbon source was not included. Investigators can not imply that microheterotroph isotopic values are accurately subsumed in the allochthonous and autochthonous primary production and/or detritus samples.

DISSOLVED INORGANIC CARBON AND AQUATIC PRIMARY PRODUCERS

Rounick and Winterbourn (1986) and Rosenfeld and Roff (1992) state that total DIC isotopic variance is the primary determinant of freshwater plant §^{13}C variability. Lazerte (1983) found a strong regression relationship between total DIC and submerged aquatic macrophyte §^{13}C in Lake Memphremagog, Quebec, but concluded that individual plant diffusion resistance was the principal factor determining isotopic fractionation in the conversion of inorganic carbon to organic carbon. Osmond et al. (1982) showed that the §^{13}C of freshwater plants depends on both individual plant metabolism and the specific DIC component ($CO_{2,}$ HCO_3^- and CO_3^{--}) that is photosynthesized at any given time. Degens (1969) and Deines et al. (1974) demonstrated that the §^{13}C of freshwater changes continuously due to isotopic fractionation between solid, dissolved, and gaseous inorganic carbon and between oxidation states and that the §^{13}C of aquatic plants is correspondingly complex.

In order to track stable carbon isotopes through food webs, an investigator must know the fractionations that occur when inorganic carbon is converted to organic carbon -- as well as the food web consumer fractionations. It would be much easier to understand these changes if variances in the isotopic values of autochthonous producers of different streams could be explained by the isotopic value of total DIC in the water. For example, Snively Springs had an annual mean isotopic value for total DIC of -4.0 ^{o}/oo, while Douglas Creek's total DIC isotopic value was -6.8 ^{o}/oo. If total DIC §^{13}C is the principal factor affecting the §^{13}C of aquatic primary producers; epilithic aufwuchs, filamentous algae, submerged macrophytes would have been more depleted at Douglas Creek than at Snively Springs. In fact, they were more enriched.

Two observations emerge from trying to link stream total DIC §^{13}C and the §^{13}C of DIC-converting autochthonous producers: 1) investigators must determine the stable carbon isotope ratio of the specific DIC component that is photosynthesized (not total DIC) and that component can change many times during a single day; 2) there is evidence that the stable carbon isotope ratio of autochthonous producers is affected strongly by many factors other than DIC, e.g., water velocity, photosynthetic rate, and plant diffusion resistance. Osmond et al. (1981) cautioned that unlike terrestrial plants, little is known about the ecology, physiology or biochemistry of photosynthesis of aquatic plants. They show that these plants use HCO^{-}_3 and CO_2 as carbon sources but the proportionality changes with individual plants, plant species, pH, temperature, water velocity, and diffusion resistance at the boundary layer. The ultimate isotopic composition of the many autochthonous carbon components depends on many factors and many complex interactions. All of these factors must be considered in attempting to explain the isotopic variability of DIC-converting autochthonous producers. Unless investigators can understand and predict the initial isotopic fractionation at the inorganic/organic carbon conversion, they are faced with continual speculations about the isotopic constituency of consumers and carbon bases in streams.

PHOTOAUTOTROPHIC AND CHEMOAUTOTROPHIC BACTERIA AND THE ISOTOPIC CONSTITUENCY OF STREAM INVERTEBRATES

Autotrophic bacteria are generally ignored in stable carbon isotope analysis of stream carbon utilization. Chemoautotrophic bacteria are not mentioned in the freshwater stream literature. Photoautotrophic bacteria are frequently mentioned as the blue-green algal component of the total algal population and have been isotopically evaluated in some aquatic ecosystems (Estep and Vigg 1985, Schidlowski 1986, Bunn et al. 1989). These organisms, however, are not usually included in the assessment of stream consumer isotopic constituency.

Whitton (1975) found that chemoautotrophic bacteria are present in all freshwater ecosystems. Schwinghammer et al. (1983) and Peterson et al. (1985) demonstrated that chemoautotrophic bacteria are major carbon contributors to tidal and estuarine ecosystems. Rau (1981) showed that the entire food web of deep sea thermal vents is based on chemosynthesis. Wetzel (1975) gave evidence that freshwater ecosystems contain a wide variety of chemosynthetic bacteria and that chemosynthesis often exceeds photosynthesis. He further illustrated the trophic importance of these bacteria by demonstrating that zooplankton migrations are often keyed to chemosynthetic production.

If chemoautotrophic bacteria are present in freshwater streams in substantial density, they would probably have a significant impact on the isotopic ratios of aquatic insects because they have been shown to have extremely depleted isotopic values of about -39.0 °/oo (Fry and Scherr 1984) and they would be readily consumed by stream invertebrates (Wetzel 1975, Suberkropp and Klug 1976). In the case of Snively Springs and Douglas Creek, for example, a carbon component with an isotopic value of about -40.0 °/oo could open up several new postulations of consumer-carbon source relationships. At this point, the freshwater literature says essentially nothing about this carbon source, and it may or may not be a significant contributor to a stream consumer's isotopic ratio; however, the possibility has not been ruled out.

Photosynthesizing blue-green bacteria are commonly found in all streams (Hynes 1970). Naiman (1976), Estep and Vigg (1985), and Fisher (1986) considered photosynthesizing blue-green bacteria to be major carbon contributors in desert stream ecosystems. Estep and Vigg (1985), Schidlowski et al. (1986) and Bunn et al. (1989), isolated blue-green bacteria in their respective study areas and determined that these bacteria have a consistently enriched isotopic value of about -16.0 °/oo. It is possible that the otherwise inexplicable sucker isotopic value at Douglas Creek (Table 2) could be explained by preferential assimilation of isotopically-enriched blue-green bacteria. Estep and Vigg (1985) did postulate a feeding relationship between suckers and blue-green bacteria in a Nevada desert stream ecosystem and Bunn et al. (1989) hypothesized a similar feeding relationship for suckers in a tundra stream; however, Fisher (1986) said that Arizona Gila Mountain suckers feed primarily on diatoms.

It is a mistake to exclude autotrophic bacteria in §^{13}C studies of autochthonous/allochthonous carbon utilization in streams. Freeden (1960, 1964) demonstrated that black fly larvae (common in samples of both streams of this study) can carry on normal life cycles on a diet of nothing but bacteria. Ivlev (1945), Hynes (1973), and Minshall (1978) contended that stream consumers only ingest detritus for the sole purpose of getting at the bacteria and fungi adsorbed to the detritus although Saunders (1969) proved that detritus is also assimilated. Certainly, some of the adsorbed bacteria would be heterotrophic, but a substantial biomass of autotrophic bacteria would be included as well. Autotrophic bacteria are significant autochthonous carbon components, especially in desert streams, with exceptionally distinctive isotopic ratios, and they should not be excluded from any analysis that purports to use stable carbon isotopes to determine autochthonous/allochthonous carbon resource utilization.

RESPIRED TERRESTRIAL CO_2 - ALLOCHTHONOUS CARBON SOURCE OR EXPLANATION FOR ISOTOPICALLY DEPLETED AUTOCHTHONOUS PRIMARY PRODUCERS

Rau (1978), Rounick et al. (1982), Rounick and James (1984), and Peterson and Fry (1987), reported extremely depleted algal isotopic values in some aquatic ecosystems. They explained these depleted values by claiming that these algae are photosynthesizing respired terrestrial plant CO_2, as opposed to other more isotopically-enriched DIC components. Frequently, this depleted algal isotopic value is cited as the basis for effectively comparing endogenous and exogenous carbon utilization in aquatic ecosystems (Rau 1978, Rosenfeld and Roff 1992).

When comparing the relative importance of allochthonous and autochthonous carbon in streams, it is ironic that an allochthonous carbon source (terrestrial plant respired CO_2) is only addressed for its causal effect in depleting the isotopic value of autochthonous algae. Why is the respired terrestrial CO_2 not shown as an allochthonous carbon resource? Its isotopic value (from C-3 plants) is fairly well established at about -24.0 ^{o}/oo (Rounick and James 1984, Schidlowski 1986, Peterson and Fry 1987), and its importance as a carbon source in streams is well documented (Lock and Williams 1981, Hynes 1983).

This paradox illustrates, perhaps better than any other, the complexity of attempting to use stable carbon isotopes to assess allochthonous/autochthonous carbon utilization in streams. Where does one divide allochthonous and autochthonous? Perhaps Goldman and Kimmel (1983) were correct in saying that the whole question of allochthonous/autochthonous energy importance in aquatic ecosystems is unresolvable, because the two carbon sources are inextricably intertwined.

THE IMPACT OF NUTRIENTS ON THE ISOTOPIC CONSTITUENCY OF STREAM BIOTA

Snively Springs is located well inside a long-established, fenced, patrolled environmental research area. There appears to be no possible source of anthropogenic impact. Douglas Creek appears to be similarly isolated, though not fenced or patrolled. During initial site selection reconnaissances and twice-monthly sampling efforts over a fifteen month period, no evidence became apparent to suggest that either of these streams might be anthropogenically nutrient enriched. There were no surface streams or irrigation water returns entering either study stretch for several kilometers upstream. Review of all stream-related literature and lengthy discussions with other investigators gave no indication of possible nutrient enrichment. Based on these observations and the fact that possible nutrient impact on freshwater stream biota isotopic values was not mentioned in the literature, it was assumed that nutrient concentrations would not be a factor in this study.

Hynes (1983) pointed out that all of the basal water flow in a stream is provided by groundwater. Groundwater is much higher in nitrate nitrogen than free flowing stream water and most desert streams are nitrogen limited (Fisher 1986), which results in groundwater nitrogen being immediately taken up by steam autotrophs (Grimm et al. 1981). The increased photosynthetic activity enabled by nutrient enrichment, causes an isotopic depletion in stream producers because ^{12}C is taken up preferentially (lighter, more mobile isotope) when photosynthetic rates are high (O'Leary 1988). Producer §^{13}C depletion, in turn, causes stream total DIC to be enriched (O'Leary 1981, 1987, Peterson and Fry 1987). These stable carbon isotope alterations illustrate that nutrient enrichment has a substantial effect on the isotopic composition of aquatic producers and stream DIC.

In retrospect there is a possibility that agricultural nutrients transported via groundwater from nearby alfalfa fields and apple orchards affected the isotopic constituency of Douglas Creek biota. Snively Springs was probably not affected. Future §^{13}C studies of carbon utilization in streams should include measurements of nutrient concentrations at each site. Peterson et al. (in press) has demonstrated immediate and dramatic isotopic changes in stream biota resulting from nitrogen and phosphorous enrichment.

STABLE CARBON ISOTOPES AND FRESHWATER STREAMS

Peterson and Fry (1987) proposed several litmus tests by which an investigator should be able to determine if stable carbon isotope analysis will be effective in any specific consumer/food source or ecosystem carbon base potential application.

1. If quantitative results are required, there can be no more than two isotopically distinct carbon sources, e.g., C-3 and C-4 plants.

2. If qualitative results are satisfactory, there can be more than two isotopically distinct carbon sources, but the results will be ambiguous, i.e., the true carbon source cannot be determined.

3. There must be a "large" isotopic difference between carbon pools. "Large" is not defined; however, as a minimum, it would seem that the two carbon pools cannot overlap in isotopic value. Where stable carbon isotope analysis has been very successful (e.g., distinguishing C-3 and C-4 plants), there has been a 10.0 °/oo to 14.0°/oo isotopic separation between carbon pools (Bender 1968).

4. There must be small variances within the carbon pools.

Applying these tests to Snively Springs and Douglas Creek, test #1 fails. At these two streams, there are at least 25 carbon components that are potentially isotopically distinct and can, therefore, affect the isotopic constituency of all stream consumers. Failing test #1, quantitative results should not be expected, and were not obtained in this study.

Test #2 can be passed. There are many more than two carbon sources, so one can expect, at best, to attain a qualitative indication of carbon dependence. This study does obtain such results; however, the ambiguity of the results is so overwhelming and the carbon relationships so speculative, it is difficult to find any advantage over any of the other traditional consumer-food source study methods discussed in the Introduction.

Test #3 fails. There is almost no difference in autochthonous and allochthonous carbon pools in Snively Springs (Table 1). The small 1.6°/oo difference falls within the error rate for sample processing and isotopic analysis (Deniro and Epstein 1978). At Douglas Creek, there is a 5.8 °/oo difference between the two carbon pools for the entire study stretch; however, there is a significant residual variance among carbon components of the autochthonous category. The autochthonous emergent macrophyte §^{13}C distinctly overlaps the allochthonous carbon pool §^{13}C ratio at both streams, and there is no existing technique for separating the impact of these two carbon sources on stream consumer isotopic constituency.

Test #4 must be considered a failure. Autochthonous carbon components varied from -18.0 °/oo to -28.0 °/oo (Table 1 and Table 3). As previously stated, some autochthonous components consistently overlapped allochthonous carbon. Allochthonous carbon appeared to have a consistent value with very little variance in the carbon pool; however, critical analysis indicates this may have been attributed to faulty sampling procedures and a misunderstanding of the live plant/detritus isotopic relationship.

From an overall perspective, stable carbon isotope technology could realistically have been expected to provide nothing but qualitative, ambiguous aquatic insect/carbon base relationships in this study. Because the various carbon components available to consumers in Snively Springs and Douglas Creek are not substantially different from those of most freshwater streams, it is suggested that the stable carbon isotope technology would yield ambiguous results in any study with similar objectives at most freshwater streams. The speculative nature of the results is such that data interpretations are of questionable validity.

5. SUMMARY AND CONCLUSION

Whether the carbon supporting stream food webs comes principally from terrestrial sources or is produced within the stream is a question of longstanding interest. Lacking data to resolve the allochthonous/autochthonous issue with any finality, stream ecologists, over the last 80 years, have alternately postulated that stream carbon was principally autochthonous (Percival and Whitehead 1929; Butcher 1932, 1940, 1946; Naiman 1976; Minshall 1978) or principally allochthonous (Hynes 1963, Minshall 1967, Fisher and Likens 1973, Cummins 1974). Others like Goldman and Kimmel (1978), argued that autochthonous and allochthonous carbon resources cannot be separated and that the allochthonous/autochthonous dependence issue is unresolvable.

The underlying concern for understanding carbon resource dependence in streams is managing our streams and their watersheds effectively. Cummins (1974) indicated that all the world's streams are in a constant state of anthropogenic perturbation and that we need to know how these perturbations affect stream carbon utilization in order to manage our freshwater resources effectively. Araujo-Lima et al. (1986) showed that the Amazon River's delicate autochthonous/allochthonous carbon balance must be managed effectively in order to sustain the detritivorous fishes that provide almost all of the protein to

Amazon basin residents. Rounick and Winterbourn (1986) emphasized that understanding sources and patterns of energy flow and material cycling in streams has great significance for managing fisheries and making environmental decisions.

In the final analysis, concern centers around what effect altering the relative inputs of allochthonous and autochthonous carbon has on stream structure and function. Winterbourn and Rounick (1985) studied the effects of forestry practices on several New Zealand streams over a period of a decade. They concluded that stream food webs do shift over time from allochthonous carbon dependence to autochthonous carbon dependence as watershed forests are cleared. However, they demonstrated that this shift in carbon dependence has no effect on stream structure and function. Chapman (1966) reached similar conclusions after evaluating the effect of commercial logging on Oregon steelhead streams. Other investigators, however, claim that logging, burning, nutrient enrichment and other man-engendered perturbations have a marked effect on stream structure and function (Ward and Cummins 1979, Webster and Patten 1979, Newbold et al. 1980 and Bird and Kauchik 1984). Obviously, the question survives and the controversy continues.

Many investigators have seized upon stable carbon isotope technology as the tool to finally resolve the issue (see reviews by Rounick and Winterbourn 1986, Peterson and Fry 1987). Unfortunately, most investigators, after attempting to apply stable carbon isotope methods to stream carbon resource analysis, have conceded that the results are rarely quantitative and that the qualitative relationships are ambiguous. After concluding that the technique is not suitable for determining specific consumer/carbon source dependencies, investigators have often ended their reports by suggesting that the technique would be effective for assessing differential utilization of two broad categories of carbon resources, i.e., autochthonous and allochthonous. These suggestions invariably reference the admonitions of Rounick and Winterbourn (1986) and Peterson and Fry (1987) that effective utilization of the stable carbon isotope method requires two and only two isotopically distinct carbon sources.

The present study points out the fallacies of trying to conjure single isotopic values for either autochthonous or allochthonous carbon. It suggests that stable carbon isotope technology is not reliable in establishing specific consumer/food source relations and that it is not suitable for assessing autochthonous/allochthonous carbon dependence in freshwater streams. Given the daunting complexity of carbon resources available to stream consumers and the number of critical carbon components that are extremely difficult to isolate and isotopically characterize, it is doubtful that stable carbon isotope technology, alone, can ever provide a valid, supportable analysis of autochthonous/ allochthonous carbon resource utilization in freshwater streams at any meaningful level of resolution.

As previously discussed, it seems that every investigator who has attempted to use stable carbon isotope technology to clarify freshwater stream carbon dependence has been less than satisfied with the results. Many of these investigators suggested that although the stable carbon isotope technology proved to be of very limited utility by itself, the study results would have been more conclusive if stable carbon isotope analysis had been supplemented with stable nitrogen isotope and stable sulfur isotope analysis. Sulfur and nitrogen isotopes were not used in this study because an objective of the study was to test the utility of stable carbon isotope technology in determining cold desert spring-stream carbon bases. It was not intended to test multiple-element stable isotope technology in attaining this objective.

Stable carbon isotope analysis has been very successful in showing cattle feeding preferences between C-3 and C-4 plants (Minson et al. 1975). In some cases, it has been successful in elucidating the carbon dependence of estuarine consumers (Fry 1981) and in postulating marine pelagic food webs (Rau et al. 1983). In a very interesting application, the technology (using bone collagen isotopic measurements) successfully determined the time period in which American Indians shifted from a hunter-gather society to a maize-cultivating society (Van der Merwe, 1982). In these cases where the technology has been successful in determining consumer/carbon relationships, the Peterson and Fry (1987) litmus tests, discussed above, have been largely satisfied.

The literature and the results of this study indicate that stable carbon isotope technology (by itself) has not been successful in meaningfully elucidating carbon dependence in freshwater streams. The carbon base of freshwater streams is a constantly changing kaleidoscope of many isotopically distinct and interacting inorganic and organic carbon components that is simply too complex for stable carbon isotope technology to effectively determine autochthonous/allochthonous carbon utilization. In the final analysis, the question must be asked, "As a result of applying stable carbon isotope technology, do we know anything more about the differential utilization of autochthonous and allochthonous carbon at Snively Springs and Douglas Creek than we did before?" Unfortunately, the answer is, "No!" and, because the carbon resources of these two streams are not markedly different from those of most freshwater streams, it is suggested the technology should be used with reservation in similar applications at any freshwater stream.

Goldman and Kimmel (1978) pointed out, "In a practical sense, aquatic systems are probably more complex trophically than most other types of ecosystems both by virtue of the inherent complexity characteristic of natural, dynamic systems and our present technical inability to clearly distinguish food sources and nutrient flow pathways." They further reminded us that technical difficulties encountered in separating particulate from dissolved matter, living from non-living particles, and autotrophs from heterotrophs often make aquatic systems more difficult to study and more complex in regard to energy flow than terrestrial systems. Evidence indicates that freshwater aquatic ecosystems in general and streams in particular are simply too trophically complex for stable carbon isotopes alone to meaningfully establish consumer/carbon base relationships.

RECOMMENDATIONS FOR IMPROVING FUTURE STABLE CARBON ISOTOPE STUDIES OF CARBON UTILIZATION IN FRESHWATER STREAMS

The following procedures would improve the validity of future studies with similar objectives, although it is suggested the results will still be disappointingly ambiguous:

1. There must be a method of developing component isotopic value weighted averages for all isotopically distinct autochthonous and allochthonous carbon components, including DOM, micro-heterotrophs, and autotrophic bacteria.

2. Ingestion/assimilation analyses should be done for each consumer. It is possible that some foods are oxidized immediately and not assimilated into tissue. These immediately oxidized foods are no less important to consumers, but the stable carbon isotope tissue analysis method denies their existence in the stream trophic economy.

3. Terrestrial animal feces and carcasses contribution to stream carbon needs to be quantified and isotopic values established so that these

stream carbon sources can be factored into the isotopic constituency of stream consumers.

4. Respired terrestrial CO_2 should be separated from respired aquatic CO_2 and isotopic values established for each. The question, "Is respired terrestrial CO_2 which is taken up by aquatic primary producers in photosynthesis, an allochthonous carbon component or an autochthonous carbon component?" should be answered. Terrestrially-derived respired CO_2 is often cited as the reason for aquatic algae being isotopically depleted; it is more to the point that terrestrially derived CO_2 should be considered an allochthonous carbon component.

5. Because of the immediate and profound effect of nutrient enrichment on the isotopic values of all freshwater steam biota, future studies should include measurements of nutrient concentrations at each sampling site.

6. Dissolved inorganic carbon (DIC) isotopic values should be broken down into components, i.e., isotopic ratios for dissolved CO_2, HCO_3^-, and CO_3^{--} should be established.

7. Some means must be found for separating emergent macrophyte (autochthonous carbon) from C-3 plants (allochthonous carbon). At this point, they both have essentially identical isotopic values, but belong in opposite carbon categories.

8. Sampling for aquatic insects must include the deeper sediment layers. Hynes (1983) showed that most stream insect biomass lies 5 cm to 10 cm below the sediment surface, and Fisher (1986) contended that this hyporheic area is also a desert stream's principal carbon storage area. It could well be that streams have a vertical stratification of carbon dependency and that sampling insects and carbon from the lower sediment strata might present an entirely different picture of carbon dependence. This possibility needs to be examined.

9. To be truly reliable, each study should include in situ analyses of individual consumer food source fractionation. Extrapolation of laboratory experiment data into field analysis belies the importance of different natural foods being fractionated at significantly different delta levels.

10. Carbon turnover time in adult consumers should be examined in

every study; otherwise, there is no assurance that the isotopic ratio of any given consumer accurately reflects its carbon assimilation for any given time period.

11. Both autochthonous and allochthonous carbon must be defined and a carbon component list established for each category. Where possible, all components of each category should be isolated and carbon ratios established. Where components cannot be isolated or separated (perhaps DOM and respired CO_2) their potential effect on consumer isotopic ratios should be addressed as quantitatively as possible. Investigators should be especially concerned that the ecological implications of the allochthonous/autochthonous carbon utilization question require consideration of entire categories -- not some component or components of either category.

12. As suggested by Peterson et al. (1985), stable carbon isotope analysis should be used in conjunction with other methods, e.g., feeding observations and gut content analysis.

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- Who's Who Among Students in American Colleges and Universities, 1960
- Danforth Fellow Nominee, 1960
- University Fellowship, Old Dominion University, 1985-1988
- Phi Kappa Delta, elected 1989
- Northwest Consortium of Colleges and Universities for Sciences (NORCUS) Fellow, 1990-1992

Mr. Mize has taught management and computer courses for George Washington University, the University of Maryland, and the University of Virginia. He was a manager with the U.S. Department of Energy's Hanford Nuclear Waste Cleanup Program. Mr. Mize served 26 years as a commissioned officer in the U.S. Marine Corps from which he is now retired.