Food-web relationships in Catamaran Brook, New Brunswick, as revealed by stable-isotope analysis of carbon and nitrogen

by

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ABSTRACT

Stable-isotope ratios of carbon and nitrogen were used to examine food-web relationships in Catamaran Brook, New Brunswick. Efforts were made to quantify trophic fractionation between macroinvertebrates and their diet, and to assess spatial and temporal isotope variability at the base of the aquatic food chain leading to juvenile Atlantic salmon production. Following this, stable-isotope data were synthesized in an attempt to investigate the utility of a two-source isotope mixing model to predict the relative importance of allochthonous and autochthonous inputs to lotic food webs.

In detail, stable-isotope ratios were used to confirm a parasitic relationship between a midge (*Nanocladius* [*Plecopteracoluthus*] undescribed sp., nr. *branchicolus*) and its stonefly host (*Pteronarcys biloba*). *Nanocladius* (*P.*) sp. always had more positive $\delta^{13}C$ and $\delta^{15}N$ values than *P. biloba*, and average fractionation factors (isotope differences between symbiont and host) were +1.2‰ and +3.5‰ for carbon and nitrogen, respectively. *Nanocladius* (*P.*) sp. were also more enriched in ^{15}N than other chironomids, and values fell within the range of other known invertebrate predators. These findings highlight the usefulness of stable-isotope technology to distinguish between phoresy and parasitism in ectosymbiotic relationships. The results also demonstrate that at least 1 of the many tenets of isotope ecology (^{13}C and ^{15}N enrichment between animal and diet) holds true in field situations.

Epilithic algae ranged from –35‰ to –19‰ and –0.8‰ to 6.5‰ for $\delta^{13}C$ and $\delta^{15}N$ respectively, and values were related to the dissolved inorganic chemistry at each site. Water velocity did not affect $\delta^{13}C$ and $\delta^{15}N$ values at all sites, suggesting that other factors (e.g., CO_2-concentrating mechanisms) may be more important in the determination of stable-isotope ratios in lotic microalgae. The grazer, *Glossosoma nigrior*, showed $\delta^{13}C$ and $\delta^{15}N$ values that correlated well with those of algae. Isotope differences among grazer species appear to be related to feeding mode and microhabitat preferences. Grazer $\delta^{13}C$ may be a better indicator of autochthonous carbon than algal $\delta^{13}C$ because of terrestrial and heterotrophic contamination in the biofilm matrix, but care should be given to the choice of isotope surrogates of primary food sources because of feeding selectivity among primary consumers.

Hydropsychid caddisflies showed highly variable $\delta^{13}C$ (–34.8‰ to –25.0‰) and $\delta^{15}N$ (0.8‰ to 8.3‰) values at 31 sites along an 18-km section between the headwater lake and the mouth of Catamaran Brook. Isotope differences were related to species-specific and seasonal changes in diet, as well as chemical influences from local tributaries. *Hydropsyche slossonae* and *Arctopsyche* sp. were isotopically more enriched than 5 other hydropsychid species, suggesting a higher trophic position for these larvae. *Cheumatopsyche aphanta* was isotopically more depleted than other species, possibly implying a greater reliance on algal food sources. Results showed that stable-isotope ratios provide a valuable means of obtaining dietary information for co-existing species. The data also illustrate that stable-isotope ratios are site-specific and are not comparable over the entire stream ecosystem.

The ability of a quantitative two-source isotope mixing model to predict the relative importance of allochthonous and autochthonous inputs to an aquatic food web was assessed using a statistical resampling technique. When differences between food sources were small (e.g., 2-3‰), the model performed poorly, assigning 95% confidence limits in the range of ± 20% to 70%. Performance was enhanced when the food-source differential was increased, but predictive power was invariably dependent on sample size. These results suggest that, at natural abundance levels, most stable-isotope data are qualitative estimates of diet, and that only robust datasets should be used for quantitative purposes. Power curves are presented to aid ecologists in future attempts to design quantitative stable-isotope studies.

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CHAPTER ONE

General Introduction

Species introductions, landscape disturbances, and other environmental perturbations can disrupt energy flow and food-web structure in many aquatic ecosystems (Rounick and Winterbourn 1985, Bilby and Bisson 1992, McClelland and Valiela 1998, Vander Zanden et al. 1999). As a result of continued alteration and exploitation of natural habitats, there is great need to accurately predict and measure the effects of environmental impacts on trophic structure and energy pathways in aquatic food webs, especially at the level of top predators such as salmon and trout (Waters 1993). However, quantitative analyses of energy pathways to higher-trophic level organisms are often impeded by detritus-based food webs and limitations of conventional methods such as gut-content analyses. Thus, food-web theory requires a more holistic, unifying, and quantitative approach to improve our understanding of linkages between primary food sources, primary consumers, and top predators.

In this thesis, food-web structure in Catamaran Brook, a small pristine forested stream in north-central New Brunswick, was examined using stable-isotope ratios of carbon and nitrogen. The main objectives of the thesis were: 1) to examine stable-isotope relationships between primary consumers and their food sources, 2) to determine the level of natural variability (on both temporal and spatial scales) in the stable-isotope ratios at the base of the stream food web, and 3) to assess the predictive power of a 2-source isotope mixing model to determine the relative importance of allochthonous inputs to a resident top predator, juvenile Atlantic salmon (*Salmo salar*). What follows in this general introduction is a brief account of stream food-web theory, stable-isotope analysis, and a quick synopsis of each thesis chapter.

Stream food-webs

Primary food sources in stream ecosystems are usually grouped into 2 main categories. "Allochthonous" sources are energy inputs which have been derived from outside the system (i.e., the terrestrial environment) and consist of falling leaves, wood, fruits, flowers, insects, etc. (Allan 1995). Allochthonous food sources may enter the stream at ground-level as wind-blown coarse particulate organic matter (CPOM) and, through subsequent abrasion and decomposition, take the form of fine particulate organic matter (FPOM) or dissolved organic matter (DOM). Terrestrial inputs may also enter the aquatic milieu directly as allochthonous DOM via transport from upstream, groundwater, surface runoff, precipitation (through-fall), or as *in-situ* leachate. Inputs which have been produced

within the stream are called "autochthonous" sources, and consist mostly of aquatic mosses, vascular macrophytes, benthic and planktonic algae.

The benthic invertebrate community has developed remarkable strategies for exploiting both allochthonous and autochthonous inputs to stream food webs (Allan 1995). In general, "shredders" such as the stoneflies (Plecoptera), *Leuctra*, *Pteronarcys*, *Taeniopteryx*, and the caddisflies (Trichoptera) *Pycnopsyche* and *Lepidostoma* are avid consumers of terrestrial CPOM. Primary consumers of autochthonous material (e.g., benthic algae), such as the caddisfly *Glossosoma*, the net-winged midge (Diptera) *Blepheracera*, the mayflies (Ephemeroptera) *Baetis*, *Cinygmula*, *Epeorus*, and the riffle-beetle (Coleoptera) *Stenelmis*, are known as "scraper-grazers". Through the action of chewing and gut-passage, larger CPOM particles are made available as smaller FPOM fragments to a diverse and opportunistic group of invertebrates known as "collector-gatherers" and "collector-filterers". Collector-gatherers, such as the mayflies *Eurylophella*, *Hexagenia*, and *Tricorythodes*, scour rock surfaces and leaf packs for nutrition usually in the form amorphous detritus, while collector-filterers, such as the blackfly (Diptera) *Prosimulium*, the caddisfly *Hydropsyche*, and the mayfly *Isonychia*, sieve food particles from the water passing overhead. "Predators" such as the stoneflies *Agnetina*, *Isoperla*, *Sweltsa*, the alderfly (Megaloptera) *Nigronia*, the dragonflies (Odonata) *Boyeria* and *Ophiogomphus*, and the dipterans *Atherix* and *Hexatoma* are generalists as well, but mainly feed on smaller invertebrate prey. Top predators in streams are usually fish, such as resident salmon and trout. Both fishes feed to some extent on drifting invertebrates, but will also forage on the stream bottom, or consume surface prey (e.g., terrestrial insects) to reduce competition (Gibson and Cunjak 1986, Power 1993).

The relative importance of allochthonous and autochthonous food sources to stream food webs has been summarized in the River Continuum Concept (RCC) (Vannote et al. 1980). The RCC states that contributions from allochthonous and autochthonous materials, and the subsequent structure of the benthic invertebrate community, will vary along the stream gradient in accordance with changes in the physical and chemical environment. Typically, in temperate North American streams, the heavy riparian canopy will shade out light needed by photo-autotrophs in headwater reaches, allowing allochthonous inputs to dominate the base of the aquatic food chain (Hynes 1975). As the canopy widens in a downstream direction, increased solar radiation stimulates algal growth and the invertebrate

community adapts to exploit this nutritious food resource. FPOM also becomes more abundant downstream due to continued mechanical abrasion and decomposition along the river continuum. Community structure usually shifts from a dependence on allochthonous sources at upstream sites, to a reliance on autochthonous sources at downstream sites.

Conventional methods used to determine relative food-source importance in stream ecosystems have relied on calculating P/R ratios, amassing organic matter budgets, and cataloging interactions between animals and their diet (Allan 1995). Ratios of gross primary production to total ecosystem respiration indicate whether a stream is a net consumer or producer of energy (Odum 1956). Organic energy budgets, usually with specific reference to carbon, provide extensive inventories of allochthonous and autochthonous inputs entering, leaving, and remaining within a certain stream reach (Fisher and Likens 1972), while mass balance equations determine whether the section is an energy source or a sink. Food-web connectivity diagrams use gut-content data to record interactions between consumers and their food, and provide detailed maps of trophic relationships (Hildrew et al. 1984). However, most of these techniques fail to some extent in their ability to provide a direct and quantitative approach to determining relative food-source importance to organisms at higher trophic levels. The P/R value does not apply to organisms, nor was it intended to (Rosenfeld and Mackay 1987). Organic energy budgets are time consuming, contain compartments which are difficult to measure, and also have no direct link to consumer diets (Cummins et al. 1983). Connectivity diagrams are extensive undertakings, and are flawed in their assumption that all interactions are of equal strength (Peters 1988). Furthermore, these diagrams rely mostly on stomach-content data, which can be of limited use due to the detrital nature of many food webs, the seasonality of food resources, and the ontogenetic shifts in diet observed for many organisms (Paine 1988).

Stable-isotope analysis

In recent years, stable-isotope ratios of common elements, such as carbon (^{13}C:^{12}C), nitrogen (^{15}N:^{14}N), sulfur (^{34}S:^{32}S), hydrogen (^{2}H:^{1}H), and oxygen (^{18}O:^{16}O) have received much attention regarding their ability to serve as chemical tracers of diet, animal migration, pollution, physiology, photosynthesis, and biogeochemical pathways in both current and paleo-ecological studies (see reviews by Fry and Sherr 1984, Peterson and Fry 1987, Rundel

et al. 1989, Coleman and Fry 1991, Knowles and Blackburn 1993, Lajtha and Michener 1994, Hobson 1999, and references contained therein). Because I concentrate on the use of stable-carbon and stable-nitrogen isotope ratios in this thesis, all subsequent explanations will be with reference to these 2 elements. For information on the stable-isotope ratios of other elements, the reader is directed to the excellent anthology by Fritz and Fontes (1980), as well as to the reviews mentioned above.

Stable-isotope ratios are expressed as delta values (δ) and are measures of a parts-per-thousand (or "per mil") difference (‰) between the isotope ratio of a sample and that of an international standard according to the formula:

$$\delta^{13}C \text{ or } \delta^{15}N = [(R_{sample} - R_{standard}) / R_{standard}] \times 1000$$

where $R = {}^{13}C/{}^{12}C$ or ${}^{15}N/{}^{14}N$. Heavier isotopes (${}^{13}C$ or ${}^{15}N$) react more slowly than lighter isotopes (${}^{12}C$ or ${}^{14}N$) in kinetic and equilibrium reactions because of slight differences in mass and bond-strength energy. Substrates with relatively more heavy isotopes are "more positive" or "enriched", whereas products with relatively fewer of the heavy isotopes are termed "more negative" or "depleted". International standards are Vienna Peedee Belemnite (VPDB) for ${}^{13}C/{}^{12}C$ (Coplen 1996) and nitrogen gas in the atmosphere for ${}^{15}N/{}^{14}N$ (Mariotti 1983). These standards are, by definition, set at 0‰.

The difference between the stable-isotope ratio of a substrate and its product is called "isotopic fractionation". Although isotopic fractionation varies greatly among physical, chemical, and biological processes, most reactions occur with some degree of predictability. For example, all plants discriminate against ${}^{13}CO_2$ in favor of ${}^{12}CO_2$ during photosynthetic carbon uptake (Fogel and Cifuentes 1993), but C_3 plants (approx. –28‰) are, on average, more depleted than C_4 plants (approx. –14‰) because the initial carboxylating enzyme in C_3 plants, ribulose bisphosphate (RUBP) carboxylase, discriminates against ${}^{13}C$ more so than phosphoenolpyruvate (PEP) carboxylase in C_4 plants (O'Leary 1988). Fractionation also occurs between the stable-isotope ratios of animals and their diet as a result of metabolic discrimination during respiration and excretion (Steele and Daniel 1978, Panteleev et al. 1999). However, isotopic discrimination in food webs is much smaller than that observed during photosynthesis, and typically at each trophic transfer of food energy, $\delta^{13}C$ values

enrich by 0-1‰, whereas $\delta^{15}N$ values increase by 3-5‰ (DeNiro and Epstein 1978, 1981, Tieszen et al. 1983, Minagawa and Wada 1984, but see Focken and Becker 1998).

These small, yet predictable, isotopic differences between animals and their diet allow $\delta^{13}C$ and $\delta^{15}N$ values to be used as chemical tracers in food-web ecology (Peterson and Fry 1987). While $\delta^{13}C$ values are used most often to distinguish between important primary food sources, such as C_3 and C_4 plants in grasslands (Cerling et al. 1999), aquatic algae and terrestrial leaf litter in streams (Rounick and Winterbourn 1986), and benthic and planktonic algae in lakes (Hecky and Hesslein 1995), $\delta^{15}N$ values are used to determine relative trophic position (Vander Zanden et al. 1996). In concert, $\delta^{13}C$ and $\delta^{15}N$ values provide effective profiles which can be used to quickly identify important pathways of energy transfer and food-web structure in many aquatic ecosystems (Fry 1991).

Stable-isotope ratios can also been used in quantitative mixing models to determine the relative importance of 2 or more food-sources (Gearing 1991). Calculations are based on simple equations, such as:

$$(X + Y)\ (\delta^{13}C_m) = (X)\ (\delta^{13}C_x) + (Y)\ (\delta^{13}C_y)$$

where X = amount of carbon from source X,

Y = amount of carbon from source Y,

$\delta^{13}C_m$ = stable-isotope ratio of the mixture,

$\delta^{13}C_x$ = stable-isotope ratio of source X, and

$\delta^{13}C_y$ = stable-isotope ratio of source Y.

Assuming only 2 food sources, this equation can be rearranged to:

$$F_x = \frac{\delta^{13}C_m - \delta^{13}C_y}{\delta^{13}C_x - \delta^{13}C_y}$$

where F_x is the relative proportion of carbon from source X.

Correction factors can also be incorporated into the model to account for isotopic fractionation due to trophic position. For example, in Catamaran Brook, the relative importance of allochthonous inputs to juvenile Atlantic salmon could be defined as:

$$\%Allochthonous = \frac{\delta^{13}C_{salmon} - \delta^{13}C_{autochthonous} - f \cdot x}{\delta^{13}C_{allochthonous} - \delta^{13}C_{autochthonous}} \times 100$$

where f = trophic fractionation in ^{13}C between an animal and its diet,

x = trophic position of salmon, measured using $\delta^{15}N$,

$\delta^{13}C_{salmon}$ = stable-isotope ratio of salmon,

$\delta^{13}C_{autochthonous}$ = stable-isotope ratio of autochthonous inputs (e.g., algae), and

$\delta^{13}C_{allochthonous}$ = stable-isotope ratio of allochthonous inputs (e.g., leaf litter).

Many studies have used quantitative isotope mixing models to determine the relative contribution of 2 or more food sources to organisms at higher trophic levels (Rau 1980, Kline et al. 1993, Junger and Planas 1994, Bilby et al. 1996, Doucett et al. 1996, Hilderbrand et al. 1996, Ben-David et al. 1997, Whitledge and Rabeni 1997, MacLeod 1998, Szepanski et al. 1999, Vander Zanden et al. 1999). However most studies-to-date have only incorporated single endpoints (i.e., average values) into their models, and have ignored isotopic variability in the primary food sources. Furthermore, robust estimates of error are rarely reported along with output from the model, raising valid concern over the reliability of results.

In summary, there appear to be many benefits regarding the use of stable isotopes in the determination of food-web relationships. Firstly, stable isotopes are chemical tracers and are not affected by the ambiguity associated with visual interpretation of detritus and other amorphous materials in gut contents. Secondly, stable isotopes refer to assimilated foods, rather than ingested materials identified in gut contents. Thirdly, stable isotopes are time-integrated measures of previous feeding history, as opposed to the single-point-in-time sample in gut contents. When multiple tissues are collected, stable-isotope ratios may reflect both short-term and long-term diet, because certain tissues (e.g., bone, muscle, blood, etc.) have different metabolic and elemental-turnover rates (Tieszen et al. 1983). Fourthly, stable isotopes can determine trophic position and account for omnivory with better precision than

other descriptors of food-web structure (Cabana and Rasmussen 1994) and can quantify the relative contribution of primary food sources using mixing model equations. Finally, stable isotopes possess the unique ability to link biogeochemistry and food-web dynamics, providing an integrative and much more holistic view of ecosystem structure and function (Wada and Yoshioka 1996, Leggett 1998).

Synopsis of thesis chapters

In chapter 2, the relationship between a stonefly host and a commensal chironomid was determined using $\delta^{13}C$ and $\delta^{15}N$ values. Trophic fractionation factors were calculated using field-collected data and values were compared to those reported in the literature. The ability of stable-isotope data to distinguish between parasitic and phoretic relationships in nature was also discussed. In chapter 3, stable-isotope relationships between a dominant scraper-grazer and epilithic algae were illustrated. The effect of water velocity on algal $\delta^{13}C$ and $\delta^{15}N$ values was reviewed, and the relationship between grazer $\delta^{13}C$ and $\delta^{15}N$ and feeding mode was explored. In chapter 4, extensive collections of hydropsychid caddisflies were made along the stream continuum in Catamaran Brook, and $\delta^{13}C$ and $\delta^{15}N$ values were used to determine spatial and temporal patterns in diet. Differences in food-source utilization and trophic position were determined for 7 co-existing hydropsychid species, and the importance of site selection was discussed in light of the heterogeneous nature of isotope profiles in streams. In chapter 5, the predictive power of a quantitative 2-source isotope mixing model was assessed using a statistical resampling technique. Consideration was given to isotopic variability in all compartments of the model. Power curves were provided to aid ecologists in future attempts to use stable-isotope data for quantitative purposes. In chapter 6, results from this thesis were briefly summarized and important contributions were highlighted. Recommendations were also made regarding the application of stable isotopes in forthcoming stream food-web studies.

CHAPTER TWO

Parasitic association of *Nanocladius* (Diptera: Chironomidae) and *Pteronarcys biloba* (Plecoptera: Pteronarcyidae): insights from stable isotopes

Abstract

Nymphs of *Pteronarcys biloba* Newman and attached chironomid larvae (*Nanocladius* [*Plecopteracoluthus*] undescribed sp., nr. *branchicolus*) were collected from Catamaran Brook, New Brunswick, in May and November 1997, for stable-carbon- and stable-nitrogen-isotope analysis. *Nanocladius* (*P.*) sp. had mean (± 1 SD) $\delta^{13}C$ and $\delta^{15}N$ values of –27.7 ± 1.0‰ and 4.9 ± 0.6‰, respectively, whereas those of *P. biloba* were –28.4 ± 1.0‰ and 1.3 ± 0.7‰, respectively. *Nanocladius* (*P.*) sp. always had more positive $\delta^{13}C$ and $\delta^{15}N$ values than *P. biloba*, and average fractionation factors (isotopic differences between symbiont and host) were +1.2‰ and +3.5‰ for carbon and nitrogen, respectively. These results suggest a parasitic relationship between *Nanocladius* (*P.*) sp. and *P. biloba*. No statistical differences were found among the $\delta^{13}C$ values of the plecopteran shredder in 4 stream reaches from headwaters to mouth, and $\delta^{13}C$ values were similar to those of their expected leaf litter diet. Pteronarcids from the headwater site (Upper Reach) were not parasitized and had $\delta^{15}N$ values distinct from those at downstream sites. However, stable-isotope ratios of parasitized stoneflies were not significantly different from those of non-parasitized individuals at the 3 other study locations. *Nanocladius* (*P.*) sp. were more enriched in ^{15}N than other chironomid genera in Catamaran Brook, including *Ablabesmyia*, a chironomid with predatory feeding habits, and fell within the range of other known invertebrate predators. These findings highlight the usefulness of stable-isotope technology to distinguish between phoresy and parasitism in ectosymbiotic relationships among aquatic organisms.

Introduction

Many chironomids live ectosymbiotically with other benthic invertebrates (Steffan 1967a). Larval associations have been documented for most aquatic insect orders including Plecoptera, Ephemeroptera, Trichoptera, Megaloptera, and Odonata (Steffan 1967b, Gotceitas and Mackay 1980, White et al. 1980, Jacobsen 1995, Dosdall and Parker 1998). Most relationships are phoretic, where the chironomid does not feed on the host's tissues, but uses the host for dispersal, protection from disturbance and desiccation, and food entrapment (Steffan 1967b, Dosdall and Mason 1981, Dosdall et al. 1986). However, parasitic feeding can occur (e.g., Jacobsen 1995), and the chironomid may obtain its nutrition directly from its host. In most cases, phoresy and parasitism are distinguished by the presence or absence of feeding scars on the host organism, the presence or absence of detrital particles in the chironomid gut, or direct observations of feeding. Alternatively, it is simply assumed that the relationship is phoretic.

Stable-isotope analysis (SIA) may help to distinguish between phoretic and parasitic relations among chironomids and their hosts. SIA is a chemical analysis of food-source origins and energy pathways, and is unencumbered by visual interpretation of ambiguous stomach contents and detrital pools (Peterson and Fry 1987). The method is based on the fact that naturally occurring stable-isotope ratios of common elements such as carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) are assimilated into primary producers with signatures characteristic of various biogeochemical processes, and are passed along food chains with relatively predictable change (reviewed in Fry and Sherr 1984, Peterson and Fry 1987, Rundel et al. 1989, Lajtha and Michener 1994). In addition, SIA provides a composite picture of feeding over a period of time, rather than the single point-in-time sample obtained through gut-content analysis. Typically, at each trophic transfer of food energy, stable-carbon-isotope ratios (expressed as $\delta^{13}C$) enrich by 0-1‰, whereas stable-nitrogen-isotope ratios ($\delta^{15}N$) increase by 3-5‰ (DeNiro and Epstein 1978, 1981, Tieszen et al. 1983, Minagawa and Wada 1984, but see Focken and Becker 1998). In aquatic ecology, $\delta^{13}C$ values have been used most often to distinguish between important primary food sources (e.g., aquatic algae versus terrestrial leaf litter; Rounick and Winterbourn 1986), whereas $\delta^{15}N$ values allow the accurate determination of trophic position (Vander Zanden et al. 1996). Stable isotopes have also been used to study biotic relationships, e.g., symbiotic relationships

among terrestrial plants (Elheringer et al. 1985), marine bivalves (Conway et al. 1989), and ascidians (Kline and Lewin 1999); and trophic relations between rabbit hosts and their endoparasites (Boag et al. 1998).

My objective was to identify the relationship between *Nanocladius* (*Plecopteracoluthus*) undescribed sp., nr. *branchicolus*, and *Pteronarcys biloba* Newman in Catamaran Brook, New Brunswick, using stable-isotope ratios of carbon and nitrogen. *Nanocladius* (*P.*) sp. is a single species in the brook and is currently being described by R. E. Jacobsen (National Park Service, Homestead, FL). The midge has a 1-y life cycle in Catamaran Brook, and spends most of the year attached to its stonefly host (Giberson et al. 1996). Midges were first noted as 2nd-instar larvae on the host in mid June, reached 4th-instar by the end of August, and overwintered as 4th-instar larvae on *Pteronarcys*. Giberson et al. (1996) reported a parasitic association between the midge and the stonefly. *Nanocladius* (P) sp. is also believed to be parasitic on other species of *Pteronarcys* in the eastern USA (R. E. Jacobsen, personal communication). However, some authors have reported that *Nanocladius*, including some on *Pteronarcys*, are phoretic (Gotceitas and Mackay 1980, Dosdall and Mason 1981, Dosdall et al. 1986, Dosdall and Parker 1998). To my knowledge, this study is the first attempt to verify ectoparasitism among freshwater benthic invertebrates using stable-isotope ratios.

Methods

Study Site

Catamaran Brook (lat. 46°52.7′N, long. 66°06.0′W) is a tributary of the Little Southwest Miramichi River, located in a pristine forested area of north-central New Brunswick (Fig. 2.1). The brook is a well buffered, circumneutral, 3rd-order stream, ~ 20.5 km long, with a drainage area of 52 km^2 (Cunjak et al. 1993). Riparian vegetation in the basin consists of 60% deciduous trees, including white birch (*Betula papyrifera*), yellow birch (*Betula lutea*), sugar maple (*Acer saccharum*), American beech (*Fagus grandifolia*), and speckled alder (*Alnus rugosa*). The remaining 40% of riparian forest cover consists of conifers, including balsam fir (*Abies balsamea*), red spruce (*Picea rubens*), and eastern white cedar (*Thuja occidentalis*). Litter fall generally peaks in late September and leaves show rapid conditioning and fungal colonization, especially in autumn (Garnett et al. 2000).

Catamaran Brook is currently the focus of several multidisciplinary studies evaluating logging impacts on the habitat and productivity of Atlantic Salmon (*Salmo salar*). Four study reaches (Upper, Middle, Gorge, and Lower) have been selected for long-term study. The Upper Reach consists of a 180-m stretch in the headwater region, and is characterized by a narrow (1-2 m) shaded stream channel, generally coarse substrates, steep slope, and rapid flow. The Middle Reach, located approximately half way down the stream channel, has a stream width of 6-8 m, riffle gradients of 2-2.3% and riffle substrates of cobble and gravel. The Gorge Reach, located ~ 2 km downstream of the Middle Reach, runs through a bedrock outcrop area and is 6-8 m wide with riffle gradients of ~ 2% and a primarily bedrock substrate with some gravel and cobble. The Lower Reach consists of the lower 2 km of the stream and is 8-12 m in width, has riffle gradients of 1.6-1.75%, and riffle substrates of gravel, cobble, and boulder. Other details on geochemistry, hydrology, and biological factors can be found in Cunjak et al. (1993) and Giberson and Caissie (1998).

Invertebrate sampling

Invertebrates were collected on 28-30 May 1997 from run-riffle habitats, using a D-frame kick net with 250-µm nylon mesh from the 4 main study reaches in Catamaran Brook. Collections of *P. biloba* and *Nanocladius* (*P.*) sp. were supplemented on 2-4 November 1997, but no isotopic differences were found between those sampled on the 2 dates (*t*-test for independent means, $p > 0.05$). On both of these dates, *Nanocladius* (*P.*) sp. were attached to *Pteronarcys* nymphs as 4th-instar larvae. Kick samples were preserved with 85% ethanol (v/v) and stored in plastic bags. Invertebrates were sorted from debris and identified to the lowest possible taxon using the keys of Merritt and Cummins (1996). *Nanocladius* (*P.*) sp. were removed from their silken tubes, which were attached to the bodies of *P. biloba*. "Free-living" chironomids (defined here as those not found attached to a host organism) were removed from debris and identified to genus by the inspection of head capsules mounted on glass slides using the keys of Wiederholm (1983).

Stable-isotope analysis

Stable-carbon- and stable-nitrogen-isotope analysis required only 0.6 mg tissue (dry mass) per sample. Invertebrates were oven-dried at constant temperature (60 °C) for 24 to 48

h and ground to a fine powder using either a mortar and pestle or a ball-mill grinder. Up to 10 individuals of smaller taxa (e.g., chironomids, simuliids, elmids, etc.) were required for each replicate sample. For larger organisms (e.g., pteronarcids, perlids, hydropsychids, tipulids, etc.), an aliquot of powdered tissue was used for each individual. All *Nanocladius* (*P.*) sp. found on a single stonefly were combined and the pooled sample was used in the analysis. Under no circumstances were *Nanocladius* (*P.*) sp. pooled among hosts.

Stable-isotope ratios are expressed as delta values (δ) and are measures of a parts-per-thousand (or "per mil") difference (‰) between the isotope ratio of a sample and that of an international standard according to the formula:

$$\delta\,^{13}C \text{ or } \delta\,^{15}N = [\,(R_{sample} - R_{standard}) / R_{standard}] \times 1000$$

where $R = {}^{13}C/{}^{12}C$ or ${}^{15}N/{}^{14}N$. Samples that are "more negative" are "depleted" and contain less ^{13}C or ^{15}N; samples that are "less negative" are "enriched" and contain more of the heavier isotopes. International standards are Vienna Peedee Belemnite (VPDB) (Coplen 1996) and nitrogen gas in the atmosphere (Mariotti 1983). These standards are set at a value of 0‰.

Isotopes were analyzed on a Micromass VG Isochrom continuous-flow isotope-ratio mass spectrometer connected to a Carlo Erba elemental analyzer at the Environmental Isotope Laboratory (EIL) (University of Waterloo, Waterloo, Ontario, Canada). Repeat analyses of common laboratory standards yielded results that were both accurate and precise (International Atomic Energy Agency [IAEA] standard CH6: $\delta^{13}C = -10.5 \pm 0.2$‰ [mean ±1 SD, $n = 32$]; IAEA-N1: $\delta^{15}N = 0.6 \pm 0.3$‰ [$n = 25$]). Replicate samples of a lipid-extracted fish standard (EIL-70) also gave reliable δ values for carbon and nitrogen ($\delta\,^{13}C = -20.7 \pm 0.2$‰ [$n = 38$], and $\delta\,^{15}N = 16.4 \pm 0.3$‰ [$n = 38$]).

Statistical analysis

Data were analyzed using SYSTAT (version 8.0, SPSS Inc., Chicago). Maximum Type-I error rates were set at $\alpha = 0.05$. Normality and homogeneity of variance assumptions were checked using plots of the residuals. Significant ANOVA results were followed by multiple comparisons using Tukey's HSD post-hoc test (Sokal and Rohlf 1995). Model-2 linear regressions (Sokal and Rohlf 1995) were used to determine the significance of relationships between *Nanocladius* (*P.*) sp. and *P. biloba* $\delta^{13}C$ and $\delta^{15}N$ values.

Results

One-hundred and ten *P. biloba* nymphs were collected, 84 of which were hosts to *Nanocladius* (*P.*) sp. Midge densities ranged from 1 to 12 midges per host (average ±1 SD = 4.4 ± 2.6). No pteronarcids in the Upper Reach were parasitized, and non-parasitized nymphs in other locations were usually smaller (<15 mm in length). Most *Nanocladius* (*P.*) sp. were attached to the femora or were located on the thoracic pleura beneath the wingpads.

The $\delta^{13}C$ and $\delta^{15}N$ values of *P. biloba* ranged from –31‰ to –26‰, and –0.5‰ to +4‰, respectively (Fig. 2.2), and were slightly more enriched than those of their presumed leaf litter diet (Table 2.1). Although no differences were noted in the $\delta^{13}C$ values of *P. biloba* among the 4 study reaches (Tukey's HSD, $p > 0.05$), $\delta^{15}N$ varied and *P. biloba* from the Upper Reach were more enriched in ^{15}N than those from all other sites (Fig. 2.3). However, stable-isotope ratios of non-parasitized individuals at 3 other locations did not differ from parasitized ones (Tukey's HSD, $p > 0.05$) (Fig. 2.3).

Model-2 linear regression showed a significant relationship between the stable-isotope ratios of *Nanocladius* (*P.*) sp. and those of *P. biloba* ($p < 0.001$; Fig. 2.4). This relationship indicated that isotopically more enriched *Nanocladius* (*P.*) sp. were found on isotopically more enriched hosts. Average isotopic differences, or fractionation factors, between *Nanocladius* (*P.*) sp. and *P. biloba* were +1.2 ± 0.7‰ and +3.5 ± 0.5‰, for carbon and nitrogen, respectively.

Nanocladius (*P.*) sp. were not isotopically distinct from most other chironomids with respect to $\delta^{13}C$, but *Eukiefferiella* and *Heterotrissocladius* were more ^{13}C-depleted than other genera (Table 2.2). *Nanocladius* (*P.*) sp. were, however, much more ^{15}N-enriched than all other midges sampled including *Ablabesmyia* (Table 2.2). Stable-nitrogen-isotope ratios showed a strong relationship with feeding type in Catamaran Brook, and *Nanocladius* (*P.*) sp. was similar to invertebrates with predatory habits (Fig. 2.5).

Discussion

Relationship between Nanocladius and Pteronarcys

Laboratory feeding experiments have shown that animals are isotopically more enriched than their diet (DeNiro and Epstein 1978, 1981, Tieszen et al. 1983). For carbon ($\delta^{13}C$), animals are usually only slightly more enriched than their food (i.e., 0 to 1‰) but, for

nitrogen ($\delta^{15}N$), trophic fractionation is much higher (i.e., 3 to 5‰; Peterson and Fry 1987). Isotopic enrichment occurs along food chains because metabolic processes, such as respiration and excretion, preferentially use lighter isotopes (^{12}C and ^{14}N), leaving more of the heavier isotopes (^{13}C and ^{15}N) to accumulate in animal tissues (Steele and Daniel 1978, Hobson and Clark 1992). With respect to my study, if *Nanocladius* (*P.*) sp. were feeding parasitically on *P. biloba*, then: 1) the chironomid should be isotopically more enriched than its host, 2) isotopic differences between the 2 animals should fall within the range of expected values for diet-tissue fractionation measured in previous laboratory studies, and 3) individual variation in stable-isotope ratios between host and parasite should be correlated. My results conform to these 3 expectations. *Nanocladius* (*P.*) sp. had $\delta^{13}C$ and $\delta^{15}N$ values that were, on average, 1.2‰ and 3.5‰ more enriched than those of *P. biloba*, similar to values reported in the literature. In addition, stable-isotope ratios correlated well between *Nanocladius* (*P.*) sp. and *P. biloba*, with isotopically enriched chironomids found on isotopically enriched hosts. Thus, stable-isotope ratios confirm that *Nanocladius* (*P.*) sp. is parasitic on *P. biloba* in Catamaran Brook.

Pteronarcys as a primary consumer

Stable-isotope ratios of *P. biloba* showed that this stonefly was feeding primarily on coarse particulate organic matter in Catamaran Brook. Deciduous leaf litter collected from the brook ranged from –31‰ to –29‰ for $\delta^{13}C$. The $\delta^{13}C$ values for *P. biloba* were similar, but slightly more enriched at –31‰ to –26‰. Because $\delta^{13}C$ values are used to elucidate primary food sources, values for *P. biloba* indicate that it obtained its energy from terrestrial leaf litter as opposed to aquatic plants (e.g., algae), which in Catamaran Brook tend to be more depleted than allochthonous inputs (Chapter 3). Trophic enrichment occurs in such a way that animals at the base of food chains possess lower $\delta^{15}N$ values than animals that feed at higher levels (Minagawa and Wada 1984). *Pteronarcys biloba* had $\delta^{15}N$ values between –0.5‰ and +4.0‰. These $\delta^{15}N$ values were more enriched than those of leaf litter (-1‰ to +1‰), and were more depleted than most other benthic invertebrates, and fish (Doucett et al. 1996) sampled at the same sites.

Although intraspecific differences in stable-isotope ratios have been previously correlated with physiological stress (Ambrose and DeNiro 1987, Hobson et al. 1993),

differences in *P. biloba* $\delta^{15}N$ values do not appear to be related to parasitism by *Nanocladius* (*P.*) sp. The most ^{15}N-enriched *P. biloba* in our study were non-parasitized individuals from the Upper Reach. Parasitized stoneflies were not isotopically distinct from non-parasitized ones. Differences in *P. biloba* $\delta^{15}N$ (and $\delta^{13}C$) may be a result of: 1) differences in the stable-isotope ratios of leaf litter at the 4 stream reaches, 2) variable assimilation of attached algal material, or 3) varying degrees of facultative omnivory. Pteronarcids are thought to be large-particle detritivores and important consumers of leaf material in headwater streams (Cummins 1974). Recent evidence suggests that pteronarcids may ingest some algae (Freilich 1991), and that the relative importance of autochthonous sources may increase with stream size (Plague et al. 1998). Gut analyses also have shown that pteronarcids ingest animal material along with their detrital diet (Freilich 1991). More information on *P. biloba* gut contents and leaf litter composition along Catamaran Brook would help clarify this issue.

Nanocladius trophic position

Isotopic comparisons with other benthic invertebrates clearly showed that *Nanocladius* (*P.*) sp. were functioning as parasites and not as detritivores. *Nanocladius* was isotopically more enriched than all other chironomids, including the predator, *Ablabesmyia*. The $\delta^{13}C$ and $\delta^{15}N$ values of other chironomids suggested that they were feeding on detritus, or fine particulate organic matter, which in Catamaran Brook had stable-isotope ratios near –27‰ for carbon and 0‰ for nitrogen (Chapter 4). *Eukiefferiella* was significantly more depleted (–30‰) than all other midges, which indicates that it consumed algae as well as detrital material. Finally, *Nanocladius* (*P.*) sp. were among some of the most ^{15}N-enriched invertebrates sampled in this study, with $\delta^{15}N$ values that were more similar to those of the predators, *Agnetina* (Plecoptera), *Atherix* (Diptera), and *Nigronia* (Megaloptera), than to those of invertebrates with herbivorous diets.

In conclusion, it was encouraging to observe field data that conformed to the isotopic fractionation factors obtained previously by others in the laboratory. Many studies using SIA to determine feeding relations among aquatic organisms presume that trophic enrichment between diet and animal is 0‰ to 1‰ for $\delta^{13}C$ and 3‰ to 5‰ for $\delta^{15}N$ because it is often too difficult to obtain exact measurements of diet-animal isotopic fractionation in the field. The validity of this underlying assumption is crucial to the success of SIA in ecology, but has

generally gone untested, prompting a call by some (Gannes et al. 1997) to retreat to the laboratory for further testing of isotopic patterns and dietary relations. Parasitic associations are ideal situations in which to test and confirm trophic enrichment of stable isotopes because the diet of the parasite is known and the fractionation factors can be measured accurately. Enrichment of +1.2‰ for $\delta^{13}C$ and +3.5‰ for $\delta^{15}N$ between *P. biloba* and *Nanocladius* (*P.*) sp. demonstrated that at least 1 of the many tenets of isotopic ecology appeared to hold true in field situations.

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Table 2.1. Stable-isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) of submerged deciduous leaves from common riparian trees in the Catamaran Brook drainage basin. Collections were made in late May 1997 from the Lower Reach. Values are means ±1 SD, with sample sizes in parentheses.

Species	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
American beech (*Fagus grandifoila*)	−31.0 ± 1.0 (4)	1.3 ± 2.0 (4)
Speckled alder (*Alnus rugosa*)	−29.5 ± 0.8 (5)	−1.1 ± 0.6 (5)
Sugar maple (*Acer saccharum*)	−30.5 ± 0.5 (4)	−0.6 ± 0.7 (4)
White birch (*Betula papyrifera*)	−30.9 ± 0.3 (3)	0.4 ± 0.6 (3)
Yellow birch (*Betula lutea*)	−30.4 ± 0.3 (3)	0.2 ± 0.8 (3)

Table 2.2. Stable-isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) of late-instar chironomids collected in late May 1997 from the Lower Reach of Catamaran Brook, New Brunswick. Values are means ±1 SD, with sample sizes in parentheses. Different superscripts represent means that are significantly different from one another (Tukey's HSD, $p < 0.05$).

Genus	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
Ablabesmyia	-27.6 ± 0.7 (8)^{a}	3.4 ± 0.3 (8)^{c}
Eukeifferiella	-30.3 ± 1.0 (5)^{b}	1.5 ± 0.7 (5)^{a}
Heterotrissocladius	-28.7 ± 2.7 (6)^{ab}	2.0 ± 0.5 (6)^{ab}
Micropsectra	-27.2 ± 0.7 (4)^{a}	3.1 ± 0.2 (4)^{bc}
Microtendipes	-27.2 ± 0.5 (14)^{a}	2.5 ± 1.0 (14)^{b}
Nanocladius (*P.*) sp.	-27.1 ± 0.8 (29)^{a}	4.6 ± 0.5 (29)^{d}

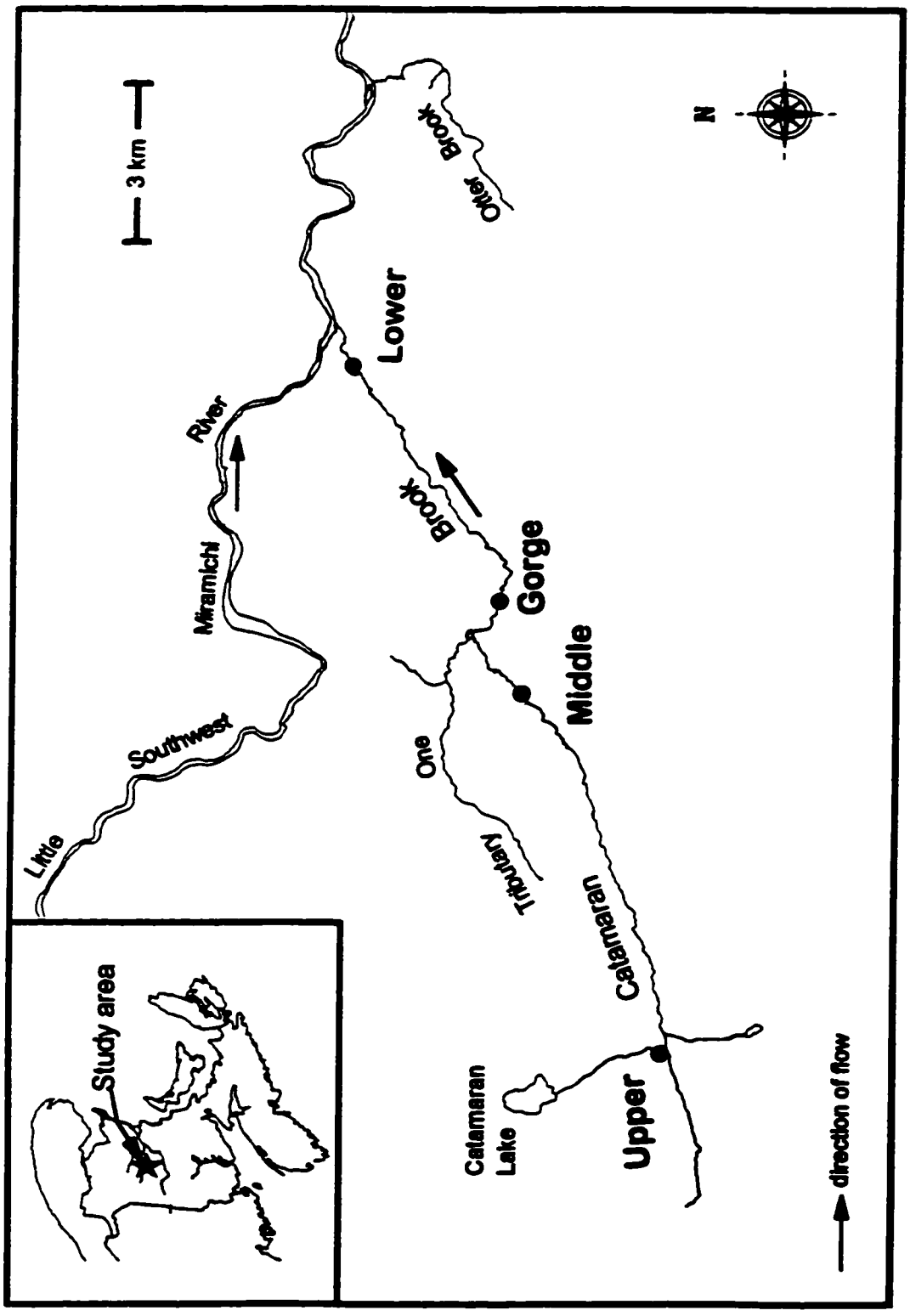

Figure 2.1. Catamaran Brook, New Brunswick, eastern Canada, showing the location of the four study sites.

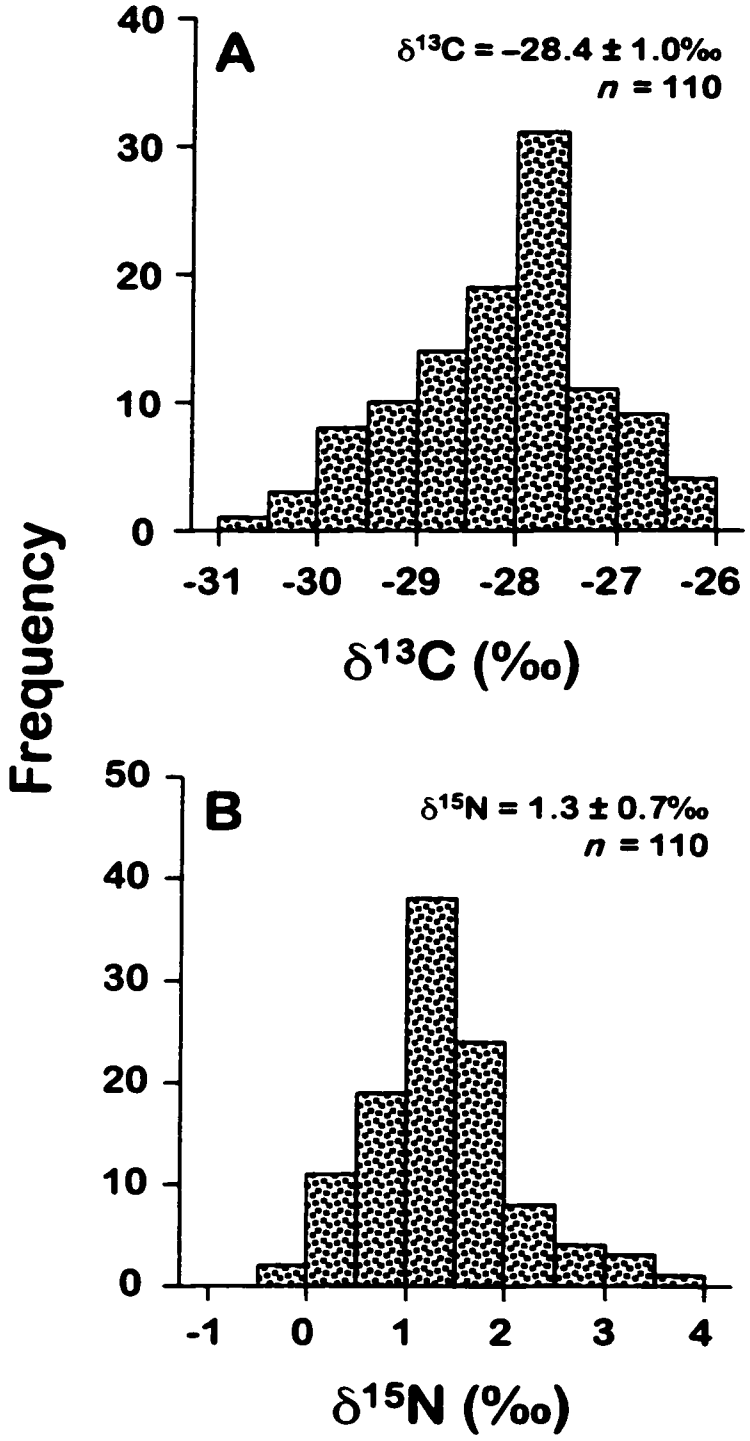

Figure 2.2. Stable-isotope ratios (A: $\delta^{13}C$ and B: $\delta^{15}N$) of *Pteronarcys biloba* collected in late May and early November 1997 from Catamaran Brook, New Brunswick (pooled across dates and locations). Average values (±1 SD) and sample sizes (*n*) are included.

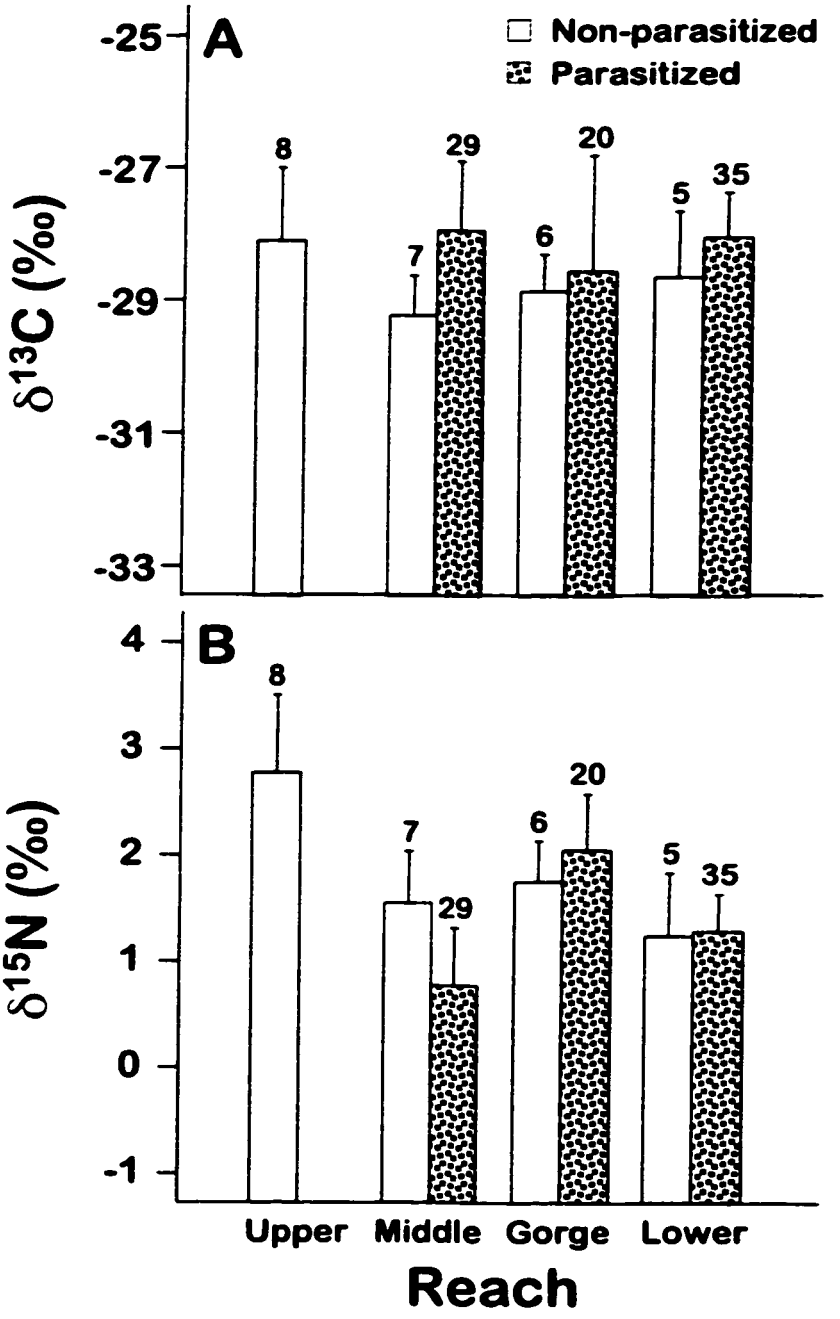

Figure 2.3. Stable-isotope ratios (A: $\delta^{13}C$ and B: $\delta^{15}N$) of parasitized and non-parasitized nymphs of *Pteronarcys biloba*, collected in late May and early November 1997 from Catamaran Brook, New Brunswick. Values are averages (+1 SD) for each reach (pooled across dates). Sample sizes are shown above the error bars.

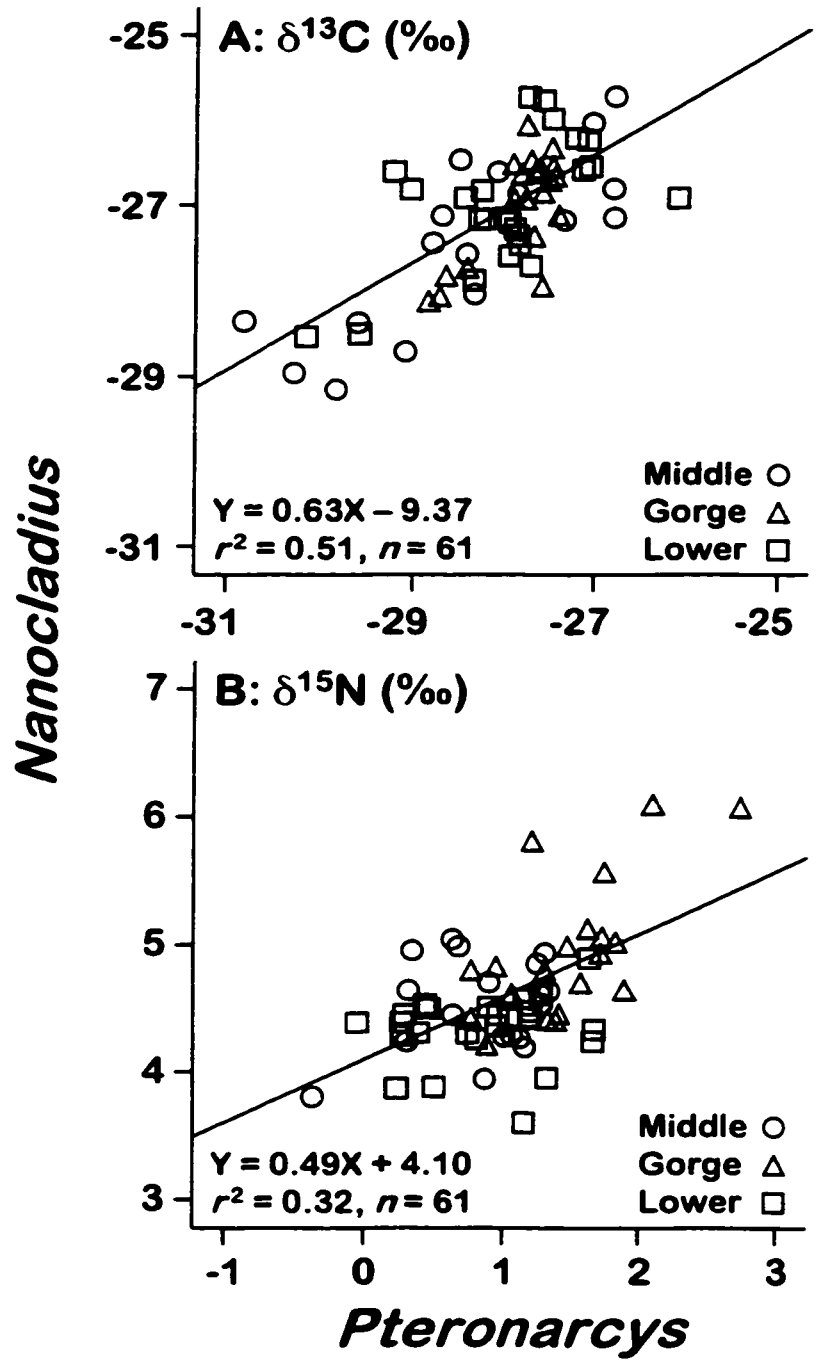

Figure 2.4. Relationship between $\delta^{13}C$ (A) and $\delta^{15}N$ (B) of *Nanocladius* (*P.*) sp. and *Pteronarcys biloba*, collected in late May and early November 1997 from Catamaran Brook, New Brunswick (samples pooled across date and location). Model-2 regression equations and coefficients of determination (r^2) are given for each relationship. Sample size (n) is 61 because 23 of 84 *P. biloba* nymphs did not host enough chironomids to reach the mass required for stable-isotope analyses.

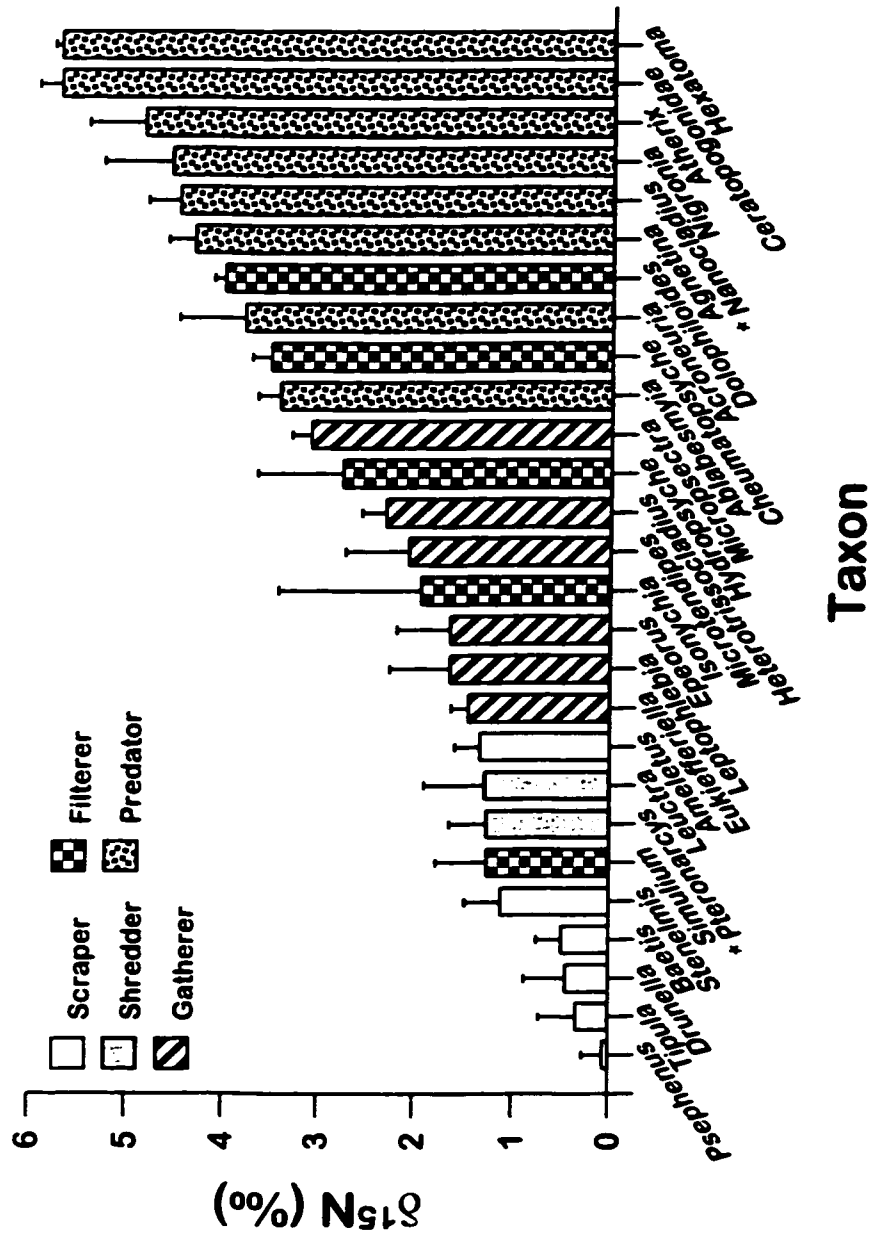

Figure 2.5. Stable-nitrogen-isotope ratios ($\delta^{15}N$) of late-instar benthic invertebrates sampled from run-riffle habitats in the Lower Reach, Catamaran Brook, New Brunswick, in late May 1997. Values are averages +1 SD. $N = 3$ to 20 replicates. Functional-feeding groups were designated according to descriptions in Merritt and Cummins (1996). Trophic patterns were similar at other sites and on other dates. Asterisks denote the 2 main study taxa, *Pteronarcys biloba* and *Nanocladius* (*P.*) sp.

CHAPTER THREE

The stable-isotope relationship between a scraper-grazer, *Glossosoma nigrior* (Glossosomatidae: Trichoptera), and its algal diet

Abstract

Epilithic algae, collected as biofilm scraped from rock surfaces, exhibited distinct $\delta^{13}C$ and $\delta^{15}N$ values in Catamaran Brook and the Little Southwest Miramichi River, New Brunswick, in August 1997. General patterns in algal $\delta^{13}C$ appeared to be related to the $\delta^{13}C$ of dissolved inorganic carbon at each site. The grazer, *Glossosoma nigrior*, also showed markedly different $\delta^{13}C$ and $\delta^{15}N$ among the 4 sites, and values were correlated with those of algae. Faster water velocities resulted in more depleted $\delta^{13}C$ and more enriched $\delta^{15}N$ values in algae and grazers than slower currents, but only at 1 of 4 sites. This suggests that other factors (e.g., carbon-concentrating mechanisms) may be more important in the determination of stable-isotope ratios in lotic microalgae. *Glossosoma nigrior* were usually more ^{13}C-depleted and ^{15}N-enriched than other grazers, and differences may be related to feeding modes (e.g., excavators vs. brushers). Grazers may be better indicators of autochthonous $\delta^{13}C$ than biofilm because of potential terrestrial and heterotrophic contamination in the epilithic matrix, but care should be given to the selection of primary consumers as isotopic surrogates of original food sources. Extensive variation in algal and grazer $\delta^{13}C$ and $\delta^{15}N$ values in this study emphasizes a need for more comprehensive sampling of autochthonous resources when using stable-isotope ratios for food-web interpretation.

Introduction

Algae are important to the diet of many stream invertebrates (Chapman and Demory 1963, Lamberti and Moore 1984), and are possibly an under-appreciated food source in some lotic ecosystems (Minshall 1978). The contribution of algae to food webs in temperate forest streams increases downstream as a result of more open canopies and better light penetration (Vannote et al. 1980). While some invertebrate species feed on detritus as early instars, and become more dependent on algae in late instars (Williams and Williams 1982, Bird and Kaushik 1984, Fuller and Desmond 1997), other grazers have evolved morphological adaptations which allow them to continually exploit algal pastures throughout their life histories (Arens 1994).

Stable-isotope analysis (SIA) may help to determine the relative importance of detrital and algal material in the diets of co-existing invertebrate grazers. SIA is a chemical analysis of food-source origins and energy pathways, and is unencumbered by visual interpretation of ambiguous stomach contents and detrital pools (Peterson and Fry 1987). The method relies on the fact that ratios of naturally occurring stable isotopes of common elements such as carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) are assimilated into primary producers with signatures characteristic of certain biogeochemical processes, and are passed along food chains with relatively predictable change (reviewed in Fry and Sherr 1984, Peterson and Fry 1987, Rundel et al. 1989, Lajtha and Michener 1994). In addition, SIA provides a composite picture of feeding over a period of time, rather than the single point-in-time sample obtained through gut-content analysis, and reflects assimilated versus ingested materials. Typically, at each trophic transfer of food energy, stable-carbon-isotope ratios (expressed as $\delta^{13}C$) enrich by 0-1‰, whereas stable-nitrogen-isotope ratios ($\delta^{15}N$) increase by 3-5‰ (DeNiro and Epstein 1978, 1981, Tieszen et al. 1983, Minagawa and Wada 1984, but see Focken and Becker 1998).

In aquatic ecology, $\delta^{13}C$ values have been used most often to distinguish between important primary food sources, such as aquatic algae and terrestrial leaf litter (Rounick and Winterbourn 1986), while $\delta^{15}N$ values allow the accurate determination of trophic position (Vander Zanden et al. 1996). Terrestrial plant $\delta^{13}C$ values remain fairly constant near –30‰ to –26‰ (O'Leary 1988), while stable-isotope ratios in aquatic plants vary extensively, resulting from differences in light intensity, growth rate, nutrient uptake and availability

(Keeley and Sandquist 1992, Goericke et al. 1994). Water velocity also affects stable-isotope ratios in aquatic plants through reductions in boundary layer diffusion (Osmond et al. 1981, Raven et al. 1982, Hecky and Hesslein 1995), but the effect of water velocity on stable-isotope ratios in lotic microalgae remains unresolved (MacLeod and Barton 1998, Finlay et al. 1999). Although numerous studies have incorporated stable-isotope ratios in their analyses of stream food webs (e.g., Rounick et al. 1982, Bunn et al. 1989, Rosenfeld and Roff 1992, Angradi 1994, Junger and Planas 1994, Mihuc and Toetz 1994, Doucett et al. 1996, Whitledge and Rabeni 1997, Finlay et al. 1999), few have detailed the relationship between invertebrate grazers and their algal food sources. The first objective of this study was to determine the effects of water velocity on $\delta^{13}C$ and $\delta^{15}N$ values in epilithic algae and an abundant trichopteran grazer, *Glossosoma nigrior* (Banks), along a stream continuum in Catamaran Brook, New Brunswick. *Glossosoma nigrior* is a dominant scraper-grazer in many stream ecosystems and feeds selectively on diatoms and other algal species in the biofilm community (Tindall and Kovalak 1979, Oemke 1984). The second objective was to determine whether morphological differences in feeding modes (e.g., brushers vs. gougers, see Arens 1994) could help explain potential differences in $\delta^{13}C$ and $\delta^{15}N$ values among various grazer species.

Methods

Study Site

Catamaran Brook (lat. 46°52.7′N, long. 66°06.0′W) is a tributary of the Little Southwest Miramichi (LSW) River, located in a pristine forested area of north-central New Brunswick (Fig. 3.1). The brook is a well buffered, circumneutral, 3rd-order stream, ~ 20 km in length, with a drainage area of 52 km^2 (Cunjak et al. 1993). The brook is currently the focus of several multidisciplinary studies evaluating logging impacts on the habitat and productivity of Atlantic Salmon (*Salmo salar*). Main stream reaches selected for long-term study include the Upper Reach: a 180-m stretch in the headwater region, characterized by a narrow (1-2 m) shaded stream channel, generally coarse substrates, steep slope, and rapid flow; the Gorge Reach, located half way up the brook, running through a bedrock outcrop area, 6-8 m wide with riffle gradients of ~ 2% and a primarily bedrock substrate with some gravel and cobble; and the Lower Reach in the lower 2 km of the stream which is 8-12 m in

width, has riffle gradients of 1.6-1.8%, and gravel, cobble, and boulder substrates. In this particular study, sampling also occurred at 1 additional site in the LSW Miramichi River, about 50 m upstream of the confluence with Catamaran Brook (Fig 3.1). Water chemistry data are provided in Table 3.1. More detail on the biology, geochemistry and hydrology of the brook can be found in Cunjak et al. (1993).

Invertebrate sampling

Larvae of *G. nigrior* were hand-picked from 15-20 randomly selected stones in run-riffle habitats at 4 sites (Upper, Gorge, Lower and LSW) (Fig 3.1). Water velocity was measured at six-tenths depth above each stone to the nearest 0.01 $m \cdot s^{-1}$ using a Marsh-McBirney flow meter. Up to 10 larvae were removed from the upper surfaces of each stone and preserved with 85% ethanol in the field (Hobson et al. 1997). Species identification was confirmed using the keys of Neubauer and Roberston (1985). Other invertebrate grazers were collected at each of the 4 sampling locations using a D-frame kick net with 250-μm nylon mesh, and were identified using keys in Merritt and Cummins (1996). Collections took place between 04-28 August 1997.

DIC and algae sampling

Stream water was collected at the 4 sites for stable-isotope analysis of dissolved inorganic carbon (DIC) at 2-week intervals in August 1997. DIC samples were stored in duplicate 500-mL glass bottles and preserved with a saturated $HgCl_2$ solution. DIC was then isolated from stream water using 2 mL of H_3PO_4 and cryogenic separation on an evacuated closed-line system. Assuming both atmospheric and chemical equilibrium, $\delta^{13}C$ values of aqueous CO_2 were calculated from those of DIC using estimates of CO_2 and HCO_3^- concentrations from pH readings (Pankow 1991) and hydration isotope effects from water temperature measurements (Mook et al. 1974). Isotopic fractionation resulting from photosynthetic carbon uptake (ε_p) was calculated as the difference between the $\delta^{13}C$ values of aqueous CO_2 and epilithic algae. At each site, biofilm was scrubbed from the upper surfaces of stones that hosted *G. nigrior*, filtered onto pre-combusted Whatman GF/F glass-fiber filters, and used as isotopic proxies for epilithic algae. Filters were inspected for large (ca. ≥1 mm) detrital material which, if found, was subsequently removed. Filters were then

acidified using 1N HCl, re-neutralized with distilled, deionized water, and air-dried, to reduce isotopic contamination by inorganic carbon.

Stable-isotope analysis

Simultaneous analysis of stable-carbon and stable-nitrogen-isotope ratios required approximately 1 mg tissue (dry mass) per sample. Individual invertebrates were oven-dried at constant temperature (60 °C) for 24-48 h and ground to a fine powder using either a mortar and pestle or a ball-mill grinder. Stable-isotope ratios are expressed as delta values (δ) and are measures of a parts-per-thousand (or "per mil") difference (‰) between the isotope ratio of a sample and that of an international standard according to the formula:

$$\delta\,^{13}C \text{ or } \delta\,^{15}N = [\,(R_{sample} - R_{standard}) / R_{standard}] \times 1000$$

where $R = {}^{13}C/{}^{12}C$ or ${}^{15}N/{}^{14}N$. Samples that are "more negative" are "depleted" and contain less ^{13}C or ^{15}N; samples that are "less negative" are "enriched" and contain more of the heavier isotopes. International standards are Vienna Peedee Belemnite (VPDB) (Coplen 1996) and nitrogen gas in the atmosphere (Mariotti 1983). These standards are, by definition, set at 0‰.

Isotope analyses of organic materials were performed on a Micromass VG Isochrom continuous-flow isotope-ratio mass spectrometer connected to a Carlo Erba elemental analyzer at the Environmental Isotope Laboratory (EIL) (University of Waterloo, Waterloo, Ontario, Canada). Inorganic materials (i.e., DIC) were analyzed on a VG Isogas (Prism Series II) stable-isotope-ratio mass-spectrometer. Repeat analyses of commercially available standards yielded results that were both accurate and precise (International Atomic Energy Agency [IAEA] standard CH6: $\delta^{13}C = -10.5 \pm 0.2$‰ [mean ± 1SD, $n = 32$]; IAEA-N1: $\delta^{15}N = 0.6 \pm 0.3$‰ [$n = 25$]). Replicates of 2 internal laboratory standards: EIL-70 (lipid-extracted fish tissue), and EIL-30 (tap water) gave reliable δ values as well ($\delta^{13}C = -20.7 \pm 0.2$‰ [$n = 38$], and $\delta^{15}N = 16.4 \pm 0.3$‰ [$n = 38$], and $\delta^{13}C = -13.4 \pm 0.3$‰ [$n = 16$], for EIL-70 and EIL-30 respectively).

Statistical analysis.

Analyses were performed using SYSTAT (version 8.0, SPSS Inc., Chicago). Maximum Type-I error rates were set at $\alpha = 0.05$. Normality and homogeneity of variance

assumptions were checked using plots of the residuals. Among-site differences in algal and *Glossosoma* $\delta^{13}C$ and $\delta^{15}N$ values were examined using fixed-effects one-way ANOVAs, followed by multiple comparisons via Tukey's HSD post-hoc tests (Sokal and Rohlf 1995). Pearson's correlation coefficients (r) were used to determine the strength of relationships between algal and *Glossosoma* $\delta^{13}C$ and $\delta^{15}N$ values. The effect of water velocity on algal and *Glossosoma* $\delta^{13}C$ and $\delta^{15}N$ was evaluated using model-2 linear regression (Sokal and Rohlf 1995).

Results

Epilithic algae

Algae showed distinct patterns at each site when both $\delta^{13}C$ and $\delta^{15}N$ values were considered (Fig. 3.2). Algal $\delta^{13}C$ differed among sites in August 1997 (ANOVA, $p < 0.001$), ranging from –34.9‰ to –19.0‰ (Fig. 3.2). Algae were more ^{13}C-depleted in Catamaran Brook (Gorge $\delta^{13}C = -31.7 \pm 1.5$‰, mean ± 1SD, [$n = 15$], Lower $\delta^{13}C = -31.3 \pm 2.2$‰, [$n = 22$], Upper $\delta^{13}C = -30.4 \pm 1.3$‰, [$n = 14$]), than at the LSW site ($\delta^{13}C = -23.8 \pm 2.6$‰, [$n = 15$]) (Tukey's HSD, $p < 0.05$). Sites in Catamaran Brook also showed more ^{13}C-depleted DIC values than at the LSW site (Table 3.2). Algal $\delta^{15}N$ also differed among sites (ANOVA, $p < 0.001$), ranging from –0.8‰ to 6.5‰ (Fig. 3.2). Algae at the Lower site ($\delta^{15}N = 1.1 \pm 1.0$‰) and LSW site ($\delta^{15}N = 1.4 \pm 0.4$‰) were more depleted than samples collected at either the Gorge site ($\delta^{15}N = 3.7 \pm 0.8$‰) or the Upper site ($\delta^{15}N = 4.2 \pm 1.1$‰) (Tukey's HSD, $p < 0.05$).

Glossosoma nigrior

Glossosoma nigrior also tended to show distinct patterns at each site when both $\delta^{13}C$ and $\delta^{15}N$ values were considered (Fig. 3.3). *Glossosoma nigrior* $\delta^{13}C$ ranged from –37.4‰ to –20.9‰, and differed among sites (ANOVA, $p < 0.001$) (Fig. 3.3). *Glossosoma nigrior* were more ^{13}C-depleted in Catamaran Brook (Gorge $\delta^{13}C = -34.4 \pm 2.0$‰, mean ± 1SD, [$n = 32$], Lower $\delta^{13}C = -30.6 \pm 2.4$‰, [$n = 52$], Upper $\delta^{13}C = -30.3 \pm 1.8$‰, [$n = 53$]), than at the LSW site ($\delta^{13}C = -24.4 \pm 1.9$‰, [$n = 24$]) (Tukey's HSD, $p < 0.05$). *Glossosoma nigrior* $\delta^{15}N$ values were also more depleted at the Lower site ($\delta^{15}N = 2.5 \pm 0.5$‰) and the LSW site

($\delta^{15}N = 2.8 \pm 0.6‰$) than at the Gorge site ($\delta^{15}N = 4.8 \pm 1.1‰$) and the Upper site ($\delta^{15}N = 5.2 \pm 0.8‰$) (Tukey's HSD, $p < 0.05$). In general, stable-isotope ratios of *G. nigrior* correlated well with those of algae ($r = 0.80$ and 0.83, for $\delta^{13}C$ and $\delta^{15}N$ respectively, $p < 0.001$, [$n = 161$]) (Fig. 3.4). On average, *G. nigrior* was $0.3 \pm 1.1‰$ more enriched in ^{13}C and $1.0 \pm 0.9‰$ more enriched in ^{15}N than its algal diet, and both fractionation factors were significantly greater than zero (one-sample t-test, $p < 0.05$, [$n = 161$]).

Effect of water velocity

Epilithic algae and *G. nigrior* both showed differences in their $\delta^{13}C$ and $\delta^{15}N$ values with respect to water velocity at the Upper site (Fig 3.5), but similar effects were not found at 3 other sites ($p > 0.05$). At the Upper site, algae and *G. nigrior* possessed $\delta^{13}C$ values that became significantly more depleted at higher flow rates than those collected at lower flows (Model-2 linear regression, $r^2 = 0.69$ and 0.50 for algae and *G. nigrior* respectively, $p < 0.001$ in both cases). On the contrary, the $\delta^{15}N$ values of algae and *G. nigrior* were significantly more enriched in faster currents than those obtained at slower speeds (Model-2 linear regression, $r^2 = 0.26$ and 0.70 for algae and *G. nigrior* respectively, $p < 0.001$ in both cases).

Other grazers

Glossosoma nigrior and *Blepheracera tenuipes* (Walker) were the most ^{13}C-depleted grazers collected at each site (Fig. 3.6). Other species, including the coleopterans (*Psephenus* sp. and *Stenelmis* spp.), a limnephilid (*Apatania* sp.), and the mayflies (*Baetis* spp., *Cinygmula subaequalis* (Banks), *Drunella* spp., and *Epeorus* spp.) showed intermediate $\delta^{13}C$ values, while the mayflies, *Ameletus* sp., *Ephemerella* spp., *Heptagenia* spp., *Rhithrogena impersonata* (McDunnough), and *Stenacron interpunctatum* (Say), were most ^{13}C-enriched. *Glossosoma nigrior* and *B. tenuipes* were also the most ^{15}N-enriched grazers at each site, while *Ephemerella* spp., *R. impersonata*, and *S. interpunctatum* showed much more depleted $\delta^{15}N$ values (Fig. 3.7).

Discussion

Isotopic fractionation in epilithic algae

In general, stable-isotope ratios of algae depend on 3 factors: 1) the isotopic composition of inorganic nutrients, 2) isotopic discrimination during photosynthetic uptake and assimilation of these nutrients, and 3) isotopic fractionation during cellular metabolism (e.g., respiration) (Goericke et al. 1994). Effects of the first 2 factors appeared to be responsible for some of the variation observed in algal $\delta^{13}C$ at the 4 sites. For example, DIC in the LSW Miramichi River was about 4‰ more ^{13}C-enriched than DIC in Catamaran Brook and, accordingly, epilithic algae were also more ^{13}C-enriched at the LSW site. Enrichment in DIC $\delta^{13}C$ along stream continua occurs as a result of increased atmospheric mixing, preferential assimilation of ^{12}C by photosynthetic organisms, and dissolution of carbonate- or silicate-bearing substrata (Kendall et al. 1992, Yang et al. 1996, Amiotte-Suchet et al. 1999). The LSW Miramichi is a much larger river system than Catamaran Brook, and the longer residence time of water at the LSW site would promote ^{13}C-enrichment of DIC through atmospheric mixing. Removal of ^{12}C via photosynthetic uptake also may have enriched the DIC signal at this site. The LSW Miramichi River is a very wide stream, and increased light penetration would have aided primary production which, under bloom conditions, can enrich the isotopic composition of the remaining DIC pool (e.g. Hollander and McKenzie 1991).

Increased photosynthetic activity may have directly affected algal $\delta^{13}C$ values by reducing isotopic fractionation during biogenic uptake and assimilation. Isotopic fractionation (ε_p) occurs during photosynthesis as a result of preferential assimilation of the lighter $^{12}CO_2$ molecule. When growth rates increase, $^{12}CO_2$ availability becomes reduced and relatively more $^{13}CO_2$ is assimilated into tissue, leading to enrichment in the algal $\delta^{13}C$ value (Calder and Parker 1973, Pardue et al. 1976, Laws et al. 1997). Effects of light intensity and growth rate on $\delta^{13}C$ have been observed in both marine macroalgae and diatoms (Wefer and Killingley 1986, Wiencke and Fischer 1990, Kübler and Raven 1996, Pancost et al. 1997). MacLeod and Barton (1998) recently reported similar results for stream algae. The LSW Miramichi River had lower ε_p values than Catamaran Brook, indicating that photosynthetic activity was greater at the LSW site where more ^{13}C-enriched values in epilithic algae were observed. In general, ε_p values in this study were lower than estimates

for other aquatic and terrestrial plants (e.g., O'Leary 1988, Laws et al. 1997, Burkhardt et al. 1999), suggesting that lotic freshwater algae may be CO_2-limited and capable of inducing active uptake mechanisms (Lucas and Berry 1985), or use HCO_3^- as an alternative source of inorganic carbon (Osmond et al. 1981, Raven et al. 1982, Fielding et al. 1998, Keller and Morel 1999).

In contrast to carbon, little is known about the mechanisms involved in the fractionation of stable-nitrogen isotopes during dissolved inorganic nitrogen (DIN) uptake and assimilation in algae (Goericke et al. 1994). Differences in algal $\delta^{15}N$ values have been associated with N_2-fixation (Gu and Alexander 1993) and the differential utilization of NO_3^- and NH_4^+ during photo-assimilation (Wada and Hattori 1978, Waser et al. 1998). In this study, total variation in algal $\delta^{15}N$ was 7‰, and the 2 upstream sites (Upper and Gorge) were, on average, 2-3‰ more enriched than the 2 downstream sites (Lower and LSW). Enrichment in $\delta^{15}N$ in stream algae may be related to reductions in enzymatic discrimination against ^{15}N, resulting from higher growth rates and light intensities (Montoya and McCarthy 1995, Pennock et al. 1996, MacLeod and Barton 1998, but see Rau et al. 1998). However, if light intensity had affected $\delta^{15}N$ values in this study, algae should have been more ^{15}N-enriched at the open-canopied downstream sites, and not at the 2 upstream sites. Instead, site-specific differences in DIN $\delta^{15}N$ were likely responsible for the differences observed in algal $\delta^{15}N$. For practical reasons, DIN $\delta^{15}N$ values were not measured in this study because volumes well in excess of 20 L per sample would have been required to obtain sufficient dissolved nitrate for isotope analysis (R. Elgood, Department of Earth Sciences, University of Waterloo, personal communication). Furthermore, high DOC concentrations at the 4 sites would have interfered with complete isolation of nitrate from water samples. Until suitable methods become available (see Chang et al. 1999), our understanding of mechanisms controlling algal $\delta^{15}N$ in nitrogen-poor waters may be limited. Future studies would also benefit from direct inspection of the biofilm for the identification of algal species.

Glossosoma nigrior

The $\delta^{13}C$ and $\delta^{15}N$ values of *G. nigrior* showed patterns similar to those observed for algae. The grazer was more ^{13}C-depleted in Catamaran Brook than in the LSW Miramichi River, and its $\delta^{15}N$ values were also more enriched upstream than at downstream sites.

Overall, the $\delta^{13}C$ and $\delta^{15}N$ values of *G. nigrior* and algae matched very well, suggesting that, if necessary, grazers could be used as proxies of stable-isotope ratios in algae. Other researchers have used primary consumers as isotopic surrogates of principal food sources (e.g., Finlay et al. 1999, Vander Zanden and Rasmussen 1999), but this assumption usually goes untested. Good agreement between the stable-isotope ratios of *G. nigrior* and its algal diet under field conditions in this study, suggests that this assumption may be valid.

In fact, grazers may be better isotopic indicators of autochthonous food sources than biofilm, which contains not only algae, but also fungi, bacteria, detritus, inorganic particles, and terrestrial organic matter (Lock et al. 1984). Non-algal material may strongly influence the stable-isotope ratios of the "inferred" autochthonous component and adversely affect food-web interpretation. For example, animals are typically 0-1‰ more ^{13}C-enriched than their food sources (DeNiro and Epstein 1978, and although this pattern was generally observed in my study (i.e., $\delta^{13}C_{grazer} - \delta^{13}C_{algae} = 0.3 \pm 1.1‰$), *G. nigrior* at the Gorge site had $\delta^{13}C$ values that were, on average, 3‰ more depleted than epilithic algae. Because *G. nigrior* is known to be a faithful consumer of diatoms (Tindall and Kovalak 1979, Oemke 1984), this grazer was likely feeding on algae with a $\delta^{13}C$ value near –35‰, and the biofilm scrape (with a $\delta^{13}C$ value near –31‰) was inappropriate as an isotopic measure of autochthonous carbon. Catamaran Brook is a well-forested stream, and terrestrial organic matter with more enriched $\delta^{13}C$ values (O'Leary 1988) could have easily contaminated the biofilm sample. Under these conditions, the $\delta^{13}C$ values of *G. nigrior* may be more representative of algal carbon than those measured in the biofilm scrape. Other techniques may improve the usefulness of biofilm as an isotopic representative of algae (Hamilton and Lewis 1992, Sachs et al. 1999), and caution should be reserved regarding the substitution of primary food sources with primary consumers for stable-isotope purposes. Isotopic fractionation between diet and animal is likely species-specific, affected by the metabolism, life-history, and the particular nutritional requirements exclusive to the organism or population under investigation (Gannes et al. 1997, Focken and Becker 1998). In some instances, it may be best to determine isotopic fractionation factors for each species, especially when stable isotopes are being used for quantitative purposes.

Effect of water velocity

Many studies have reported isotope effects of water velocity on macrophytes and planktonic algae (Smith and Walker 1980, Osmond et al. 1981, Raven et al. 1982, Keeley and Sandquist 1992, Hecky and Hesslein 1995). Turbulent flows improve boundary layer diffusion and increase the relative availability of $^{12}CO_2$ at cell membranes, allowing for greater photosynthetic discrimination against $^{13}CO_2$ and leading to more depleted algal $\delta^{13}C$ values. On the contrary, MacLeod and Barton (1998) found negligible effects of water velocity on stable-isotope ratios in stream microalgae, and suggested that diffusion and internal recycling of CO_2 within the epilithic matrix may have more influence on algal $\delta^{13}C$ and $\delta^{15}N$ values in running waters. Finlay et al. (1999) claimed to have observed water velocity effects on $\delta^{13}C$ in riverine algae, though only at sites with high in-stream productivity. In my study, water velocity affected the $\delta^{13}C$ and $\delta^{15}N$ values of epilithic algae and *G. nigrior* at only 1 of 4 sites. The lack of any consistent effect of water velocity in these studies, suggests that it plays a minor role in the determination of stable-isotope ratios in lotic microalgae. However, attempts to understand mechanisms leading to isotopic fractionation in algae have been based on the presumption that carbon assimilation occurs via passive uptake of aqueous CO_2, since there is an energetic cost associated with the active transport of HCO_3^- across the plasmalemma (Beardall 1985, Raven and Lucas 1985). If algae utilize active transport mechanisms when CO_2 concentrations are low, isotopic fractionation resulting from water velocity (and other diffusional effects) will be minimized (Sharkey and Berry 1995). Certainly, more research is needed before water velocity is negated as a possible mechanism controlling stable-isotope ratios in lotic microalgae.

Other grazers

Fourteen genera of co-existing invertebrate grazers showed isotopic segregation in Catamaran Brook. The trichopteran, *G. nigrior*, and the dipteran, *B. tenuipes*, were always more ^{13}C-depleted and more ^{15}N-enriched than other grazers, especially the mayflies, *Ephemerella* spp., *Heptagenia* spp., *R. impersonata*, and *S. interpunctatum*. Morphological adaptations may have played a role in determining the stable-isotope ratios of these grazers. For example, *Glossosoma* and *Blepheracera* both use shovel-like mandibles to gouge and excavate firmly attached algae, while many heptageniid species possess modified maxillae to

brush loosely attached algae and detritus from rock surfaces (McShaffrey and McCafferty 1986, 1988, Arens 1994). This suggests that *Glossosoma* and *Blepheracera* are better isotopic indicators of algal food sources than other species (e.g., *Rhithrogena* and *Stenacron*) which remove only superficial materials from the epilithon, calling into question the general utility of functional feeding groups in aquatic ecology (Mihuc 1997).

Summary

Stable-isotope ratios of carbon and nitrogen in epilithic algae and *G. nigrior* were distinct in Catamaran Brook and the LSW Miramichi River, New Brunswick, in August 1997, and likely resulted from site-specific differences in water chemistry and light availability. Water velocity may affect stable-isotope ratios in epilithic algae and their invertebrate consumers, but results from this study were inconclusive and future research should focus on isolating algal materials from biofilm samples, or assessing the ability of lotic microalgae to actively uptake CO_2 or HCO_3^-. The grazing invertebrate community had highly variable stable-isotope ratios, partly owing to differences in the morphological strategies adopted by certain species to exploit an important food resource. Some grazers may serve as better isotopic surrogates of algae than others, and considerable effort should be given to the selection of primary consumers as isotopic surrogates of original food sources.

Acknowledgements

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Table 3.1. Selected water chemistry and stream catchment characteristics measured at Catamaran Brook and the LSW Miramichi River, New Brunswick, in August 1997. Sites are as in Figure 3.1

	Catamaran Brook		LSW Miramichi
	Upper	Lower	River
Catchment area (km^2)	12	52	1200
Altitude (m)	250	75	70
Slope (%)	3.6	0.6	0.4
Width (m)	3.9	9.3	90
Mean depth (m)	0.26	0.40	0.68
Mean velocity ($m \cdot s^{-1}$)	0.10	0.32	0.76
Discharge ($m^3 \cdot s^{-1}$)†	0.03	0.22	10.4
Temperature (°C)†	13.7	16.5	18.5
pH*	7.2	7.3	6.9
Conductivity ($\mu S \cdot cm^{-1}$)*	23.3	62.5	27.3
Alkalinity ($mg \cdot L^{-1}$)*	7.1	28.8	9.9
DOC ($mg \cdot L^{-1}$)*	6.8	5.6	5.8
$NO_3^- - N$ ($mg \cdot L^{-1}$)*	0.05	0.04	0.04
TP ($mg \cdot L^{-1}$)*	0.011	0.004	0.006

* Data were made available by P. Hardie (Fisheries and Oceans, Moncton, NB), and are estimates measured from monthly water samples. Values were unavailable for the Gorge site, as it is not a routinely sampled location.

† Data are monthly averages taken from continuously monitoring data-loggers.

Table 3.2. Stable-carbon-isotope ratios ($\delta^{13}C$) of dissolved inorganic carbon (DIC), aqueous CO_2 and epilithic algae collected from Catamaran Brook and the LSW Miramichi River, New Brunswick, in August 1997. Photosynthetic discrimination (ε_p) refers to the difference between the $\delta^{13}C$ values of aqueous CO_2 and epilithic algae. Sites are as in Figure 3.1.

	Site			
	Upper	**Gorge**	**Lower**	**LSW**
DIC $\delta^{13}C$ (‰)	–11.7	–13.1	–12.0	–8.1
$CO_{2\,aq.}$ $\delta^{13}C$ (‰)	–19.1	–19.8	–19.4	–14.4
Algal $\delta^{13}C$ (‰)	–30.4	–31.7	–31.3	–23.8
ε_p (‰)	–11.3	–11.9	–11.9	–9.4

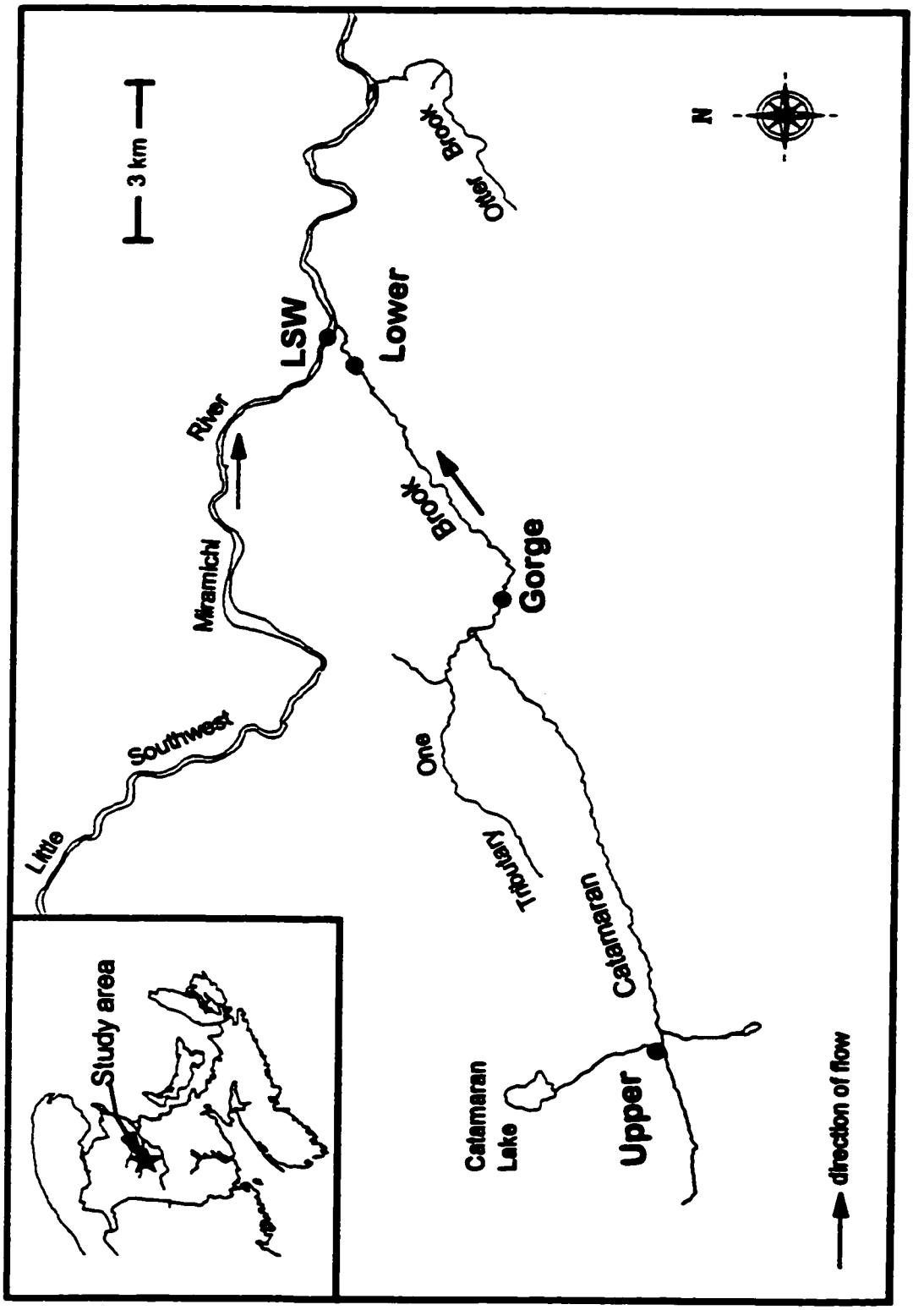

Figure 3.1. Catamaran Brook, New Brunswick, eastern Canada, showing the location of the four study sites.

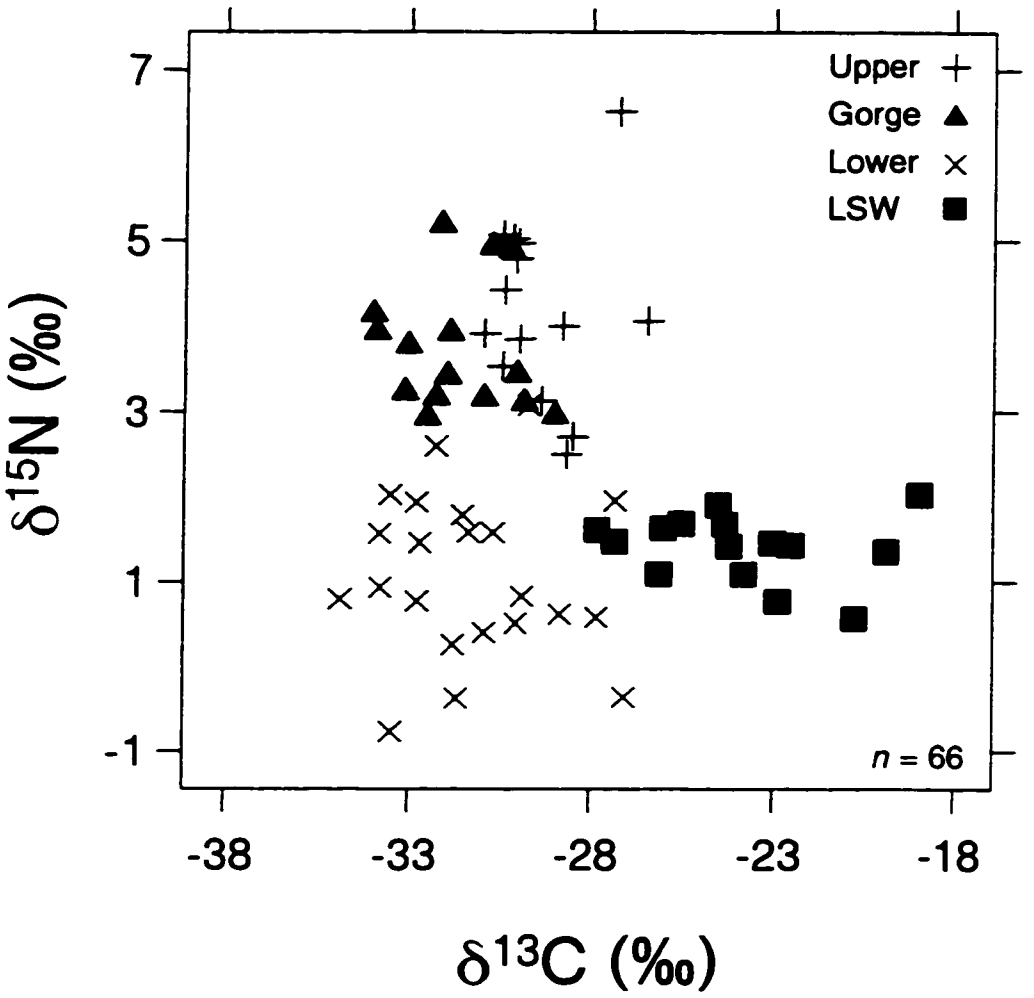

Figure 3.2. Stable-carbon ($\delta^{13}C$) and stable-nitrogen-isotope ratios ($\delta^{15}N$) of epilithic algae collected from 3 sites in Catamaran Brook and 1 site in the LSW Miramichi River, New Brunswick, in August 1997. Sites are as in Figure 3.1.

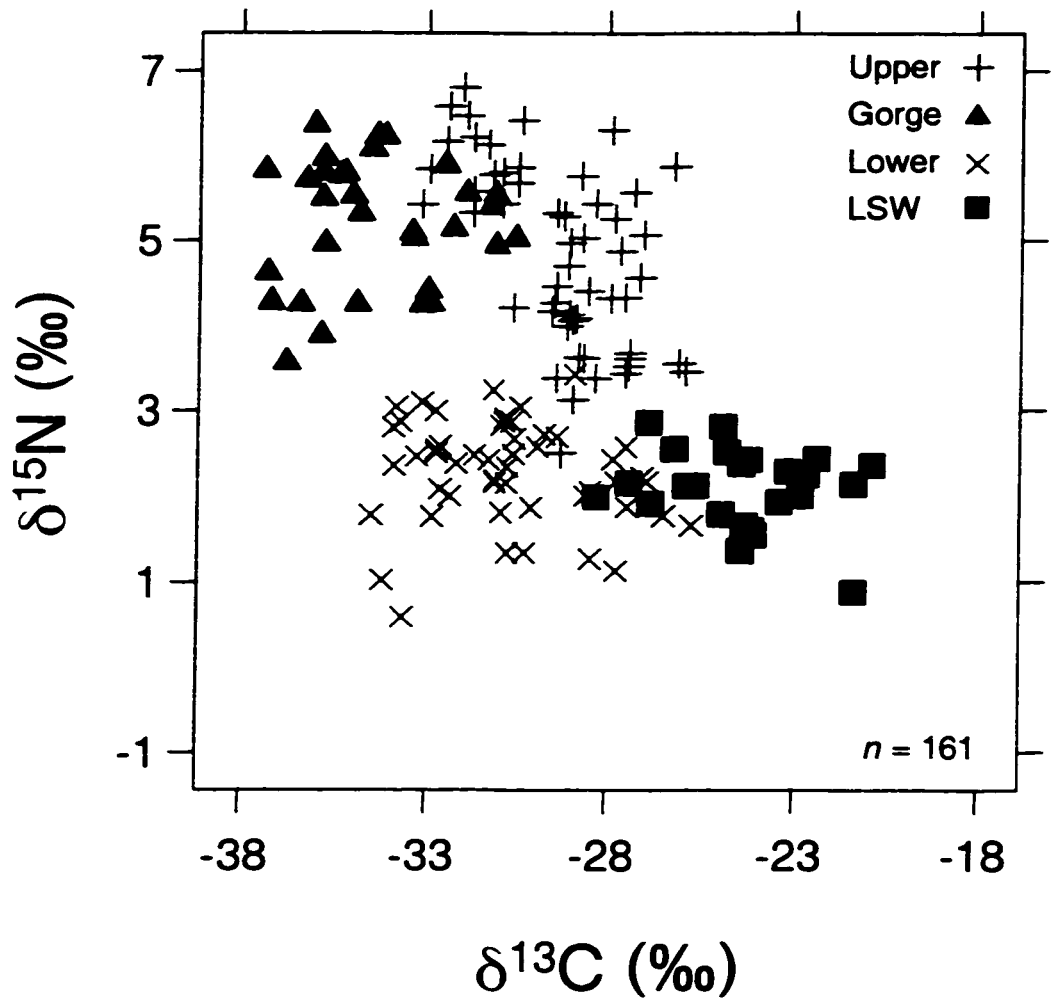

Figure 3.3. Stable-carbon ($\delta^{13}C$) and stable-nitrogen-isotope ratios ($\delta^{15}N$) of *Glossosoma nigrior* collected from 3 sites in Catamaran Brook and 1 site in the LSW Miramichi River, New Brunswick, in August 1997. Sites are as in Figure 3.1.

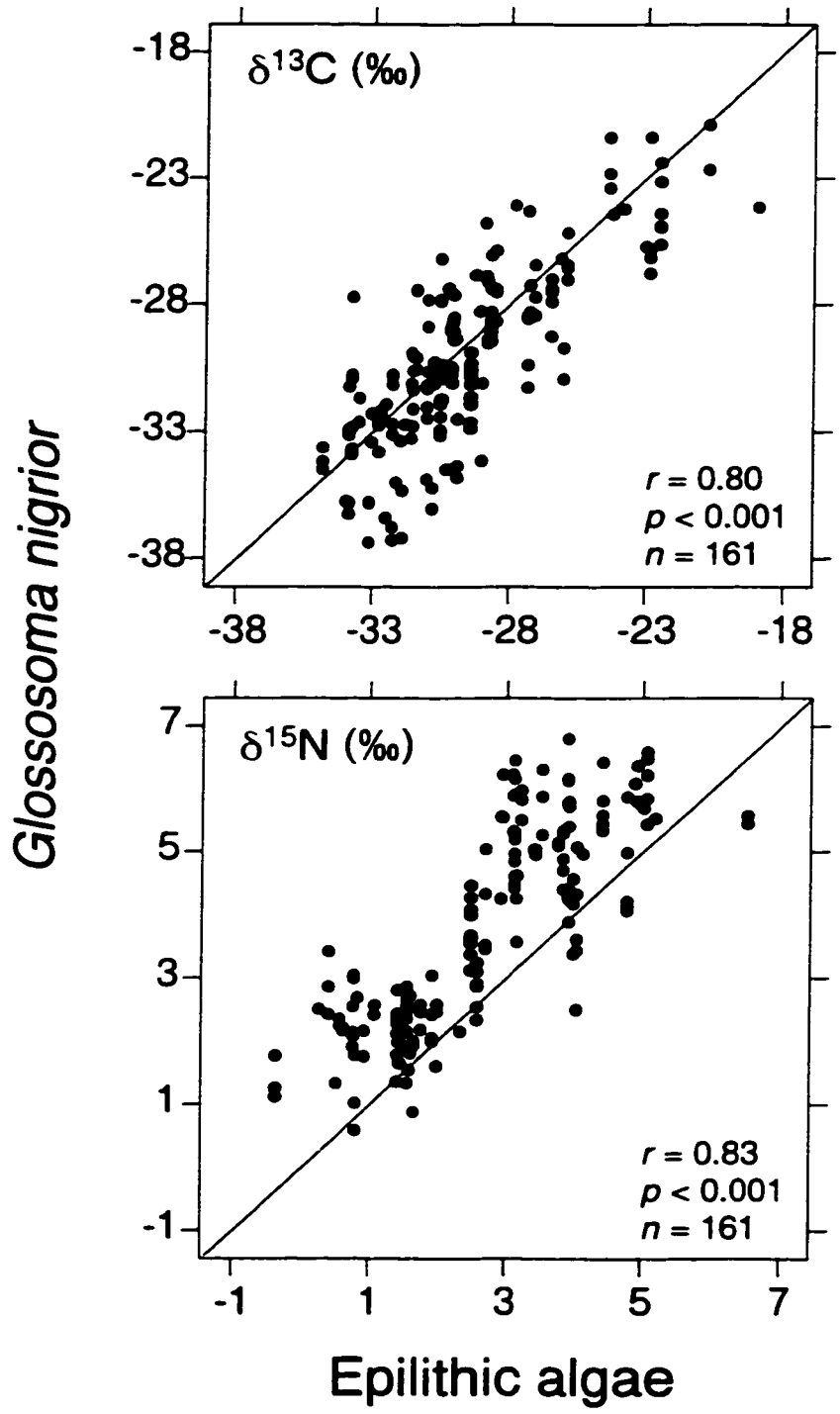

Figure 3.4. Relationship between the $\delta^{13}C$ and $\delta^{15}N$ values of epilithic algae and *Glossosoma nigrior* collected at Catamaran Brook and the LSW Miramichi River, New Brunswick, in August 1997. The diagonal line represents a 1:1 relationship.

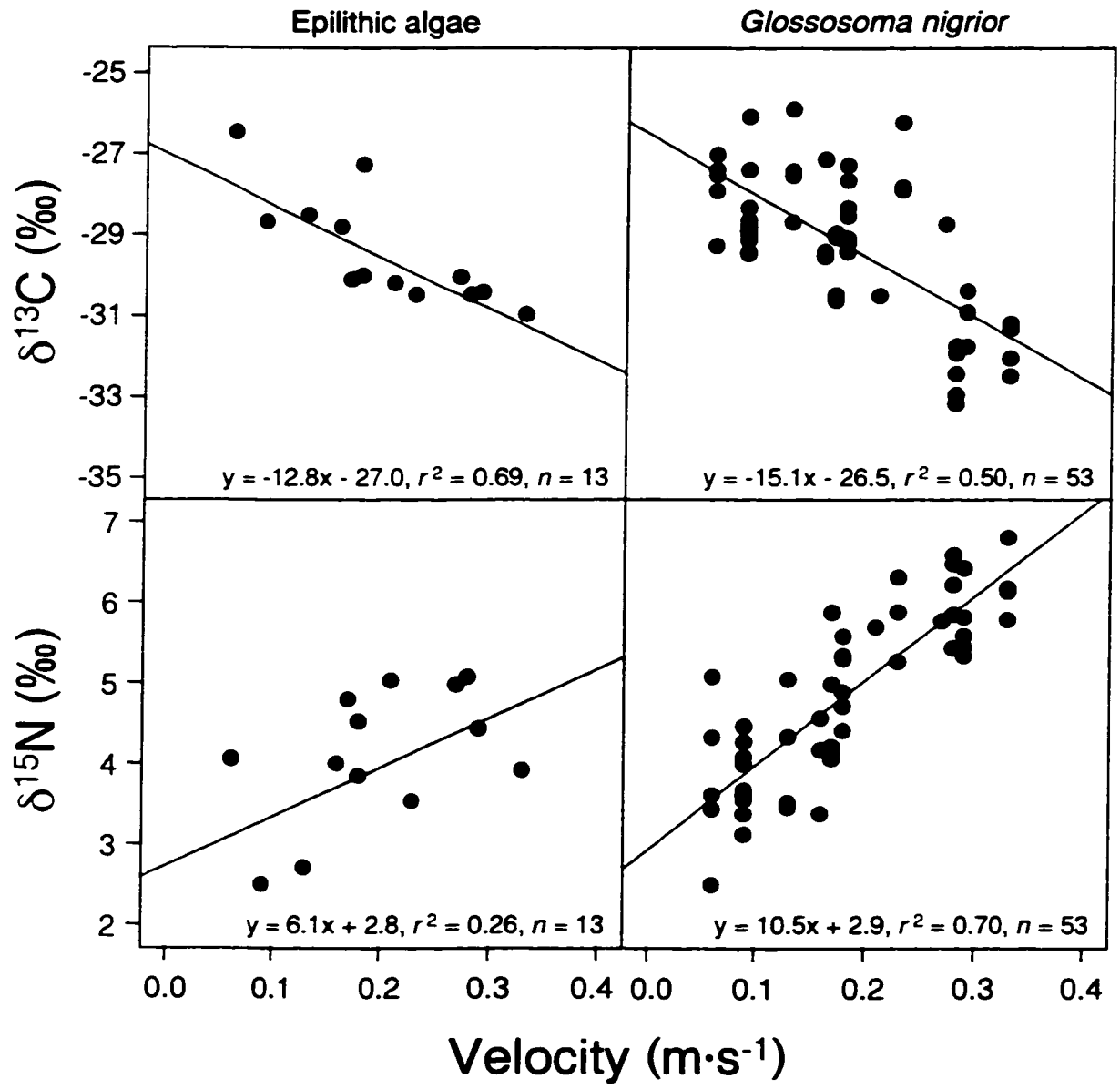

Figure 3.5. The effect of water velocity on the $\delta^{13}C$ and $\delta^{15}N$ of algae and *Glossosoma nigrior* at the Upper site in Catamaran Brook, New Brunswick, in August 1997. Model-2 regression equations, coefficients of determination (r^2), and sample size (n) are given.

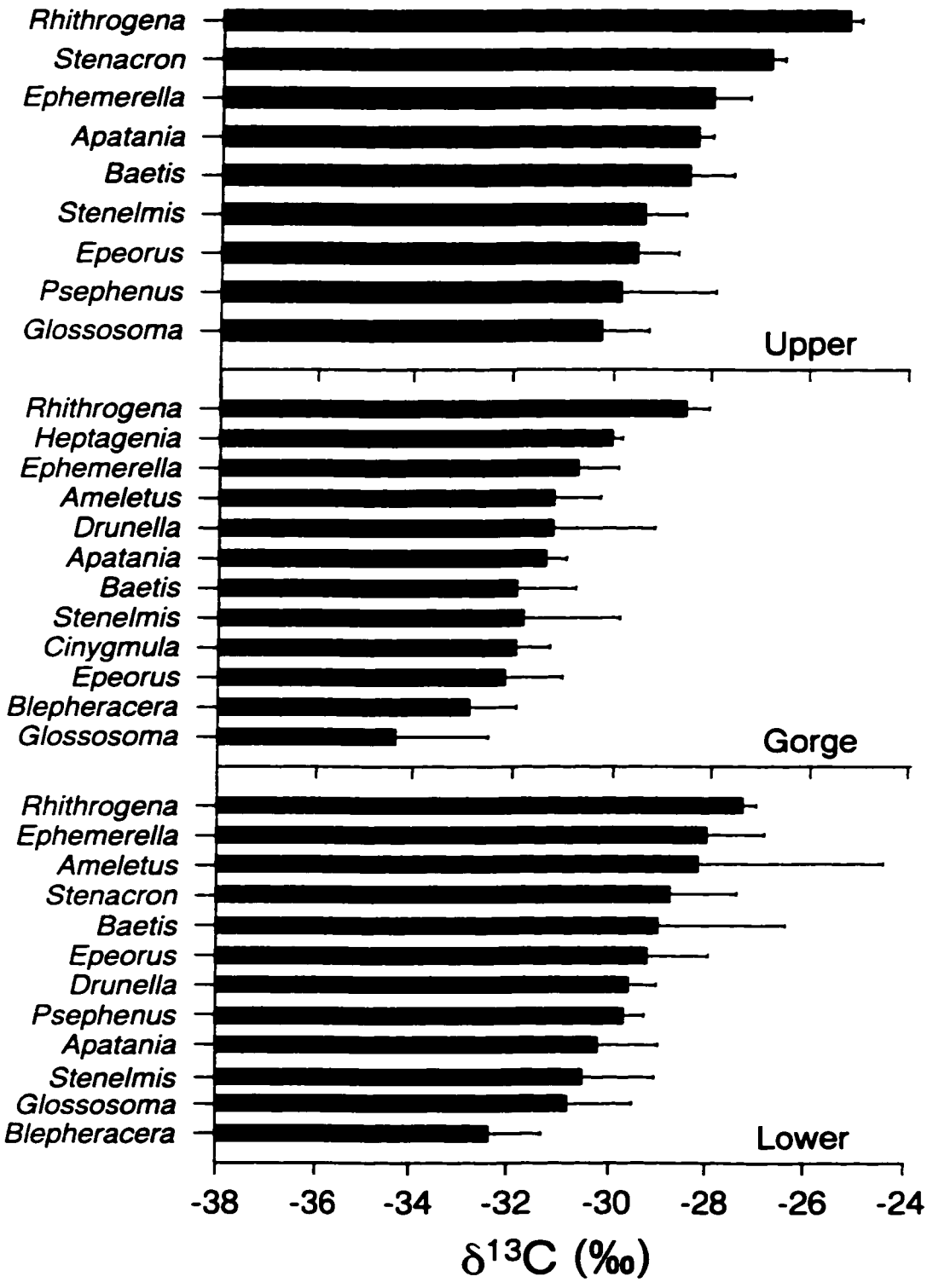

Figure 3.6. Comparison of $\delta^{13}C$ values among benthic invertebrate grazers in Catamaran Brook, New Brunswick in August 1997. Values are means + 1SD. Sites are as in Figure 3.1.

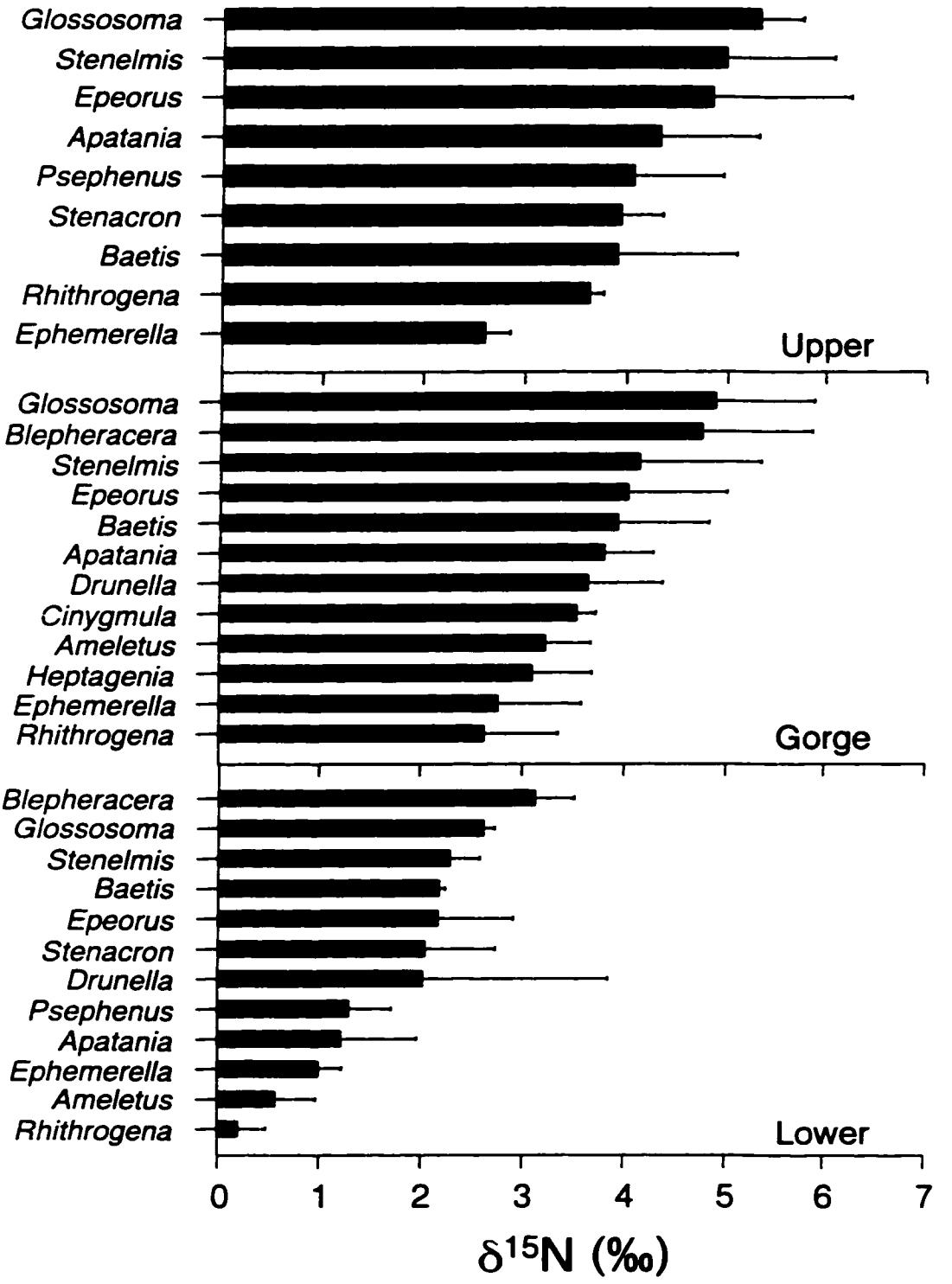

Figure 3.7. Comparison of $\delta^{15}N$ values among benthic invertebrate grazers in Catamaran Brook, New Brunswick in August 1997. Values are means + 1SD. Sites are as in Figure 3.1.

CHAPTER FOUR

Elucidating energy sources for hydropsychid caddisflies (Trichoptera) along a stream continuum using stable-isotope analysis

Abstract

Hydropsychid caddisflies were collected every 500 m along an 18-km length of stream from the headwater lake to the mouth of Catamaran Brook, New Brunswick, for stable-isotope ratios of carbon and nitrogen in June and October 1998. Approximately 600 late-instar larvae, comprising one species of *Arctopsyche*, one species of *Cheumatopsyche*, and five species of *Hydropsyche*, were obtained from 31 different sites. Stable-carbon-isotope ratios ($\delta^{13}C$) of larval hydropsychids generally ranged between –30‰ and –28‰, but larvae were more depleted below major tributaries (–35‰ to –31‰), and were more enriched in the headwaters (–27‰ to –25‰). Stable-nitrogen-isotope ratios ($\delta^{15}N$) mirrored patterns observed in $\delta^{13}C$, and were generally more enriched upstream (6-8‰) than downstream (0-2‰). Larvae collected in October were consistently 2-3‰ more enriched in ^{13}C and 1-2‰ more enriched in ^{15}N than those sampled in June. Species composition did not differ along the stream. *Hydropsyche sparna* was the most abundant species on both dates, whereas *H. slossonae* was important in June and *H. morosa* and *Arctopsyche* sp. were numerous in October. *Hydropsyche slossonae* and *Arctopsyche* sp. were isotopically more enriched in both ^{13}C and ^{15}N than other hydropsychids, suggesting a higher trophic position for these larvae. *Cheumatopsyche aphanta* was isotopically more depleted in ^{13}C and ^{15}N than other species, implying a greater reliance on algal food sources. This study shows that stable-isotope ratios provide a valuable means of obtaining dietary information on co-existing hydropsychid species.

Introduction

Filter-feeding caddisflies of the family Hydropsychidae are important processors of food energy in many stream ecosystems (Wallace et al. 1977, Mackay and Wiggins 1979, Wallace and Merritt 1980). Hydropsychids spin silken nets to passively filter suspended material from running waters, and partition habitat according to current velocity and resource concentration (Malas and Wallace 1977, Alstad 1982, Georgian and Thorp 1992). Species composition changes along longitudinal stream gradients in response to individual preferences in temperature, oxygen, seston particle size, and current speed (Hildrew and Edington 1979, Fuller and Mackay 1980a, Ross and Wallace 1983, Becker 1987, Camargo 1992, Tachet et al. 1992, Mihuc et al. 1996). Larval hydropsychids are facultative omnivores and can influence the quality of seston flowing past their nets (Parker and Voshell 1983, Valett and Stanford 1987). These caddisflies ingest detrital, animal, and algal material to varying degrees depending upon capture net-mesh size, larval instar, season, and location (Mecom 1972, Benke and Wallace 1980, Fuller and Mackay 1980b), and can graze biofilm from rock surfaces surrounding their retreats (Williams and Hynes 1973, Fuller and Mackay 1980b).

Stable-isotope analysis (SIA) may help to distinguish between the relative importance of detrital, algal, and animal material in the diets of co-existing hydropsychid larvae. SIA is a chemical analysis of food sources and energy pathways, and does not suffer from the ambiguities associated with visual interpretation of stomach contents and detrital pools (Peterson and Fry 1987). The method relies on the fact that ratios of naturally occurring stable isotopes of common elements such as carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) are assimilated into primary producers with signatures characteristic of certain biogeochemical processes, and are passed along food chains with relatively predictable change (reviewed in Fry and Sherr 1984, Peterson and Fry 1987, Rundel et al. 1989, Lajtha and Michener 1994). In addition, SIA provides a composite picture of feeding over a period of time, rather than the single point-in-time sample obtained through gut-content analysis, and reflects assimilated versus ingested materials. Typically, at each trophic transfer of food energy, stable-carbon-isotope ratios (expressed as $\delta^{13}C$) enrich by 0-1‰, whereas stable-nitrogen-isotope ratios ($\delta^{15}N$) increase by 3-5‰ (DeNiro and Epstein 1978, 1981, Tieszen et al. 1983, Minagawa and Wada 1984, but see Focken and Becker 1998). In aquatic ecology, $\delta^{13}C$ values have

been used most often to distinguish between important primary food sources, such as aquatic algae and terrestrial leaf litter (Rounick and Winterbourn 1986), while $\delta^{15}N$ values allow the accurate determination of trophic position (Vander Zanden et al. 1996). The main objective of this study was to elucidate habitat partitioning and resource utilization (allochthonous versus autochthonous inputs) among co-existing hydropsychid species along a stream continuum using $\delta^{13}C$ and $\delta^{15}N$ values as indicators of diet and trophic status. Another objective was to compare larval hydropsychid diets (inferred from stable-isotope ratios) with those of the benthic invertebrate community at each site.

Methods

Study Site

Catamaran Brook (lat. 46°52.7′N, long. 66°06.0′W) is a tributary of the Little Southwest

Miramichi River, located in a pristine forested area of north-central New Brunswick (Fig. 4.1). The brook is a well buffered, circumneutral, 3rd-order stream, ~ 20 km in length, with a drainage area of 52 km^2 (Cunjak et al. 1993). Riparian vegetation in the basin consists of 60% deciduous trees, including white birch (*Betula papyrifera*), yellow birch (*Betula lutea*), sugar maple (*Acer saccharum*), American beech (*Fagus grandifolia*), and speckled alder (*Alnus rugosa*). The remaining 40% consists of conifers, including balsam fir (*Abies balsamea*), red spruce (*Picea rubens*), and eastern white cedar (*Thuja occidentalis*). Litter fall peaks in late September and leaves show rapid conditioning and fungal colonization in autumn (Garnett et al. 2000).

Catamaran Brook is currently the focus of several multidisciplinary studies evaluating logging impacts on the habitat and productivity of Atlantic Salmon (*Salmo salar*). Four main stream reaches (Upper, Middle, Gorge, and Lower) have been selected for long-term study. The Upper Reach consists of a 180-m stretch in the headwater region, and is characterized by a narrow (1-2 m) shaded stream channel, generally coarse substrates, steep slope, and rapid flow. The Middle Reach is located approximately half way down the stream channel, and has a stream width of 6-8 m, riffle gradients of 2-2.3% and riffle substrates of cobble and gravel. The Gorge Reach, located ~ 2-3 km downstream of the Middle Reach, runs through a bedrock outcrop area and is 6-8 m wide with riffle gradients of ~ 2% and a primarily bedrock

substrate with some gravel and cobble. The Lower Reach consists of the lower 2 km of the stream and is 8-12 m in width, has riffle gradients of 1.6-1.75%, and riffle substrates of gravel, cobble, and boulder. Some water chemistry data are provided in Table 4.1. More detail on the biology, geochemistry and hydrology of the brook is found in Cunjak et al. (1993) and Giberson and Caissie (1998).

Invertebrate sampling

Larval hydropsychids were collected from 31 sites at approximately 500-m intervals from the headwater lake to the mouth of Catamaran Brook. At each site, larvae were hand-picked from 10 randomly selected stones in run-riffle habitats and were preserved with 85% ethanol in the field (Hobson et al. 1997). For comparison, other invertebrates were sampled using a D-frame kick net with 250-μm nylon mesh from 6 locations (sites A-F) (Fig. 4.1). Collections took place on 21-23 June 1998 and 14-17 October 1998. Hydropsychids were identified to species using setal characteristics (Schefter & Wiggins 1986) and head coloration patterns (Schuster and Etnier 1978). Other invertebrates were identified to the lowest practical taxon, and assigned to functional-feeding groups using Merritt and Cummins (1996).

DIC, DOM, FPOM, algae, and leaf litter sampling

Stream water was collected for stable-isotope analysis of dissolved inorganic carbon (DIC) and dissolved organic matter (DOM) at sites A-F in June 1998. In the field, DIC samples were stored in duplicate 500-mL glass bottles and preserved with a saturated $HgCl_2$ solution. DOM samples were filtered through pre-combusted Whatman GF/F filters (nominal pore size = 0.7 μm), stored in duplicate 1-L glass bottles, and acidified to pH ≈ 2-3 with concentrated HCl. Filters clogged during the process of obtaining DOM were used to isotopically analyze seston, or fine particulate organic matter (FPOM). At sites A-F, epilithon was scrubbed from the upper surfaces of rocks and filtered onto pre-combusted glass-fiber filters. FPOM and algal filters were visually inspected for large (ca. ≥1 mm) material, which was subsequently removed. Leaf litter was hand-picked from the stream at sites A-F and rinsed to remove attached detritus. In the laboratory, DIC was isolated from stream water using H_3PO_4 and cryogenic separation. DOM samples were freeze-dried for 72 h and stored in glass vials to await stable-isotope analysis.

Stable-isotope analysis

Simultaneous analysis of stable-carbon and stable-nitrogen-isotope ratios required approximately 1 mg tissue (dry mass) per sample. Invertebrates were oven-dried at constant temperature (60 °C) for 24-48 h and ground to a fine powder using either a mortar and pestle or a ball-mill grinder. Five to ten individuals of smaller taxa (e.g., chironomids, simuliids, elmids, etc.) were required for each replicate sample. For larger organisms (e.g., most stoneflies and caddisflies), an aliquot of powdered tissue was used for each individual.

Stable-isotope ratios are expressed as delta values (δ) and are measures of a parts-per-thousand (or "per mil") difference (‰) between the isotope ratio of a sample and that of an international standard according to the formula:

$$\delta\,^{13}C \text{ or } \delta\,^{15}N = [\,(R_{sample} - R_{standard}) / R_{standard}] \times 1000$$

where $R = {}^{13}C/{}^{12}C$ or ${}^{15}N/{}^{14}N$. Samples that are "more negative" are "depleted" and contain less ^{13}C or ^{15}N; samples that are "less negative" are "enriched" and contain more of the heavier isotopes. International standards are Vienna Peedee Belemnite (VPDB) (Coplen 1996) and nitrogen gas in the atmosphere (Mariotti 1983). These standards are set at a value of 0‰.

Isotopic analyses of organic materials were performed on a Micromass VG Isochrom continuous-flow isotope-ratio mass spectrometer connected to a Carlo Erba elemental analyzer at the Environmental Isotope Laboratory (EIL) (University of Waterloo, Waterloo, Ontario, Canada). Inorganic materials (i.e., DIC) were analyzed on a VG Isogas (Prism Series II) stable-isotope-ratio mass-spectrometer. Repeat analyses of commercially available isotope standards yielded results that were both accurate and precise (International Atomic Energy Agency [IAEA] standard CH6: $\delta^{13}C = -10.5 \pm 0.2$‰ [mean ± 1SD, $n = 32$]; IAEA-N1: $\delta^{15}N = 0.6 \pm 0.3$‰ [$n = 25$]). Replicates of 2 internal laboratory standards: EIL-70 (lipid-extracted fish tissue), and EIL-30 (tap water) gave reliable δ values as well ($\delta^{13}C = -20.7 \pm 0.2$‰ [$n = 38$], and $\delta^{15}N = 16.4 \pm 0.3$‰ [$n = 38$], and $\delta^{13}C = -13.4 \pm 0.3$‰ [$n = 16$], for EIL-70 and EIL-30 respectively).

Statistical analysis

Statistical analyses were performed using SYSTAT (version 8.0, SPSS Inc., Chicago). Maximum Type-I error rates were set at $\alpha = 0.05$. Normality and homogeneity of

variance assumptions were checked using plots of the residuals. Differences in $\delta^{13}C$ and $\delta^{15}N$ values among hydropsychid species were examined using a fixed-effects one-way ANOVA, followed by multiple comparisons via Tukey's HSD post-hoc tests (Sokal and Rohlf 1995). Isotope differences between months were examined using *t*-tests for independent means.

Results

DIC, DOM, FPOM, algae, and leaf litter

Dissolved inorganic carbon (DIC) was most ^{13}C-depleted just below the headwater lake (Site A= –20.6‰), and most ^{13}C-enriched in the Lower Reach (Site F = –11.5‰), and a similar pattern was observed for algae (–38.9‰ to –31.0‰) (Table 4.2). Fine particulate organic matter (FPOM) and dissolved organic matter (DOM) were consistently more ^{13}C-enriched than epilithic algae and were similar to values obtained for terrestrial detritus (–28.6‰ to –26.1‰) at all sites, except below the headwater lake. Algal $\delta^{15}N$ (–3.2‰ to +0.9‰) was much less variable than algal $\delta^{13}C$, and tended to overlap with that of terrestrial $\delta^{15}N$ (–1.4‰ and +0.1‰). The low $\delta^{15}N$ of FPOM (–0.6‰ and +0.6‰)and DOM (–2.4‰ and +0.9‰) reflected the $\delta^{15}N$ of allochthonous and autochthonous inputs.

Hydropsychid larvae

Seven hydropsychid species were found among the 596 late-instar larvae collected from Catamaran Brook in June and October 1998: *Hydropsyche sparna* Ross, *H. walkeri* Betten and Mosely, *H. morosa* Hagen, *H. slossonae* Banks, *Arctopsyche* sp. McLachlan, *H. bronta* Ross (Appalachian form), and *Cheumatopsyche aphanta* Ross (Fig. 4.2). Late-instar *H. sparna* and *H. walkeri* were common in both the June and October samples. The maximum abundance of *H. slossonae* and *C. aphanta* occurred in June, whereas *H. morosa*, *H. bronta*, and *Arctopsyche* sp. were more common in October.

Hydropsychid $\delta^{13}C$ values varied considerably between the headwater lake and the mouth of Catamaran Brook, ranging from –34.8‰ to –25.0‰ (Fig. 4.3). In June, these filter-feeders became progressively more ^{13}C-enriched between the lake outlet (Site A = –30.3 ± 0.8‰, average ± 1SD) and the Upper Reach (Site B = –26.9 ± 0.5‰). Below the confluence of the Lake Outflow and the East-West tributaries, $\delta^{13}C$ values became more depleted (Site C

= –33.0 ± 0.5‰) and then enriched to about –30‰ for the next 4 km (including Site D). Hydropsychid $\delta^{13}C$ then shifted to more depleted values below the outfall of Tributary One (Site E = –33.2 ± 0.8‰), before returning to values near –30‰ in the Lower Reach (including Site F). A similar pattern was observed in October, though $\delta^{13}C$ values were significantly more enriched (–27.9 ± 1.5‰, n = 313) than in June (–30.3 ± 1.6‰, n = 283) (t-test, $p < 0.001$).

Hydropsychids were also more ^{15}N-enriched in October (4.9 ± 1.3‰) than in June (3.4 ± 0.9‰) (t-test, $p < 0.001$), and $\delta^{15}N$ varied longitudinally with changes occurring at the same points as observed in $\delta^{13}C$, though this pattern was less apparent in June than October (Fig. 4.4). The most ^{15}N-enriched hydropsychids were found below the two headwater tributaries (Site C = 7.6 ± 0.5‰, October), while the most ^{15}N-depleted individuals were located in the Lower Reach (Site F = 2.8 ± 0.7‰, June).

To evaluate isotopic differences between hydropsychid species, $\delta^{13}C$ and $\delta^{15}N$ values were compared to those of *H. sparna* at each site, and tested against a hypothesized difference of zero. Stable-isotope ratios of *H. sparna* were used for comparison because this was the only species present at all locations. *Hydropsyche slossonae*, and *Arctopsyche* sp. were significantly more ^{13}C-enriched and ^{15}N-enriched than *H. sparna* (Tukey's HSD, $p <$ 0.05) (Fig. 4.5). *Cheumatopsyche aphanta* was the only species significantly more ^{13}C-depleted and ^{15}N-depleted than *H. sparna* (Tukey's HSD, $p < 0.05$).

Other Invertebrates

Many other taxa also exhibited widely variable $\delta^{13}C$ values in Catamaran Brook in June 1998 (Table 4.3). For example, *Glossosoma nigrior* Banks showed very depleted $\delta^{13}C$ values below the headwater lake (Site A = –38.4 ± 0.7‰), below the confluence of the upper tributaries (Site C = –34.6 ± 0.4‰), and below Tributary One (Site E = –34.2 ± 0.6‰), but this scraper was much less depleted at Sites B, D, and F (~ –31‰). Other scrapers, such as *Cinygmula subaequalis* (Banks), *Epeorus vitreus* (Walker), and *Stenelmis* spp., as well as the collector-gatherers *Baetis* spp., *Ephemerella rotunda* Morgan, and *Rhithrogena impersonata* (McDunnough) also exhibited variable $\delta^{13}C$ values, but these taxa were less depleted than *G. nigrior*. *Lepidostoma togatum* (Hagen), *Leuctra ferruginea* Walker, *Pycnopsyche guttifer* (Walker), and *Taeniopteryx parvula* Banks, had $\delta^{13}C$ values between –28.3‰ and –25.5‰,

and these shredders were isotopically similar to deciduous leaf litter. As expected, the filter-feeders, *Dolophiloides distinctus* (Walker) and *Prosimulium mixtum* Syme and Davies, showed $\delta^{13}C$ values similar to FPOM and DOM. Invertebrate predators such as *Ablabesmyia* sp., *Atherix* sp., *Hexatoma* sp., and *Nigronia serricornis* Say, had $\delta^{13}C$ values between –27‰ and –24‰, except for *Agnetina capitata* (Pictet) and those that were sampled just below the lake outlet (–30‰ to –28‰).

Invertebrate $\delta^{15}N$ also varied considerably in Catamaran Brook in June 1998 (Table 4.4). Taxa collected at Site A (–1.0‰ to +4.6‰) were noticeably more ^{15}N-depleted than those found at other sites (+0.2‰ to +7.9‰). Scrapers and shredders generally showed more ^{15}N-depleted values than other organisms, while predators were the most ^{15}N-enriched invertebrates at each site. Taxa sampled in October showed slight isotope enrichment over individuals of the same species collected in June. For example, the scraper, *E. vitreus*, was consistently 1-2‰ more enriched in both $\delta^{13}C$ and $\delta^{15}N$ at 3 sites sampled in October (Figs. 4.6 and 4.7). *Stenelmis* sp., *D. distinctus*, and *Paraleptophelbia mollis* (Eaton) were also isotopically more enriched in October. However, stable-isotope ratios of the shredder, *T. parvula*, and the predators, *Atherix* sp., *Hexatoma* sp., and *N. serricornis*, were unchanged between seasons.

Discussion

Hydropsychid distribution

Five species (*Hydropsyche sparna*, *H. walkeri*, *H. morosa*, *H. slossonae*, and *Arctopsyche* sp.) were present at most sampling locations along the stream, and only 2 species, *Cheumatopsyche aphanta* and *H. bronta*, were restricted to the Lower Reach. Because very few individuals of *C. aphanta* and *H. bronta* were actually recovered, it is difficult to say whether these species preferred downstream habitats, or were missed at upper sites due to their low abundance. Although differences in species composition along stream gradients have been recorded previously (Gordon and Wallace 1975, Hildrew and Edington 1979, Ross and Wallace 1983, Tachet et al. 1992), it was unlikely that the distance sampled in this study (i.e., ~18 km) was of great enough length to observe these effects. Catamaran Brook is a small, clean headwater stream without physical barriers or permanent human habitation in its basin, and there is a lush riparian canopy along its length. Differences in

species distribution resulting from eutrophication (Camargo 1992), oxygen deficits (Becker 1987), or physical impoundments (Parker and Voshell 1983, Mackay and Waters 1986) would not occur along this brook.

Temporal differences in species composition were apparent in Catamaran Brook. *Hydropsyche sparna* was the most abundant species collected on both sampling dates, while other species, such as *H. slossonae* and *Arctopsyche* sp., were most common in either June or October, respectively. Though, detailed life-history information is missing from this study, *H. sparna* was likely bivoltine (Mackay 1979, Rutherford and Mackay 1986), since it was abundant as late-instar larvae on both sampling dates. *Hydropsyche slossonae* and *Arctopsyche* sp. were probably univoltine, using life-history strategies that differed temporally to maximize habitat use and availability (Wallace et al. 1977, Mackay 1979).

Spatial and temporal patterns in $\delta^{13}C$

Changes in hydropsychid $\delta^{13}C$ along Catamaran Brook resulted from variation in the relative use of algal and terrestrial carbon resources. Leaf litter $\delta^{13}C$ values were consistently between –28‰ and –26‰ in Catamaran Brook, and were similar to values expected for temperate C_3 plants (O'Leary 1988). *Lepidostoma togatum*, *Leuctra ferruginea*, *Pycnopsyche guttifer*, and *Taeniopteryx parvula*, the dominant shredders in the brook, possessed $\delta^{13}C$ values that were comparable to those of leaf litter inputs. If larval hydropsychids had assimilated carbon solely from terrestrial sources, their $\delta^{13}C$ values should have mimicked those of the invertebrate shredders. However, hydropsychid $\delta^{13}C$ ranged from about –35‰ to –25‰, and did not faithfully resemble that of leaf litter, FPOM, or DOM. Thus, it was likely that hydropsychids were consuming some algal material, since it was the only sampled material more ^{13}C-depleted than bulk seston. Hydropsychids are quite capable of digesting epilithic diatoms (Boon 1985), and it is possible that they selectively utilized algal carbon unavailable to competitors such as philopotamid caddisflies which do not possess a gastric mill (Williams and Hynes 1973).

Two other filter-feeding insects, *Dolophiloides distinctus* and *Prosimulium mixtum*, showed $\delta^{13}C$ values that were similar to terrestrial carbon sources. Except for Site A, $\delta^{13}C$ values of *D. distinctus* and *P. mixtum* were invariably between –29‰ and –27‰, and resembled values obtained for invertebrate shredders. It was likely that *D. distinctus* and *P.*

mixtum obtained their ^{13}C-enriched carbon from FPOM or DOM strained from the passing water. These 2 food sources, which had $\delta^{13}C$ values similar to those of terrestrial inputs, are known to be important components of collector-filterer diets (Wallace and Merritt 1980). Philopotamid caddisflies spin fine-mesh nets and are located under rocks where small particles accumulate (Williams and Hynes 1973, Wallace and Malas 1976), while black fly larvae sieve micro-seston (FPOM and colloidal DOM) from the water column in rapidly flowing currents (Wooton 1988).

The peculiar $\delta^{13}C$ pattern displayed by hydropsychids along Catamaran Brook is explained by variation in algal $\delta^{13}C$ between sites. Algal $\delta^{13}C$ ranged between –39‰ and –31‰, and was most depleted below the lake and below the two tributaries (i.e., Sites A, C, and E). Scraper-grazer $\delta^{13}C$ closely matched algal $\delta^{13}C$ at all sites, as did the $\delta^{13}C$ of a few collector-gatherers. Because larval hydropsychid $\delta^{13}C$ varied in a manner similar to that of scrapers and gatherers, it seems plausible to suggest that these filter-feeders relied heavily on algal carbon resources. Changes in DIC $\delta^{13}C$ correlated with changes in algal $\delta^{13}C$. More negative $\delta^{13}C$ values in DIC at Site A likely resulted from the addition of ^{13}C-depleted respired CO_2 from organic matter in the headwater lake (Rau 1978). Depletion in the $\delta^{13}C$ of DIC at Sites C and E probably occurred for similar reasons. These 2 sites are located below tributaries that drain areas inundated with debris dams built by beaver (*Castor canadensis*). Beaver dams are known to alter carbon cycling and increase rates of microbial respiration in streams and rivers (Ford and Naiman 1988), and were the most probable causes of ^{13}C-depletion in streamwater DIC and algae in Catamaran Brook. It was surprising to see effects of ^{13}C-depletion in hydropsychids for up to 2 km beyond the outfall of these tributaries. This observation should serve as a cautionary note to ecologists regarding the importance of site selection when using stable isotopes to discern food-web patterns in streams.

Temporal changes in hydropsychid $\delta^{13}C$ are difficult to interpret, but may have resulted from increased utilization of terrestrial carbon. Leaf fall in the drainage basin of Catamaran Brook generally peaks in late September and fungal colonization of submerged leaves occurs rapidly at this time (Garnett et al. 2000). Because $\delta^{13}C$ values of larval hydropsychids were 2-3‰ more enriched in October than in June, it is possible that they were feeding on this very abundant source of ^{13}C-enriched material. However, scraper $\delta^{13}C$

also became more enriched in October, suggesting that algal $\delta^{13}C$ was changing between sampling dates as well. Temporal shifts in algal $\delta^{13}C$ have been reported in streams (MacLeod and Barton 1998) and lakes (Zohary et al. 1994, Leggett et al. 1999), and result from changes in temperature, light intensity, species composition, seasonal fluxes in nutrient availability, and changes in the isotope composition of DIC. Differences in hydropsychid $\delta^{13}C$ between June and October in Catamaran Brook were likely affected by both processes (i.e., increased availability of ^{13}C-enriched terrestrial inputs and ^{13}C-enrichment in algal resources).

Spatial and temporal patterns in $\delta^{15}N$

Hydropsychid $\delta^{15}N$ also varied considerably along Catamaran Brook in June 1998. Similar to the effects of DIC and algal $\delta^{13}C$ on hydropsychid $\delta^{13}C$, spatial differences in dissolved inorganic nitrogen (DIN) and algal $\delta^{15}N$ were likely responsible for the observed trend in hydropsychid $\delta^{15}N$. For practical reasons, DIN $\delta^{15}N$ was not measured in this study. There is very little dissolved nitrogen in Catamaran Brook (Table 4.1), and under such conditions it would be difficult to obtain sufficient nitrate in the presence of high concentrations of dissolved organic carbon for $\delta^{15}N$ analysis (see Chang et al. 1999). Despite this shortcoming, the $\delta^{15}N$ of epilithic algae did vary between sites, and assimilation of this material could have resulted in site-specific differences in hydropsychid $\delta^{15}N$. At 2 sites sampled below tributaries (Sites C and E), hydropsychid $\delta^{15}N$ values were enriched, while their $\delta^{13}C$ values were depleted. Microbial alteration of organic matter may have given rise to ^{15}N-enriched DIN and algae at these 2 sites. Microbes can produce large changes in the isotope composition of organic compounds (Macko and Estep 1984), and remineralization of processed organic material may have released ^{15}N-enriched DIN to downstream locations.

Differences in hydropsychid $\delta^{15}N$ along Catamaran Brook may also have resulted from spatial changes in diet, because $\delta^{15}N$ is intimately associated with trophic position. Stable-nitrogen-isotope ratios enrich by 3-5‰ at each trophic transfer of food energy, such that primary consumers have lower $\delta^{15}N$ values than top predators (DeNiro and Epstein 1981, Minagawa and Wada 1984). Enrichment in $\delta^{15}N$ occurs along food chains because metabolic processes, such as respiration and excretion, preferentially use the lighter ^{14}N-isotope, leaving the heavier ^{15}N-isotope to accumulate in body tissues (Gaebler et al. 1966,

Steele and Daniel 1978). Data from this study supported this notion, because invertebrate predators in Catamaran Brook maintained higher $\delta^{15}N$ values than invertebrate scrapers and shredders. Hydropsychids at Site C were, on average, 3-4‰ more ^{15}N-enriched than larvae collected downstream at Site F, suggesting higher trophic positions for larvae in the Upper Reach. A greater proportion of carnivorous habits and coarse-mesh nets are thought to be more prevalent in hydropsychids at headwater versus downstream sites (Wallace et al. 1977, Ross and Wallace 1983). However, this issue remains unclear, because the scrapers, *E. vitreus*, *G. nigrior*, and *Stenelmis* sp. were also more ^{15}N-enriched at Site C than at Site F. It is likely that both carnivory and differences in algal $\delta^{15}N$ were responsible for higher $\delta^{15}N$ values in hydropsychids in the headwaters. Researchers need to be aware that variation in invertebrate $\delta^{15}N$ along stream continua can exist as a result of both changes in trophic status and biogeochemical modification at the base of the food chain (see Cabana and Rasmussen 1996, Vander Zanden and Rasmussen 1999).

Differences in $\delta^{15}N$ at the base of the food chain may have been responsible for seasonal changes in hydropsychid $\delta^{15}N$ as well. Much like $\delta^{13}C$, hydropsychid $\delta^{15}N$ was more positive in October than in June, and this enrichment was apparent in scrapers and collectors as well, but not in shredders or predators. Higher $\delta^{15}N$ in scrapers implies higher $\delta^{15}N$ in algae, and hydropsychids collected in autumn were likely reflecting this more ^{15}N-enriched algal food source. Enrichment in $\delta^{15}N$ can also be related to increased carnivory. However, a suggestion of more carnivory in the fall is contrary to what has been reported previously for hydropsychid diets, since they generally ingest more animal material in spring and summer when invertebrate drift is at its highest level (Mecom 1972, Williams and Hynes 1973, Fuller and Mackay 1980b). More information on hydropsychid gut contents and on the $\delta^{15}N$ values of DIN in Catamaran Brook would help to clarify this issue.

Species-specific differences in $\delta^{13}C$ and $\delta^{15}N$

Stable-isotope ratios of carbon and nitrogen suggest that there is some dietary overlap among the 7 hydropsychid species collected in Catamaran Brook. Resources (e.g., optimal space to construct nets) are thought to be limited for hydropsychid species, and conspecifics partition habitat according to larval instar stage, capture net-mesh size, and preferences for

oxygen, temperature, and water velocity, in order to minimize effects of interspecific competition (Malas and Wallace 1977, Wallace et al. 1977, Hildrew and Edington 1979, Mackay and Wiggins 1979, Rutherford and Mackay 1986). In Catamaran Brook, larvae of *Cheumatopsyche aphanta* were more ^{13}C- and ^{15}N-depleted than all other species, suggesting greater reliance on algae and less on detrital or animal material than other hydropsychids. On the other hand, *Arctopsyche* sp. were significantly more ^{13}C- and ^{15}N-enriched than larvae of *H. sparna*, suggesting a higher trophic position and little dietary overlap with *H. sparna* or *C. aphanta*. Previous work has shown that late-instar larvae of *Arctopsyche* sp. are more carnivorous than other hydropsychids (Wallace 1975). However, *H. slossonae* was also more ^{13}C- and ^{15}N-enriched than *H. sparna* and *C. aphanta*, and this may imply strong competition between *Arctopsyche* sp. and *H. slossonae*. It was not surprising to observe late-instar larvae of *H. slossonae* mostly in June, while larvae of *Arctopsyche* sp. were prevalent in October. Competition between these 2 species may have lead to their temporal segregation through the alteration of life-history, allowing their co-existence in the same habitats.

In summary, spatial and temporal patterns in the stable-carbon and stable-nitrogen-isotope ratios of larval hydropsychid caddisflies in Catamaran Brook resulted from: 1) changes in the relative utilization of terrestrial and aquatic resources, 2) changes in trophic status, and 3) changes in the baseline isotope signature of aquatic algae. Baseline isotope signatures were different at sites located below the lake and the 2 major tributaries. These results highlight the need to cautiously select sampling locations when using stable isotopes to interpret stream food-webs. Despite biogeochemical effects, $\delta^{13}C$ and $\delta^{15}N$ values were successful at identifying dietary overlap between con-specific hydropsychids. Stable-isotope ratios should also benefit ecologists studying co-existing taxa of other generalist-feeding guilds, such as the collector-gatherers.

Acknowledgements

I thank P. Hardie for helping to collect invertebrates. R. Cunjak, Fisheries and Oceans Canada, Moncton, New Brunswick, provided logistic support. R. Drimmie and W. Mark, Environmental Isotope Laboratory, University of Waterloo, Waterloo, Ontario, provided technical assistance. Funding was provided by a Natural Science and Engineering Research Council (NSERC) operating grant to G. Power, and by a NSERC-Industrial Post-Graduate Scholarship to R. Doucett, in which the industrial partner was Noranda Inc.

Table 4.1. Selected water chemistry parameters and stream catchment characteristics measured at 3 study reaches in Catamaran Brook, New Brunswick, in 1998. Parameters with two values show estimates for June and October, respectively.

	Catamaran Brook		
	Upper Reach	**Middle Reach**	**Lower Reach**
Catchment area (km^2)	12	25	52
Altitude (m)	250	155	75
Slope (%)	3.6	1.1	0.6
Width (m)	3.9	6.9	9.3
Mean depth (m)	0.26	0.28	0.40
Mean velocity ($m \cdot s^{-1}$)	0.10 / 0.08	0.35 / 0.28	0.32 / 0.30
Discharge ($m^3 \cdot s^{-1}$)	0.20 / 0.17	0.71 / 0.61	1.35 / 1.16
Temperature (°C)	10.2 / 5.8	11.4 / 6.5	13.1 / 7.1
pH	6.7 / 6.8	7.1 / 7.5	7.3 / 7.5
Conductivity ($\mu S \cdot cm^{-1}$)	21.9 / 30.6	47.7 / 58.4	57.4 / 63.9
Alkalinity ($mg \cdot L^{-1}$)	6.0 / 8.4	18.9 / 24.1	27.0 / 23.3
DOC ($mg \cdot L^{-1}$)	6.4 / 6.6	7.1 / 7.0	6.3 / 7.8
NO_3^- - N ($mg \cdot L^{-1}$)	0.04 / 0.03	0.02 / 0.02	0.05 / 0.02
TP ($mg \cdot L^{-1}$)	0.014 / 0.029	0.010 / 0.005	0.006 / 0.005

Table 4.2. Stable-isotope ratios (‰) of dissolved inorganic carbon (DIC), dissolved organic matter (DOM), fine particulate organic matter (FPOM), epilithic algae, and deciduous leaf litter collected in Catamaran Brook in June 1998. All DIC, DOM, and FPOM values are averages of duplicate samples. Algae and leaves were analyzed in triplicate. Sites are as in Figure 4.1.

		Site A	Site B	Site C	Site D	Site E	Site F
DIC	$\delta^{13}C$	-20.6	-12.7	-16.1	-12.3	-14.7	-11.5
DOM	$\delta^{13}C$	-28.9	-26.6	-26.4	-26.5	-26.7	-26.6
	$\delta^{15}N$	-1.2	-2.4	-1.1	-1.7	-0.8	0.9
FPOM	$\delta^{13}C$	-30.1	-26.9	-27.0	-26.2	-26.3	-25.9
	$\delta^{15}N$	0.1	0.3	-0.2	-0.6	0.2	0.6
Algae	$\delta^{13}C$	-38.9	-33.1	-35.8	-30.9	-34.7	-31.0
	$\delta^{15}N$	-3.2	-0.7	0.5	-0.6	0.9	-1.0
Leaves	$\delta^{13}C$	-28.4	-27.4	-27.8	-28.6	-27.7	-26.1
	$\delta^{15}N$	-1.4	0.1	-1.6	-1.8	-0.8	-1.1

Table 4.3. Benthic invertebrate $\delta^{13}C$ for taxa collected in Catamaran Brook in June 1998. Values are averages of 3-10 replicate samples. Precision was better than ± 1‰ (SD).

		$\delta^{13}C$ (‰)					
FFG	**Taxa**	**Site A**	**Site B**	**Site C**	**Site D**	**Site E**	**Site F**
Shredders	*Lepidostoma togatum*		-25.7			-27.4	-26.5
	Leuctra ferruginea		-25.8		-25.8	-25.5	-25.6
	Pycnopsyche guttifer		-27.0	-27.8	-26.6	-26.6	
	Taeniopteryx parvula		-27.0			-28.3	-27.7
Scrapers	*Cinygmula subaequalis*				-31.4	-32.8	-31.3
	Epeorus vitreus	-34.0	-30.1	-33.1		-33.0	-30.6
	Glossosoma nigrior	-38.4	-30.8	-34.6	-31.4	-34.2	-31.1
	Stenelmis spp.	-35.0	-30.4	-33.7	-29.2		-30.8
Gatherers	*Baetis* spp.	-34.6	-29.5	-32.1		-33.3	
	Ephemerella rotunda	-32.8	-27.5	-31.4	-30.8	-31.2	-29.6
	Eukiefferiella sp.	-30.3	-25.1	-33.5		-29.3	-27.6
	Micropsectra sp.	-27.7	-25.5	-25.3	-26.4	-26.2	-26.7
	Microtendipes sp.	-27.0	-25.4		-27.4	-26.9	-27.2
	Paraleptophelbia mollis			-25.0		-26.4	-27.8
	Rhithrogena impersonata		-28.2	-30.4	-29.1	-28.5	
Filterers	*Dolophiloides distinctus*		-28.4	-26.8		-27.4	-26.9
	Prosimulium mixtum	-30.5	-26.8	-27.4	-28.7	-27.0	-27.6
Predators	*Ablabesmyia* sp.	-27.6	-24.5		-27.3	-25.3	
	Agnetina capitata	-29.0	-27.4	-28.6	-28.7	-30.3	-29.0
	Atherix sp.	-29.9	-26.0	-27.3		-28.4	-26.9
	Hexatoma sp.			-24.2	-25.0	-25.8	-25.0
	Nigronia serricornis	-29.6		-25.1	-26.2	-26.8	-25.9

Table 4.4. Benthic invertebrate $\delta^{15}N$ for taxa collected in Catamaran Brook in June 1998. Values are averages of 3-10 replicate samples. Precision was better than ± 1‰ (SD).

		$\delta^{15}N$ (‰)					
FFG	**Taxa**	**Site A**	**Site B**	**Site C**	**Site D**	**Site E**	**Site F**
Shredders	*Lepidostoma togatum*		3.7			2.3	3.3
	Leuctra ferruginea		3.9		3.0	3.1	3.5
	Pycnopsyche guttifer		2.3	2.2	2.8	2.8	
	Taeniopteryx parvula		3.3			3.7	3.1
Scrapers	*Cinygmula subaequalis*				2.6	3.5	2.0
	Epeorus vitreus	-0.3	2.8	3.8		3.1	1.6
	Glossosoma nigrior	-1.0	2.7	2.4	3.4	3.4	1.5
	Stenelmis spp.	-0.1	1.8	3.7	2.8		1.2
Gatherers	*Baetis* spp.	0.3	2.0	3.5		3.4	
	Ephemerella rotunda	0.1	0.2	3.4	2.5	2.2	1.3
	Eukiefferiella sp.	1.5	1.5	4.2		3.6	2.3
	Micropsectra sp.	2.9	4.7	5.0	4.7	4.8	3.2
	Microtendipes sp.	2.4	3.6		4.0	3.7	1.4
	Paraleptophelbia mollis			4.2		3.4	2.1
	Rhithrogena impersonata		3.6	2.0	3.3	2.6	
Filterers	*Dolophiloides distinctus*		2.7	3.0		4.1	4.0
	Prosimulium mixtum	2.1	3.1	3.2	3.5	3.7	2.6
Predators	*Ablabesmyia* sp.	3.4	5.4		4.8	6.2	
	Agnetina capitata	3.3	6.1	5.9	6.0	6.1	4.3
	Atherix sp.	4.6	5.7	6.4		5.6	5.3
	Hexatoma sp.			7.7	7.1	7.9	5.7
	Nigronia serricornis	4.1		6.9	5.2	5.4	5.4

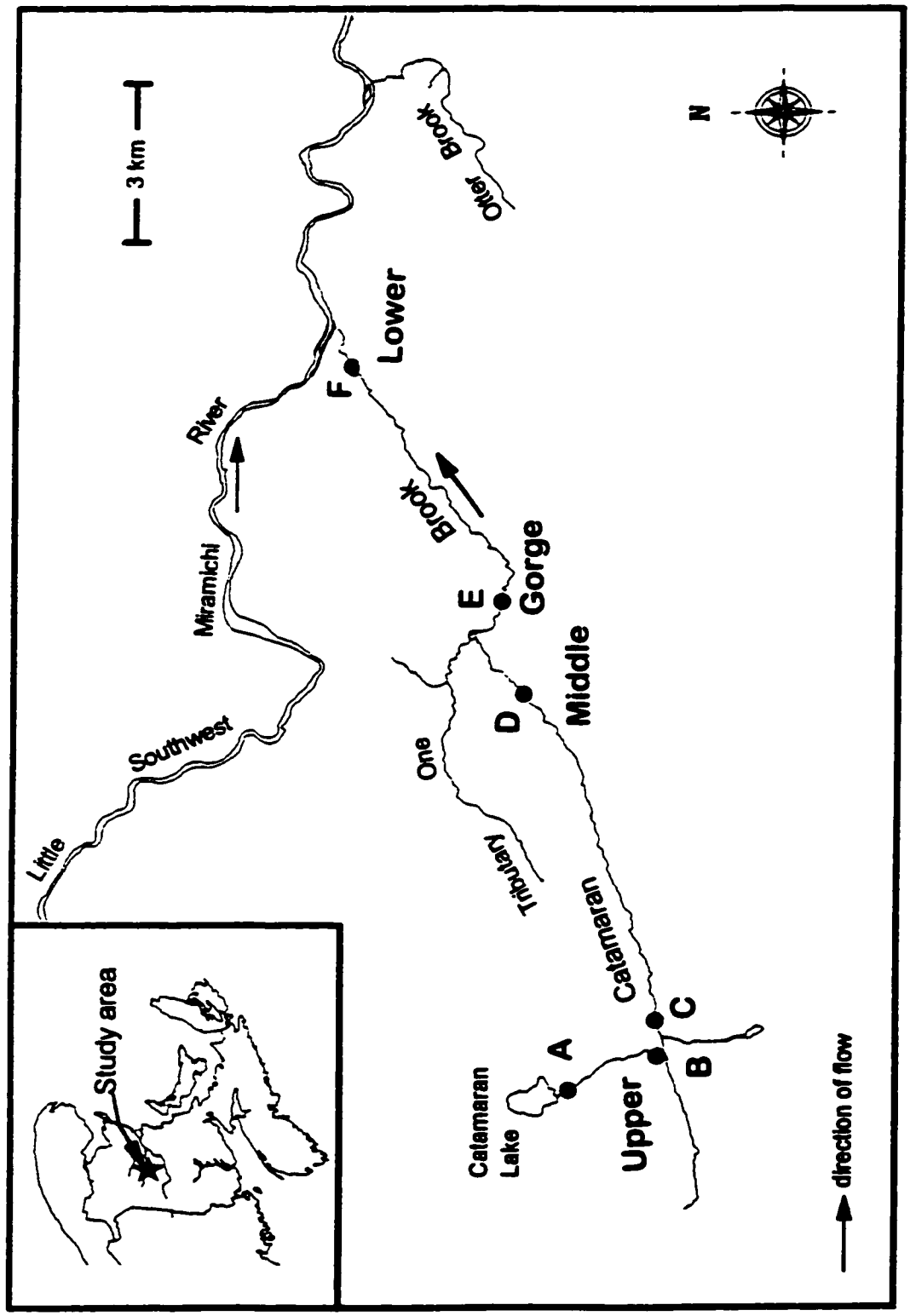

Figure 4.1. Catamaran Brook, New Brunswick, eastern Canada, showing the location of the six study sites.

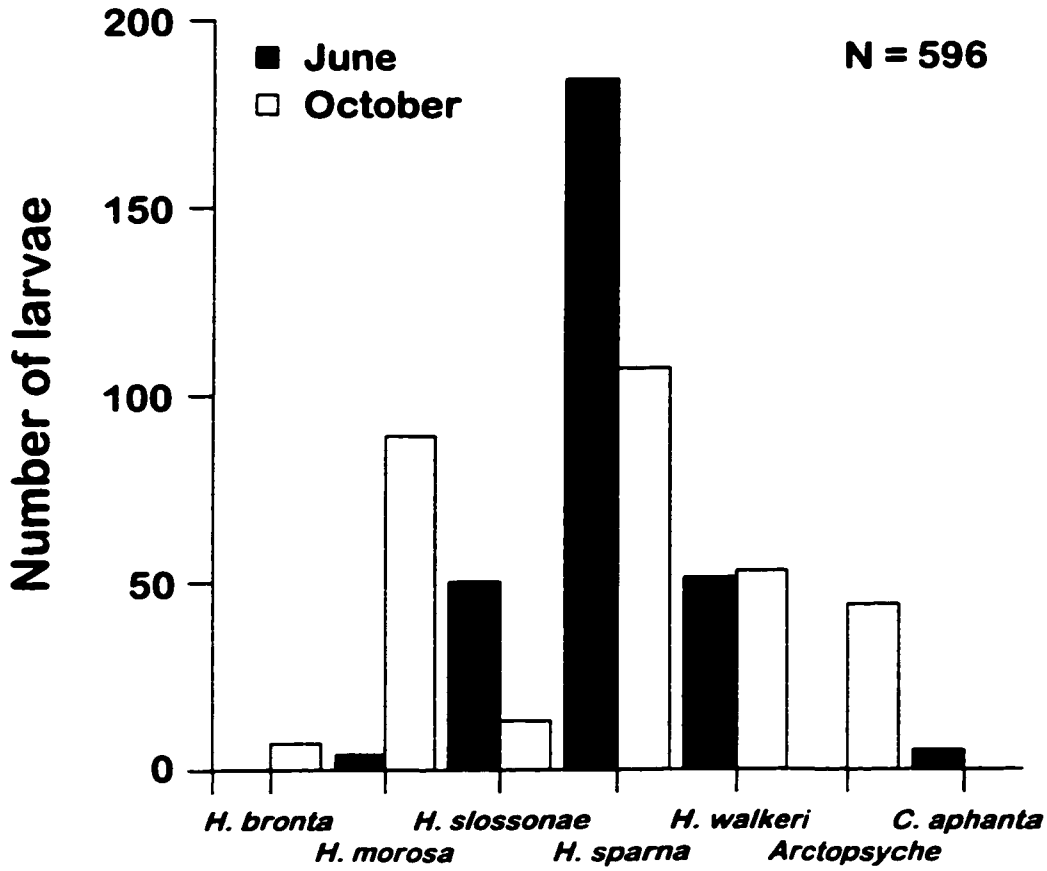

Figure 4.2. Total abundance of 7 species of late-instar hydropsychid caddisfly larvae collected in Catamaran Brook, New Brunswick, in June and October 1998, for stable-isotope ratios of carbon and nitrogen.

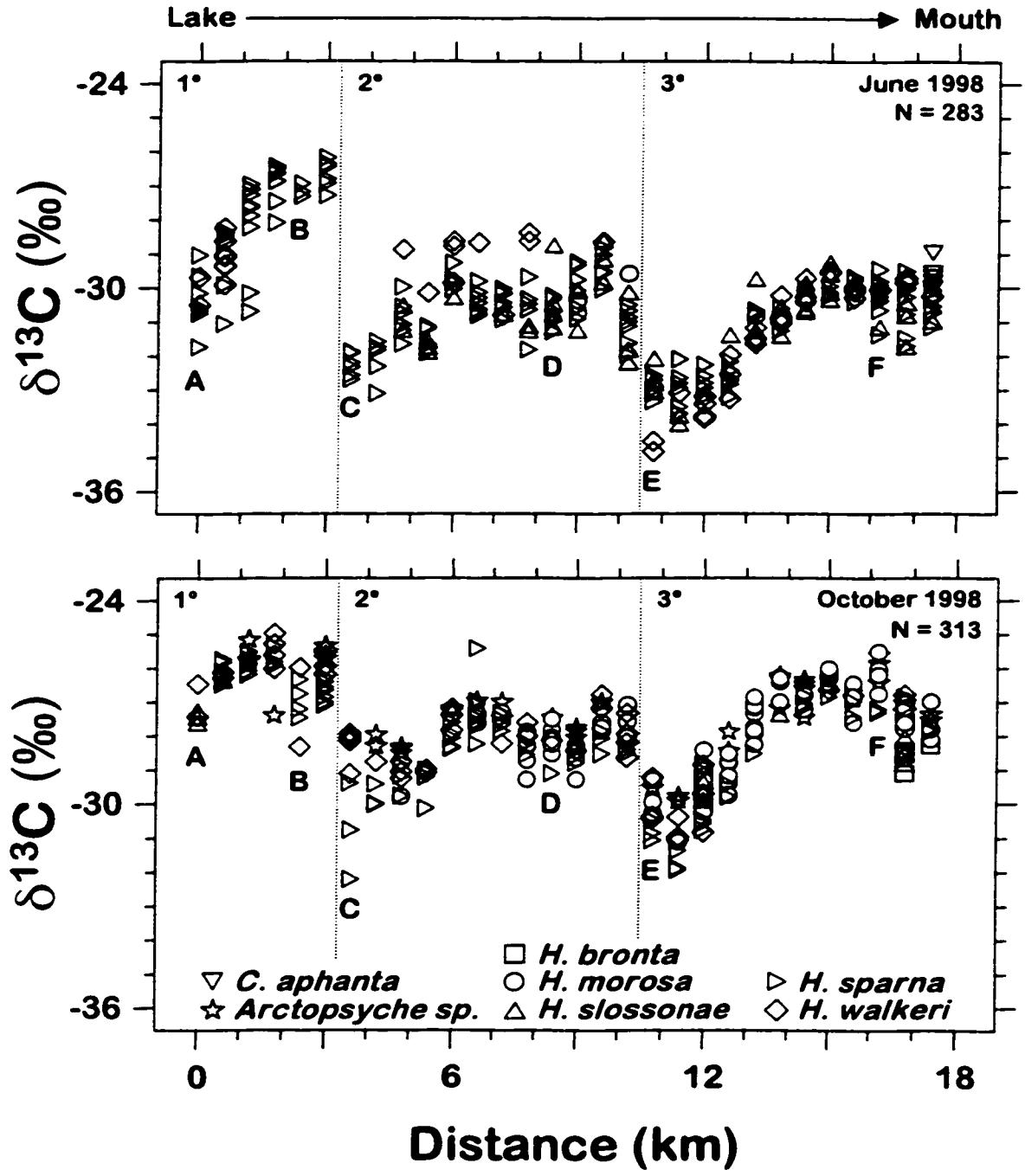

Figure 4.3. Stable-carbon-isotope ratios ($\delta^{13}C$) of late-instar hydropsychid larvae collected along an 18-km section from the headwater lake to the mouth of Catamaran Brook, New Brunswick, in June and October 1998. Stream order and site locations are also given.

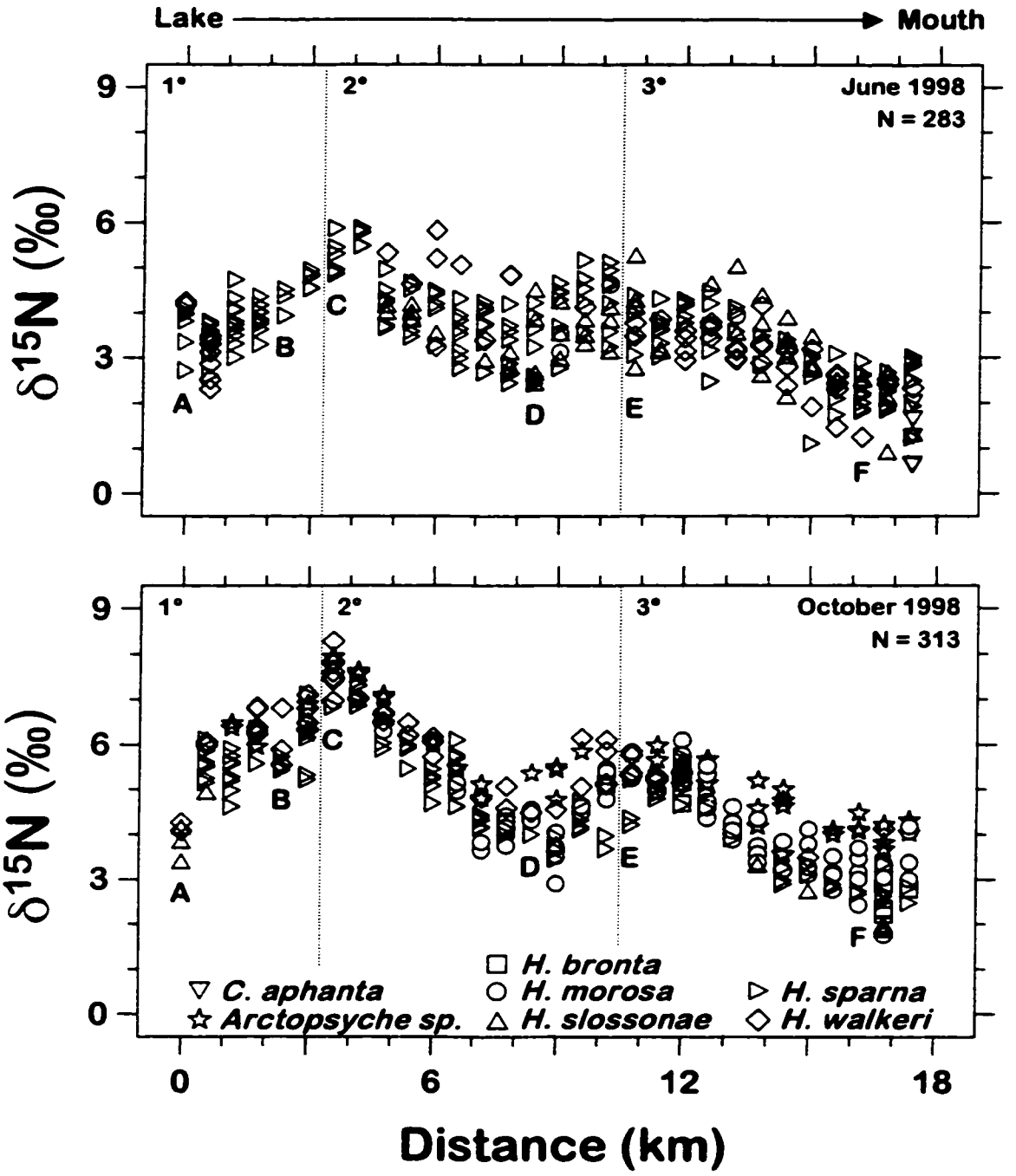

Figure 4.4. Stable-nitrogen-isotope ratios ($\delta^{15}N$) of late-instar hydropsychid larvae collected along an 18-km section from the headwater lake to the mouth of Catamaran Brook, New Brunswick, in June and October 1998. Stream order and site locations are also given.

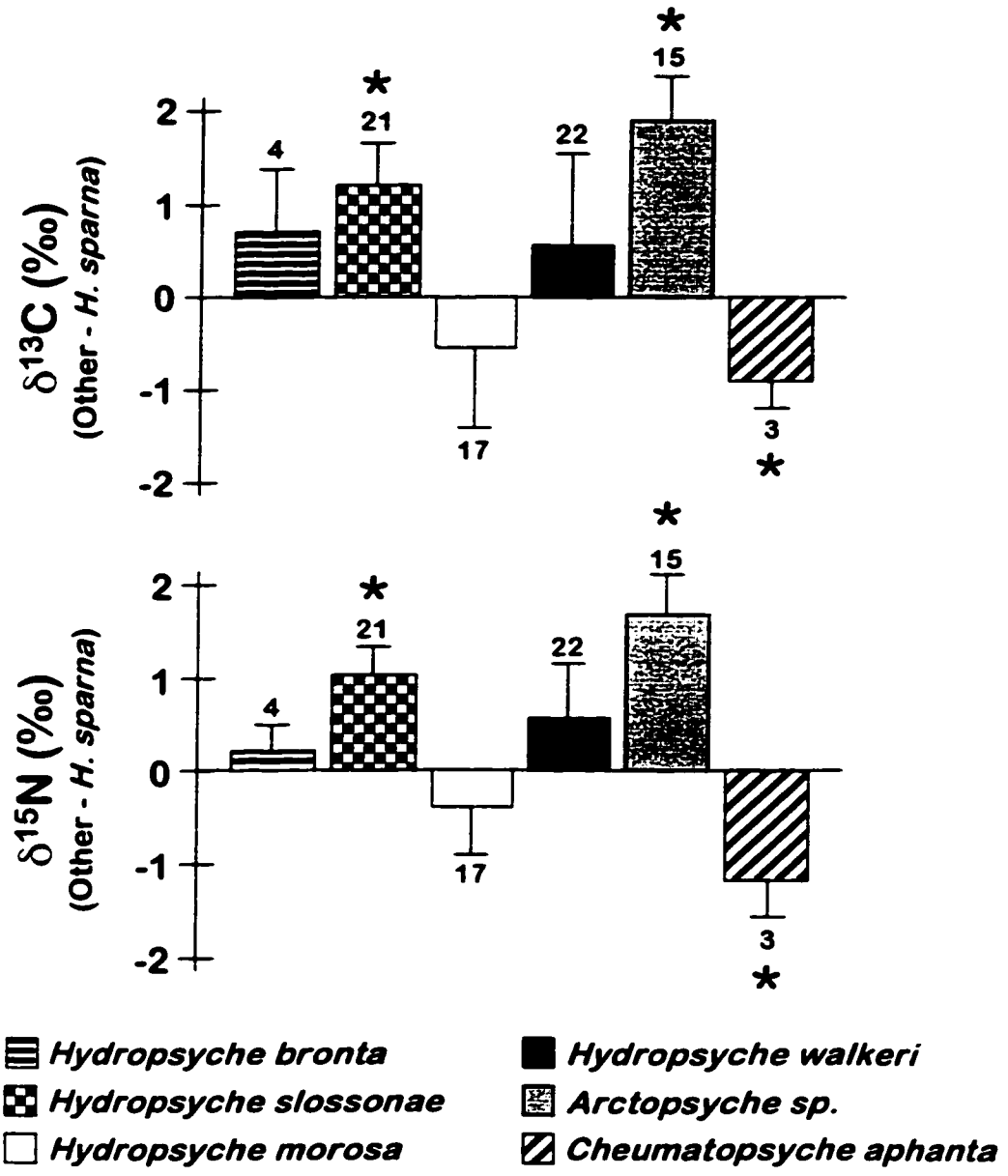

Figure 4.5. Relative difference in $\delta^{13}C$ and $\delta^{15}N$ values between *Hydropsyche sparna* and six other hydropsychid species in Catamaran Brook, New Brunswick, in 1998. Samples were pooled between dates (June and October). Asterisks (*) denotes statistically significant differences between species (Tukey's HSD, $p < 0.05$). Sample sizes are shown above (or below) the bars.

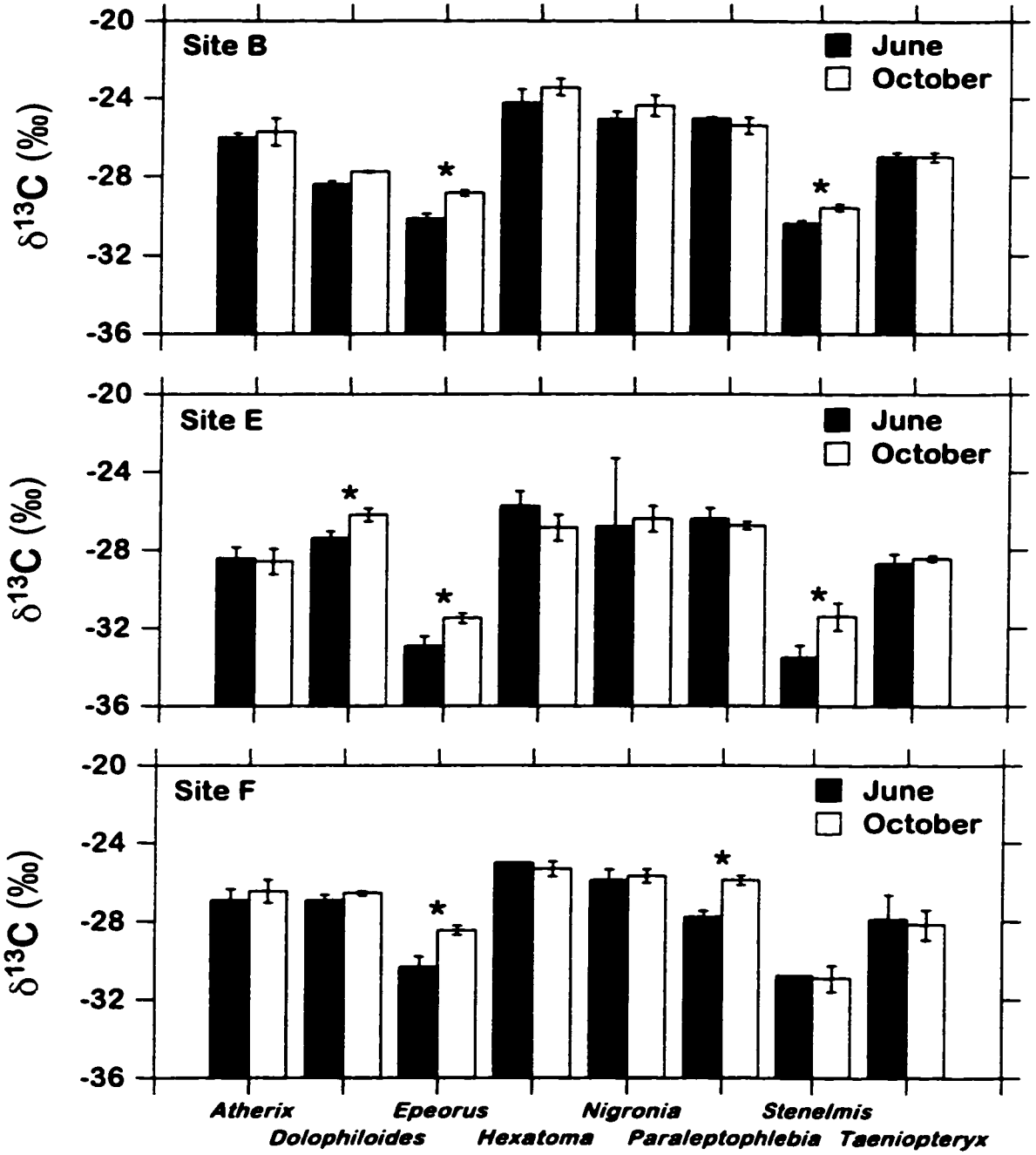

Figure 4.6. Stable-carbon-isotope ratios ($\delta^{13}C$) of 8 benthic species present in samples collected in both June and October 1998, in Catamaran Brook, New Brunswick. Asterisks (*) denote statistically significant differences between sampling dates (*t*-test, $p < 0.05$).

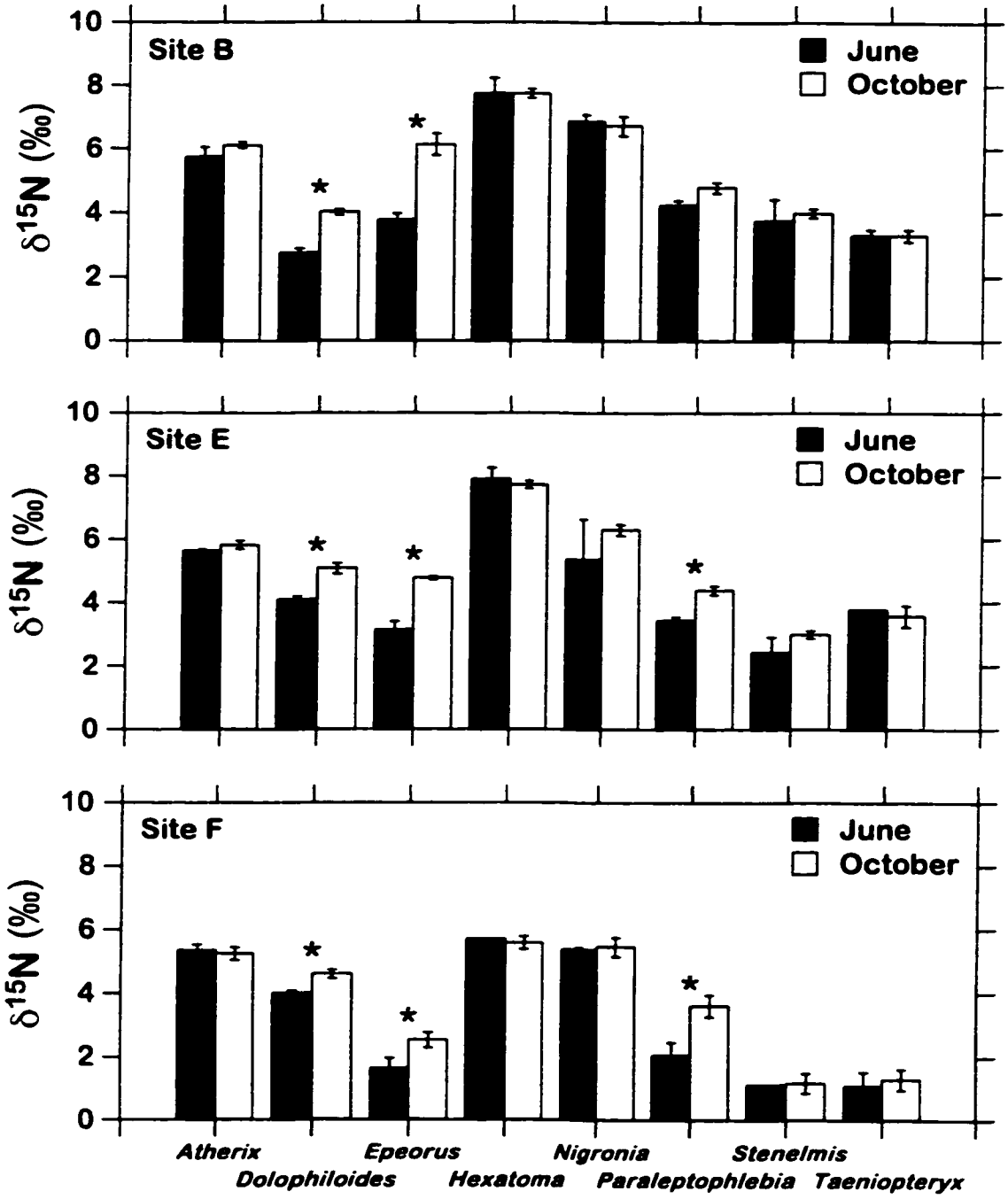

Figure 4.7. Stable-nitrogen-isotope ratios ($\delta^{15}N$) of 8 benthic species present in samples collected in both June and October 1998, in Catamaran Brook, New Brunswick. Asterisks (*) denote statistically significant differences between sampling dates (*t*-test, $p < 0.05$).

CHAPTER FIVE

Assessing the predictive power of a two-source isotope mixing model in aquatic ecology using a statistical resampling technique

Abstract

The ability of a quantitative two-source isotope mixing model to predict the relative importance of allochthonous and autochthonous inputs to an aquatic food web leading to juvenile Atlantic salmon was assessed using a statistical resampling approach and stable-isotope data ($\delta^{13}C$ and $\delta^{15}N$) obtained from Catamaran Brook, New Brunswick, in August, 1997. When the difference between the 2 primary food sources was small (e.g., 2-5‰), the model performed poorly and assigned 95% confidence limits in the range of ± 20% to 70%. Performance was enhanced when the difference between the 2 food sources was increased (e.g., 6-10‰), but the predictive power of the model was invariably dependent on sample size. Altering the degree of trophic fractionation showed only minor effects on the predictive capacity of the model. These results suggest that, at natural abundance levels, most stable-isotope data should be used as qualitative estimates of diet, and that only robust isotope datasets are amenable to quantitative interpretation. Statistical power curves are presented to aid ecologists in future attempts to design successful stable-isotope studies.

Introduction

Measuring the relative contribution of primary food sources to higher trophic-level organisms, such as commercially important fish species, is an important concern in current fisheries science (Waters 1993). Species introductions, landscape disturbances, and other environmental perturbations can disrupt energy flow and disturb food-web structure in many aquatic ecosystems (Rounick and Winterbourn 1985, McClelland and Valiela 1998, Vander Zanden et al. 1999), highlighting a need to assess the magnitude of these impacts. However, quantitative analyses of energy pathways are often impeded by detritus-based food webs, and conventional methods such as gut-content analyses are often limited by their instantaneous inventories of consumed prey, requiring intensive sampling efforts to obtain meaningful dietary estimates. Furthermore, ethical limitations are usually placed on the sampling of top predators and other ecologically-sensitive species.

Stable-isotope analysis (SIA) may help to discern the relative importance of primary food sources to top predators in aquatic food webs. SIA is a chemical analysis of food-source origins and energy pathways, and does not suffer from the ambiguities associated with visual interpretation of stomach contents and detrital pools (Peterson and Fry 1987). The method relies on the fact that ratios of naturally occurring stable isotopes of common elements such as carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) are assimilated into primary producers with signatures characteristic of certain biogeochemical processes, and are passed along food chains with relatively predictable change (reviewed in Fry and Sherr 1984, Peterson and Fry 1987, Rundel et al. 1989, Lajtha and Michener 1994). In addition, stable-isotope data are continuous variables, making them conducive to rigorous statistical testing. SIA also provides a composite picture of feeding over a period of time, rather than the single point-in-time sample obtained through gut-content analysis, and refers to assimilated versus ingested materials.

In freshwater ecology, stable-carbon-isotope ratios (expressed as $\delta^{13}C$) have been used most often to distinguish between important primary food sources, such as aquatic algae and terrestrial leaf litter in streams (Rounick and Winterbourn 1986), or benthic and planktonic algae in lakes (Hecky and Hesslein 1995). On the other hand, stable-nitrogen-isotope ratios ($\delta^{15}N$) have allowed for the accurate determination of trophic position

(Minagawa and Wada 1984). Typically, at each trophic transfer of food energy, $\delta^{13}C$ values enrich by 0-1‰, whereas $\delta^{15}N$ values increase by 3-5‰ (DeNiro and Epstein 1978, 1981, Tieszen et al. 1983, Minagawa and Wada 1984, but see Focken and Becker 1998). Although many studies have used quantitative isotope mixing models to determine the relative contribution of 2 or more food sources to organisms at higher trophic levels (Rau 1980, Kline et al. 1993, Junger and Planas 1994, Bilby et al. 1996, Doucett et al. 1996, Hilderbrand et al. 1996, Ben-David et al. 1997, Whitledge and Rabeni 1997, MacLeod 1998, Szepanski et al. 1999, Vander Zanden et al. 1999), most studies incorporate single endpoints (i.e., average values) into the model, ignoring variability in the stable-isotope ratios of the primary food sources. Furthermore, robust estimates of error are rarely reported along with output from the mixing model, raising concern over the validity of results.

The first objective of this study was to determine the relative importance of allochthonous inputs to juvenile Atlantic salmon (*Salmo salar*) in Catamaran Brook and the Little Southwest (LSW) Miramichi River, New Brunswick, using stable-isotope ratios of carbon and nitrogen and a 2-source isotope mixing model. The second objective was to place 95% confidence limits on the output of the mixing model using a statistical resampling technique (Simon and Bruce 1991). The final objective was to examine the predictive capability of the mixing model under different scenarios and generate power curves which could aid researchers in future attempts to use stable-isotope data for quantitative purposes.

Methods

Study Site

Catamaran Brook (lat. 46°52.7′N, long. 66°06.0′W) is a tributary of the LSW Miramichi River, located in a pristine forested area of north-central New Brunswick (Fig. 5.1). The brook is a well buffered, circumneutral, 3rd-order stream, ~ 20.5 km long, with a drainage area of 52 km^2 (Cunjak et al. 1993). Riparian vegetation consists of 60% deciduous trees, including white birch (*Betula papyrifera*), sugar maple (*Acer saccharum*), and speckled alder (*Alnus rugosa*). The remaining 40% of riparian forest cover consists of conifers, including balsam fir (*Abies balsamea*), red spruce (*Picea*

rubens), and eastern white cedar (*Thuja occidentalis*). Litter fall generally occurs in late September and leaves show rapid conditioning and fungal colonization, especially in autumn (Garnett et al. 2000).

Catamaran Brook is currently the focus of several multidisciplinary studies evaluating logging impacts on Atlantic Salmon habitat and productivity. Four study reaches (Upper, Middle, Gorge, and Lower) have been surveyed and characterized in the brook for long-term study. The Upper Reach consists of a 180-m stretch in the headwater region, and is characterized by a narrow (1-2 m), highly shaded stream channel, generally coarse substrates, steep slopes, and rapid flow. The Middle Reach, located approximately half way down the stream channel, has a stream width of 6-8 m, riffle gradients of 2-2.3% and riffle substrates of cobble and gravel. The Gorge Reach, located ~ 2 km downstream of the Middle Reach, runs through a bedrock outcrop area and is 6-8 m wide with riffle gradients of ~ 2% and a primarily bedrock substrate with some gravel and cobble. The Lower Reach consists of the lower 2 km of the stream and is 8-12 m in width, has riffle gradients of 1.6-1.75%, and riffle substrates of gravel, cobble, and boulder. Other details on geochemistry, hydrology, and biological factors can be found in Cunjak et al. (1993) and Giberson and Caissie (1998).

Field sampling

Sampling occurred in August 1997 at 4 study sites in Catamaran Brook (Upper, Middle, Gorge, and Lower), and at one additional site in the LSW Miramichi River, about 50 m upstream of the confluence with Catamaran Brook (Fig. 5.1). At each site, epilithic algae were scrubbed from the upper surfaces of 15-20 randomly selected rocks in run-riffle habitats and filtered onto pre-combusted Whatman GF/F glass-fiber filters. Filters were inspected for large (ca. ≥1 mm) detrital material which, if found, was subsequently removed. Filters were then acidified using 1N HCl, re-neutralized with distilled, deionized water, and air-dried, to reduce isotope contamination by inorganic carbon. Leaves from common riparian tree species were hand-picked from the stream, rinsed of attached detritus, air dried, and stored in plastic bags to await isotope analysis. Age-1+ Atlantic salmon parr were collected using a backpack electrofisher from all sites

by Federal Fisheries personnel and dorsal muscle tissue samples were provided by Peter Hardie (Fisheries & Oceans Canada, Moncton, NB).

Stable-isotope analysis

Fish muscle and leaf materials were oven-dried at constant temperature (60 °C) for 24-48 h and ground to a fine powder using either a mortar and pestle or a ball-mill grinder. One milligram aliquots of powdered tissue were used as individual samples. Algal filters were cut into thin sections and tightly packed into tin weighing boats using a small press.

Stable-isotope ratios are expressed as delta values (δ) and are measures of a parts-per-thousand (or "per mil") difference (‰) between the isotope ratio of a sample and that of an international standard according to the formula:

$$\delta\,^{13}C \text{ or } \delta\,^{15}N = [\,(R_{sample} - R_{standard}) / R_{standard}] \times 1000$$

where $R = {}^{13}C/{}^{12}C$ or ${}^{15}N/{}^{14}N$. Samples that are more negative are depleted and contain less ^{13}C or ^{15}N; samples that are less negative are enriched and contain more of the heavier isotopes. International standards are Vienna Peedee Belemnite (VPDB) (Coplen 1996) and nitrogen gas in the atmosphere (Mariotti 1983). These standards are, by definition, set at 0‰.

Isotopic analyses were performed on a Micromass VG Isochrom continuous-flow isotope-ratio mass spectrometer connected to a Carlo Erba elemental analyzer at the Environmental Isotope Laboratory (EIL) (University of Waterloo, Waterloo, Ontario, Canada). Repeat analyses of commercially available isotope standards yielded results that were both accurate and precise (International Atomic Energy Agency [IAEA] standard CH6: $\delta^{13}C = -10.5 \pm 0.2$‰ [mean ± 1SD, $n = 32$]; IAEA-N1: $\delta^{15}N = 0.6 \pm 0.3$‰ [$n = 25$]). Replicates of an internal laboratory standard: EIL-70 (lipid-extracted fish tissue) also gave reliable δ values ($\delta^{13}C = -20.7 \pm 0.2$‰ [$n = 38$], and $\delta^{15}N = 16.4 \pm 0.3$‰ [$n = 38$], and $\delta^{13}C = -13.4 \pm 0.3$‰ [$n = 16$]).

Two-source isotope mixing model

Because allochthonous carbon can contribute to stream food webs in more than one way (e.g., it may be consumed directly by herbivores, in which case it can be

considered allochthonous production, or it may be respired to CO_2 and form part of autochthonous production before being utilized by herbivores), I have defined allochthonous carbon in this study as allochthonous production and report the relative importance of this food source (e.g., terrestrial leaf litter) to fish at each site using the following equation:

$$\%Allochthonous = \frac{\delta^{13}C_{salmon} - \delta^{13}C_{autochthonous} - f \cdot x}{\delta^{13}C_{allochthonous} - \delta^{13}C_{autochthonous}} \times 100$$

where f is the average enrichment of ^{13}C between an animal and its food, and x is the trophic position of the fish determined using $\delta^{15}N$. The model was run using either f = 0‰ (no trophic fractionation), f = 1‰ (a commonly accepted value for trophic fractionation in ^{13}C; DeNiro and Epstein 1978), or $f = 1.2 \pm 0.7$‰ (Doucett et al. 1999). The model was also run using $x = \delta^{15}N_{fish} / 3.4$‰, where 3.4‰ is a commonly accepted value for trophic fractionation in ^{15}N (Minagawa and Wada 1984, Vander Zanden et al. 1999), or $x = \delta^{15}N_{fish} / 3.5 \pm 0.5$‰ (Doucett et al. 1999). This model assumes that both primary food sources have $\delta^{15}N$ values of 0‰. The $\delta^{13}C_{allochthonous}$ value is the average stable-carbon-isotope ratio of the sampled leaf litter, which was considered constant at all sites. The $\delta^{13}C_{autochthonous}$ is the site-specific average stable-carbon-isotope ratio of epilithic algae. This two-source mixing model assumes that leaf litter and algae are the two most important food sources in Catamaran Brook. This hypothesis is based on the general acceptance that mosses and macrophytes are not often used as food by stream consumers (Hynes 1970).

Statistical analysis

Data were analyzed using Resampling Stats (version 4.0.7, Resampling Stats Inc., Arlington, VA), a software package that performs randomization tests (Simon and Bruce 1991). Manly (1997) states that in the absence of any knowledge about a population, the distribution of values found in a random sample of size n from the population is the best guide to the distribution in the population. Thus, it is sensible to "resample' the sample in order to approximate what would happen if the population could be sampled

repeatedly. Resampling approaches are commonly adopted to assign valid error terms to population estimates, and are also used to test hypotheses when datasets do not meet the strict assumptions of standard parametric procedures (Manly 1997). In this study, the "bootstrap" method (Efron 1979) was used to place 95% confidence limits on output from the 2-source isotope mixing model. Assuming that researchers want to be able to determine relative food-source importance to within some degree of reliability, a confidence interval of "± 10%" was considered the desirable effect size (e.g., Robson and Regier 1964). For each parameter in the mixing model (e.g., $\delta^{13}C$ of algae, leaf litter, and fish), a sample of size *n* was randomly selected (with replacement) from existing datasets, and the mean value of each "resample" was incorporated into the mixing model equation. Simulations were repeated 10 000 times for each site, averaging ~12 s on a standard PC computer (PIII 500 MHz) (Appendix 5.1).

Results

Stable-isotope ratios of epilithic algae, leaf litter, and 1+ Atlantic salmon are presented in Table 5.1. Incorporating these values into the mixing model equation, the relative importance of allochthonous carbon in Catamaran Brook and the LSW Miramichi River, New Brunswick, in August 1997 varied considerably, with average values ranging from 9% to 72% (Table 5.2). Using the bootstrap procedure, 95% confidence limits were determined for each estimate, and the size of the error terms were related to the magnitude of the difference between the 2 primary food sources (Table 5.2). For example, at the LSW site, where the $\delta^{13}C$ values of epilithic algae and leaf litter differed by 5.0 ± 1.1‰ (Fig. 5.2A), salmon were 9.0 ± 19.3% dependent on allochthonous inputs. However, at the Upper site, where the food-source differential was only 1.6 ± 0.8‰, the model gave a much less reliable estimate of 38.2 ± 71.8% (Fig. 5.2B).

Assuming that the stable-isotope distributions of algae, leaf litter, and salmon could be used as reliable estimates of each population, the isotope mixing model was re-run to determine its predictive capability under different scenarios. Varying the degree of trophic fractionation showed only minor effects on the predictive power of the model (Fig 5.3A), and a food-source differential of about 7-10‰ was necessary in all cases to

reduce the 95% confidence interval to a reasonable effect size of ± 10%. Varying sample size had a much larger impact on the predictive capacity of the model (Fig. 5.3B). For 5 samples, a food-source differential of 15‰ was necessary to obtain a 95% confidence interval of ± 10%, but for n = 50 a difference of only 5‰ was needed to achieve the same power. Similarly, if the food-source differential was 10‰, a sample size of 15-20 was adequate to produce a 95% confidence interval of ± 10% (Fig. 5.4). However, when the difference between food sources was only 3‰, close to 150 samples were required to achieve similar power.

Discussion

The temptation to use stable-isotope data for quantitative purposes remains large, in light of a need to accurately predict and measure the effects of environmental impacts on trophic structure and energy pathways in aquatic food webs (Waters 1993). Unlike other descriptors of food-web structure (Pimm 1982), stable-isotopes are continuous variables and are amenable to rigorous statistical testing. Incorporating stable-isotope data into quantitative mixing model equations (Peterson and Fry 1987), allows the relative importance of 2 or more food sources to be determined for species of interest, such as top predators and other higher-trophic-level organisms (Kline et al. 1993, Hilderbrand et al. 1996, Ben-David et al. 1997, Vander Zanden et al. 1999). However, results from this study suggest that quantitative isotope mixing models should be used with caution. In Catamaran Brook, where isotopic differences between epilithic algae and terrestrial leaf litter were small (e.g., 2-3‰), the mixing model generated results with prohibitively large error terms, making among-site comparisons difficult. In the LSW Miramichi River, where the food-source differential was 5‰, the model yielded results that were accurate to only ± 20%. Under these conditions, it appears that stable-isotope ratios should not be incorporated into mixing model equations, but should be used as qualitative estimates of diet.

Improving the quantitative ability of isotope mixing models may be possible through adjustments to either the number of samples collected or to the size of the isotopic difference between food sources. In most field studies, however, the food-source differential will be "fixed" or under the control of local environmental and

biogeochemical conditions (e.g., Wada and Yoshioka 1996). In these situations, sample size should be modified so that the isotope mixing model has sufficient power to interpret potential differences between estimates. Using power curves generated in this study, one can see that the most effective sample size will depend on the magnitude of the difference between the 2 food sources. Assuming that researchers want to be able to predict relative food-source importance to within ± 10%, a sample size of 20 to 40 replicates would be necessary to achieve this power when the food-source differential is between 6‰ to 10‰. If the difference between food sources is 5‰ or less, sample sizes may be considered too large (i.e., $n > 100$) to carry out quantitative studies using stable-isotope data, especially when research is being conducted on ecologically-sensitive species.

If large sample sizes are impracticable or unethical, attempts should be made, where possible, to increase the isotope difference between food sources. Using the power curves, one can see that a difference of at least 15‰ would give the mixing model sufficient power at low sample sizes. Isotope enrichment studies (e.g., Peterson et al. 1997, Hall et al. 1998) are quite capable of increasing food-source differentials by 15‰ or more. In these studies, ^{15}N-labelled ammonium can be dripped into the aquatic environment to alter the original isotope signature of aquatic autotrophs. The cost of labelling a small stream with an enriched stable isotope appears relatively inexpensive (Hershey and Peterson 1996), but similar efforts in large rivers and lakes may be impractical. Stable-isotope ratios may become more variable at enriched levels, requiring larger sample sizes in order to maintain sufficient statistical power.

Other methods that may help increase the "natural" isotope difference between food sources in aquatic ecosystems involve isolating algal components from bulk samples (Bidigare et al. 1991, Hamilton et al. 1994, Sachs et al. 1999). Stream epilithon contains not only algae, but fungi, bacteria, detritus, inorganic particles, and terrestrial organic matter (Lock et al. 1984). If methods to remove these non-algal materials from the biofilm are unsuccessful, contamination may alter the $\delta^{13}C$ value of the "inferred" autochthonous component because terrestrial inputs are usually more ^{13}C-enriched than algal inputs (Rounick and Winterbourn 1986, O'Leary 1998). Under these conditions, the $\delta^{13}C_{autochthonous}$ value will shift towards the $\delta^{13}C_{allochthonous}$ value and the food-source differential will become smaller. This situation likely occurred in Catamaran Brook,

where grazers were often more ^{13}C-depleted than biofilm samples (Chapter 3). Certainly, more research is required to determine whether sampling methods should be refined for the determination of stable-isotope ratios in epilithic algae.

In summary, small differences between the $\delta^{13}C$ values of epilithic algae and leaf litter interfered with the use of a quantitative isotope mixing model to determine the relative importance of allochthonous inputs to juvenile Atlantic salmon in Catamaran Brook, New Brunswick. Using a statistical resampling technique, estimates from the mixing model were found to be associated with large error terms, making among-site comparisons difficult. It is likely that small differences exist between the stable-isotope ratios of primary food sources in many other aquatic environments, suggesting that quantitative mixing models be used with caution. However, the predictive power of these models can be greatly improved by intensifying sampling efforts or increasing the food-source differential. Given the need to quantify energy flow between primary producers and top predators in aquatic ecosystems, further refinements to stable-isotope techniques are essential.

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Table 5.1. Stable-isotope ratios of epilithic algae, leaf litter, and 1+ Atlantic salmon parr collected from Catamaran Brook and the LSW Miramichi River, New Brunswick, in August 1997. Values are means ± 95% confidence limits. Sample sizes are in parentheses. Sites are as in Figure 5.1.

	Algae	Leaf	Salmon parr	
Site	$\delta^{13}C$ (‰)	$\delta^{13}C$ (‰)	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
Upper	-30.4 ± 2.7 (14)	–28.8 ± 2.7 (51)	–28.0 ± 2.2 (8)	8.3 ±1.2 (8)
Middle	-30.9 ± 2.9 (15)	–28.8 ± 2.7 (51)	–26.7 ± 2.5 (41)	7.7 ± 2.1 (41)
Gorge	-31.7 ± 2.3 (15)	–28.8 ± 2.7 (51)	–27.9 ± 2.0 (99)	8.7 ± 1.4 (99)
Lower	-31.3 ± 3.6 (22)	–28.8 ± 2.7 (51)	–27.3 ± 2.2 (94)	7.5 ± 1.8 (94)
LSW	-23.8 ± 2.0 (15)	–28.8 ± 2.7 (51)	–21.4 ± 2.2 (32)	8.4 ± 0.7 (32)

Table 5.2. The relative importance of allochthonous inputs to 1+ Atlantic salmon parr in Catamaran Brook and the LSW Miramichi River, New Brunswick, in August 1997. Values are means ± 95% confidence limits. Results were obtained using data in Table 5.1 and 10 000 simulations of the 2-source isotope mixing model (f = 1.0‰ and $x = \delta^{15}N_{fish}$ / 3.4‰). Sites are as in Figure 5.1.

Site	Difference ($\delta^{13}C_{algae} - \delta^{13}C_{leaf}$)	% Allochthonous
Upper	1.6 ± 0.8	38.2 ± 71.8
Middle	2.1 ± 1.4	71.9 ± 47.9
Gorge	2.9 ± 0.9	44.4 ± 24.8
Lower	2.5 ± 1.0	57.5 ± 27.9
LSW	5.0 ± 1.1	9.0 ± 19.3

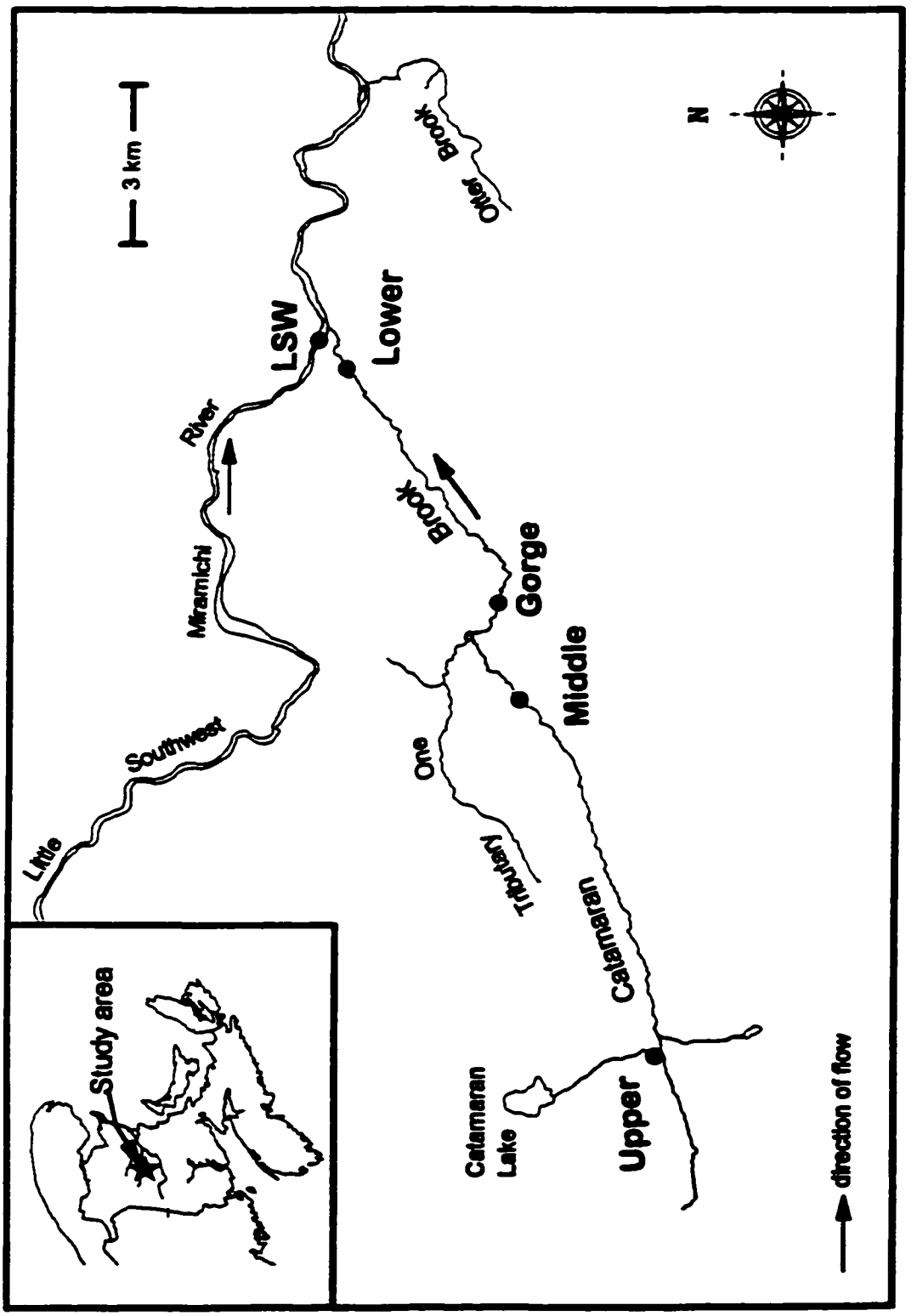

Figure 5.1. Catamaran Brook, New Brunswick, eastern Canada, showing the location of the five study sites.

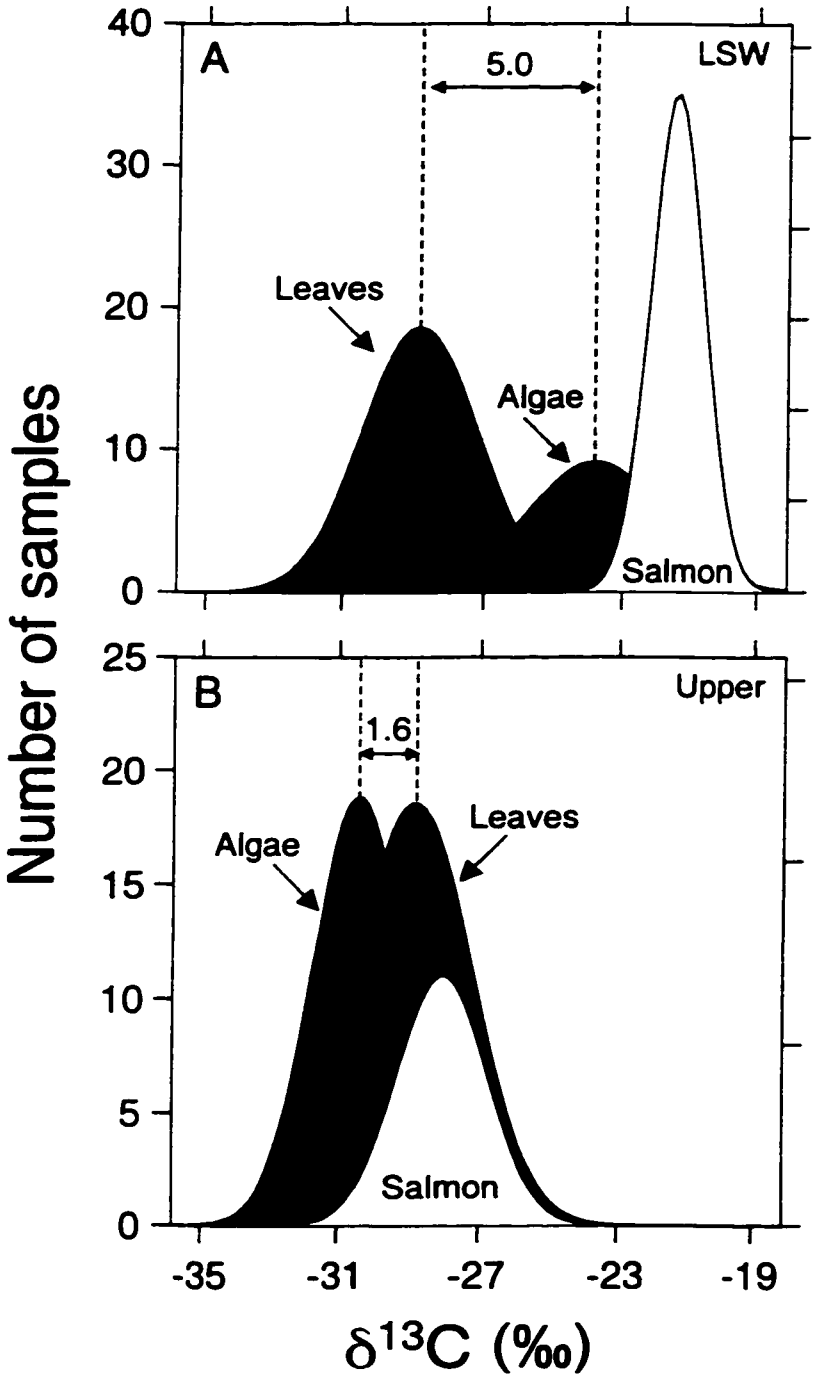

Figure 5.2. The $\delta^{13}C$ distributions for epilithic algae, leaf litter, and 1+ Atlantic salmon at the LSW Miramichi River (A) and at the Upper site in Catamaran Brook (B), New Brunswick, in August 1997. The dashed lines highlight the relative difference between the 2 primary food sources.

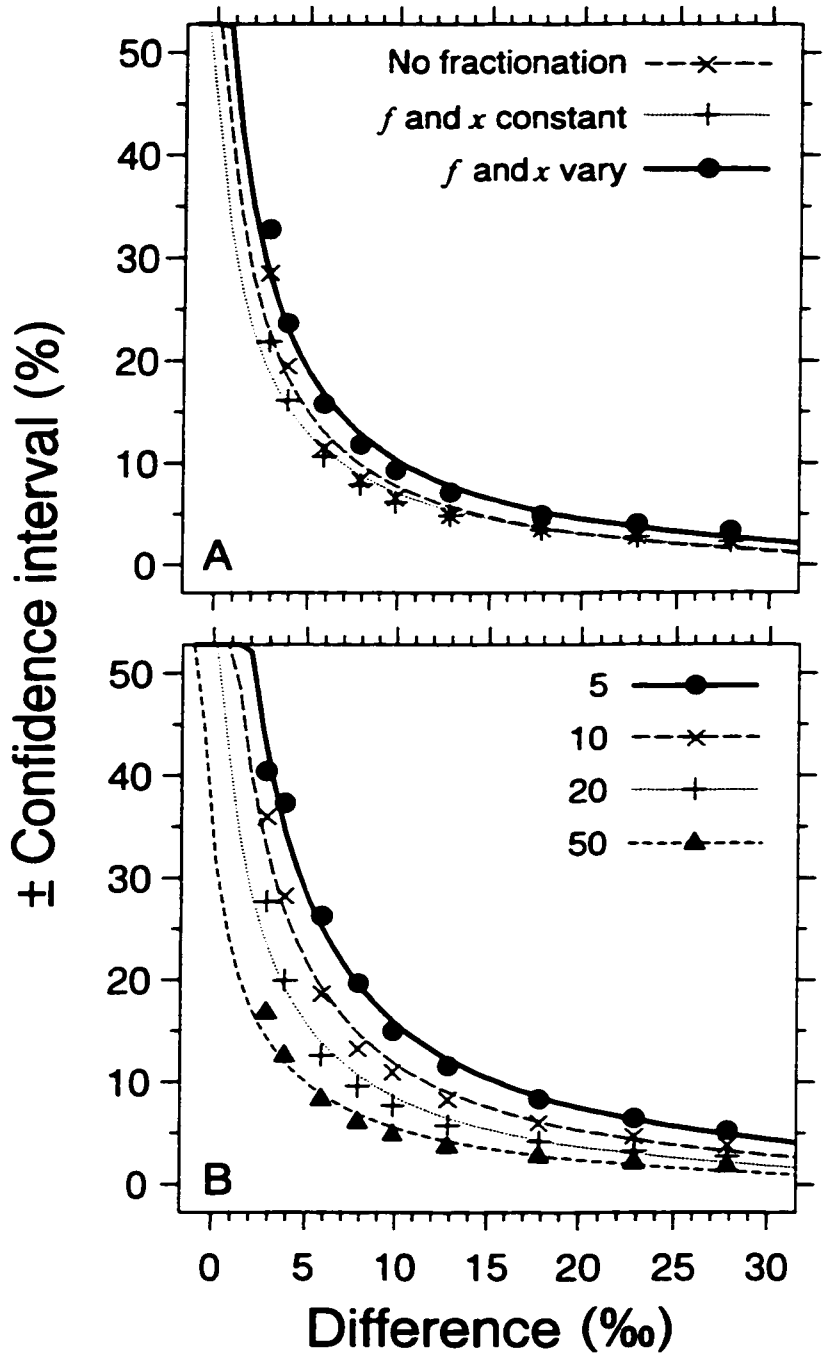

Figure 5.3. Relationship between the 95% confidence interval and the food-source differential. Panel A shows effects of trophic fractionation (no fractionation: f = 0‰; f and x constant: f = 1.0‰ and $x = \delta^{15}N_{fish} / 3.4‰$; and f and x vary: $f = 1.2 \pm 0.7‰$ and $x = \delta^{15}N_{fish} / 3.5 \pm 0.5‰$). Panel B shows effects of sample size (n = 5, 10, 20, 50), with f = 1.0‰ and $x = \delta^{15}N_{fish} / 3.4‰$. Power curves were fitted using SYSTAT.

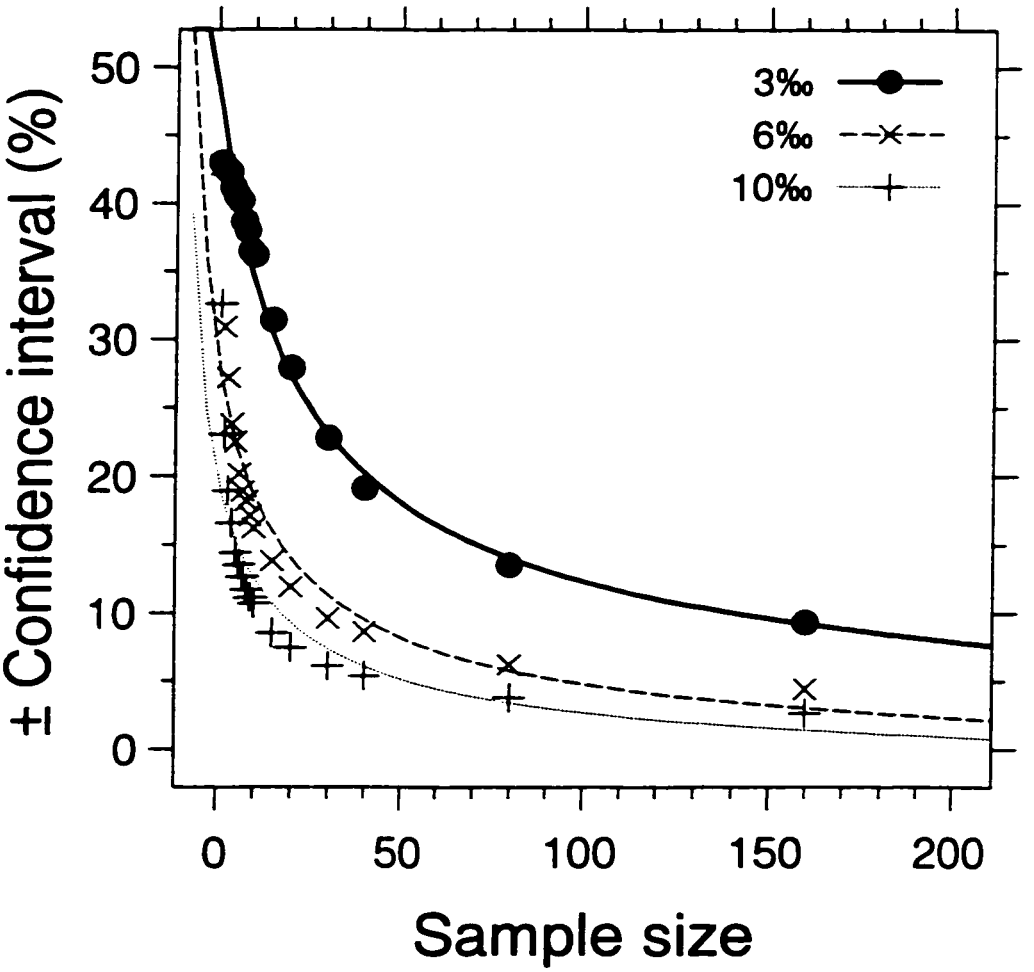

Figure 5.4. The relationship between the 95% confidence interval and sample size for various primary food-source differentials (i.e., 3‰, 6‰, 10‰) with f = 1.0‰ and x = $\delta^{15}N_{fish}$ / 3.4‰. Curves were fitted using a statistical power function.

Appendix 5.1. Sample code written for the RESAMPLING STATS program in order to test the predictive power of a 2-source isotope mixing model.

```
read file "gorge" a b c
d e f

maxsize default 10000

repeat 10000

  sample 50 a aa
  sample 50 b bb
  sample 50 c cc
  sample 50 d dd
  sample 50 e ee
  sample 50 f ff

  mean aa aaa 'algae
  mean bb bbb 'leaf
  mean cc ccc 'fish c13
  mean dd ddd 'fish n15
  mean ee eee 'frac c13
  mean ff fff 'frac n15

  subtract ccc aaa top
  divide ddd fff f2
  multiply f2 eee frac
  subtract top frac top
  subtract    bbb    aaa
bottom
  divide    top    bottom
model
  multiply    model    100
answer
   score answer z
   score bottom ddiff
   score aaa aalgae
   score bbb bleaf
   score ccc cfishc13
   score ddd dfishn15
   score eee efracc13
   score fff ffracn15
   score frac gfrac

end

mean z zz
mean aalgae algae
mean bleaf leaf
mean cfishc13 fishc13
mean dfishn15 fishn15
mean efracc13 fracc13
mean ffracn15 fracn15
mean gfrac frac

      mean ddiff diff

      percentile z (2.5) CLz
      subtract zz CLz CLz
      percentile aalgae (2.5) CLalgae
      subtract algae CLalgae CLalgae
      percentile bleaf (2.5) CLleaf
      subtract leaf CLleaf CLleaf
      percentile cfishc13 (2.5) CLfishc
      subtract fishc13 CLfishc CLfishc
      percentile dfishn15 (2.5) CLfishn
      subtract fishn15 CLfishn CLfishn
      percentile efracc13 (2.5) CLfracc
      subtract fracc13 CLfracc CLfracc
      percentile ffracn15 (2.5) CLfracn
      subtract fracn15 CLfracn CLfracn
      percentile gfrac (2.5) CLfrac
      subtract frac CLfrac CLfrac
      percentile ddiff (2.5) CLdiff
      subtract diff CLdiff CLdiff

      'histogram z

      print zz
      print CLz
      print algae
      print CLalgae
      print leaf
      print CLleaf
      print fishc13
      print CLfishc
      print fishn15
      print CLfishn
      print fracc13
      print CLfracc
      print fracn15
      print CLfracn
      print frac
      print CLfrac
      print diff
      print CLdiff
```

CHAPTER SIX

General Conclusions

In this thesis, feeding relationships between stream invertebrates and their diets were examined using stable-isotope ratios of carbon and nitrogen in a small pristine forested stream, Catamaran Brook, in north-central New Brunswick. Variability was documented for $\delta^{13}C$ and $\delta^{15}N$ values at the base of the aquatic food chain, and isotope distributions were incorporated into a quantitative 2-source mixing model to determine the relative importance of allochthonous inputs to juvenile Atlantic salmon. The predictive power of the model was assessed using a statistical resampling technique, and suggestions were made regarding future use of stable-isotope ratios as quantitative tools in aquatic food-web ecology.

Summary of important findings

Trophic enrichment in ^{13}C and ^{15}N, between a commensal chironomid (*Nanocladius P.* sp.) and its stonefly host (*Pteronarcys biloba*), agreed with values documented in the literature (Chapter 2), implying that at least one of the many tenets of isotope ecology holds true in field situations. This report was the first to verify ectoparasitism among aquatic invertebrates using stable-isotope ratios, and offers exciting opportunities to investigate other relationships where the diet of the symbiont remains unconfirmed, or where both phoretic and parasitic forms occur on the same host.

The stable-isotope ratios of a dominant invertebrate grazer, *Glossosoma nigrior*, showed good agreement with those of its algal diet (Chapter 3). Site-specific differences in algal $\delta^{13}C$ and $\delta^{15}N$ were attributed to changes in water chemistry, light availability, and growth rate along the stream continuum. Effects of water velocity on algal $\delta^{13}C$ and $\delta^{15}N$ were observed at only 1 of 4 sites, suggesting that it plays only a minor role in the determination of stable-isotope ratios in lotic microalgae. *Glossosoma nigrior* and *Blepheracera tenuipes* were isotopically distinct from other grazers, such as *Rhithrogena impersonata* and *Stenacron interpunctatum*, and differences appeared to be related to the feeding-mode adopted by these species. This result highlights that some scraper-grazers are more selective than others in their ability to feed directly on algae within the biofilm, suggesting that care should be given to the choice of isotopic surrogates of primary food sources in food-web studies.

Large gradients in the stable-isotope ratios of hydropsychids collected along the stream continuum resulted from tributary influences (Chapter 4). This implied that site

selection should be an important concern in studies using stable-isotope ratios to interpret food-web relations. In general, hydropsychid $\delta^{13}C$ and $\delta^{15}N$ mirrored patterns in algal $\delta^{13}C$ and $\delta^{15}N$, suggesting that algae played an important role in the nutrition of these filter-feeding caddisflies. *Arctopsyche* sp. had more enriched $\delta^{15}N$ values than most other hydropsychids, resulting from its larger capture-net mesh size and more carnivorous diet. *Hydropsyche slossonae* was also more ^{15}N-enriched than other conspecifics, suggesting it filled a niche space similar to that of *Arctopsyche* sp., and that segregated life history strategies allowed for their co-existence within the same habitats. These results emphasize the usefulness of stable-isotope ratios to help elucidate dietary overlap and trophic position among co-existing competitive species.

Stable-isotope ratios of juvenile Atlantic salmon (Chapter 5) were synthesized with data on trophic fractionation (Chapter 2), isotope variability in algae (Chapter 3) and leaf litter (Chapters 2 and 4), and were used to determine the relative importance of allochthonous inputs to an aquatic top predator. Small differences in the $\delta^{13}C$ values of algae and leaf litter resulted in large confidence limits being placed on estimates generated from the isotope mixing model, hindering the ability to quantitatively define relative food-source importance. Using a statistical resampling technique, the model was reanalyzed to determine conditions under which performance could be improved. Predictive power was invariably dependent on both sample size and the isotope differential between food sources, but not trophic fractionation. These results suggest that quantitative isotope mixing models should be used with caution and that it is important to place robust error terms on output from the model. Power curves were provided so that researchers could determine *a-priori* the conditions necessary to achieve sufficient statistical power when stable-isotope ratios are used for quantitative purposes.

Recommendations and implications for future study

Some alterations in methodology could improve the effectiveness of stable-isotope ratios in stream food-web ecology. Of obvious need is an ability to separate algae from terrestrial detritus, fungi, microbes, and other extra-cellular material contained within the biofilm matrix. Non-algal components may strongly influence the isotope signal of the "inferred" autochthonous component, leading to false overlap between the stable-isotope

ratios of autochthonous and allochthonous food sources. Techniques that facilitate the purification of algae include those that isolate chlorophyll and other specific compounds from bulk particulate organic matter. These methods have been used in stable-isotope studies of marine food webs (Bidigare et al. 1991, Sachs et al. 1999), and similar attempts should be made to use chlorophyll as an isotopic proxy of autochthonous sources in streams.

Application of an enriched isotope is another potential means of separating the stable-isotope ratios of allochthonous and autochthonous sources in stream food webs. Isotope enrichment studies have been carried out in only a few stream ecosystems (Peterson et al. 1997, Hall et al. 1998), and attention has been directed more towards understanding nutrient processes than interpreting food-web relations. The method uses ^{15}N-enriched ammonium as a tracer of in-stream nutrient cycling and produces very heavy stable-isotope ratios (e.g., 400‰) in aquatic autotrophs and their consumers. Enriched stable-isotope ratios of aquatic algae would be very distinct from those of terrestrial leaf litter, facilitating quantitative interpretation of food-web patterns. Use of this technique in a small stream is relatively inexpensive, costing less than $500 CAN to enrich a small study reach for a 2-month period (Hershey and Peterson 1996), and it is my intention to carry out this type of study at Catamaran Brook in the near future.

More attention should be given to the identification of species within the epilithic algal community and the influence of particular forms on stable-isotope ratios in the biofilm sample. For example, the ability of certain species to actively uptake dissolved CO_2 and HCO_3^- may affect $\delta^{13}C$ values, while the presence of N_2-fixers could change $\delta^{15}N$ values. Given that stream algae grow under a wide variety of environmental conditions, intensive sampling efforts are still warranted in order to ascertain the extent to which stable-isotope ratios vary. Additional information on catchment geology, hydrology, other water chemistry parameters, and the stable-isotope ratios of both dissolved inorganic carbon and nitrogen would also improve study designs.

No study is perfect and scientists must continually reflect on ways to improve their research. Stable-isotope ecology is a relatively new field of science, owing in part to the recent development and increased availability of continuous-flow isotope-ratio mass spectrometers, and the reasonably inexpensive cost of current analyses. Due to the complexity of many ecosystems and the diversity of organisms that inhabit them, several

assumptions in stable-isotope ecology still remain to be tested (Gannes et al. 1997). In comparison to the number of stable-isotope studies that have been carried out in marine environments, relatively few investigations have occurred in freshwater, and only a small number of these have been conducted in streams. Contemporary research in stable-isotope ecology suggests that $\delta^{13}C$ and $\delta^{15}N$ values are quite variable at the base of the food chain (Leggett 1998, MacLeod 1998, data in this thesis), suggesting that stable-isotope analysis (SIA) is not the "quick and easy" method initially proposed by some authors (Rounick and Winterbourn 1986, Fry 1991). However, this is not to say that SIA should be disregarded as a potentially useful method in food-web ecology. Advantages of SIA far outweigh its disadvantages. SIA uses naturally existing chemical tracers that have the facility to resolve many issues in food-web ecology that remain hindered by limitations in other techniques. SIA also possesses a unique ability to link biogeochemistry to ecosystem structure and function, and can provide quantitative estimates of diet when suitable conditions exist. If used in concert with other techniques (e.g., gut-content data, secondary production), data can be obtained to rigorously test certain ecological hypotheses. Future endeavors will improve the value of SIA for use in ecological research, so long as the validity of the assumptions upon which SIA is based are rigorously tested.

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