Factors Affecting Stable Carbon and Nitrogen Isotopic Signatures in Food Webs of the Ste. Marguerite River (Quebec, Canada)

Véronique Trudeau
Department of Biology
McGill University
M.Sc. Thesis

February 2nd 2005

General introduction

Stable isotopic analyses are becoming an important tool for freshwater ecologists studying trophic interactions in communities. These analyses are mainly based on carbon and nitrogen isotopic signatures ($d^{13}C$ and $d^{15}N$), although isotopes of other elements have long been recognised to reflect biogeochemical processes as well. For example, $d^{18}O$ and dD have been used to study paleotemperatures and the origin of geothermal water and brines of various kinds, while $d^{34}S$ have been used to determine the natural (e.g. metabolic H_2S released by sulfate-reducing bacteria) or anthropogenic (S compounds from combustion of fossil fuels) sources of S in the atmosphere (Kendall and McDonnell 1998; Faure 1986). In ecology, $^{13}C/^{12}C$ ratio is mostly used to examine C source relationships and $^{15}N/^{14}N$ ratio to look at food web structure (Vander Zanden and Rasmussen 1999; 2001). This type of analysis has considerable potential in conservation biology, since minute amounts of animal tissue can often be obtained without animal sacrifice, which is important when studying rare species. Stable isotopic signatures of organisms are influenced by a wide range of factors, and their usefulness ultimately depends on understanding the whole suite of factors that determine them for both primary producers and consumers. In this thesis, I will discuss the potential importance of water velocity - an abiotic factor that is currently poorly understood.

In nature, most biogeochemical reactions and physical processes that involve elements with multiple stable isotopes exhibit isotopic fractionation to some degree. Molecules containing lighter isotopes form weaker bonds and are thus more reactive. These are more readily used in chemical reactions than molecules with heavier isotopes (e.g. photosynthetic carbon fixation). In terms of physical processes, lighter isotopes also diffuse faster than heavy ones (e.g. plant CO_2 boundary layer diffusion). These

properties cause different isotopes of the same element to be partitioned unequally between substances or phases, commonly called isotopic fractionation (Hoefs 1997). The more an organism fractionates the smaller the isotopic ratio (heavy isotope/light isotope) will be in its tissues. Understanding the underlying processes of isotopic fractionation is essential for stable isotopic signatures to be useful in determining the function of each trophic level and basically how ecosystems function. We believe water velocity is involved in important underlying processes of isotopic fractionation, as well as algal biomass.

It has been shown that factors controlling the rates of primary production affect $d^{13}C$ of aquatic plants. MacLeod and Barton (1998), as well as Wienke and Fisher (1990), found that light intensity and temperature positively affected primary producer $d^{13}C$. Differences in algal photosynthetic mechanisms can also affect primary producer $d^{13}C$ (Fogel et al. 1992). Fry and Wainright (1991) have shown that cell size can influence marine phytoplankton $d^{13}C$. Species composition has also been suggested to increase epiphyte $d^{13}C$ variability (Osmond et al. 1981). France (1999) showed that increasing DOC concentration results in more negative epilithon $d^{13}C$. These are all examples of factors that have been reported to contribute to primary producer $d^{13}C$ variability, yet there is still a large proportion unexplained.

Aquatic plants, both unicellular and multicellular, have a boundary layer which forms at their surface where the flow is laminar, almost stagnant, and that is disrupted by water turbulence. The thickness of the boundary layer is thus delimited by the turbulence flow (Dingman 1984). Turbulence in the water facilitates the diffusion of dissolved gases, which slows down when molecules diffuse through the boundary layer of plants. It has been generally hypothesized that the degree of mixing of inorganic

carbon between the boundary layer and surrounding medium of aquatic plants influences their $d^{13}C$ – the boundary layer/diffusion hypothesis.

Fractionation by aquatic plants likely leads to build-up of the heavier isotope in the boundary layer, which should be greater at low velocities and where biomass is greater (and presumably greater C uptake rates). That is, in high energy environments, greater water movements should result in thinner or more turbulent boundary layers, enhancing C exchange and minimizing depletion in the boundary layer. Preferential uptake of light isotopes from the boundary layer should lead to the accumulation of heavier isotopes and tend to reduce the degree of apparent fractionation relative to the surrounding water mass. Thinner or more turbulent boundary layers will not be as readily depleted of nutrient elements and would be expected to accumulate heavy isotopes to a lesser extent. Plants exposed to high water velocities, a turbulent medium, and/or poor growing conditions, should thus appear to fractionate to a greater extent relative to the surrounding medium. Conversely, plants growing in low water velocities, less turbulent medium, and/or favourable growing conditions should appear to fractionate less between heavier and lighter isotopes.

We have provided evidence from a controlled laboratory study that water velocity had a significant effect on periphyton $d^{13}C$ and $d^{15}N$ (Trudeau and Rasmussen 2003). Both signatures tended to get more negative at higher water velocities. In Chapter 1 of this thesis, we specifically examine the effect of water velocity and periphyton biomass on the $d^{13}C$ and $d^{15}N$ of periphyton in a natural environment. Both factors significantly affected periphyton $d^{13}C$, yet water velocity had the strongest influence. No relationship was found for periphyton $d^{15}N$. In Chapter 2, we determined to what extent the water velocity signal was transmitted up the food web. Due to the complexity of freshwater

ecosystems, we expected other processes to reduce the effect of water velocity on consumers' signatures. We briefly introduce two biological processes potentially obscuring this signal; diet and spatial averaging. These questions could not be fully answered with the available data, yet preliminary analyses suggest these factors should be explored in further research.

Water velocity is an important factor to consider in the Ste. Marguerite River system, especially since the construction of highway 172 in the 1960's, which altered the ecosystem of the river. Channelization has been shown by Parker and Andres (1976) to increase the slope of a river, thereby increasing water velocity. Supporting evidence by Talbot and Lapointe (2002) demonstrate that meander straightening in the Ste. Marguerite River has in fact increased the slope of the rectified reach significantly, thus increasing water velocity and the rate of sediment transport until the next equilibrium is reached. As well, improperly installed culverts and accelerated rates of erosion are other examples of disturbances that altered the river and its tributaries, including modification and loss of habitats preferred by fish. Studies on aquatic organisms of all trophic levels and geomorphology are currently in progress on the Ste. Marguerite River system to understand the consequences of these river changes. Better knowledge of the fish species inhabiting the river system (e.g. natural and anthropogenic variations in abundance over time, morphology, genetics, habitat requirements), and their interactions with lower trophic levels is essential. We believe stable isotope ratios can help understand some crucial biogeochemical processes. This thesis refines the stable isotope method and makes it a better tool for freshwater ecologists. It provides useful information on the feeding behaviours of invertebrates and fish, and thus, can contribute

to the protection and management practices that need to be applied to this and other river systems to maintain diversity and sustain fish populations.

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Chapter 1

Patterns in river and stream periphyton $d^{13}C$ and $d^{15}N$: the role of water velocity and periphyton biomass

By

Véronique Trudeau and Joseph B. Rasmussen.

Biology Department, McGill University

1205 Dr. Penfield Ave., Montreal, Quebec, Canada, H3A 1B1

Running head: Water velocity and isotopic signatures

Acknowledgements

We thank Geneviève Morinville, David Browne, and other graduate students of the McGill University Biology Department for helpful suggestions on earlier versions of the manuscript. We also thank Ian Popple and Jamie Luce for proof-reading and comments. Financial support was provided by an NSERC Strategic grant to J.B.R.

Abstract

The use of stable C and N isotopes in food web studies, and as environmental indicators, requires deeper understanding of the factors that affect the signatures of primary producers. A laboratory experiment by Trudeau and Rasmussen (2003) demonstrated the negative effect of water velocity on periphyton C and N signatures ($d^{13}C$ and $d^{15}N$). Under a range of water velocities normally found in lotic systems (0.05-0.62 m s^{-1}), periphyton had significantly more negative signatures in faster currents. The present field study was conducted in the Ste. Marguerite River system and compared periphyton signatures found in sites with different flow regimes (0-1.27 m s^{-1}). Regression analyses on periphyton $d^{13}C$ in the eight sections sampled yielded similar patterns to those found in the laboratory. The tendency of periphyton to have more negative signatures in high water velocity sites was statistically significant for $d^{13}C$, which ranged from -17.7‰ to -31.5‰. In addition, regression analyses showed that periphyton biomass (chlorophyll *a* concentration ranging from 0.3 to 31.0 mg m^{-2}) had a positive effect on periphyton $d^{13}C$. Our field study however, failed to replicate the pattern for $d^{15}N$ previously found in the laboratory, and showed no significant trends for this element. All of the periphyton communities collected were dominated by a few diatom species and varied in the thickness of the biofilm. Fractionation in favour of ^{12}C by the periphyton communities likely leads to build-up of the heavier isotope in the boundary layer. At lower water velocities, as well as higher biomass (presumably greater C uptake rates), this build-up eventually results in periphyton having less negative $d^{13}C$. Failure of the $d^{15}N$ to reflect this process may result either from N limitation, which would prevent isotopic fractionation altogether, or possibly from local variability in the N source signature from groundwater. This study

allows a better understanding of aquatic primary producer $d^{13}C$ fluctuations and may potentially be useful in explaining those at higher trophic levels.

Introduction

Stable C and N isotopic signatures ($d^{13}C$ and $d^{15}N$), even though not fully understood, are becoming an important tool in freshwater ecology to study trophic interactions in communities. Starting at the base of the food chain, factors controlling the thickness of the boundary layer of aquatic plants may be responsible for some of the variability in the $d^{13}C$ and $d^{15}N$ data observed in streams and rivers. We believe water velocity may be important in this process, and primary producers should reflect this effect first. Related studies looking at the effect of water movement on $d^{13}C$ of aquatic plants have already showed ^{13}C-enriched signatures in low-energy versus high-energy systems. For example, a meta-analysis by France (1995) showed that planktonic algae had more negative $d^{13}C$ than benthic algae in both marine and freshwater environments. Similarly, Hecky and Hesslein (1995) found that phytoplankton from open-water turbulent environments are also often depleted in ^{13}C compared to littoral benthic algae. Even freshwater macrophytes have been reported to be enriched in ^{13}C in low-energy lakeshores, compared to the same species found in fast water currents (Osmond et al. 1981). High turbulence conditions created in a laboratory setting have also resulted in decreased $d^{13}C$ of individual algal species (Degens et al. 1968; France and Holmquist 1997).

Whole food webs appear to be influenced by this abiotic factor. Finlay et al. (1999) found a strong negative relationship between water velocity and herbivore $d^{13}C$, which reflect algal $d^{13}C$, in productive rivers. Results from this comparative study support the idea that boundary layer thickness strongly influences the supply rate of inorganic carbon like CO_2 to aquatic plants, which in turn affects the baseline signature passed on to higher trophic levels in the community. While most results tend to support

the boundary layer/diffusion hypothesis, some studies do not. In a comparative study, MacLeod and Barton (1998) found that isotopic fractionation of both C and N isotopes in periphyton was strongly influenced by light intensity and temperature, which affect the rate of metabolic activity, but detected no effect of water velocity (ranging from 0.02-0.57 m s^{-1}). The sampling method may be responsible for some of the contradicting results previously observed; it is possible that the water velocity signal may not be noticeable at all temporal and spatial scales, especially if the velocity range is not sufficiently large.

Recently, we showed that periphyton $d^{13}C$ and $d^{15}N$ both decreased with increasing water velocity as expected from the boundary layer/diffusion hypothesis (Trudeau and Rasmussen 2003). In this controlled laboratory study, water velocities in artificial streams ranged from 0.1-0.6 m s^{-1} similarly to most natural rivers and streams. Although the negative relationships between water velocity and isotopic signatures were significant ($P = 0.001$) for both elements, relationships tended to be stronger for $d^{13}C$ than for $d^{15}N$ (r^2 of 0.66 versus 0.53, respectively, for diatoms and 0.36 versus 0.28, respectively, for filamentous green algae). These results were of great interest since information in the literature is limited, especially with reference to aquatic plant $d^{15}N$ in relation to water movement.

Results from this study also showed that for the same water velocity, diatoms had more negative $d^{13}C$ relative to filamentous green algae. Thus, we hypothesized that a second factor - the thickness (density) of the algal mat - might determine the C uptake flux. At high periphyton density, the low diffusive rate of CO_2 will cause the inorganic C pool to be increasingly depleted from top to bottom in algae mat resulting in enriched algal $\delta^{13}C$. Support for this hypothesis can be seen in a lake study by Doi et al. (2003)

who found that POM (particulate organic matter) mainly composed of phytoplankton had more negative $d^{13}C$ relative to high-density algal mats of diatoms assimilating the same C source (dissolved CO_2).

The present research focused on the importance of water velocity and periphyton biomass in explaining isotopic signature variability of periphyton in a natural environment - the Ste. Marguerite River. The primary objective was to determine if the effect of water velocity on periphyton $d^{13}C$ and $d^{15}N$ was strong enough to be detected and significant, as observed in laboratory artificial streams. Periphyton $d^{13}C$ and $d^{15}N$ were expected to be more negative at high versus low water velocity sites. The second objective was to determine if periphyton $d^{13}C$ was positively affected by the biomass of the algal mat. The relationships between $d^{13}C$ and $d^{15}N$ of aquatic plants and water velocity and plant biomass will allow a better understanding of the high signature variability of aquatic plants in streams and rivers that is not observed in terrestrial plants. Eventually, they may also be used to explain some of the variability observed in periphyton consumer signatures, namely benthic invertebrates and fish, in different habitats.

Materials & methods

Study site

The study area is the Ste. Marguerite River in the Saguenay region, Quebec, Canada, and two of its tributaries (Figure 1). The river is composed of three branches - the main branch of about 95 km in length, the north east branch and north west branch. The forested drainage basin releases no agricultural fertilizers or industrial wastes contaminating the waters of that river system. Sunlight at the riverbed downstream is not affected by riparian vegetation since riverbanks are mostly cobble bars.

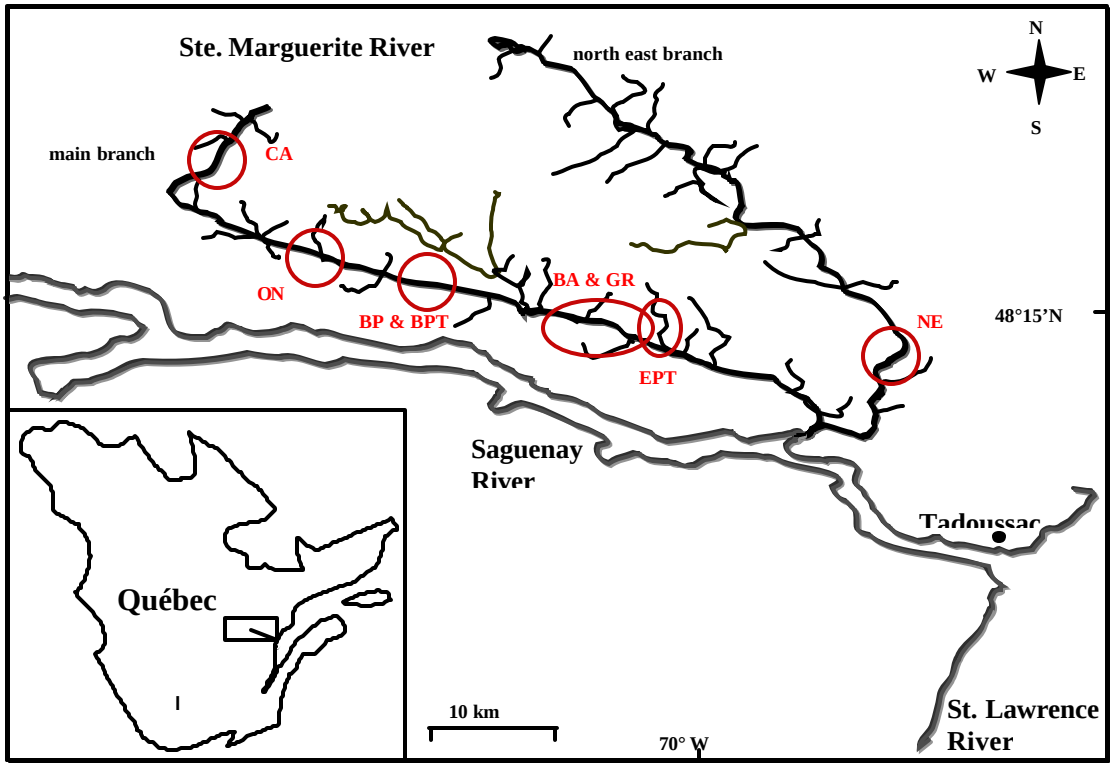

Figure 1. Map of the Ste. Marguerite River system. Circles represent reaches sampled.

Upstream, small trees and bushes on the riverbanks and a narrower channel slightly reduce sunlight at the riverbed. At streambeds, sunlight becomes highly variable due to the greater and patchy canopy cover.

Species collected

The main component of the periphyton communities growing on rocks were diatoms (Bacillariophyceae), which included mainly *Tabellaria spp., Eunotia spp., Novicula spp., Synedra spp., Gomphonema spp.*. At time of sampling, almost no green filamentous algae (Chlorophyta) was found in the river and associated streams. Some species were found in low proportion in a few samples (*Tetraspora spp., Bulbochaete spp., Draparnaldia spp.,* and *Ulothrix spp*.). A few species of cyanobacteria also accounted for a small proportion of the communities (*Gloeotrichia spp., Phormidium spp., Leptothrix crassa*). Blue green filamentous algae were also present at some sites. Finally, pollen (coniferous mainly) and fine particulate detritus were often entrapped in the periphyton communities. Since each component contributed to the $d^{13}C$ and $d^{15}N$ of the periphyton communities, the latter were analysed as a whole.

Water velocity measurements

Since we could not sample the whole river system, a total of eight reaches (river and stream sections) were chosen for detailed sampling (Table 1). The five reaches in the main branch of the river are listed in order from downstream to upstream in Table 1. Over this approximately 45 km distance, the river narrows from roughly 40 m to 10 m. BA reach is a wide and generally deep area of the main branch with no canopy cover. GR reach is a wide and fast flowing section of the river (steepest slope) with lots of boulders and no canopy cover. BP and ON reaches are narrower, and small trees and bushes along the riverbanks slightly increased the canopy cover. CA reach is the

Section	Reach	# of sites
Main branch	Bardsville (BA)	11
	Grand Rapide (GR)	7
	Big Pool (BP)	9
	Onesime (ON)	8
	Cascade (CA)	15
North east branch	North East (NE)	13
Tributaries	Epinette (EPT)	15
	Big Pool Tributary (BPT)	14

Table 1. Reaches sampled in the different branches and streams of the Ste. Marguerite River system and the number of sites sampled in each reach. Only abbreviations of reaches in brackets are used in the text.

narrowest of the main branch with little, yet continuous canopy cover. In the north east branch of the river, the NE reach is comparable to BA reach; wide and deep with no canopy cover. In the two streams sampled, EPT and BPT reaches have distinct riffles and pools. They are very narrow (2-4 m wide) and shallow, with a high percent canopy cover.

In every reach, 7 to 15 sites (cross-sections) were chosen according to their overall water velocity; either a pool (< 0.1 m s^{-1}), a run (0.1-0.3 m s^{-1}), or a riffle (> 0.3 m s^{-1}). Consecutive sites within a reach were always different in terms of overall water velocity. Reaches varied in length and were generally between 100 m in streams, where sites were closer together, up to 1 km in the river, where the length of individual sites were sometimes up to 100 m. We sampled at sites where the water was maximum 70 cm deep and rock size varied between 5-30 cm in diameter. Within each site, three different water velocities were measured using a Gurley Pygmy current meter (625DF8N – Wading Rod Suspended Pygmy type current meter outfit with Model 1100 digital indicator). Water velocity measurements were taken 1.5 cm above the surface of the periphyton layer and in the middle of a 0.1 m^2 surface area delimited by a container of the same size. Rocks substrate covering this area were removed, and periphyton were completely brushed and washed off the rocks with a known volume of water. A subsample of this water-algae mix was filtered using Whatman 47 mm glass microfibre filters and kept in the dark for subsequent chlorophyll *a* analysis. Another subsample was kept for stable isotopic analysis. All samples were frozen at -20°C until analysed.

$d^{13}C$ and $d^{15}N$ have been reported to vary seasonally (Finlay 2004; Doi et al. 2003; Cabana and Rasmussen 1996). Preliminary results of the present study site had shown that isotopic values varied slightly from the beginning of June until the end of

August, yet not significantly (paired t-test; $P = 0.55$ for $\delta^{13}C$ and $P = 0.28$ for $\delta^{15}N$). The water level in rivers and streams also varies throughout the year, which affects the water velocity. The water level discharge is usually highest during snowmelt and rain events during the spring. Summer thunder showers and frontal systems also lead to periodic high water levels. To avoid possible biases due to small temporal isotopic variations and changes in the water level, all reaches were sampled over a short three-week period in July during the high productivity season. Sampling of individual reaches took no more than 2-3 consecutive days during which the water level stayed fairly constant (approximately ± 2 cm).

Sample analyses

Stable isotope analyses

All samples of periphyton were oven-dried, pulverised, and placed into tin capsules. Stable C and N isotopic analyses were performed by mass-spectrometry (Finnigan-Mat Delta-Plus continuous flow isotope-ratio mass spectrometer coupled to a Carlo-Erba elemental analyser on line; G.G. Hatch Isotope Laboratories, University of Ottawa, Ontario, Canada, and GEOTOP Laboratory, University of Quebec in Montreal, Quebec, Canada). The analytical precision of these apparatus is typically 1 SD for C and N and is in the range of 0.05 to 0.2‰, which is small relative to the range of values found in nature (Kendall and Caldwel 1998). Stable isotopic signatures were expressed using the following standard equation:

$$d^{13}C \text{ and } d^{15}N = ([R_{sample}/R_{standard}] - 1) \times 1000$$

where R is the ratio of $^{13}C/^{12}C$ and $^{15}N/^{14}N$, and the standards are Pee Dee Belemnite limestone and atmospheric nitrogen for C and N respectively. The units are in permil (‰).

Chlorophyll a analysis

Chlorophyll *a* was extracted for 24 h from each periphyton sample using 95% acetone. Chlorophyll *a* concentrations in mg m^{-2} were then measured by spectrophotometry (Parson et al. 1984), since microalgal chlorophyll *a* concentration is a good indicator of microalgal biomass (Wetzel and Likens 2000).

Data analyses

Box plots were used to show the distribution and range of the data to be used in the regressions. As well, the per reach mean of each variable was calculated to look at between reach variation.

A regression was performed at the river system level to determine if water velocity had an effect on periphyton biomass. To avoid redundancy in the amount of periphyton $d^{13}C$ variability explained by each factor, multiple linear regressions were performed at the river system and reach levels to estimate the effect of water velocity and biomass on the periphyton $d^{13}C$. Chlorophyll a values were logged for normality. Finally, linear regressions were performed at the river system and reach levels to determine the effect of water velocity on periphyton $d^{15}N$.

Results

Basic statistics of variables measured

Figure 2 shows that a large range of water velocities was measured per reach with a similar number of pools, runs, and riffles sampled in individual reaches. In total, water velocities ranged from 0 m s^{-1} to 1.27 m s^{-1}, which is representative of ranges

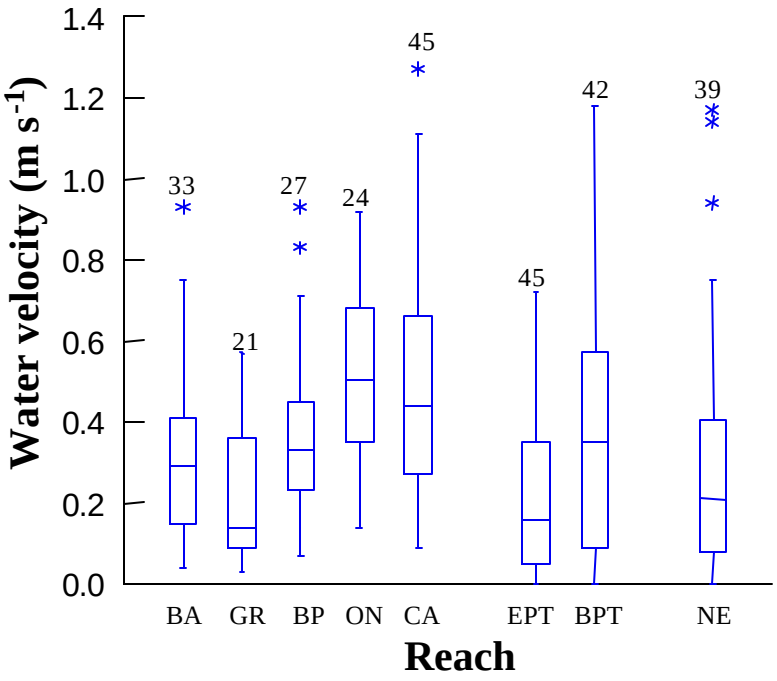

Figure 2. Box plot of water velocities measured in each reach. Sample sizes are marked above each reach.

normally found in rivers and streams. Water velocity means tended to increase from downstream to upstream in the main branch as the river width diminishes (Table 2). The chlorophyll *a* concentrations varied between 0.3 mg m^{-2} to 31.1 mg m^{-2} for a total range of 30.8 mg m^{-2} (Figure 3). The range was large in most reaches. Mean chlorophyll *a* concentrations were similar between reaches, from 4.7 mg m^{-2} to 8.1 mg m^{-2}, except in the NE reach which had the highest mean biomass of 12.4 mg m^{-2} (Table 2).

Periphyton $d^{13}C$ varied from -31.5‰ to -17.7‰ over all the reaches sampled for a total range of 13.8‰ (Figure 4). Within reaches, periphyton generally showed high variability with $d^{13}C$ ranges between 3.6‰ and 7.8‰. All reaches in the main river, as well as BPT reach, had similar mean $d^{13}C$ between -25.3‰ and -26.7‰ (Table 2). The EPT reach had the most negative $d^{13}C$ mean (-29.7‰) and the smallest range, while the NE reach had the least negative $d^{13}C$ mean (-21.0‰) and the largest range. Finally, periphyton also showed a relatively large range of $\delta^{15}N$ per reach, 2.4‰ to 5.7‰, for a total range of 5.9‰ (Figure 5). The mean $\delta^{15}N$ of individual reaches were very similar and varied from 1.1‰ to 3.1‰.

Periphyton $d^{13}C$ versus velocity & biomass

A regression between the two independent variables was initially performed at the river system level to determine if water velocity affected periphyton biomass. A linear regression gave the best fit (Figure 6). The resulting relationship was weak, but highly significant (r^2 of 0.07, $P < 0.0005$), suggesting that water velocity negatively affected periphyton growth. The appearance of the graph, however, suggested that a curvilinear regression would be more appropriate. A logarithmic regression gave the second best

Variable means	BA	GR	BP	ON	CA	EPT	BPT	NE
Velocity (m s^{-1})	0.32	0.22	0.36	0.51	0.49	0.21	0.37	0.30
Biomass (mg m^{-2})	7.5	7.3	4.7	5.2	5.3	8.1	7.3	12.4
Periphyton $d^{13}C$ (‰)	-26.1	-26.1	-26.7	-26.8	-25.3	-29.7	-26.7	-21.0
Periphyton $d^{15}N$ (‰)	1.8	2.0	2.4	2.9		2.1	1.1	3.1

Table 2. The mean of each variable measured per reach.

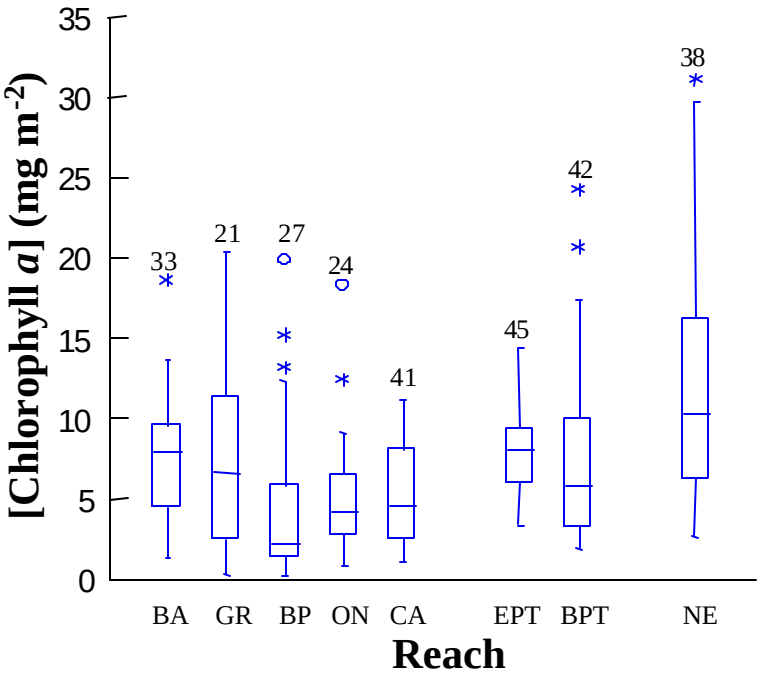

Figure 3. Box plot of chlorophyll *a* concentrations in each reach. Sample sizes are marked above each reach.

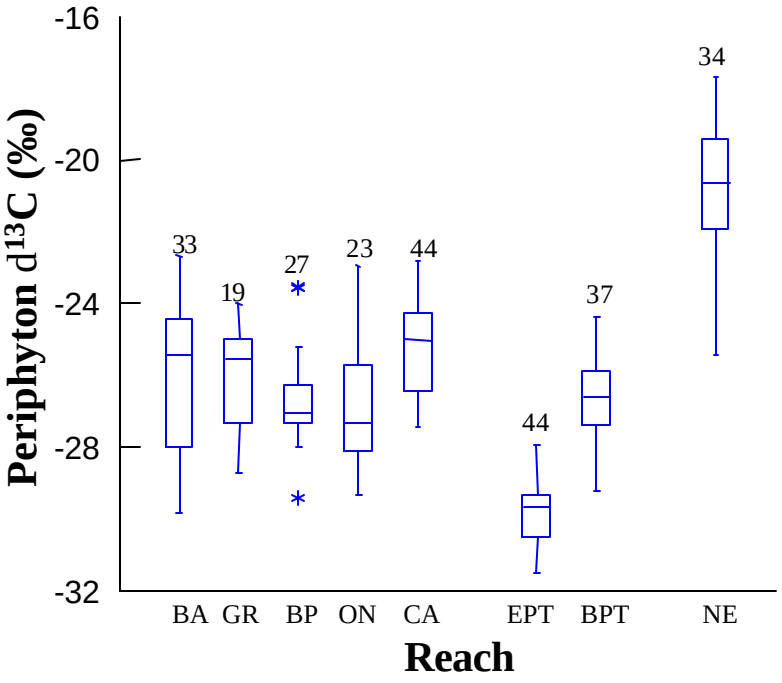

Figure 4. Box plot of periphyton $d^{13}C$ in each reach. Sample sizes are marked above each reach.

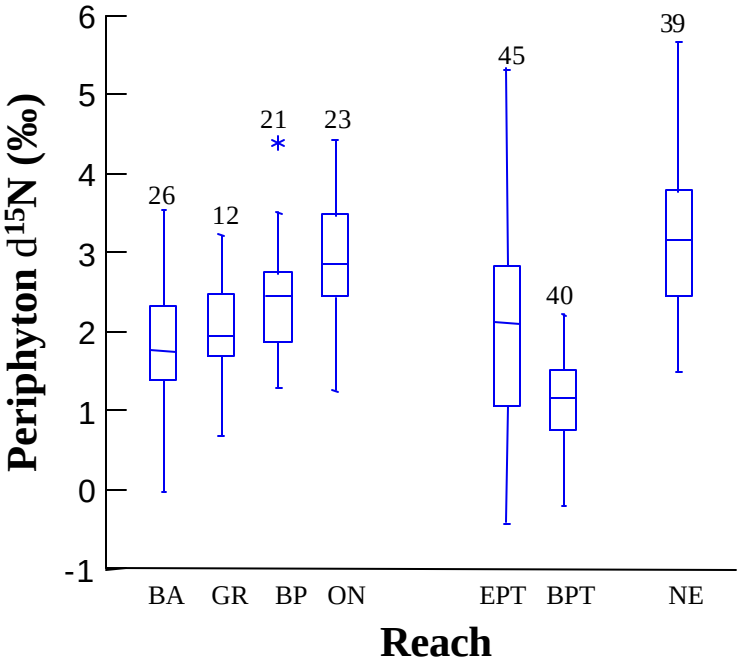

Figure 5. Box plot of periphyton $d^{15}N$ in each reach. Sample sizes are marked above each reach. CA samples for $\delta^{15}N$ were lost.

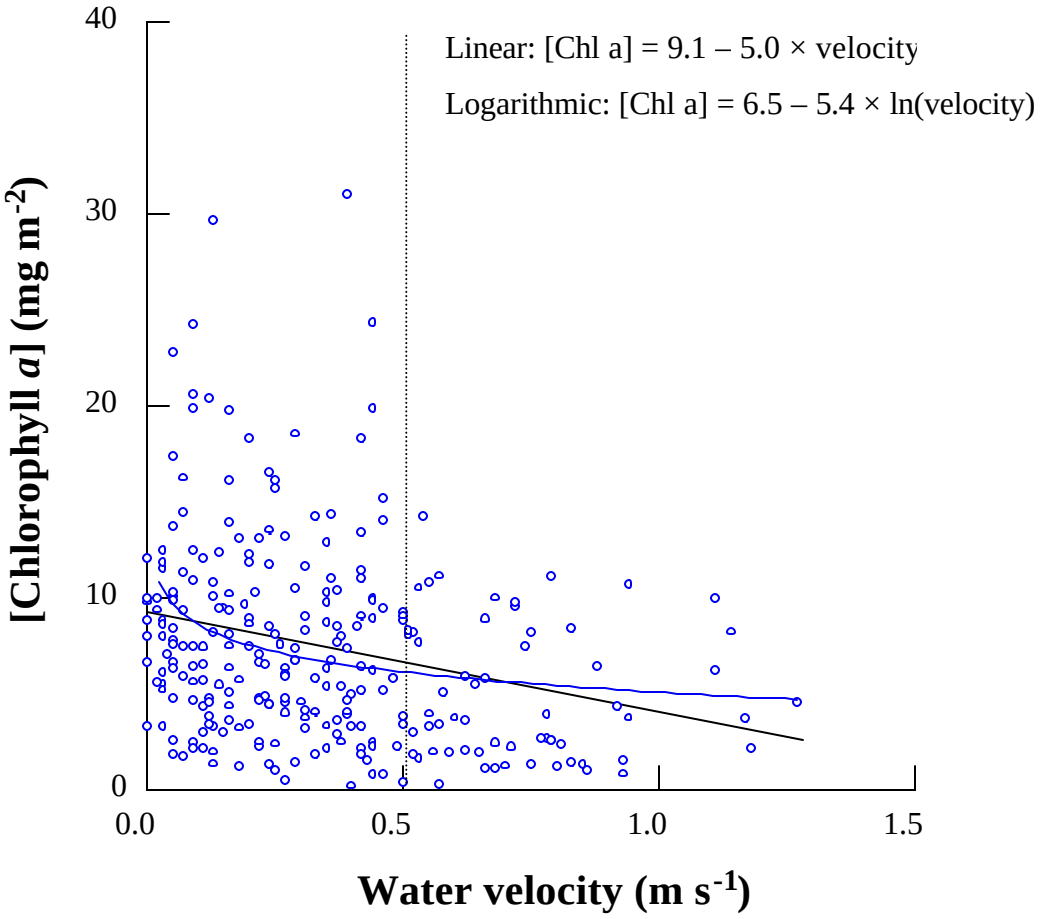

Figure 6. Linear and logarithmic regressions between water velocity and periphyton biomass. The linear regression had a r^2 of 0.7, $P < 0.0001$, and $F = 19.1$, while the logarithmic regression had a r^2 of 0.02, $P < 0.007$, and F = 7.4. The dashed line shows around which water velocity periphyton biomass appears to be negatively affected.

fit. It appears from the data that periphyton biomass becomes negatively affected by water velocity at around 0.5 m s^{-1}.

To avoid redundancy in the proportion of periphyton $d^{13}C$ variability explained by each independent variable, a multiple forward stepwise linear regression including both water velocity and log periphyton biomass was performed on each reach. All models accepted both independent variables, r^2 ranged from 0.29 to 0.71, and all $P = 0.001$ (Table 3). Partial $r^2_{velocity}$ varied from 0.21 to 0.55 (for comparison with laboratory results in Trudeau and Rasmussen (2003)). Slopes for water velocity were all negative and varied from –1.7 to -6.0, while for biomass, they were all positive and varied from 0.9 to 5.4. Intercepts ranged from –25.2‰ to –30.6‰.

At the river system level, different baseline signatures (intercepts) from reach to reach reduced the strength of the multiple regression. To eliminate this effect, in each reach we subtracted the respective mean periphyton $d^{13}C$ from all individual data points. The multiple regression using residuals (z-scores) had similar strength and significance level as those found at the reach level (Table 3; r^2 of 0.40 and $P < 0.0005$). The slopes were also within the ranges found at the reach level.

Periphyton $\delta^{15}N$ versus velocity

Periphyton $\delta^{15}N$ fluctuations showed no strong or consistent relationship with water velocity. Results in Table 4 show that only BPT reach out of the seven reaches (N.B. CA samples for $\delta^{15}N$ were lost) showed a significant and positive linear regression with water velocity. Of the six insignificant relationships, half were positive (in BA, GR, and ON reaches) and half were negative (in BP, EPT, and NE reaches).

Spatial scale		Slope Velocity	Slope LogChl *a*	Intercept (‰)	SE of estimate (‰)	Total r^2	Partial r^2 velocity	*n*
System		-1.2	2.5	-27.6	2.68	0.13	0.04	260
System (z-scores)		-2.6	1.5	-0.25	1.25	0.38	0.27	260
Reach	**BA**	-6.0	1.8	-25.6	1.41	0.59	0.55	33
	GR	-5.3	0.9	-25.5	1.05	0.58	0.51	19
	BP	-2.7	1.7	-26.4	0.72	0.71	0.31	27
	ON	-3.0	3.0	-27.1	1.12	0.64	0.36	23
	CA	-2.4	1.8	-25.2	1.09	0.41	0.29	41
	NE	-3.3	5.4	-25.5	1.36	0.69	0.35	34
	EPT	-3.4	1.7	-30.6	0.69	0.49	0.42	44
	BPT	-1.7	1.1	-26.9	1.03	0.29	0.21	39

Table 3. Multiple stepwise linear regressions of periphyton $d^{13}C$ against water velocity and periphyton biomass at the reach and river system levels. At the river system level, residual periphyton $d^{13}C$ were used in one of the regressions. Regressions for GR and BPT reaches had a significance level of $P < 0.002$, while the others had a significance level of $P < 0.0005$.

Regression data	BPT
n	40
r^2	0.11
P	0.04
SE of estimate (‰)	0.52
Intercept (‰)	0.87
Slope	0.61

Table 4. Linear regression results between periphyton $d^{15}N$ and water velocity for the only regression with a significance level of $P < 0.05$. The other regressions had a significance level of P between 0.11 and 0.84.

Discussion

Periphyton $\delta^{13}C$ and $\delta^{15}N$ variability

As can be observed from Figures 4 and 5, periphyton $d^{13}C$ and $d^{15}N$ can vary a lot throughout a river system. Even on a smaller scale (i.e. reach level here), isotope signature variability can be large, especially for $d^{13}C$. This indicates that it is almost impossible to get a good grasp of the periphyton $d^{13}C$ and $d^{15}N$ in a river or stream from only a few samples. Stable isotopic analyses are pricey, which often limits researchers to use fewer samples in their studies. Nonetheless, the impact of sample size on sampling efficiency appears important. The interpretation of the literature data should consider this factor and use the data more cautiously.

Periphyton $d^{13}C$ versus velocity & biomass

The negative relationship between periphyton biomass and water velocity (Figure 2) shows that at water velocities below 0.5 m s^{-1}, periphyton biomass covered the full range from almost 0.3 to 31.0 mg m^{-2}, and at water velocities close to or greater than 0.5 m s^{-1}, periphyton biomass never exceeded 14.5 mg m^{-2}. This suggests that periphyton biomass, and consequently periphyton $d^{13}C$, begins to be negatively affected when water velocities are around 0.5 m s^{-1}. At high water velocities, surface layers of periphyton may detach from the algae mat if they cannot attach strongly enough to the substrate. However, we would expect the threshold velocity for long filamentous algae to be lower than for tightly attached diatom-dominated periphyton communities. Other studies have also reported that phytoplankton (Gu et al. 1999) and periphyton (Clausen and Biggs 1997; Quinn et al. 1996; Ghosh and Gaur 1994) biomass were negatively affected by water velocity.

The effect of water velocity alone on $d^{13}C$ of diatom-dominated communities was strong enough to be detected, yet less pronounced in the field compared to in the laboratory (partial $r^2_{velocity}$ from 0.29 to 0.55 versus 0.66 respectively). This was not surprising given the number of uncontrolled factors known to influence plant stable isotopic signatures operating under field conditions. The multiple linear regression performed on each reach showed that water velocity and biomass together explain a significant proportion of the variability in the periphyton $d^{13}C$. These results reinforce the idea of the boundary layer/diffusion hypothesis. As water velocity increases, the increasing turbulence causes the boundary layer surrounding the algae to get thinner, thus a more efficient gas exchange. This facilitates C molecules like CO_2 and HCO_3^- to diffuse through the boundary layer and be assimilated by the algae. This process avoids both nutrient depletion and heavy isotope build-up in the boundary, and results in more negative periphyton $d^{13}C$. Conversely, under slow water velocities, the boundary layer thickens, which favours nutrient depletion and heavy isotope build-up, thus less negative periphyton $d^{13}C$. Similarly, higher C uptake flux associated with higher algal biomass contributes to nutrient depletion and heavy isotope build-up in the boundary layer. All of the regression results were supportive of our hypotheses, since water velocity negatively affected the periphyton $d^{13}C$, while periphyton biomass affected them positively.

The water velocity and periphyton biomass signals were significant at both spatial scales observed, yet results tended to be stronger at the reach level, except for BPT reach (Table 3; r^2_{reach} between 0.41 to 0.71 (excluding BPT reach) versus r^2_{system} of 0.38). We did not have sufficient data to do regressions at the cross-section level, but we believe a larger sample size would have generated similar results. Results were also on average

stronger in the river compared to in streams. Water velocity and periphyton biomass explained 41% to 71% of the periphyton $d^{13}C$ variability in the river compared to 29% to 49% in streams (Table 4). BPT reach, the most narrow and shaded, had the lowest r^2 (0.29), while EPT reach, wider and less shaded, had a r^2 of 0.49. Increasing canopy cover along with decreasing width of the water channel can lower the strength of these relationships, since they cause greater variability in light intensity at the streambed. Under high light intensity, plant productivity is greater, causing signatures to be ^{13}C-enriched as plants require more C and fractionate less, while the opposite is observed under low light intensity (MacLeod and Barton 1997). CA reach also had one of the lowest r^2 (0.41). This reach was less shaded than the ones in the streams, however, it was the narrowest of the reaches in the river with the most vegetation on both shores. Where there was no riparian vegetation (BA, GR, BP, ON, NE reaches), light intensity was more constant, and the proportion of the variability explained was greater (r^2 between 0.58 and 0.71). Thus, variability in light intensity may partially obscure the water velocity and biomass signals, which in turn may explain the lower regression strength at the river system level that combined reaches varying in light intensity. Nonetheless, water velocity and periphyton biomass still explained a significant proportion of periphyton $d^{13}C$ variability in both streams and the river.

Differences in mean periphyton $d^{13}C$ (Table 2) and intercepts (Table 3) between reaches indicate differences in the signature values of the C sources taken up by the periphyton. In the main branch, mean periphyton $d^{13}C$ and intercepts were fairly similar from one reach to another (-25.3‰ to –26.7‰ and –25.2‰ to -27.1‰, respectively), thus the C sources, mainly dissolved CO_2, but also HCO_3^-, must have had similar $d^{13}C$. The BPT reach sampled was close to the junction with the main branch and had a mean

and intercept also within the range found in the main branch. The EPT reach, however, was sampled farther upstream in the tributary relative to the main branch. Its mean and intercept of –29.7‰ and –30.6‰, respectively, were much more negative relative to all the other reaches, indicating that the C sources had more negative $d^{13}C$. This may be a sign of groundwater sources that usually contain CO_2 depleted in C^{13} from the decomposition of terrestrial vegetation, which can have $d^{13}C$ as low as -32‰ (Boutton 1991). On the opposite end, the NE reach had an intercept of –25.5‰ (due to the much more positive biomass slope relative to other reaches), but a mean of –21.0‰. It is reasonable to believe that C sources assimilated by the periphyton in the NE reach had quite different $d^{13}C$. The soil in that area was mainly composed of clay, which contains more bicarbonate (HCO_3^-) than granite. Dissolved bicarbonate originating from clay can be ^{13}C-enriched relative to dissolved CO_2 by up to 8‰ (Fogel and Cifuentes 1993), which could explain the increase in ^{13}C of the periphyton in this region of the river. The periphyton biomass may also have contributed to the less negative $d^{13}C$ in that region as it had by far the highest mean biomass - 1.5-2 fold greater on average than in all other reaches. Overall, periphyton $d^{13}C$ found in the Ste. Marguerite River system were within the range normally found in freshwater ecosystems where primary producer $d^{13}C$ typically vary from -15‰ to -35‰ (Boutton 1991).

Other important factors

Increasing DOC concentration had been found by France (1999) to cause epilithon $d^{13}C$ to decrease. DOC concentrations were not measured in this experiment, yet this factor may have added noise to the periphyton $d^{13}C$ data. The effect of biomass might also be underestimated if periphyton had been recently heavily grazed upon by benthos. Some experiments suggested grazers control periphyton biomass (Steinman

1996; Feminella and Hawkins 1995), while another showed no significant effect of grazers on periphyton biomass (Hall and Likens 2001). We do not have the data to test it, yet it would be useful to study this grazer effect. Differences in algal photosynthetic mechanisms can also affect primary producer $d^{13}C$ (Fogel et al. 1992). Fry and Wainright (1991) have shown that cell size influences marine phytoplankton $d^{13}C$. Species composition has also been suggested to increase epiphyte $d^{13}C$ variability (Osmond et al. 1981). Together, these factors must have contributed to the remaining periphyton $d^{13}C$ variability not explained by water velocity and periphyton biomass.

Periphyton biomass is a biotic factor influenced also by many abiotic variables. Substrate size may influence periphyton biomass. Certain rock sizes may present better growth conditions for periphyton that would in turn partially control their biomass and $d^{13}C$. Our observations suggested periphyton growing on rock substrate of at least medium size and larger (>15 cm diameter), were the ones with the highest biomass. Smaller rocks generally supported only a very thin layer of periphyton. Two underlying factors may cause this effect. During sediment transport, smaller rocks are moved to a greater extent than larger rocks, which may enhance detachment of algae and reduce periphyton biomass on rock substrate. Also, smaller rocks are more prone to abrasion by sand (sand blasting) than larger ones. The abrasive property of sand may limit periphyton biomass accumulation on smaller rocks, which are closer to the riverbed. In fact, BP, ON, and CA reaches had the lowest mean biomass and also had small rocks at most sites. They also experienced on average the fastest flows, which can themselves negatively affect periphyton biomass and increase abrasion by transport of fine sediments. Accordingly, both BP and ON reaches had the lowest mean $d^{13}C$ of the reaches in the main branch. The CA reach, however, had the highest mean $d^{13}C$ of the

main branch. As previously mentioned, a different baseline signature of the C sources may be responsible for the results in CA reach.

Periphyton $\delta^{15}N$ and velocity & biomass

Periphyton $\delta^{15}N$ values showed no constant trend with water velocity. Some correlations were negative, while other ones were positive, and only one was significant (Table 4). It does not appear that water velocity can explain periphyton $\delta^{15}N$ fluctuations in this river system. Water velocity could not even predict the direction in which periphyton $\delta^{15}N$ varied. These results were opposite to empirical evidence from the previously controlled laboratory experiment where periphyton $\delta^{15}N$ were strongly and negatively correlated with water velocity (Trudeau and Rasmussen 2003).

One or many other factors must be obscuring the signal of water velocity on periphyton $\delta^{15}N$ in the Ste. Marguerite River. Nitrogen is often a limiting nutrient in freshwater ecosystems. If a nutrient becomes strongly limiting to the point where it is entirely depleted within the boundary layer, this would inhibit fractionation altogether. Under such conditions there would be no effect of any other factors on $d^{15}N$. Moreover, isotopic signatures of groundwater depend on physical and chemical processes in the soil, thus $d^{15}N$ values can vary a lot independently of river and stream water $d^{15}N$. Drifting detritus originating from terrestrial vegetation in the reaches sampled had depleted $d^{15}N$ ranging from -2.3‰ to -0.9‰ and dead leaves from the banks ranging from -2.5‰ to -0.2‰ (similar to $d^{15}N$ values found by McCutchan and Lewis 2002). It may be that after decomposition of organic matter in the soil, the N that ends up in groundwater is ^{15}N-depleted. In places where groundwater mixes with the river and stream water, periphyton $\delta^{15}N$ may be strongly influenced. While there are patterns to

groundwater recharge and discharge zones, spatial variability is high within river systems and hard to predict. The groundwater signal may override that of the water velocity and be responsible for a portion of the noise in this data set. Unfortunately, detecting all groundwater sources to test this hypothesis would be very difficult and time-consuming. This makes it very difficult in nature to predict primary producer $d^{15}N$, which is probably the reason why information related to $d^{15}N$ is so rudimentary.

Conclusion

The key points that can be extracted from this study are that water velocity and periphyton biomass are important factors contributing to the variation of periphyton $d^{13}C$ in the Ste. Marguerite River system. The baseline $d^{13}C$ of aquatic primary producers is determined by factors such as $d^{13}C$ of inorganic material, light intensity, and temperature. Variability around this $d^{13}C$ baseline arises mainly from variations in secondary factors, such as the two examined in this study. Terrestrial plants are not affected by these factors and, in fact, drifting terrestrial detritus and dead leaves from the shore showed very little $d^{13}C$ variability. In the present study, water velocity appears to be a major factor creating large fluctuations in the periphyton $d^{13}C$ data. The basic $d^{13}C$ patterns were consistent with those found in the laboratory. Algal biomass also played a role, albeit to a lesser extent, for the $d^{13}C$ fluctuations. Unfortunately, we did not succeed in demonstrating the effect of water velocity on periphyton $d^{15}N$ as predicted from our previous laboratory experiment.

Periphyton $d^{13}C$ patterns observed here support the boundary layer/diffusion hypothesis. Water velocity and periphyton biomass should be taken into account in C stable isotopic studies related to aquatic plants. Since periphyton represents a major food source for many grazing invertebrates, which are in turn important for fish, studies

on $d^{13}C$ of higher trophic levels should also consider these two factors to explain variability in consumer $d^{13}C$. Since stable isotopic signatures are increasingly used in freshwater ecology, it becomes mandatory to understand and consider factors regulating the isotopic signatures of organisms of all trophic levels. We believe our study contributes to this and also leads to other research, since the variation in the stable isotopic ratios of $^{15}N/^{14}N$ in aquatic plants remains unsolved.

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Chapter 2

Spatial patterns in macroinvertebrate and salmonid $d^{13}C$ along the Ste. Marguerite River system (Quebec, Canada): relationship to periphyton signatures and water velocity

By

Véronique Trudeau and Joseph B. Rasmussen.

Biology Department, McGill University

1205 Dr. Penfield Ave., Montreal, Quebec, Canada, H3A 1B1

Running head: Patterns in aquatic consumer $d^{13}C$

Acknowledgements

Special thanks to David Browne, and Michel Lapointe for useful input to this manuscript. Also thanks to Geneviève Morinville, Jamie Luce, Ian Popple, and other graduate students of the McGill University Biology Department for helpful suggestions on earlier versions of the manuscript. Financial support was provided by an NSERC Strategic grant to J.B.R.

Abstract

The use of stable C isotopic signatures ($d^{13}C$) to study freshwater consumers is limited, since we do not understand all underlying factors controlling them. Water velocity has been shown to significantly affect periphyton both in the laboratory and in the field (Trudeau and Rasmussen 2003; Chapter 1). Periphyton $d^{13}C$ tend to decrease with increasing water velocity. It remains unclear, however, how strongly the effect of this abiotic factor is reflected in consumers' $d^{13}C$, namely benthic macroinvertebrates and salmonids. In the present field study, we demonstrate the degree to which the water velocity signal is transmitted to higher trophic levels in the food web. We chose eight series of sites with different flow regimes (0-1.27 m s^{-1}) in the Ste. Marguerite River, Quebec, Canada, and two of its tributaries. Consumers tended to have more negative $d^{13}C$ in high velocity sites, but the strength and significance level of regressions varied with the spatial scale observed; usually stronger at the smaller scale, but with a higher significance level at the larger scale. We discuss factors that may be responsible for the loss of the water velocity signal with increasing trophic level, such as diet and spatial averaging. Diet averaging appeared important for both consumer levels, while spatial averaging may be important mostly for fish. This study gives an indication of how much variability in aquatic consumer $d^{13}C$ can be explained by water velocity in lotic systems.

Introduction

The Ste. Marguerite River system, Saguenay, Quebec, has been the home and spawning ground of Atlantic salmon (*Salmo salar*) and sea brook trout (*Salvelinus fontinalis*) since the last glaciation. Decreasing stocks of salmon and increasing interest in trout sport fishing makes this river system an area of choice to study factors related to food web ecology and river management. The present study focuses on the effect of water velocity in river and stream food webs via trophic interactions between periphyton at the base of the food web, benthic invertebrates, and fish. One way to examine trophic interactions in freshwater ecology is through the use of stable carbon isotopes (^{12}C and ^{13}C), which are most often used to determine the diet of consumers. Isotopic signatures are increasingly used in ecology, yet there is still a lot of unexplained variability in consumers' $d^{13}C$ within and between systems.

We have shown in a laboratory study that water velocity explained a significant proportion (66%) of the $d^{13}C$ variability of periphyton communities dominated by diatoms (Trudeau and Rasmussen 2003). In natural river conditions, the same negative relationship was also shown significant (Chapter 1). Now that this relationship has been established, we hypothesise that collecting periphyton and consumers across a series of sites differing in flow regime will allow a better understanding of the effect of water velocity on aquatic consumer $d^{13}C$. It may explain some of the $d^{13}C$ variability in the literature data on aquatic consumers and provide an indication as to how important the water velocity effect is at all trophic levels. Results may further help confirm the importance of periphyton as a major food source in lotic systems. Combined with others relevant studies, this information would provide better knowledge on habitat types to be protected for fish and improve aquatic habitat management.

It has been generally accepted that the baseline $d^{13}C$ signature of primary producers is passed on to consumers with 0-1‰ increase per trophic level (DeNiro and Epstein 1978). Benthic macroinvertebrates and fish have isotopic signatures that reflect a weighted average of their different food sources - here referred to as diet averaging. If given a choice, grazers preferentially feed on higher quality food such as periphyton and associated microbial films, which are nutritionally richer than terrestrial detritus. In fact, a study by McCutchan and Lewis (2002) in a mountain stream showed that algae could contribute up to 80% of macroinvertebrate production, even though algal production accounted for less than half of the total organic matter available in the stream. The second major food source for grazers is generally fine detritus of terrestrial origin (Mulholland et al. 2000). Since spatial variability of terrestrial plant and detrital $d^{13}C$ is much less than that of aquatic primary producers (Boutton 1991), variability in grazer $d^{13}C$ should mostly result from periphyton $d^{13}C$. Mayflies, caddisflies, chironomids, and blackflies are the most common invertebrates in the Ste. Marguerite River system and the main components of the diet of salmon parr (hereafter parr) and juvenile trout. Keeley and Grant (1997) have also shown that these invertebrates made up most of the diet of juvenile Atlantic salmon. Parr and juvenile trout are thus expected to have $d^{13}C$ reflecting an average of these taxa.

Summer habitat selection by salmonids depends in part on hydro-physical conditions such as water velocity, depth, and substrate size (Morantz et al. 1987; Heggenes 1996). Morantz et al. (1987) found that parr preferentially chose microhabitats with nose velocities between 0.5 m s^{-1} and 0.35 m s^{-1}. Fish are subject to water level variations during the summer, and consequent changes in water velocity forces them to move laterally and/or longitudinally to find their preferred water

velocities. Benthic invertebrates and fish also drift, which allows them to feed over a larger spatial scale than they otherwise would. Therefore, in addition to diet averaging, isotopic signatures of consumers reflect the average of their food sources over space - here referred to as spatial averaging.

The objective of this study was to determine whether or not the effect of water velocity previously observed on periphyton $d^{13}C$ is transmitted up the food web, and over which spatial scale such an effect can be detected. We predicted the water velocity signal would be reflected in the $d^{13}C$ of closely interrelated aquatic consumers, namely grazers and young fish. We also predicted the water velocity signal to be stronger at the primary producer level and decrease with increasing trophic level due to either or both diet and spatial averaging by consumers. Finally, we expected consumer $d^{13}C$ to be significantly correlated with that of their main food sources along the course of the river.

Materials & methods

Study area and site selection

The study area is the Ste. Marguerite River system in the Saguenay region, Quebec, Canada (Figure 1). This river has three branches; the main, the north east, and the north west branches. Many streams feed the river and there are no polluting industrial sources within the drainage system.

Since we could not sample the whole river system, we performed detailed sampling in a total of eight reaches (sections of a water channel); five in the main branch of the river, as well as one in the north east branch and two in important tributaries inhabited by parr or trout (Table 1). The five reaches in the main branch are listed in order from downstream to upstream in Table 1. Over this approximately 45 km

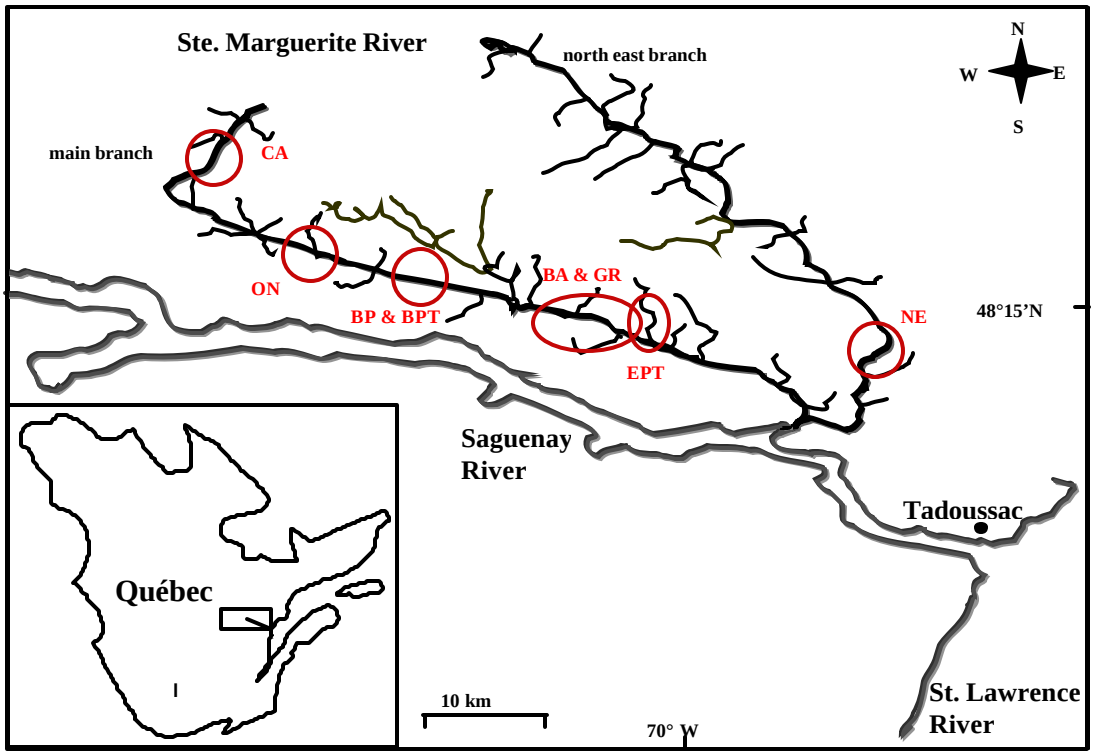

Figure 1. Map of the Ste. Marguerite River system. Circles represent reaches sampled.

Section	Reach	# of sites
Main branch	Bardsville (BA)	11
	Grand Rapide (GR)	7
	Big Pool (BP)	9
	Onesime (ON)	8
	Cascade (CA)	15
North east branch	North East (NE)	13
Tributaries	Epinette (EPT)	15
	Big Pool Tributary (BPT)	14

Table 1. Reaches sampled in the different sections of the river system. Reaches in the main branch of the river are from downstream to upstream. Only abbreviations in brackets will be used to refer to reaches in the text.

distance, the river narrows from roughly 40 m to 10 m. The BA reach, is a wide section with no canopy cover. Just upstream, GR reach is also wide with no canopy cover, but also has the steepest slope and lots of boulders. The next two, BP and ON reaches, are 10 km and 20 km, respectively, upstream of GR. Small trees and shrubs are found almost continuously along both banks of these reaches. The most upstream section sampled, CA reach, was the narrowest and shallowest with the highest canopy cover of the main branch. In the north east branch, NE reach is comparable to BA reach; wide with no canopy cover. In streams, EPT and BPT reaches are very narrow, on average 4 m and 2 m wide respectively, shallow, and with a continuously high percent canopy cover.

Velocity measurements & sampling method

In each reach, 7 to 15 sites (cross-sections) were sampled on the basis of the overall water velocity: pools (< 0.1 m s^{-1}), runs (0.1-0.3 m s^{-1}), and riffles (> 0.3 m s^{-1}). Consecutive sites were always different in terms of water velocity. Thus, if the first site sampled in a reach was in a pool section, the following site sampled upstream would be either in a run or a riffle section, and so on. In the river, the distance between sites varied from 25 to 100 m, while they varied from 5 to 15 m in streams. Within each site, three different water velocities were measured using a Gurley Pygmy current meter (625DF8N – Wading Rod Suspended Pygmy type current meter outfit with Model 1100 digital indicator). At each sampling point, the water velocity measurement was taken 1.5 cm above the periphyton. Using a 0.1 m^2 container, rock substrates covering this surface area and surrounding the measurement point were then removed from the water. Invertebrates were collected using forceps and periphyton samples were brushed off the rocks. Fish were captured by electro-fishing. All samples were frozen at -20°C until

identification and stable isotope analysis. Water depth (maximum 70 cm deep) and substrate size (rocks between 5 and 30 cm in diameter) were also considered qualitatively in site selection.

After the winter flood, the water level in rivers and streams is still subject to variations throughout the summer depending on the precipitation patterns, which in turn affect water velocity. To avoid biases due to water level changes, individual reaches were sampled over a very short time span of 2-3 d in July, when the water level stayed relatively constant (approximately ± 2 cm).

Sample collection

Periphyton (diatom-dominated communities) and the most common benthic invertebrates, mostly grazers (mayflies; Ephemeroptera, caddisflies; Tricoptera, chironomids; Diptera), were collected where each water velocity measurement was taken. Where present, filter-feeding benthic invertebrates (blackflies; Diptera) were also collected. Parr were found in all reaches except in EPT reach where, due to an improperly installed culvert, salmon were prevented from ascending this stream. Instead, resident juvenile brook trout were electro-fished in this reach. Terrestrial drifting detritus was collected with drift nets at all sites.

Stable isotope analyses

Invertebrate samples were soaked in 75% ethanol for 24 h to remove lipids before preparation for stable isotope analysis. All periphyton, invertebrate, fish, and detritus samples were oven-dried, pulverised, homogenised, weighed, and packed into tin capsules. Stable C isotopic analyses were done by mass-spectrometry (Finnigan-Mat Delta-Plus continuous flow isotope-ratio mass spectrometer coupled to a Carlo-Erba elemental analyser on line; G.G. Hatch Isotope Laboratories, University of Ottawa,

Ottawa, Ontario, Canada and GEOTOP Laboratory, UQAM University, Montreal, Quebec, Canada). The analytical precision of these apparatus is typically 1 SD (0.05 to 0.2‰), which is small relative to the range of values found in nature (Kendall and Caldwel 1998). Stable isotopic signatures were expressed using the following standard equation:

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

where R stands for the ratio of $^{13}C/^{12}C$ and the standard is Pee Dee Belemnite limestone. The units are in permil (‰).

Fish caudal fin $d^{13}C$ versus white muscle $d^{13}C$ and stomach contents

Fish white muscle tissues are commonly used for stable isotopic analyses (Pinnegar and Polunin 1999). Considering the decreasing density of Atlantic salmon in the Ste. Marguerite River, we sacrificed only 13 fish during preliminary sampling to establish the relationship between $\delta^{13}C$ of muscle and caudal fin tissues. White muscle tissue samples were cut from the dorsal side of the fish, just below the dorsal fin. Caudal fin tissue samples were cut from the lower part of the fin. Results showed there were no significant differences between the signatures of both tissue types (paired t-test; $P = 0.80$). The correlation between the $d^{13}C$ of fish white muscle and caudal fin tissues was strong and positive with a slope of 1.2. The r^2 was of 0.91 and the $P < 0.0005$. Therefore, only caudal fin isotopic signatures were used in further analyses. Stomach contents of sacrificed fish were also analysed and confirmed that the invertebrates sampled contributed to about 90% of the fish's diet. Parr and juvenile trout fed mostly on mayflies and caddisflies, but also on blackflies and chironomids.

Data analyses

Linear Regressions

Linear regressions were performed to determine if the water velocity effect on periphyton $\delta^{13}C$ is transmitted up the food chain. We performed linear regressions at the river system level (all reaches together), and at the reach level to determine at which spatial scale(s) the water velocity effect is noticeable. Since some reaches had significantly different baseline $\delta^{13}C$, we used residuals (z-scores) from reach means in regressions with water velocity at the river system level. Linear regressions were also performed between $\delta^{13}C$ of all directly interacting species (periphyton and invertebrates, as well as invertebrates and fish) at both the river system and reach levels to help indicate the principal food sources of consumers (strongest trophic links). Since blackflies were only found in one third of the sites along the river and tributaries, regressions with this taxon were performed only at the river system level. Trout on the other hand were only found in EPT reach, and thus were only considered in regressions at the reach level. Since fish were not necessarily captured exactly where invertebrates were sampled, we used averages per site in regressions with fish.

? $\delta^{13}C$ between trophic levels

The ? $\delta^{13}C$ between trophic levels gave additional information on the potential food sources and their importance in the diet of consumers. We compared the average ? $\delta^{13}C$ between each invertebrate taxon and their expected preferred food source, periphyton. The same was done between fish and each invertebrate taxon.

Spatial pattern comparisons

We compared the $\delta^{13}C$ spatial pattern of consumers to that of their potential food sources from downstream to upstream in all reaches. Plotting the per site average signature of each invertebrate taxon downstream to upstream in each reach allowed us to visualize how they varied along the river and tributaries. It also allowed visualization of how well spatial patterns of invertebrates correlated with those of periphyton and detritus (e.g. coinciding peaks, enrichment or depletion of consumer $\delta^{13}C$ relative to both food sources, consistency among and within reaches). The spatial patterns of fish were compared to that of the average of all invertebrates together.

Mixing models

We used mixing models to estimate the percentage of periphyton and detritus in the diet of each invertebrate taxon, making the reasonable assumption that grazers had no major food sources other than periphyton (including associated biofilm) and drifting detritus. Invertebrates were also assumed to have $\delta^{13}C$ showing a 0.5‰ increase relative to the average of their food sources, which is the mid-point between 0‰ and 1‰, the range of increase per trophic level found by Deniro and Epstein (1978). We used the following mixing model equation:

$$I_P = ([\delta^{13}C_I - 0.5‰] - \delta^{13}C_D)/(\delta^{13}C_P - \delta^{13}C_D)$$

where P stands for periphyton, I for invertebrate, D for detritus, and I_P is the estimated percent periphyton in the invertebrate's diet.

$$I_D = 100 - I_P$$

where I_D is the estimated percent detritus in the invertebrate's diet. We could not do mixing models for fish since they had more than two potential food sources.

Unequal variances and smoothing

Diet averaging was not always sufficient to explain the lower fish $\delta^{13}C$ variance relative to their food sources. In such cases, we first used Bartlett's test to determine whether or not there was a significant difference between the per reach $\delta^{13}C$ variance of fish and that of their food sources. Only for cases where variances were significantly different (significance level set to 0.1 due to the low number of sites in some reaches), smoothing of the invertebrate data was done until the variance level of the fish was obtained. Smoothing consisted of averaging invertebrate $\delta^{13}C$ from each site with those of the upstream and downstream sites using weighing factors (WF) to reduce the invertebrate variance to that of fish in each reach. The degree of smoothing should provide a rough estimate of how much spatial averaging would be necessary to explain the lower fish $\delta^{13}C$ variance when diet averaging could not explain it all.

Results

Linear regressions

At the river system level, regressions with $d^{13}C$ residuals from reach means were significant for periphyton, grazers, and parr (Table 2). Water velocity explained 27% of the variability in the periphyton $\delta^{13}C$ data, and regression strengths greatly decreased with increasing trophic level ($0.04 = r^2_{grazers} = 0.09$, $r^2_{parr} = 0.03$). Only blackfly $d^{13}C$ showed no significantly correlation with water velocity ($r^2 = 0.02$, $P = 0.34$, $n = 52$). Periphyton $\delta^{13}C$ were highly correlated with those of each invertebrate taxa. Parr $d^{13}C$ were strongly correlated with larger grazers, namely mayflies and caddisflies. The regression was also significant with blackflies albeit much weaker. Finally, parr $d^{13}C$ were not significantly correlated with those of chironomids ($r^2 = 0.01$, $P = 0.51$, $n = 38$).

Independent variable	Dependent variable ($d^{13}C$)	Slope (± SE)	Intercept (‰) (± SE)	r^2	*P*	*n*
Velocity	**Periphyton**	-3.11 ± 0.32	1.07 ± 0.14	0.27	*	264
	Mayflies	-6.73 ± 1.47	3.49 ± 0.63	0.08	*	230
	Caddisflies	-1.22 ± 0.40	0.45 ± 0.18	0.04	0.003	238
	Chironomids	-1.83 ± 0.54	0.65 ± 0.24	0.09	0.001	122
	Parr	-0.40 ± 0.17	0.17 ± 0.08	0.03	0.016	168
Periphyton $d^{13}C$	**Mayflies**	0.97 ± 0.07	-1.54 ± 1.72	0.71	*	90
	Caddisflies	0.80 ± 0.07	-4.84 ± 1.77	0.61	*	90
	Chironomids	0.64 ± 0.09	-8.79 ± 2.35	0.47	*	63
	Blackflies	0.67 ± 0.10	-7.39 ± 2.69	0.64	*	29
Mayflies $d^{13}C$	**Parr**	1.71 ± 0.10	15.02 ± 2.48	0.84	*	56
Caddisflies $d^{13}C$	**Parr**	1.57 ± 0.10	12.63 ± 2.40	0.83	*	55
Blackflies $d^{13}C$	**Parr**	0.45 ± 0.16	-14.26 ± 3.96	0.35	0.015	16

Table 2. Linear regression results at the river system level. $d^{13}C$ residuals of all trophic levels were used only for regressions with water velocity. Only significant results are shown in the table. Regressions with a significance level of $P < 0.0005$ are represented by an *. Regressions between water velocity and blackflies, as well as between parr and chironomids were not significant ($P > 0.05$).

At the reach level, results were less consistent than at the river system level (Table 3). Sample size per reach of individual invertebrate taxon varied from three to 45. Correlations were performed only for sample sizes of five or more. We first correlated water velocity with organisms' $d^{13}C$. In all reaches, periphyton $d^{13}C$ was significantly correlated with water velocity. For mayflies, four correlations were significant ($P < 0.05$) and two were marginally significant ($P < 0.1$). Caddisfly $d^{13}C$ were significantly correlated with water velocity in two reaches. Chironomids only had one significant correlation with water velocity, while two others were marginally significant. Correlations between fish $d^{13}C$ and water velocity were significant in two reaches, while two others were marginal.

Correlations between the $d^{13}C$ of directly interacting trophic levels showed that mayflies were the invertebrates that correlated best with periphyton (three significant and two marginally significant correlations). Chironomids followed with two, and caddisflies with one significant correlation. Parr were significantly correlated with mayflies and caddisflies in two reaches out of seven. Only one correlation between parr and chironomids was marginally significantly. In EPT reach, juvenile trout were significantly correlated only with caddisflies.

? $\delta^{13}C$ between trophic levels

According to Deniro and Epstein (1978), if periphyton was the main food source of invertebrates, we would expect them to show a 0-1‰ increase in $d^{13}C$ relative to periphyton. Mayfly $d^{13}C$ were on average more negative (–0.63‰) than periphyton over the whole river and tributaries. At the reach level, only in CA and NE reaches did

Independent Variable	Dependent Variable ($d^{13}C$)	BA	GR	BP	ON	CA	EPT	BPT	NE
Velocity	**Periphyton**	0.74	0.71	0.56	0.60	0.54	0.65	0.47	0.56
	Mayfly	0.40	0.44*		0.40*		0.66	0.66	0.45
	Caddisfly				0.41				0.36
	Chironomid	0.55				0.40*	0.36*		
	Fish	0.35*	0.55	0.45*			0.39	0.80	
Periphyton $d^{13}C$	**Mayfly**	0.33*	0.42*		0.71		0.66		0.39
	Caddisfly				0.57				
	Chironomid	0.42			0.62				
Mayfly $d^{13}C$	**Fish**	0.62	0.78*						0.75
Caddisfly $d^{13}C$			0.36				0.66		0.80
Blackfly $d^{13}C$		0.57*							

Table 3. Correlation results (r) for the $d^{13}C$ data at the reach level. Only correlations with a significance level of $P < 0.05$ or $P < 0.1$ (marked by an asterisk) are shown in this table. Most correlations were performed with all data points (three per site for each organism). Averages per site were used only for correlations with fish. Sample sizes varied from 5 to 45.

mayflies show the expected increase compared to periphyton (Figure 2a). Caddisfly $d^{13}C$ were overall 0.65‰ enriched relative to periphyton. At the reach level, increases were as expected in GR, BP, BPT, and NE reaches (Figure 2b). Chironomids $d^{13}C$ were overall 0.67‰ enriched relative to periphyton and showed a 0-1‰ increase in BA, BP, and ON reaches (Figure 2c).

The per reach $?d^{13}C$ average between invertebrates and fish were always positive relative to invertebrates, except for parr and caddisflies in the NE reach. Parr and trout $d^{13}C$ mean increases were generally much greater than 1‰ relative to mayflies (Figure 2a). Similar results were observed for caddisflies, yet the $?d^{13}C$ were within the expected range in CA and BPT reaches (Figure 2b). Compared to chironomids, parr from BA, BP, ON, and BPT reaches were on average 0-1‰ enriched in ^{13}C (Figure 2c). Blackflies were not consistently present along the river, thus we used the overall averaged signatures to compare them with fish. Parr and trout $d^{13}C$ were on average 0.80‰ and 1.73‰ greater than those of blackflies, respectively.

Spatial pattern comparisons

Detritus originating mostly from riparian vegetation had $d^{13}C$ varying from –32.57‰ to –28.30‰ (SD = 0.48, v = 0.23). These $d^{13}C$ values fluctuated much less and were consistently more negative than those of periphyton, which ranged from –31.53‰ to –17.66‰ (SD = 2.87, v = 8.22).

The spatial patterns of mayfly $d^{13}C$ generally tracked well those of periphyton (Figure 3ai, aii). Mayfly $d^{13}C$ were generally half way between those of periphyton and detritus, except in CA reach where they clearly increased and were much closer to those of periphyton. In EPT reach, there was a clear decrease in periphyton and grazers $d^{13}C$

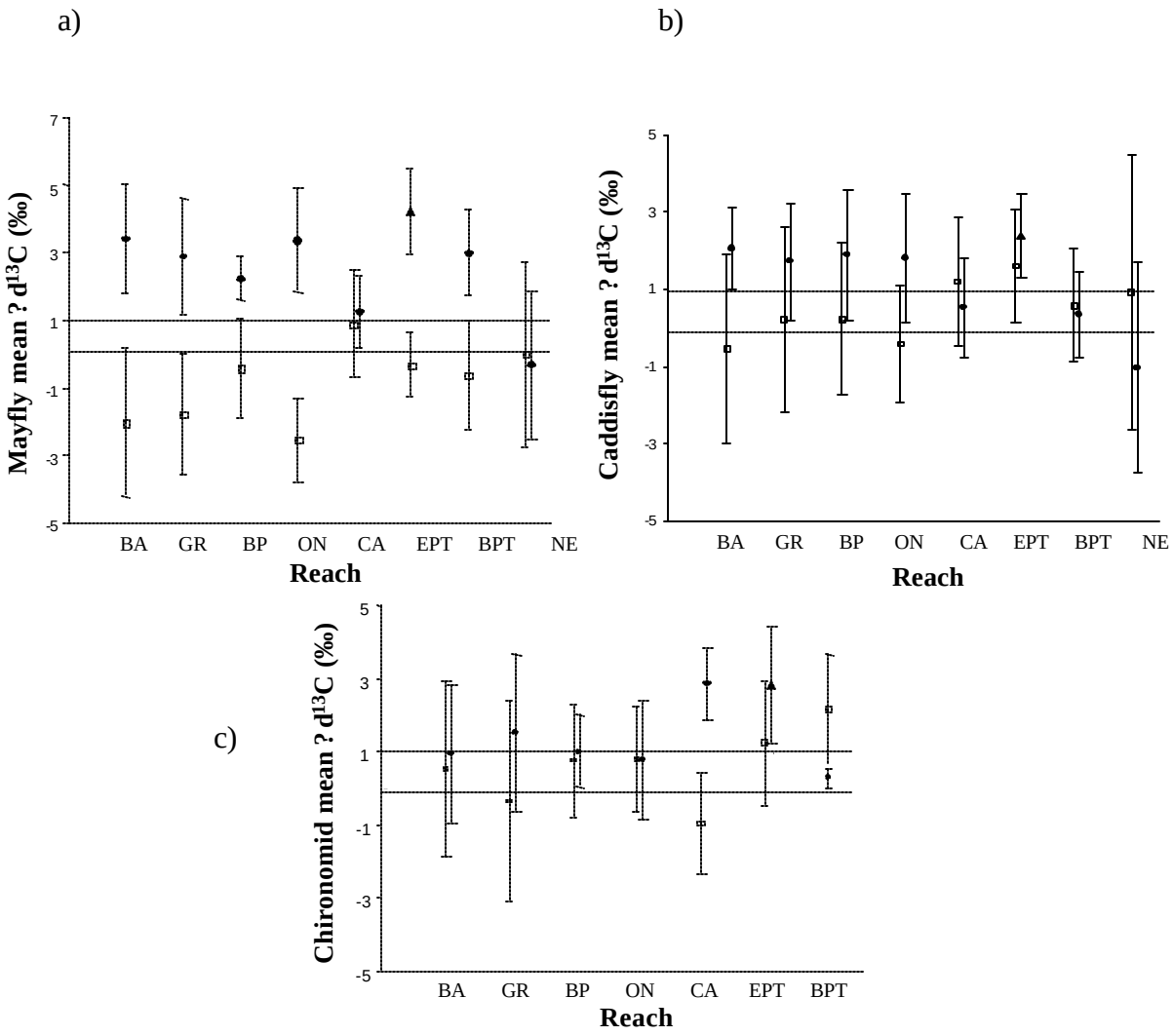

Figure 2. Mean ?$d^{13}C$ between periphyton and grazers (open squares), as well as between grazers and parr (solid circle) or juvenile trout (solid triangle). Error bars represent 1 SD from the mean. (a) mayflies, (b) caddisflies, and (c) chironomids. The two dotted lines represent the range in which the mean ?$\delta^{13}C$ should fall in if taxa in question predominantly fed on periphyton and if fish predominantly fed on this taxa.

Figure 3. $d^{13}C$ spatial pattern of periphyton (dark and thick continuous line), mean detritus (straight continuous line), invertebrates (light and thin continuous line), and fish (dashed line) from downstream to upstream in reaches of the river and streams. (ai) mayflies main branch, (aii) mayflies streams and north east branch, (bi) caddisflies main branch, (bii) caddisflies streams and north east branch, (ci) chironomids main branch, (cii) chironomids streams, (di) average invertebrates main branch, (dii) average invertebrates streams and north east branch.

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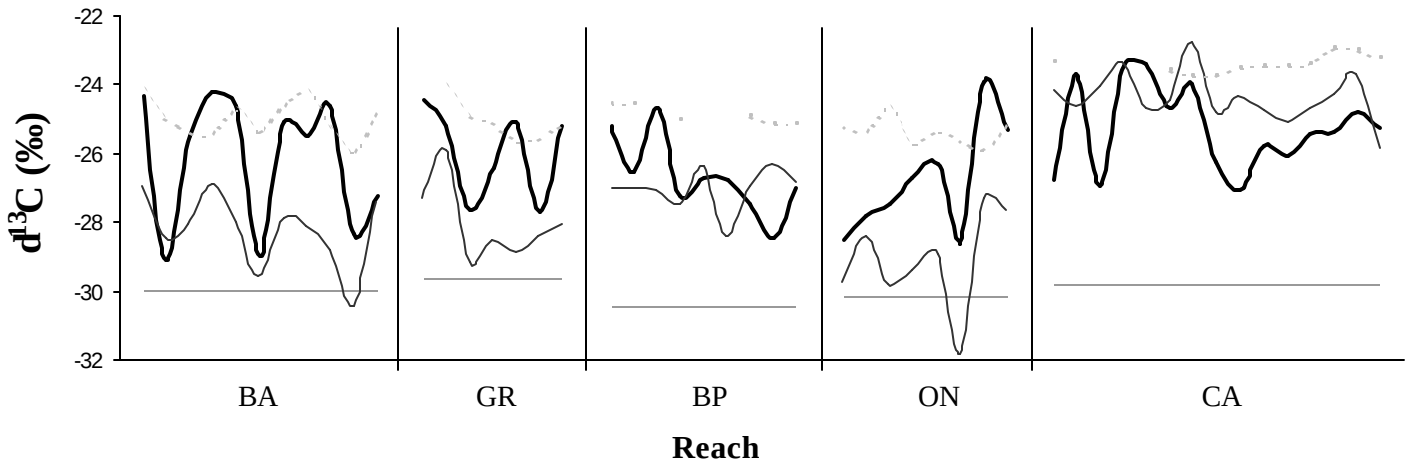
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d13C (‰)
BA
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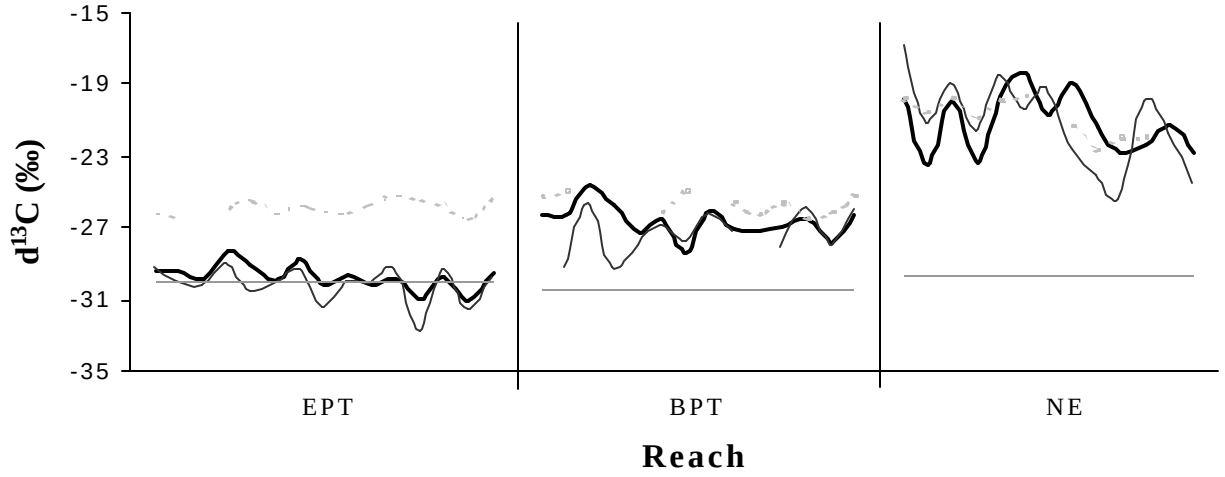
aii)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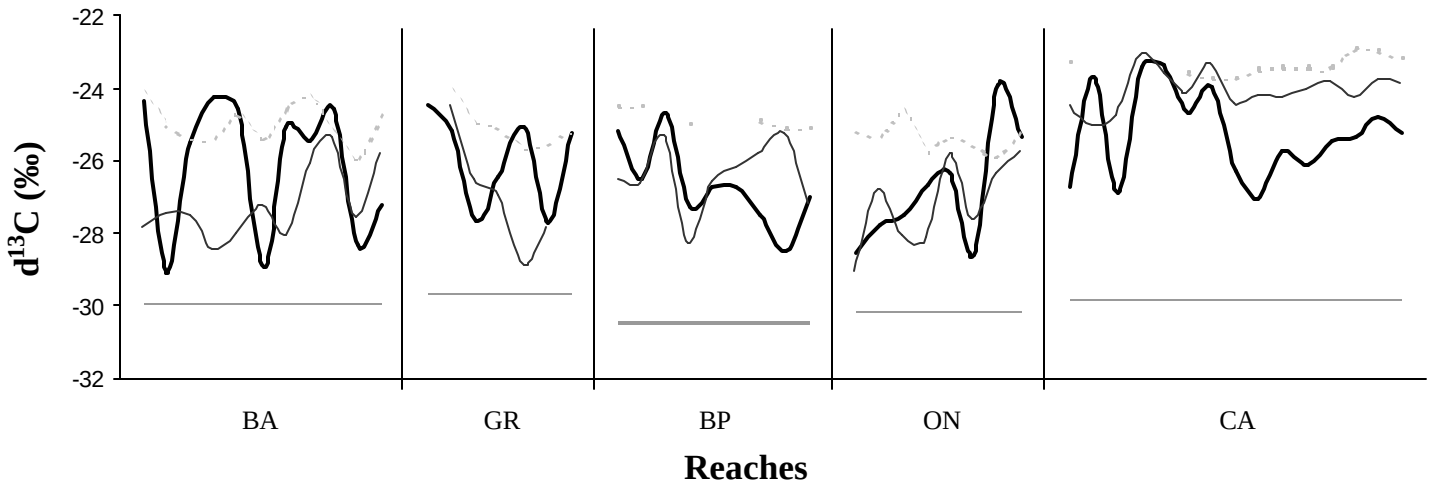
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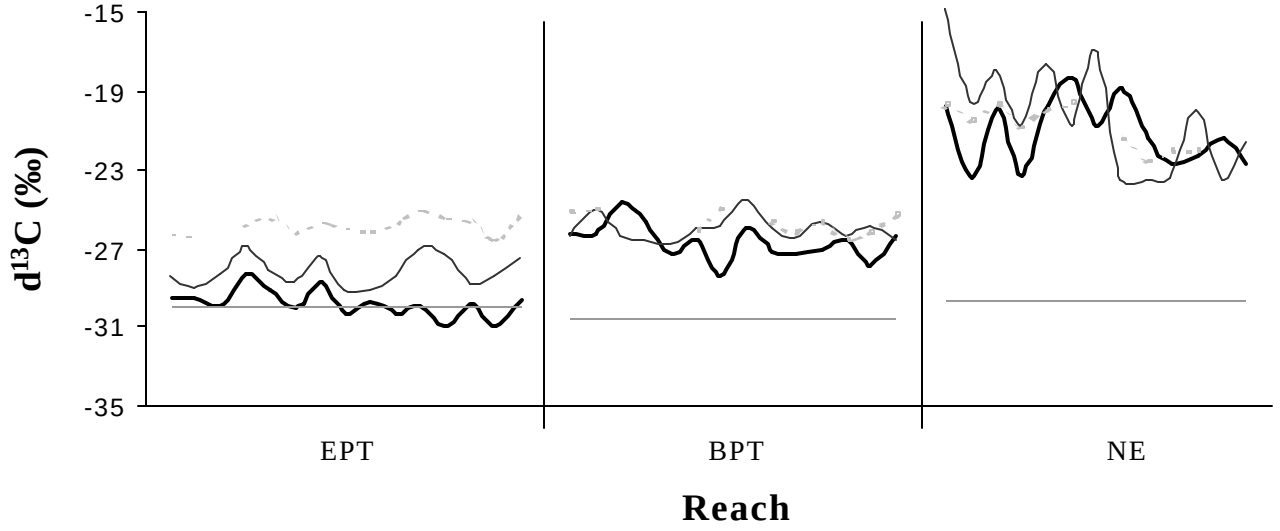
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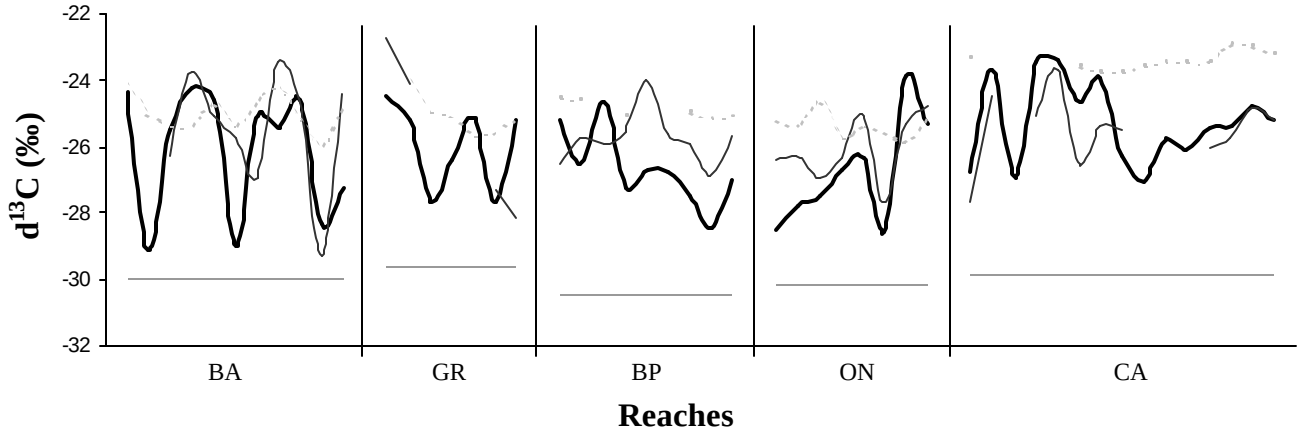
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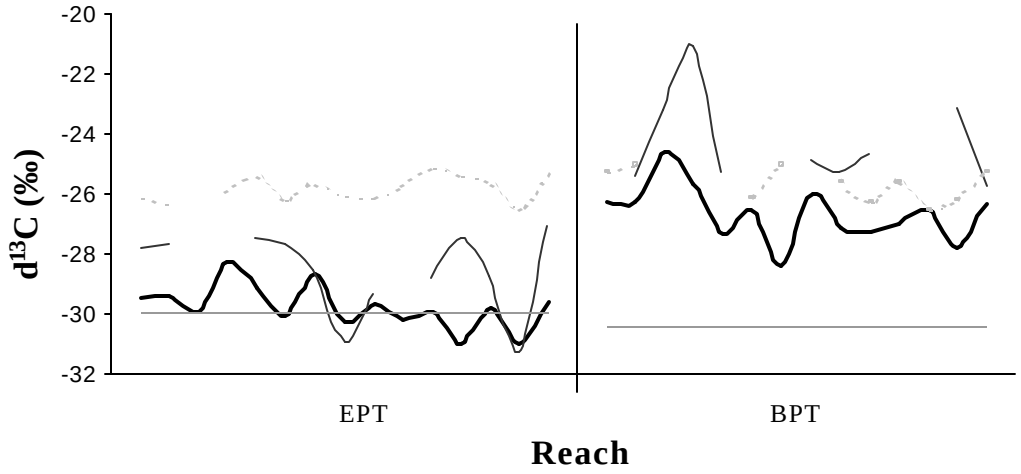
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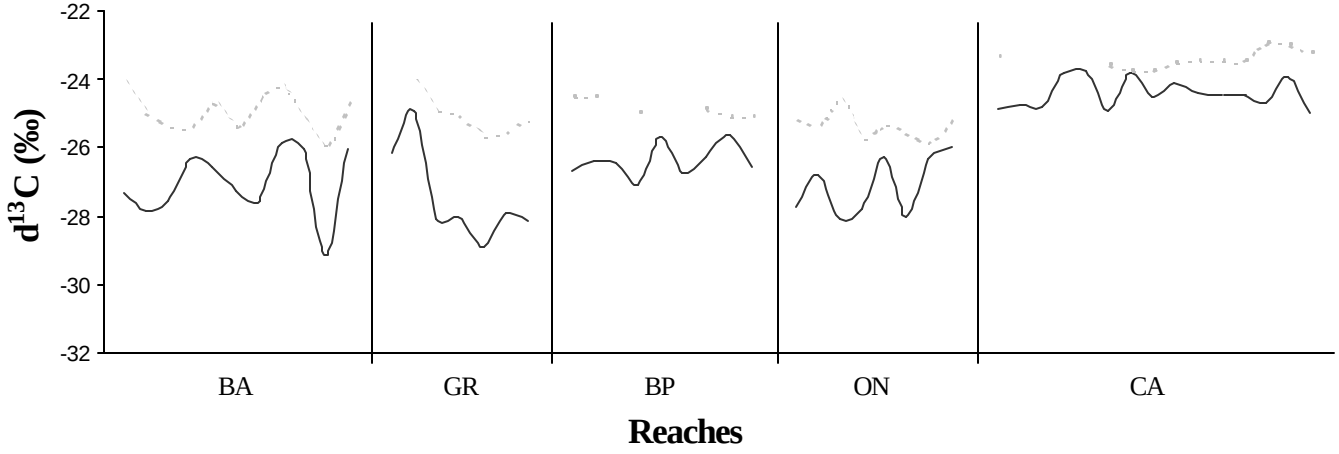
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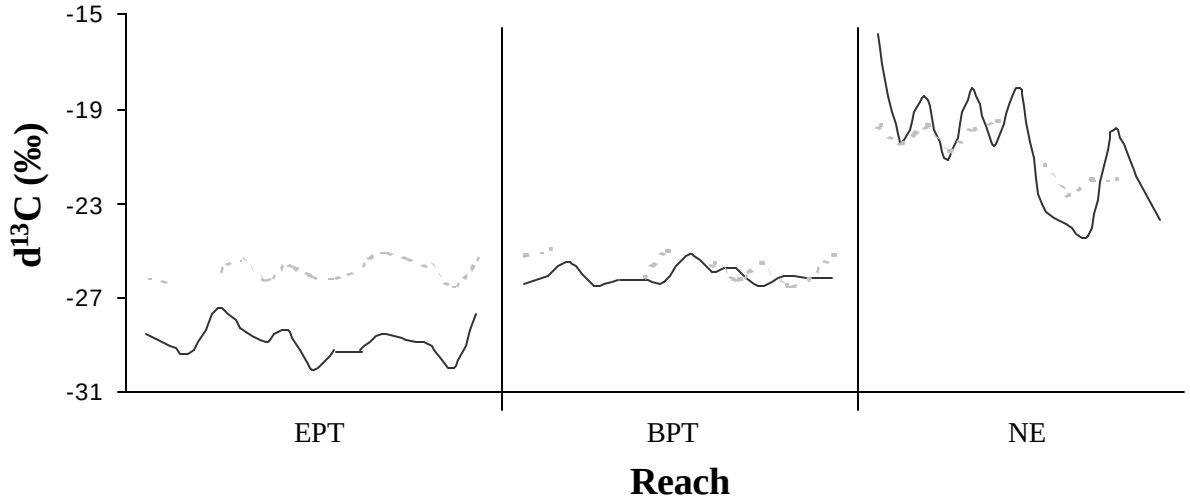
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d13C (‰)
EPT
BPT
NE
Reach

relative to all other reaches, thus mayfly $d^{13}C$ were in the same range as those of periphyton and detritus in that reach. In the NE reach, the mayfly spatial pattern appeared to be shifted to the left in the upstream portion.

Compared to mayflies, caddisfly $d^{13}C$ were on average more positive and their spatial patterns generally did not track as well those of periphyton (Figure 3bi, bii). Caddisfly $d^{13}C$ were generally closer to those of periphyton than detritus. The $d^{13}C$ of these grazers also clearly increased in CA reach. In EPT reach, caddisfly $d^{13}C$ were more negative than anywhere else, yet were slightly more positive than periphyton $d^{13}C$.

Where present, chironomids had fluctuations in their $d^{13}C$ spatial patterns that often followed closely those of periphyton (Figure 3ci, cii). Chironomid $d^{13}C$ were always more positive than those of detritus and usually within –1 to +1‰ relative to periphyton. Unlike mayflies and caddisflies, chironomid $d^{13}C$ did not clearly increase in CA reach, and in fact were almost always more positive than larger grazers.

No continuous spatial pattern could be done for blackfly $d^{13}C$ due to their absence at many sites. However, where present, their $d^{13}C$ were usually ^{13}C-enriched compared to periphyton, thus always much more positive than those of detritus.

Fish $d^{13}C$ were generally more positive than those of invertebrates. Their spatial patterns were generally also less variable than that of individual invertebrate taxon. Fish $d^{13}C$ were even less variable than those of the average of all invertebrates (the average of the four major food sources of fish in this river system), except in the BPT reach. Despite the lower variability, the spatial patterns of fish generally tracked well those of average invertebrates (Figure 4di and dii).

Mixing models

Mixing model results using $d^{13}C$ suggested mayflies ate a mixture of periphyton and detritus in all reaches (Table 4). Periphyton seemed to represent only one third to one fifth of the diet of mayflies in three reaches of the main branch, BA, GR and ON. In the remaining reaches of the main branch (BP and CA), as well as BPT and NE reaches, these grazers appeared to switch to periphyton as a primary food source. In EPT reach, the mixing model suggested mayflies fed equally on periphyton and detritus. Caddisflies seemed to rely on periphyton predominantly in six out of eight reaches. In the remaining two reaches (BA and EPT), caddisflies appeared to rely equally on both food sources. Mixing models for chironomids suggested periphyton was their principal food source everywhere, except in EPT reach where again, there was no significant difference between the percent periphyton and detritus in their diet. In reaches where blackflies were found, mixing models suggested they fed almost solely on periphyton, except in EPT reach like the other invertebrate taxa. Overall, our results suggest that invertebrates rely primarily on detritus and periphyton downstream in the river, and switch to largely periphyton upstream and in tributary streams.

Unequal variances and smoothing

As predicted and shown in Figure 4di and ii, per reach fish $\delta^{13}C$ variance was lower than that of the average of all invertebrates, except in BPT reach, yet the difference was often small. Barlett's test for comparing variances confirmed that fish $\delta^{13}C$ variances were significantly lower (significance level set at 0.1 due to the low number of sites in certain reaches) in five out of eight reaches of the river. Using WF, the average invertebrate $\delta^{13}C$ variances were reduced to those of fish in respective

Reach	Estimated % periphyton in diet			
	Mayflies	**Caddisflies**	**Chironomids**	**Blackflies**
BA	34	*64	81	
GR	29	70	83	
BP	78	83	93	100
ON	20	71	96	100
CA	94	97	77	95
EPT	*45	*53	*60	*46
BPT	67	91	100	100
NE	86	88		

Table 4. Invertebrate mixing model results. Gives the estimated % periphyton in the diet of each invertebrates taxa in individual reaches (% detritus = 1-% periphyton). Stars signify that the % periphyton and detritus did not differ significantly (t-test; $P > 0.05$).

reaches (Table 5). The smoothing analysis suggested that in BA and CA reaches, parr ate 2/3 of their food at the home site (where sampled) and 1/3 from the upstream and downstream sites. In GR, ON, and NE reaches, more smoothing was required to account for the fish signature variability; here the analysis suggested that parr ate 1/5 to 1/4 of their food at the home site and 3/4 to 4/5 from the upstream and downstream sites.

Discussion

At the river system level, linear regressions with $\delta^{13}C$ residuals clearly showed that the relationships between water velocity and organisms' $\delta^{13}C$ were negative and significant for periphyton, grazers, and fish. The signal was negative, but not significant for blackflies, which was not surprising due to the lower number of individuals collected relative to other taxa. Sampling across a series of sites differing considerably in water velocity resulted in large fluctuations in the periphyton $d^{13}C$ data (Chapter 1), and as predicted, the strongest water velocity effect was on periphyton $\delta^{13}C$. Regression strengths, however, weakened sharply at higher trophic levels, and regression slopes accordingly tended to decrease from periphyton to fish (mayflies excepted due to the extremely low and high $\delta^{13}C$ from EPT and NE reaches, respectively). Even though significant, the proportion of the variability in $\delta^{13}C$ explained by water velocity dropped considerably from periphyton to grazers and fish, from a quarter to less than a tenth.

At the reach level, the water velocity signal was stronger on periphyton $d^{13}C$ than at the river system level (except for BPT reach) with the same level of significance. For significant regressions, the water velocity signal was also stronger for grazers and fish (r^2 from 0.12 to 0.43 at the reach level versus 0.03 to 0.09 at the river system level). Finlay et al. (1999) also found significant negative relationships between water velocity

	BA	GR	BP	ON	CA	EPT	BPT	NE
Invertebrate $d^{13}C$ variance	0.95	2.51	0.31	0.82	0.25	0.34	0.20	5.03
Fish $d^{13}C$ variance	0.30	0.35	0.07	0.14	0.07	0.18	0.32	1.72
***P* of Bartlett's test**	0.08	0.08	0.20	0.03	0.05	0.30	0.60	0.10
$WF_{adjacent\ sites}$ (%)	34	74		74	34			80

Table 5. Fish smoothing results. Shows the per reach fish and invertebrate $d^{13}C$ variances. Invertebrate $d^{13}C$ variances represent that of the average of all four taxa combined. $WF_{adjacent\ sites}$ represents the estimated percentage of invertebrates eaten from the adjacent upstream and downstream sites.

and herbivore $d^{13}C$ in productive rivers. Relationships were also negative in their less productive streams, but insignificant. Their comparative study supported the idea that boundary layer thickness affects the baseline $d^{13}C$ passed on to higher trophic levels, since herbivore $d^{13}C$ should reflect algal $d^{13}C$. Morinville & Rasmussen (2003) in a study on bioenergetic differences also reported significant $\delta^{13}C$ differences between resident and anadromous juvenile brook trout. Resident trout using slower currents had $\delta^{13}C$ on average 1‰ less negative relative to anadromous trout using faster currents. Overall, water velocity seems to be an abiotic factor partially responsible for the $d^{13}C$ fluctuations of organisms of all trophic levels in rivers and streams (Table 2). It can explain a considerable proportion of the $d^{13}C$ variability of primary producers, yet the signal weakens fast in consumers possibly due to averaging of consumers over different food sources and space.

At the river system level (Table 2), regressions between the $\delta^{13}C$ of periphyton and each grazer taxa were positive, strong, and significant, with periphyton explaining close to half or more of the variability in grazer $\delta^{13}C$. Even blackflies, which are filter feeders, were strongly correlated with periphyton. Blackfly larvae are small and remain in proximity to the rock surface when filtering water. They probably filter diatoms detaching from the periphyton layer. The regressions support the notion that periphyton is very important in the diet of benthic invertebrates. Similarly, regressions between parr and invertebrate $\delta^{13}C$ were all positive and significant, except for chironomids. $\delta^{13}C$ of the larger taxa, mayflies especially followed by caddisflies, were the most strongly correlated with those of parr, suggesting these invertebrates are important in the diet of parr. At the reach level (Table 3), most regressions were not significant, which

we believe resulted from much lower sample sizes combined with a diminished water velocity effect with increasing trophic level. Even so, the regression results did not dispute our hypothesis that periphyton and macroinvertebrates are important food sources for macroinvertebrates and fish, respectively, in this river system. As well, mayflies had on average the strongest and most significant regressions with water velocity, periphyton $d^{13}C$ and parr $d^{13}C$, suggesting that they probably represent the strongest trophic link in this food web between primary producers and fish. Results from a study conducted by Mookerji et al. (2004) in the Ste. Marguerite River reported that parr feed mainly on Ephemeroptera (35-60% of their diet), which supports our findings. The next strongest links were caddisflies and chironomids equally. The $\delta^{13}C$ regressions between trophic levels were all positive as expected, yet more data points would be required to obtain a greater number of significant regressions at the reach level.

Grazers and fish were expected to have $\delta^{13}C$ enriched on average by 0-1‰ relative to the average of their food sources. Mean $?\delta^{13}C$ should therefore indicate the principal food source(s) of consumers. It appears that grazers which relied predominantly on periphyton changed from reach to reach (Figures 2a, b, c). Only chironomids seemed to feed primarily on periphyton in BA reach and only caddisflies in GR reach. Further upstream, in BP reach, periphyton appeared to be the predominant food source for both caddisflies and chironomids. In ON reach, only chironomids had a $?\delta^{13}C$ between 0-1‰. For the first time, in CA reach, mayflies appeared to feed primarily on periphyton. In streams, results suggested only caddisflies fed primarily on

periphyton in BPT reach. Finally, periphyton appeared as the main food source in the diet of mayflies and caddisflies in NE reach.

Grazer ? $\delta^{13}C$ values were often below the expected 0-1‰ range relative to periphyton. In such cases, detritus could have led to the lower invertebrate $d^{13}C$, since detritus was almost always ^{13}C-depleted relative to periphyton in this study. Vander Zanden and Rasmussen (2001) also found similar negative ? $\delta^{13}C$ values between herbivores and periphyton. They showed that herbivorous invertebrates (mainly amphipods and polychaetes) raised in a laboratory had $d^{13}C$ on average –0.41‰ relative to their food source. Their results, along with ours, suggest that herbivorous invertebrates fractionate more than generally expected. Conversely, grazer ? $\delta^{13}C$ were sometimes above 1‰. The only other food source that could explain the higher grazer ? $\delta^{13}C$ are filamentous algae, since they are usually ^{13}C-enriched relative to periphyton (Trudeau & Rasmussen 2003). However, this type of algae was uncommon at the time of sampling. Slight deviations from the 0-1‰ increase from periphyton to grazers may also be partially explained by failure to capture the ends of the water velocity range at every site, thus periphyton with the most positive and negative $\delta^{13}C$.

? $\delta^{13}C$ between grazers and fish tended to be greater than expected in many reaches (Figures 2a, b, c). In BA, BP, and ON reaches in the main branch, only chironomids appeared to be eaten by parr. In CA and NE reaches, mayfly ? $\delta^{13}C$ were only slightly above and below 0-1‰ respectively, suggesting parr may be eating a lot of these invertebrates here. In BPT reach, caddisflies and chironomids appeared to be preferentially eaten by parr, while in GR and EPT reaches none of the grazer taxa had ? $\delta^{13}C$ in the 0-1‰ range. Since we captured all the main invertebrate taxa in the

sections of the river and streams sampled, we cannot explain the ? $\delta^{13}C$ between fish and invertebrates greater than 1‰ with other taxa having more positive $\delta^{13}C$. Dragonfly and stonefly larvae were found in very few fish stomachs and sites along the reaches and thus, were not expected to account for a large enough proportion of the fish diet to change their signatures significantly. Fish may just be fractionating more than predicted.

Downstream to upstream trends in isotopic signatures allowed qualitative assessment of linkages between trophic levels (Figure 3). For invertebrates, we used spatial patterns in combination with mixing models to determine which taxa potentially ate which primary producer and where in the river system. Many mayfly $d^{13}C$ peaks coincided with those of periphyton (Figures 3ai, aii). Fluctuations of mayfly $d^{13}C$ were on average less pronounced relative to periphyton in the main branch, while the opposite was observed in streams and the NE reach. Larger invertebrate fluctuations in some sections may simply be due to the lack of periphyton sampling in the slowest and fastest water velocities. The mixing model indicated that detritus was the main food source of mayflies downstream in the main branch. In the middle section of the main branch (BP and ON reaches), mayflies seemed to shift their diet to mainly periphyton and back to mainly detritus. Finally, upstream in the main branch, they definitely switched to periphyton as a principal food source. In the one reach sampled in the north east branch, mayflies appeared to rely mainly on periphyton. Similarly, in streams, mayflies seemed to feed principally on periphyton. In EPT reach, periphyton $d^{13}C$ were in the same range as those of detritus. The mixing model suggested mayflies ate both food sources about

equally, yet the spatial pattern of mayfly $d^{13}C$ tracked that of periphyton so well that the latter appears to be more important in their diet than predicted from the mixing model.

Caddisfly $d^{13}C$ spatial patterns showed fluctuations generally less pronounced than those of periphyton, except in GR and NE reaches probably for similar reasons than mayflies (Figures 3bi, bii). Caddisfly $d^{13}C$ fluctuations coincided less well with those of periphyton, yet their $d^{13}C$ were generally closer to those of periphyton than detritus. In downstream reaches of the main branch, both types of primary production appeared important for caddisflies, yet the mixing model suggested a slight preference for periphyton. Caddisflies switched mainly to periphyton in the following upstream reaches of the main branch, especially in CA. Such as for mayflies, caddisflies in the north east branch and in streams seemed to rely mainly on periphyton.

Where present in consecutive sites, chironomid $d^{13}C$ fluctuations were less pronounced than those of periphyton only in about half the reaches (Figures 3ci, cii). Chironomid $d^{13}C$ spatial patterns and mixing models suggested periphyton was their predominant food source everywhere. Blackflies were only found sparsely in five out of eight reaches. Consequently, no spatial pattern showing their $d^{13}C$ fluctuations could be done. However, blackfly $d^{13}C$ were almost always more positive than those of periphyton, thus much more positive than those of detritus, suggesting they relied mostly on periphyton as a major food source.

As predicted, fish $d^{13}C$ values always fluctuated less than for individual invertebrates (Figures 3di, dii). Parr and juvenile trout $d^{13}C$ were generally similar or more positive than all invertebrates. Parr $d^{13}C$ tended to be on average slightly more negative than those of caddisflies only in NE reach, suggesting caddisflies were probably not the main food source of parr in that reach. Overall, fish $d^{13}C$ spatial

patterns tracked well that of averaged invertebrates, while the per reach fish $d^{13}C$ variance was generally lower. This indicated that diet averaging was not a factor sufficient to explain the lower fish $d^{13}C$ variances in some reaches as it was for invertebrates. The second factor may be spatial averaging, which we estimated using a smoothing technique. Bartlett's test for unequal variances showed that in streams and BP reach, fish $d^{13}C$ variances did not significantly differ from that of averaged invertebrates. Parr and juvenile trout in these reaches probably did not move much over the summer, and found most of their food within the site where they were fished. This much movement may be sufficient to explain the slightly lower fish $d^{13}C$ variances relative to invertebrates. In the remaining reaches, the smoothing technique roughly estimated how much spatial averaging would be necessary to explain the significantly lower fish $d^{13}C$ variances. In BA and CA reaches, the smoothing analyses suggested 2/3's of the parr diet came from the site where they were found versus 1/5 to 1/4 in GR, ON, and NE reaches. The rest of the diet came from the adjacent upstream and downstream sites. These estimates seem reasonable, since parr may be forced to move and drift to find their preferred nose velocities or thermal refuge after flooding events. Thus, a fish captured in one site may have spent much of its summer further upstream. Moreover, invertebrates normally have limited mobility and a small foraging range, but could have drifted to downstream sites in fast waters. Fish can therefore consume invertebrates from upstream sites. These two situations can significantly increase the amount of spatial averaging fish can reflect.

Our results pertaining to the relative importance of periphyton versus detritus along the river are not in agreement with the predictions of the river continuum concept (RCC) presented by Vannote et al. (1981). They suggested that the diet of lotic

macroinvertebrates should change gradually from being detritus-dominated upstream (in 1st and 2nd order tributaries), where detrital input and shading are greatest, to an algae-dominated diet downstream, where the river gets larger and the relative importance of detrital input and shading have diminished. Findings from our study suggest that algae is generally an important component in the diet of all invertebrate taxa sampled. Its importance seems to increase as the water channel narrows, such as upstream in the river and in tributary streams. Detritus consumption appears important only for mayflies and only at downstream sites in the river. These results contradict the predictions of Vannote et al. (1981), and if our inferences based on $d^{13}C$ are indeed valid, they cast doubt on the fundamental mechanisms they proposed. No other studies to our knowledge have attempted to test these predictions of the RCC using stable isotopic signatures.

Conclusion

The present study allowed us to determine the effect of water velocity on grazers and fish $d^{13}C$ in the Ste. Marguerite River system, through its effect on periphyton. We found that the negative effect of water velocity is significantly transmitted up the food web at the river system level, yet the strength of the signal decreases rapidly with increasing trophic position. At the reach level, the signal was always negative and generally stronger, but not always significant, which most likely resulted from smaller sample sizes. As expected, $d^{13}C$ variability tends to diminish in consumers. For grazers, diet averaging between periphyton and detritus seem sufficient to understand their lower $d^{13}C$ variances. However, diet averaging between grazers and blackfly larvae was not always sufficient to explain the lower fish $d^{13}C$ variances. We believe fish spatial averaging is another factor to be considered.

More research is required to understand the underlying processes causing $d^{13}C$ variability in freshwater primary producers and consumers, as well as the tendency for $d^{13}C$ variability to decrease with increasing trophic level. Diet and spatial averaging of grazers and fish should be considered and studied in more detail as they appear to be important processes. In fact, there is currently little information on distances travelled by invertebrates and fish during drift, and such information is essential to properly estimate spatial averaging. Overall, this paper provides interesting insights on the use of $d^{13}C$ heterogeneity of aquatic consumers to study their diet. It also refines the stable isotope technique, an increasingly important tool for freshwater ecologists.

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General Conclusion

Stable isotope ratios are an important tool in freshwater ecology - they can help understand some crucial biogeochemical processes. Therefore, it becomes mandatory to understand and consider factors regulating stable isotopic signatures of organisms of all trophic levels. We have shown that water velocity is a major factor contributing to the $d^{13}C$ variability around the baseline signatures of periphyton in rivers and streams. These $d^{13}C$ patterns are consistent with those previously found in the laboratory and support the boundary layer/diffusion hypothesis. To a lesser extent, algal biomass also appears to be responsible for periphyton $d^{13}C$ fluctuations. We did not succeed, however, in demonstrating similar patterns between water velocity and periphyton $d^{15}N$ as predicted from our previous laboratory results. Our results demonstrate that the water velocity effect is significantly transmitted up the food web, yet less strongly than at the primary producer level. As well, consumer $d^{13}C$ variability tends to diminish relative to periphyton and we believe diet and spatial averaging are the main processes involved. More research is required to properly understand the underlying processes causing $d^{13}C$ and $d^{15}N$ variability in freshwater organisms, as well as the tendency for $d^{13}C$ variability to decrease with increasing trophic level.

Stable isotopic information arising from this study also confirmed the importance of periphyton in the food web related to parr and trout in this ecosystem. This type of information can contribute to the proper management of microhabitats used by fish in this river system to maintain the trout population and help the salmon population recover. It also provides essential basic information on stable isotopic analyses, a technique with great potential in ecology at large, from the study of food webs to issues in conservation biology.

Appendice a. Data used in the regressions and correlations of both chapters. The $d^{13}C$ data were also used in the mixing models and Bartlett's test for unequal variances.