Determination of food sources for benthic invertebrates and brook trout *Salvelinus fontinalis* in Canadian Boreal Shield lakes using stable isotope analysis

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ABSTRACT: Allochthonous inputs can be an important contribution of organic matter in lake ecosystems. Yet, our understanding of the patterns of energy dependence of littoral invertebrates and fish is poor. We measured carbon (δ^{13}C) and nitrogen (δ^{15}N) stable isotope values for primary producers, terrestrial detritus, benthic macroinvertebrates, zooplankton and brook trout *Salvelinus fontinalis* in 8 oligotrophic Canadian Boreal Shield lakes to determine food sources that support benthic consumers and brook trout. Mixing models used to determine animal diets from stable isotope analysis showed leaf litter to be the principal food source for benthic primary consumers in 6 out of 8 lakes. Brook trout derived its carbon mainly from benthic predatory macroinvertebrates in all lakes, with a contribution ranging from 60 to 90%. Zooplankton also contributed to brook trout diet in 3 of 8 lakes, ranging from 28 to 37%. δ^{15}N increased from primary producers to consumers at different trophic positions in all lakes. Nitrogen isotopic signatures of brook trout became more positive with increasing fish length, indicating a change in fish foraging strategy related to size. Our study (1) suggests that carbon from terrestrial habitat (leaf litter) may contribute significantly to the food web of oligotrophic Canadian Boreal Shield lakes and (2) highlights the importance of carbon originating from benthic habitats in supporting brook trout in these lakes.

KEY WORDS: Allochthonous subsidies · Benthic macroinvertebrates · Zooplankton · Brook trout · Diet breadth · Size-related diet shift · Stable isotopes · Boreal lakes

INTRODUCTION

Organic matter that supports consumers in lakes can be produced within the ecosystem (autochthonous matter) or imported into the systems from adjacent terrestrial habitats (allochthonous matter). In low-productivity lakes that dominate in the boreal ecoregion, the relative availability of these resources is controlled by allochthonous terrestrial inputs (i.e. leaf litter falling from riparian vegetation; Ask et al. 2009, Karlsson et al. 2009). Thus, lakes are coupled to watershed inputs of organic matter as movements of

organic matter connect terrestrial ecosystems with aquatic food webs (Pace et al. 2004). Moreover, because the vast majority of lakes in this region are small and shallow and their perimeter to area ratios are relatively high (Schindler & Scheuerell 2002), they can be highly influenced by allochthonous organic matter of terrestrial origin (Weidel et al. 2008, Premke et al. 2010, Solomon et al. 2011).

Littoral zones are known to receive large inputs of allochthonous matter from riparian vegetation (Schindler & Scheuerell 2002). Most current studies have focused on the pelagic food webs. However, lit-

toral benthic pathways often play a key role in lake production and food web dynamics (Vadeboncoeur et al. 2002). Indeed, littoral benthic macroinvertebrates are an important heterotrophic component of lake ecosystems and constitute a link between basal resources and upper trophic levels in the food web such as fishes (Vander Zanden & Vadeboncoeur 2002, Vander Zanden et al. 2006). Knowledge of the relative importance of allochthonous matter sources supporting benthic macroinvertebrates and ultimately fish is still poor (Carpenter et al. 2005, Solomon et al. 2008). However, Cole et al. (2006) showed that in oligotrophic lakes between 60 and 85% of benthic macroinvertebrates used allochtonous organic matter to meet their energy requirements.

A variety of processes at multiple trophic levels create linkages among terrestrial, pelagic and benthic energy pathways in lakes (Schindler & Scheuerell 2002, Vadeboncoeur et al. 2002). For instance, benthic consumers may utilize dissolved or particulate detritus of terrestrial origin, and fishes may consume benthic, pelagic, or terrestrial prey (Solomon et al. 2011). Many fishes use both benthic and pelagic habitats or undergo ontogenic habitats shifts, integrating benthic and pelagic food webs (Vadeboncoeur et al. 2002). Brook trout *Salvelinus fontinalis* is a widely distributed and economically important fish species of eastern Canadian Boreal Shield lakes (Power 1980). It has been identified as a generalist carnivore fish that feeds mainly on benthic macroinvertebrates (Tremblay & Magnan 1991, Lacasse & Magnan 1992), but also on zooplankton, terrestrial insects (Power et al. 2002, Tremblay-Rivard 2007) and fish, including brook trout (Magnan 1988, Lacasse & Magnan 1992). Recent studies have shown that stable isotope signatures could be used to identify intraspecific shifts in feeding strategies of brook trout (Power et al. 2002, Tremblay-Rivard 2007). However, little is known about brook trout diet and size-related diet shifts in lakes of the Canadian Boreal Shield.

In this study, we measured naturally occurring carbon and nitrogen stable isotope ratios of basal resources (terrestrial detritus, macrophytes, periphyton, sediment organic matter), benthic organisms, zooplankton and brook trout in 8 oligotrophic Canadian Boreal Shield lakes. Using stable isotope analysis we examined feeding relationships among consumers. Our objectives were to: (1) determine the ultimate sources from which benthic primary consumers derive their energy in order to evaluate the relative importance of allochthonous organic matter for these consumers and (2) identify brook trout diet breadth and size-related diet shifts.

MATERIALS AND METHODS

Study area

The study was conducted in the province of Quebec, on the Boreal Shield, in the Mistassibi River drainage basin. The study area is dominated by virgin black spruce *Picea mariana* forests. Eight oligotrophic lakes with similar morphometric characteristics were selected in a territory covered by pristine boreal forest that was recently accessible by a network of forestry roads (Table 1). In all lakes, the only fish species found was brook trout, except for Lake 8, in which brook trout coexists with white sucker *Catostomus commersoni*.

Sampling

Eight lakes were sampled in July 2008. Water samples and pH measurements were taken at 5 littoral sampling stations set uniformly around each lake. At each sampling station, pH was measured at the subsurface using a YSI 556 MPS probe. Water transparency was estimated with a Secchi disk at the deepest part in each lake. Water samples (4 l) were collected at each littoral station at 50 cm below the surface with an Alpha bottle for the determination of chlorophyll *a* (chl *a*), dissolved organic carbon (DOC), total phosphorus (TP) concentrations and isotopic analysis of particulate organic matter (POM). Terrestrial detritus (leaf litter, woody debris), macrophytes, periphyton (epilithon and epixylon), surface sediment organic matter (SOM), benthic macroinvertebrates, zooplankton and brook trout were sampled for stable isotope analysis. Terrestrial detritus, macrophytes and SOM were hand-collected in the littoral zone of lakes and placed in scintillation vials. Periphyton was collected by scraping from rocks and woody debris in the littoral zone and placed in scintillation vials. Benthic invertebrates were collected in the littoral zone using a Turtox D-net with a mesh size of 500 μm (n = 3 replicates per station) and placed in cooler boxes. Zooplankton samples were obtained by towing a plankton net (mouth opening 20 cm, mesh 53 μm) mounted alongside a boat for 5 to 10 min. Zooplankton were then placed in Ziploc® bags containing water. Fish were captured with 6 experimental gill nets, according to the Quebec Ministry of Natural Resources and Wildlife standard protocol (mesh sizes of 1, 1.5, 2, 2.5, 3, and 3.5 inches [25 to 90 mm]) set simultaneously around the lake, perpendicular to the shore with the

small mesh always set toward the shore. Gill nets were left overnight for 12 h. All fish samples were placed in cooler boxes and transported to the laboratory for processing.

Sample preparation and analyses

Water samples for the determination of chl *a* were filtered (200 ml or more) onto Whatman GF/F filters. Pigments were extracted for 24 h in 90% acetone, at 5°C in the dark without grinding. Chl *a* was determined using the Welschmeyer et al. (1993) method. DOC was determined by filtering subsamples of water through precombusted (500°C, 5 h) Whatman GF/F filters. The filtrate was placed in glass vials with teflon-lined caps and acidified with 25% v/v H_3PO_4 (10 µl ml^{-1}). DOC values were obtained using a TOC-5000A analyzer (Shimadzu), following the protocol of Whitehead et al. (2000). DOC reference standards were produced by the Hansell's Certified Reference Materials (CRM) program. Total phosphorus (TP) was measured using the molybdenum blue method (Stainton et al. 1977) after autoclaving 50 ml samples with 0.5 g of potassium persulfate for 1 h at 120°C. For the determination of isotopic signatures of POM, subsamples of water were filtered through precombusted (500°C, 5 h) Whatman GF/F filters and stored frozen at −20°C until analysis. Terrestrial detritus, macrophytes, periphyton and SOM samples were cleared and separated with the aid of a dissecting microscope. Benthic macroinvertebrates and zooplankton samples were identified at the order or family level using Merritt & Cummins (1996) and Edmondson (1959) respectively. After identification, benthic macroinvertebrates were frozen separately at −20°C while zooplankton was frozen as a pool of organisms at −20°C. The total length of each brook trout was recorded. A 2 cm square and 1 cm deep block of dorsal white muscle without skin sample was taken from 25 brook trout in each lake. All muscle samples were kept frozen at −20°C in scintillation vials until analysis.

Stable isotope analyses

POM filters, terrestrial detritus, macrophytes, periphyton, SOM, whole benthic invertebrates, zooplankton, and brook trout samples were dried at 60°C for 48 h. Mortar and pestle were thereafter used to grind samples into a fine powder (except for POM filters). Powder and filter samples were then encapsulated in pressed tin capsules (5 × 9 mm) and tin foil cups (Costech Analytical Technology), respectively. Encapsulated dry mass was approximately 1 mg for terrestrial detritus, macrophytes, periphyton, benthic invertebrates, zooplankton, and brook trout muscle tissue and 3 mg for SOM. Lipid correction techniques were not considered in our analyses because of low carbon to nitrogen (C:N) ratio values (Vander Zanden & Rasmussen 1999). Previous studies showed that lipids are lower in animals analyzed as muscle tissue, as in our study for fish, than animals analyzed as whole (McCutchan et al. 2003). C:N ratio values for fish varied between 3.9 (Lake 7) and 4.7 (Lake 6) in our study.

Analyses of stable isotopes ratios of C (δ^{13}C) and N (δ^{15}N) were carried out at the Institut des sciences de la mer (ISMER, Rimouski, Quebec, Canada) using a COSTECH ECS 4010 Elemental Analyser coupled with a DeltaPlus XP Isotope Ratio Mass Spectrometer (IRMS, Thermo Electron). System control as well as acquisition and treatment of the data were carried out using the Isodat 2 software. Stable isotope ratios were expressed in δ notation as parts per thousand (‰) according to the equation:

$$\delta X = [(R_{sample}/R_{standard}) - 1] \times 1000 \qquad (1)$$

where X is ^{13}C or ^{15}N and R is the corresponding ^{13}C:^{12}C or ^{15}N:^{14}N ratios.

International standards used for the measurement were Vienna Pee Dee Belemite (VPDB) limestone for ^{13}C and atmospheric nitrogen for ^{15}N. Regional standards for in-lab normalization regressions to determine sample δ values were anhydrous caffeine (Sigma Chemical), Mueller Hinton Broth (Becton Dickinson) and *Nannochloropsis*, respectively. These homemade standards were calibrated once a year using standards from the National Institute of Standards and Technology (NIST). Replicate analyses of standards gave analytical errors (SD) of ±0.30‰ for C and ±0.18‰ for N.

Data analyses

To estimate the relative contribution of the different food sources to the diet of benthic invertebrates and brook trout, we adopted a Bayesian multi-source stable isotope mixing model, available as an open source R package (SIAR, Parnell et al. 2010). SIAR takes data on organism isotopes and fits a Bayesian model to their dietary habits based on a Gaussian likelihood with a dirichlet distribution prior mixture on the mean. SIAR offers a number of advantages over earlier mixing models; it can incorporate trophic

enrichment factors within the model and known C and N concentrations dependence, assuming that for each element, a source's contribution is proportional to the contributed mass times the elemental concentration in that source. We assumed a trophic enrichment of +1‰ for ^{13}C (De Niro & Epstein 1978) and +3.4‰ for ^{15}N (Minagawa & Wada 1984). To assess these trophic enrichment assumptions, we combined organisms into main groups of consumers: zooplankton, benthic primary consumers and predatory macroinvertebrates and calculated average $\delta^{13}C$ and $\delta^{15}N$ for each group.

Using *Daphnia* spp. as the baseline trophic level, trophic position of consumers was calculated using the formula given in Vander Zanden & Rasmussen (2001):

$$\text{Trophic position}_{consumer} = (\delta^{15}N_{consumer} - \delta^{15}N_{baseline})/f + 2 \quad (2)$$

where $\delta^{15}N_{consumer}$ is the $\delta^{15}N$ value of the consumer for which the trophic position is estimated, $\delta^{15}N_{baseline}$ is the $\delta^{15}N$ of the baseline organism, 2 is the expected trophic position of the organism used to estimate baseline $\delta^{15}N$, and f is the fractionation factor between a predator and its prey which corresponds to 1 trophic level. We used the fractionation factor of 3.4‰ as proposed by Minagawa & Wada (1984) and generally used in studies estimating trophic position of aquatic consumers (Vander Zanden et al. 1997).

Stable isotopes of carbon and nitrogen were also individually plotted against fish length in each lake. Simple linear regressions were then calculated for each lake.

RESULTS

Physico-chemical data for the 8 lakes are outlined in Table 1. The lakes are small (0.031 to 0.288 km²), as are their catchments (0.202 to 2.895 km²). All the lakes are relatively shallow (maximum depth < 9 m) and exhibited low levels of phytoplankton biomass (chl *a*) but high DOC concentrations (Table 1). This is typical of oligotrophic Canadian Shield lakes, which are slightly acidic, with high concentrations of humic materials (Power et al. 2002, Glaz et al. unpubl.).

Benthic consumers and potential food sources

Mayflies (Ephemeroptera), corixids (Corixidae), amphipods (Amphipoda) and caddisflies (Trichoptera), all of them non-predatory macroinverte-

Table 1. Lake characteristics of the 8 studied Canadian Boreal Shield lakes. pH, chl a, dissolved organic carbon (DOC) and total phosphorus (TP) are reported as means (SD) over the 5 sampling stations in the photic zone. na: not available

	Lake 1	Lake 2	Lake 3	Lake 4	Lake 5	Lake 6	Lake 7	Lake 8
Latitude (°N)	50°25'44"	50°29'22"	50°23'13"	50°28'34"	50°30'9"	50°31'25"	50°30'40"	50°28'11"
Longitude (°W)	71°57'28"	71°57'32"	72°1'24"	71°57'15"	71°47'1"	71°56'26"	71°56'5"	71°46'51"
Lake area (km²)	0.170	0.169	0.063	0.031	0.288	0.090	0.277	0.043
Catchment area (km²)	0.916	2.799	0.586	0.202	2.895	1.761	2.416	0.339
Drainage area (km²)	0.746	2.630	0.523	0.171	2.606	1.671	2.138	0.296
Maximum depth (m)	5	2	0.5	2	9	4.5	7.5	2
Secchi depth (m)	1.25	1.50	naᵃ	1.75	1.50	1.65	1.40	1.40
pH	5.92 (0.10)	5.75 (0.02)	5.94 (0.05)	5.87 (0.07)	5.92 (0.06)	5.02 (0.05)	5.62 (0.15)	5.38 (0.28)
Chl a (mg m⁻³)	0.427 (0.065)	0.390 (0.061)	0.617 (0.200)	0.363 (0.050)	0.982 (0.212)	0.546 (0.060)	0.681 (0.161)	0.486 (0.072)
DOC (mg l⁻¹)	10.78 (0.57)	12.06 (0.58)	12.56 (1.01)	12.33 (0.48)	11.91 (0.73)	9.82 (0.40)	8.98 (0.43)	13.73 (1.04)
TP (µg l⁻¹)	5.05 (0.26)	4.95 (0.52)	5.77 (0.50)	5.13 (1.01)	5.09 (0.70)	4.69 (0.47)	5.26 (0.70)	4.65 (0.55)

ᵃNot measured because lake was too shallow

brates, were grouped as benthic primary consumers. Leeches (Hirudinea), water mites (Hydracarina), dragonflies (Anisoptera), damselflies (Zygoptera), dysticids (Dysticidae) and alderflies (Sialidae), all of them active predators, and Empidids (Empididae) and Chironomids (Chironomidae), which are mainly predators, were enriched in ^{15}N relative to primary consumers in all lakes (Fig. 1). Consequently, these organisms were pooled as predatory macroinvertebrates. A mixing model was performed to evaluate basal resource contribution to benthic primary consumers. Benthic primary consumers derive their carbon mainly from leaf litter in all lakes (Table 2). However, in Lakes 1 and 2 epilithon and epixylon also contributed to their diet, with mean contributions of 43 and 46% respectively (Table 2).

Brook trout diet breath

Brook trout mean carbon isotope ratios ranged from −31.1 (Lake 8) to −26.2‰ (Lake 3) and mean nitrogen isotopes ratios ranged from 5.6 (Lake 1) to 6.4‰ (Lake 4). Both $\delta^{13}C$ and $\delta^{15}N$ signatures for brook trout were well separated from their potential prey items in all lakes. Brook trout were enriched in ^{15}N relative to all other sources in all lakes (Fig. 1). The mixing model confirmed that brook trout derive their carbon biomass mainly from predatory benthic macroinvertebrates in all lakes, with feasible mean contributions ranging from 60 (Lake 2) to 90% (Lake 3) (Table 3). However, in Lakes 1, 2 and 8, zooplankton also appeared as a potentially important food resource with feasible mean contributions of 28, 37 and 32% respectively. The mixing model also indicated that brook trout do not depend on benthic primary consumers for food (Table 3).

Trophic position

$\delta^{15}N$ increased from primary producers to consumers at different trophic positions in all lakes (Fig. 1), indicating that the heavy nitrogen isotope (^{15}N) was preferentially retained during nutrient assimilation and incorporation into animal tissues. Brook trout were enriched in ^{15}N by 3.77‰ relative to predatory macroinvertebrates when all lakes were pooled (Table 4). However, predatory macroinvertebrates were only enriched by 2.60‰ in ^{15}N relative to benthic primary consumers (Table 4).

Mean trophic position of consumers was significantly different among the 3 main trophic groups examined in all lakes (Table 5). The *a posteriori* Tukey test confirmed the existence of significant differences for all paired combinations in all lakes. Predatory macroinvertebrates had a mean trophic position that was significantly higher than that of benthic primary consumers and fish had a significantly higher trophic position than both benthic primary consumers and predatory macroinvertebrates in all lakes (Table 5).

Size-related diet shift of brook trout

Brook trout mean length ranged from 192 (Lake 8) to 245 mm (Lake 2). Brook trout showed great variability in $\delta^{13}C$ values and they became significantly more depleted in ^{13}C with increasing length in Lakes 2, 4 and 8 (although in Lake 4 sampling size was low, n = 8, in comparison to the other lakes, n = 25). Lakes 1, 3, 5, 6 and 7 did not show any particular pattern in relation to increasing fish length (Fig. 2a, Table 6). Brook trout became significantly more enriched in ^{15}N with increasing body length in all lakes but Lake 4, and this relationship was also significant when all lakes were pooled (Fig. 2b, Table 7).

DISCUSSION

Leaf litter appeared to be the principal carbon source for benthic primary consumers in 7 out of our 8 lakes, suggesting a significant contribution of allochthonous matter to the littoral food web. Brook trout seemed to derive their food mainly from benthic predatory macroinvertebrates in all lakes, although zooplankton also contributed to brook trout diet in 3 out of 8 lakes. A size-related diet shift was also observed for brook trout as they became ^{15}N-enriched with increasing body length. However, no consistent pattern was observed for $\delta^{13}C$ values.

Carbon flow in lake food webs

In oligotrophic lakes, humic matter originating in the catchment can be an important contribution of allochthonous matter for aquatic organisms (France 1997, Glaz et al. unpubl.). Moreover, the loading of allochthonous matter can greatly exceed autochthonous primary production (Carpenter et al. 2005). High DOC concentrations strongly influence light penetration in lakes (Carignan et al. 2000, Karlsson et al. 2009) and present an unfavorable environment
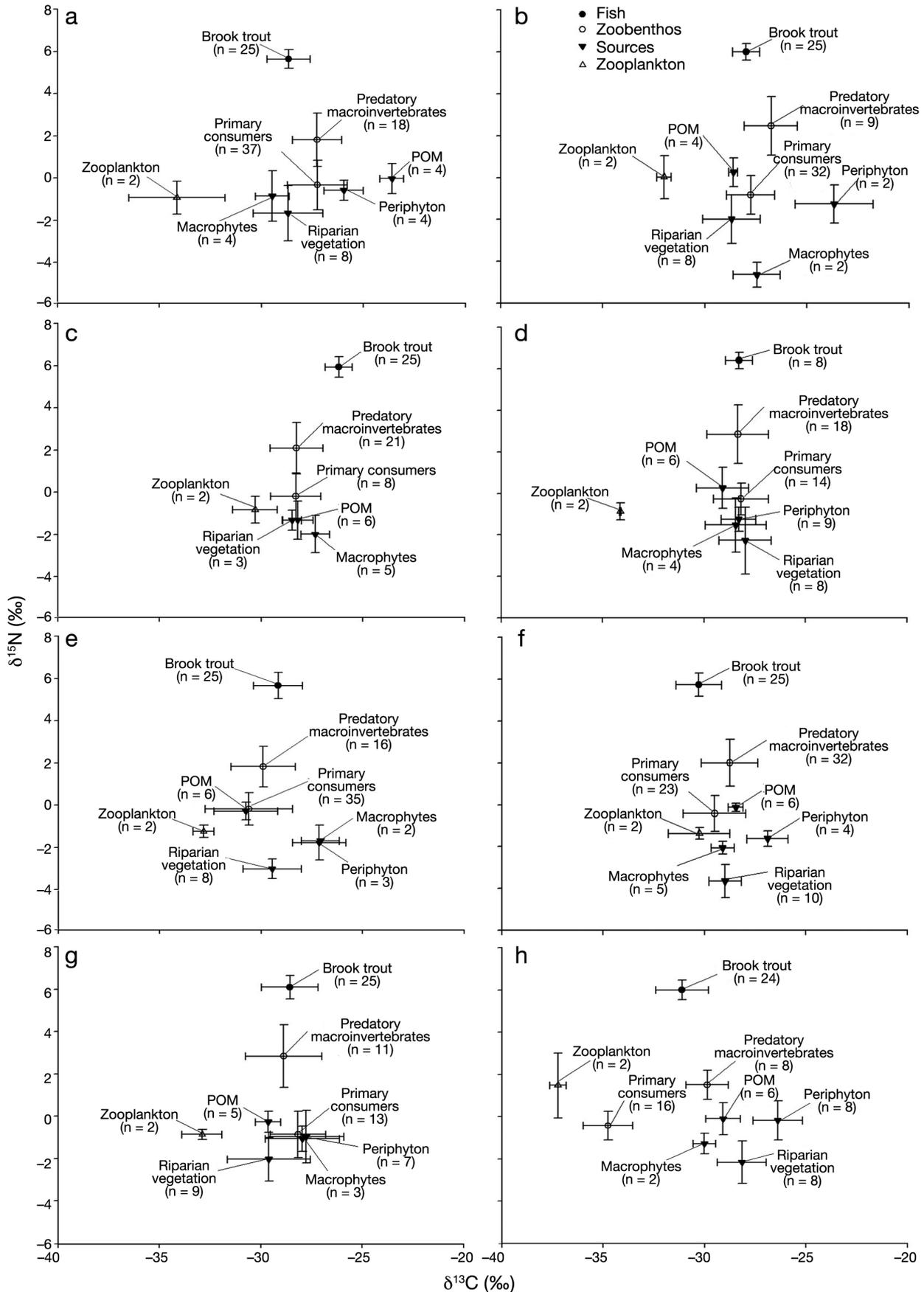
Fig. 1. Stable isotope signatures of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) for potential food sources and brook trout for 8 lakes: (a) Lake 1; (b) Lake 2; (c) Lake 3; (d) Lake 4; (e) Lake 5; (f) Lake 6; (g) Lake 7 and (h) Lake 8. Error bars represent the mean ± SE. POM: particulate organic matter

Table 2. Bayesian mixing model (SIAR) results for benthic primary consumers in 8 lakes. Percentiles show the 1st to 99th range of potential contribution. Sources with a necessary contribution (minimal potential contribution > 0%) in **bold**. abs: absent

Lake	Leaf litter Mean	Percentile	Epilithon Mean	Percentile	Epixylon Mean	Percentile	Macrophytes Mean	Percentile	POM Mean	Percentile
1	**43**	**26−59**	abs		**43**	**29−56**	7	0−20	6	0−19
2	**36**	**10−58**	10	0−20	abs		**46**	**28−66**	7	0−19
3	**44**	**25−63**	3	0−9	abs		27	0−49	26	0−49
4	**52**	**40−66**	17	0−35	10	0−26	13	0−31	7	0−20
5	**95**	**90−99**	2	0−5	abs		1	0−4	1	0−3
6	**78**	**68−87**	abs		4	0−11	6	0−17	8	0−17
7	**47**	**23−71**	7	0−20	22	0−44	11	0−27	11	0−31
8	**57**	**45−70**	7	0−20	10	0−25	12	0−28	13	0−28

Table 3. *Salvelinus fontinalis.* Bayesian mixing model (SIAR) results for brook trout in 8 lakes. Percentiles show the 1st to 99th range of potential contribution. Sources with a necessary contribution (minimal potential contribution > 0%) in **bold**

Lake	Zooplankton Mean	Percentile	Benthic primary consumers Mean	Percentile	Predatory macroinvertebrates Mean	Percentile
1	**28**	**20−36**	3	0−9	**68**	**58−79**
2	**37**	**30−45**	2	0−7	**60**	**51−68**
3	2	0−5	8	0−17	**90**	**80−99**
4	13	0−24	14	0−35	**73**	**48−93**
5	5	0−13	8	0−19	**86**	**74−97**
6	6	0−14	7	0−17	**86**	**76−97**
7	13	0−23	6	0−15	**80**	**70−89**
8	**32**	**23−42**	3	0−8	**64**	**55−73**

Table 4. Mean (± SD) nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) stable isotope signatures of principal groups of consumers. All lakes were pooled

	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)
Fish	5.93 (0.48)	−28.70 (1.02)
Predatory macroinvertebrates	2.16 (1.20)	−28.52 (1.40)
Benthic primary consumers	−0.44 (0.93)	−29.33 (1.45)
Zooplankton	−0.57 (0.64)	−32.97 (0.91)

Table 5. Mean (± SD) trophic-position estimates in all lakes and analysis of variance (ANOVA) results comparing estimates between benthic primary consumers, predatory macroinvertebrates and fish in 8 lakes. The *a posteriori* Tukey test confirmed the existence of significant differences for all paired combinations in all lakes. Significance level α = 0.05

Lake	Benthic primary consumers	Predatory macroinvertebrates	Fish	F	p
1	2.31 (0.34)	2.87 (0.41)	4.09 (0.13)	240.71	<0.001
2	2.21 (0.43)	2.97 (0.52)	3.97 (0.12)	168.10	<0.001
3	2.63 (0.33)	3.23 (0.38)	4.41 (0.14)	172.34	<0.001
4	2.25 (0.21)	3.18 (0.53)	4.22 (0.12)	90.04	<0.001
5	2.40 (0.24)	3.11 (0.30)	4.09 (0.18)	446.22	<0.001
6	2.23 (0.25)	2.91 (0.34)	4.03 (0.16)	269.27	<0.001
7	1.90 (0.26)	2.99 (0.44)	4.00 (0.16)	323.14	<0.001
8	2.08 (0.32)	2.73 (0.25)	3.85 (0.14)	324.13	<0.001
Pooled mean	2.25 (0.30)	2.99 (0.40)	4.08 (0.14)		

for photosynthetic fixation of carbon *in situ* by autotrophic phytoplankton (Jones 1992), limiting autochthonous production. This is consistent with the low chl *a* and high DOC values encountered in our studied lakes.

This study demonstrates that allochthonous matter (leaf litter) is the main food source contributor of benthic primary consumers, although benthic algae also contributed to their diet in Lakes 1 and 2. Also, since primary consumers are prey for predatory invertebrates (Merritt & Cummins 1996) that are, in turn, principal dietary items for fish, allochthonous matter assimilated by primary consumers is transferred up the food web. Benthic invertebrates would be then the primary link between terrestrial carbon and fish. Our results support an emerging consensus that terrestrial and benthic support of lake food webs can be substantial (Vadeboncoeur et al. 2002, Premke et al. 2010, Solomon et al. 2011).

Trophic position

Our results enabled the description of the vertical structure of the food web using $\delta^{15}N$. Benthic primary consumers, predatory macroinvertebrates and fish had mean trophic positions of 2.25, 2.99 and 4.08 respectively. If we consider primary producers as Trophic level 1, then trophic levels of these groups would be 1.25, 1.99 and 3.08 respectively. These results are
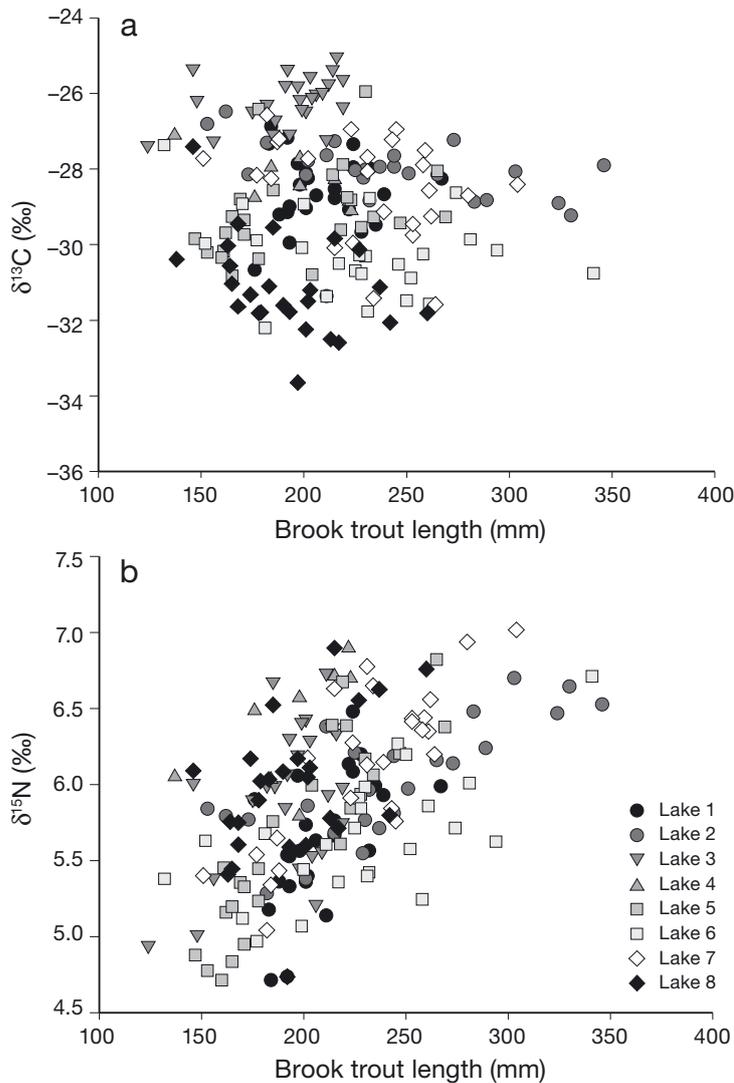
Fig. 2. *Salvelinus fontinalis*. Changes in brook trout (a) carbon ($\delta^{13}C$) and (b) nitrogen ($\delta^{15}N$) stable isotope signatures with increasing fish length in 8 lakes

Table 6. *Salvelinus fontinalis*. Simple regression results of carbon stable isotope ratio ($\delta^{13}C$) against brook trout length in 8 lakes. ** $p < 0.001$, * $p < 0.05$

Lake	n	Slope	r^2	F	p
1	25	0.0008	0.0003	0.0061	0.9383
2	24	−0.0081	0.4058	15.0264	0.0008**
3	25	0.0104	0.1508	4.0847	0.0551
4	8	−0.0173	0.5643	7.7710	0.0317*
5	25	0.0125	0.1436	3.8573	0.0617
6	24	−0.0072	0.0911	2.2056	0.1517
7	25	−0.0125	0.1176	3.0645	0.0934
8	24	−0.0185	0.1899	5.3916	0.0294*
All lakes	180	−0.0054	0.0150	2.7473	0.0992

Table 7. *Salvelinus fontinalis*. Simple regression results of $\delta^{15}N$ vs. brook trout length in 8 lakes. ** $p < 0.001$, * $p < 0.05$

Lake	n	Slope	r^2	F	p
1	25	0.0117	0.3302	11.3376	0.0027**
2	24	0.0045	0.5807	30.4728	<0.0001**
3	25	0.0095	0.2344	7.0408	0.0142*
4	8	0.0086	0.3880	3.8047	0.0990
5	25	0.0152	0.7891	86.0649	<0.0001**
6	24	0.0054	0.3904	14.0915	0.0011*
7	25	0.0114	0.6383	40.5921	<0.0001**
8	24	0.0075	30.2704	8.1535	0.0092*
All lakes	180	0.0064	0.2490	59.3456	<0.0001**

very consistent with traditional food-chain models, which attribute trophic positions of 1, 2 and 3 relative to primary consumers, predatory macroinvertebrates and fish (Vander Zanden & Rasmussen 1999). Our re-sults are also consistent with previous isotopic studies in which predatory macroinvertebrates had higher ^{15}N ratios than primary consumers and fish were en-riched in $\delta^{15}N$ relative to invertebrates (Vander Zan-den & Rasmussen 1999, Herwig et al. 2004, Anderson & Cabana 2007). Brook trout exhibited slightly higher $\delta^{15}N$ enrichment (3.77‰) than the typical assumed shift of 3.4‰ per trophic level (DeNiro & Epstein 1978, Minagawa & Wada 1984). However, Post (2002) calcu-lated a standard deviation associated with this typical value of ±1‰. For predatory macroinvertebrates, the $\delta^{15}N$ fractionation value was lower than expected (2.60‰). Minagawa & Wada (1984) reported a wide range in $\delta^{15}N$ trophic enrichment values across taxa (0 to 9‰). Moreover, $\delta^{15}N$ trophic enrichment was shown to differ between herbivores (mean = 2.5‰) and carnivores (mean = 3.4‰) (Vander Zanden & Ras-mussen 2001), suggesting that in our study omnivory (feeding at more than one trophic level) may be im-portant for predatory macroinvertebrates as well. Brook trout, however, is corroborated as carnivorous.

Brook trout diet breadth

Stable isotopes analyses and mixing models showed that brook trout fed primarily on benthic predatory macroinvertebrates in the 8 Canadian Boral Shield lakes, with a contribution ranging from 60 to 90% of fish diet. While zooplankton contributed 28 to 37% in 3 of 8 lakes, it does not appear to be a main source of energy for fish in our lakes. Brook trout, as with other salmonids, are visual predators and are known to feed on the most abundant and vis-ible food, selecting the largest prey items (Allan

1978). The larger body size of benthic invertebrates relative to pelagic prey makes feeding on benthos more energetically attractive to fish, increasing the efficiency of this trophic link (Vander Zanden et al. 2006). This may explain the greater proportion of benthic macroinvertebrates in fish diet compared to zooplankton in our study, as also shown by other studies (Lacasse & Magnan 1992, Power et al. 2002, Herwig et al. 2004). Brook trout are known to coexist in many lakes with other species, especially the white sucker *Catostomus commersonii*, a specialist benthivore (Scott & Crossman 1973). When living in sympatry with white sucker, brook trout shift their feeding niche from benthic macroinvertebrates to zooplankton in response to interspecific competition for resources (Magnan 1988, Lacasse & Magnan 1992). In this study, only Lake 8 contained both brook trout and white sucker. In this lake, zooplankton represents 32 % of the diet of brook trout according to mixing models. This is consistent to a certain extent with the brook trout niche shift when living in sympatry with white sucker. However, this may not be the only explanation, as in Lakes 1 and 2 zooplankton also contributed to brook trout diet (28 and 37 % respectively), but in these 2 lakes there was no presence of white sucker. Other lake characteristics may influence brook trout planktonic feeding behavior.

In addition, other studies on brook trout have estimated that they consume between 10 (Tremblay-Rivard 2007) and up to 30 % (Vander Zanden et al. 2006) of terrestrial prey. We have not taken this food source into account and as a result may have underestimated the allochthonous matter source contribution to brook trout diet coming in the form of terrestrial insects. Future studies on brook trout feeding should consider sampling terrestrial prey as a potential food source. Invertebrate isotopic signatures vary in the short term, possibly confounding diet comparisons with fish that have much slower tissue turnover rates. As our sampling was conducted in July, stable isotope results would be limited to the understanding of the feeding behavior of brook trout during spring or early summer.

Brook trout size-related diet shifts

Ontogenetic diet shifts and size-selective predation are common among fish (Werner & Gilliam 1984). In our study, δ^{13}C signatures exhibited a complex pattern. δ^{13}C values became significantly more depleted with increasing fish length in 3 lakes, likely indicating a diet shift related to size. Other studies have

shown a significant ^{13}C-enrichment with increasing fish length (Grey 2001, Xu et al. 2007), but they considered both alevin and juvenile stages, which was not the case in our study. When all trout stages were considered, Grey (2001) did not find a particular pattern of ^{13}C values in relation to increasing fish length. Furthermore, a change in δ^{13}C values is indicative of a shift in benthic or pelagic feeding (Vander Zanden et al. 1998) and expecting that a change in diet (to a different trophic level) can occur without a significant change in δ^{13}C may be reasonable. In contrast, in our study, δ^{15}N ratios of brook trout became more positive with increasing fish length in 7 out of 8 lakes. This was also shown in other studies on brook trout (Power et al. 2002, Tremblay-Rivard 2007) and other fish species (Grey 2001, Xu et al. 2007) and is typical of an ontogenetic dietary shift (Power et al. 2002). As a fish increases in length and age, it is capable of handling larger prey items and ingesting later instars of macroinvertebrates and terrestrial insects that fall into the lake (Power et al. 2002).

In conclusion, our study suggests than carbon originating from terrestrial habitat, particularly leaf litter, may significantly contribute to the food web in eastern Canadian Boreal Shield lakes. This study also highlights the importance of carbon produced in benthic habitats to support brook trout in small oligotrophic boreal lakes. Our stable isotope study indicates that benthic macroinvertebrates are important contributors to fish diet. It supports a growing body of literature suggesting that benthic rather than pelagic habitats are the primary energy pathway contributing to fish production (Vadeboncoeur et al. 2002, Herwig et al. 2004, Weidel et al. 2008).

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