Aquatic and terrestrial organic matter in the diet of stream consumers: implications for mercury bioaccumulation

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Abstract. The relative contribution of aquatic vs. terrestrial organic matter to the diet of consumers in fluvial environments and its effects on bioaccumulation of contaminants such as mercury (Hg) remain poorly understood. We used stable isotopes of carbon and nitrogen in a gradient approach (consumer isotope ratio vs. periphyton isotope ratio) across temperate streams that range in their pH to assess consumer reliance on aquatic (periphyton) vs. terrestrial (riparian vegetation) organic matter, and whether Hg concentrations in fish and their prey were related to these energy sources. Taxa varied in their use of the two sources, with grazing mayflies (Heptageniidae), predatory stoneflies (Perlidae), one species of water strider (*Metrobates hesperius*), and the fish blacknose dace (*Rhinichthys atratulus*) showing strong connections to aquatic sources, while *Aquarius remigis* water striders and brook trout (*Salvelinus fontinalis*) showed a weak link to in-stream production. The aquatic food source for consumers, periphyton, had higher Hg concentrations in low-pH waters, and pH was a much better predictor of Hg in predatory invertebrates that relied mainly on this food source vs. those that used terrestrial C. These findings suggest that stream biota relying mainly on dietary inputs from the riparian zone will be partially insulated from the effects of water chemistry on Hg availability. This has implications for the development of a whole-system understanding of nutrient and material cycling in streams, the choice of taxa in contaminant monitoring studies, and in understanding the fate of Hg in stream food webs.

Key words: acidity; allochthonous; autochthonous; benthic invertebrates; diet; mercury; New Brunswick, Canada; pH; streams.

INTRODUCTION

Aquatic ecologists have long sought to determine the importance of in-stream aquatic sources and terrestrial inputs to food webs (Hynes 1975). The strength of terrestrial inputs, particularly in small headwater systems, is believed to regulate community composition and biomass (Vannote et al. 1980), yet the functional importance of these inputs and the implications for higher-order consumers remain relatively unresolved (Wallace et al. 1997, Hall et al. 2001), especially given the presence of the "microbial loop" that may consume a large fraction of C entering the stream as detritus (Hall and Meyer 1998, Lewis et al. 2001).

Analysis of stable isotopes of C ($\delta^{13}C$) have proved useful in delineating the reliance of consumers on the two sources of organic matter in streams, C fixed within the stream, and that fixed outside the stream, which enters as detritus (Finlay 2001). However, the broad range in $\delta^{13}C$ of aquatic vegetation, coupled with the narrow range of $\delta^{13}C$ for terrestrial C and similar average values for both types across systems, has limited

the application of source tracing to specific sites (France 1995, Hall et al. 2001, Jardine et al. 2008b). Recently, however, a new approach has been advanced that uses changes in consumer $\delta^{13}C$ in response to changing periphyton $\delta^{13}C$ across sites as a means to assess the relative contribution of dietary C sources (Rasmussen 2010). This technique allows dominant food source pathways to be attributed to consumers even if there is isotopic overlap among sources, and has the potential to allow further testing of the implications of variable C source use on other end points such as contaminant exposure. Contaminant biomagnification in stream food webs has only recently been explored (Walters et al. 2008a, Chasar et al. 2009, Ward et al. 2010), despite the large number of rivers and streams with health advisories related to contamination of fish with persistent substances like Hg (U.S. EPA 2009).

The fate of Hg is affected by a suite of biological and biogeochemical processes, but is much better understood in aquatic than terrestrial systems. Hg becomes available for uptake into aquatic food webs following atmospheric deposition, weathering, or export from wetlands in association with dissolved organic C (Mierle and Ingram 1991, Munthe et al. 2007). It is subsequently transformed by sulphur-reducing bacteria into the more toxic form, methyl Hg, that biomagnifies through food

webs (Gilmour et al. 1992, Watras et al. 1998). Traditionally it has been assumed that little methylation of Hg occurs in the terrestrial environment (Rudd 1995) and, as such, consumers of terrestrial organic matter have low Hg concentrations; however, recent work has challenged this belief by demonstrating high methyl Hg concentrations in birds that forage in terrestrial environments (Rimmer et al. 2005). Although some recent studies have shown higher contaminants in riparian predators consuming aquatic than terrestrial prey (e.g., Walters et al. 2008*b*), it is not yet known whether aquatic organisms with a reliance on terrestrial energy sources would have different Hg concentrations than those feeding mainly on in-stream sources of production. Critical in our understanding of Hg cycling in streams, therefore, is an evaluation of the role of C sources in determining Hg exposure for stream consumers (Cristol et al. 2008).

Catchment characteristics and resultant water chemistry can have a strong influence on the amount of Hg available to aquatic organisms. Low pH waters that are high in organic C content often originate from wetlands where Hg is readily methylated and exported downstream (Driscoll et al. 1995, Watras et al. 1995). The availability of methyl Hg is further enhanced by acidic conditions due to changes in chemical speciation and resultant solubility at lower trophic levels (Mason et al. 1996). Hg desorbs from soils and forms neutral complexes with chloride ions at low pH (Gabriel and Williamson 2004), making it more available to pass through biological membranes (Bienvenue et al. 1984, Block et al. 1997, Kelly et al. 2003). This in turn leads to higher concentrations in aquatic consumers in acidic waters (Watras et al. 1998, Greenfield et al. 2001), which may also be exacerbated by physiological stress and poorer growth (Greenfield et al. 2001). However, the influence of Hg availability to primary producers on its subsequent bioaccumulation through the food web may depend largely on the route of exposure to the organisms. Because the diet is presumed to be the dominant source of Hg for consumers such as fish (Hall et al. 1997), animals that feed on C sources from outside the stream may not be susceptible to the higher concentrations induced by low pH waters.

Our goals for the current study were to evaluate the use of terrestrial C by stream consumers using the newly developed gradient approach (Rasmussen 2010), and to assess if variability in terrestrial C use affected Hg concentrations in fish and invertebrates. We hypothesized that consumer groups more tightly connected to in-stream C and N sources would be more susceptible to Hg uptake as evidenced by (1) their higher Hg concentrations, and (2) stronger links between their Hg and stream-water pH. We tested this by comparing the strength of periphyton–consumer isotope associations with the strength of the effect of pH on consumer Hg concentrations.

METHODS

Sample collection

Water and biotic samples were collected in August and September from 60 forested first- to fourth-order streams in New Brunswick, Canada (Appendix). Fish were collected with a backpack electroshocker, and two common species were retained (blacknose dace, *Rhinichthys atratulus* and brook trout, *Salvelinus fontinalis*), both insectivores (Garman and Moring 1993, Mookerji et al. 2004). For the purposes of statistical analyses and assuming limited flexibility in feeding, invertebrates were grouped according to functional feeding mode (Merritt and Cummins 1996). Primary consumers included grazing mayflies (Heptageniidae and other Ephemeroptera) and water pennies (Psephenidae), shredding stoneflies (Pteronarcyidae), and filter-feeding mussels (Unionoida). Omnivores included two families of caddisflies, Philopotamidae and Hydropsychidae. Predatory invertebrates included stoneflies (Perlidae), dobsonflies (Megaloptera), dragonflies (Gomphidae, Cordulegastridae, and Aeshnidae grouped as Odonata), and two species of water striders (*Aquarius remigis* and *Metrobates hesperius*). These invertebrates were collected with a D-frame kick net or hand net, coarsely live sorted, and identified to family (or species for water striders) in the laboratory. Periphyton consisted of three composite samples of biofilm, each scrubbed from a minimum of three rocks within a given site. All samples were stored on ice and frozen upon return to the laboratory. Water quality samples were taken in high-density polypropylene bottles and submitted to an analytical laboratory for analysis of pH and total organic C (New Brunswick Department of Environment, Fredericton, New Brunswick, Canada). These two variables were strongly correlated ($r = -0.84$; Appendix), suggesting that both reflect the proportion of wetlands in the catchment (Watras et al. 1995). For the purposes of the current study, we focused on pH in our analyses.

Analysis of stable C and N isotopes and Hg concentrations

Samples were freeze dried and ground to a homogenous powder for analysis of C and N stable isotope composition and total and methyl Hg concentrations using methods detailed in Jardine et al. (2008*b*, 2009). Briefly, C and N isotope data were obtained by combusting small quantities of material (0.2 mg for animals, 1 mg for plants) in a Carlo Erba elemental analyzer (Carlo Erba, Milan, Italy), and resultant gases were delivered to a Thermo Finnigan Delta XP mass spectrometer (Finnigan, Palo Alto, California, USA). Data are reported as delta values relative to International Atomic Energy Agency standards CH6, CH7, N1, and N2 that are calibrated against Peedee belemnite carbonate and atmospheric N. Accuracy and precision were measured using commercially available standards and sample repeats, with standard deviations of 0.2‰

and 0.3‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively (Jardine et al. 2008b). Because of relatively high C/N in some taxa (4 to 7), indicative of moderate lipid loads, all consumer $\delta^{13}C$ data were lipid-corrected using formulas in Logan et al. (2008). All fish and most invertebrates were analyzed for total Hg, a subset of these samples was analyzed for methyl Hg, and all periphyton samples were analyzed for both total and methyl Hg. Fish and invertebrate samples were analyzed for total Hg using a Direct Mercury Analyzer (DMA-80, Milestone Microwave Laboratory Systems, Shelton, Connecticut, USA). Calibration curves were made by plotting the absorbances of certified reference materials (CRMs) DORM-2 (dogfish muscle) and TORT-2 (lobster hepatopancreas) (National Research Council, Ottawa, Ontario, Canada) of varying masses against the mass of Hg in nanograms. These CRMs for calibration covered the range of Hg yielded (0.3–50 ng Hg) from samples with concentrations between 30 and 5000 ng/g weighed at ~0.01 g on a high-precision microbalance (±0.00001 g). For methyl Hg, these samples were extracted in dichloromethane using a procedure outlined in Al-Reasi et al. (2007) and analyzed by GC-MS on a HP 6890 series with HP injector series 7863 (Hewlett Packard Company, Palo Alto, California, USA) (Cai et al. 1997). For the periphyton samples, methyl Hg was measured by GC-AFS on a Brooks Rand Model III (Brooks Rand Labs, Seattle, Washington, USA) following digestion in 25% KOH/MeOH and ethylation with $NaB(C_2H_5)_4$ This method also generates Hg(II) data, and thus total Hg can be calculated from the sum (Liang et al. 1994). Terrestrial leaves were not analyzed for Hg.

All data are reported as micrograms per gram of dry mass. For total Hg data on the DMA-80, recoveries of CRMs analyzed alongside the samples were 93.7% ± 2.9% and 104.0% ± 3.9% (mean ± SD) for DORM-2 (n = 47) and TORT-2 (n = 27), respectively. Blanks were analyzed after every 10 samples and were consistently <10% of sample concentrations; blank values were not used to adjust sample values, and samples were reanalyzed if blanks were high relative to sample concentrations. Sample repeats had a mean difference of 8.7% ± 8.2% (n = 16) and 10.1% ± 6.5% (n = 18) within and across analytical runs, respectively. For methyl Hg analysis of fish and invertebrates, recovery of Hg in the CRM (DORM-2) was 94.1% ± 11.5% (mean ± SD; n = 21). For periphyton, recoveries of the CRM (DOLT) was 97.4% ± 14.1% for methyl Hg and 103.1% ± 11.2% for total Hg (n = 4). Sample repeats had a mean difference of 26.2% ± 11.2% and 18.3% ± 10.5% for methyl Hg and total Hg, respectively. These higher values likely reflected the difficulty in homogenizing samples, and they necessitated the analysis of multiple replicates within each site.

Data analysis

Following the approach detailed in Rasmussen (2010), we used two metrics to evaluate the linkage to aquatic organic matter sources by consumers. The first was the slope of the regression between the $\delta^{13}C$ of periphyton and the $\delta^{13}C$ of the consumer. The second was the coefficient of determination (r^2) for these regressions. Because there is limited trophic fractionation of $\delta^{13}C$ (Post 2002), a species, family, or order that is tightly connected to aquatic C sources will have a slope approaching (or exceeding) one and a high r^2 (Rasmussen 2010). This approach makes the assumption that dietary habits within a given taxon are consistent across sites, evidenced by a failure of higher-order polynomial regressions to account for more of the variance than first-order equations (suggesting a consistent linear response along the gradient [Rasmussen 2010]). We separated taxa at the species (fish and water striders), family (Heptageniidae, Hydropsychidae, Philopotamidae, Psephenidae, Pteronarcyidae, and Perlidae) or order (Odonata, Megaloptera, Ephemeroptera other than Heptageniidae, Unionoida) level depending on their availability across sites, with a minimum n = 5 for isotope regressions.

In addition to the gradient approach, for the fish only, we calculated proportion of aquatic C (P[aquatic]) in the diet using IsoError software (Phillips and Gregg 2001) with one isotope ($\delta^{13}C$) and two sources (aquatic and terrestrial), where $\delta^{13}C$ (terrestrial) was a literature value for $\delta^{13}C$ of terrestrial particulate organic matter (−28.2‰ ± 0.9‰ [mean ± SD], n = 22 [Finlay 2001]) and $\delta^{13}C$ (aquatic) was the $\delta^{13}C$ of periphyton at each site. We used the literature value as the end-member for terrestrial material in our mixing models rather than the mean for fresh alder leaves from our sites (−30.3‰ ± 1.8‰, n = 92; Fig. 1A) because conditioned litter with higher $\delta^{13}C$ is most likely to enter aquatic food webs. Sites where periphyton $\delta^{13}C$ was within 2‰ of terrestrial $\delta^{13}C$ were excluded from this particular analysis (31 of 60 sites [Jardine et al. 2008b]). Fish $\delta^{13}C$ was corrected for diet-tissue fractionation, using a literature value (0.4‰ [Post 2002]) for dace and a laboratory-derived value of 0.7‰ for brook trout that was corrected for lipids in the diet (Jardine et al. 2008a).

To further validate the $\delta^{13}C$ data, we also examined correlations between the $\delta^{15}N$ of periphyton and the $\delta^{15}N$ of consumers. The $\delta^{15}N$ exhibits greater fractionation than $\delta^{13}C$ as it passes through food webs (Post 2002), thus generating more uncertainty in the gradient approach for higher-order consumers. However, in these streams, terrestrial $\delta^{15}N$ is lower and less variable than $\delta^{15}N$ of periphyton (Fig. 1B). Thus, a similar gradient approach can be used across sites despite most having little impact from agriculture and low human density (Anderson and Cabana 2005), with the caveat that some of the variation in consumer–periphyton $\delta^{15}N$ associations may stem from omnivory (i.e., this approach assumes a fixed trophic level for consumers).

We then used regression analyses to examine how Hg concentrations within taxa were related to stream pH, predicting higher Hg concentrations in low pH waters
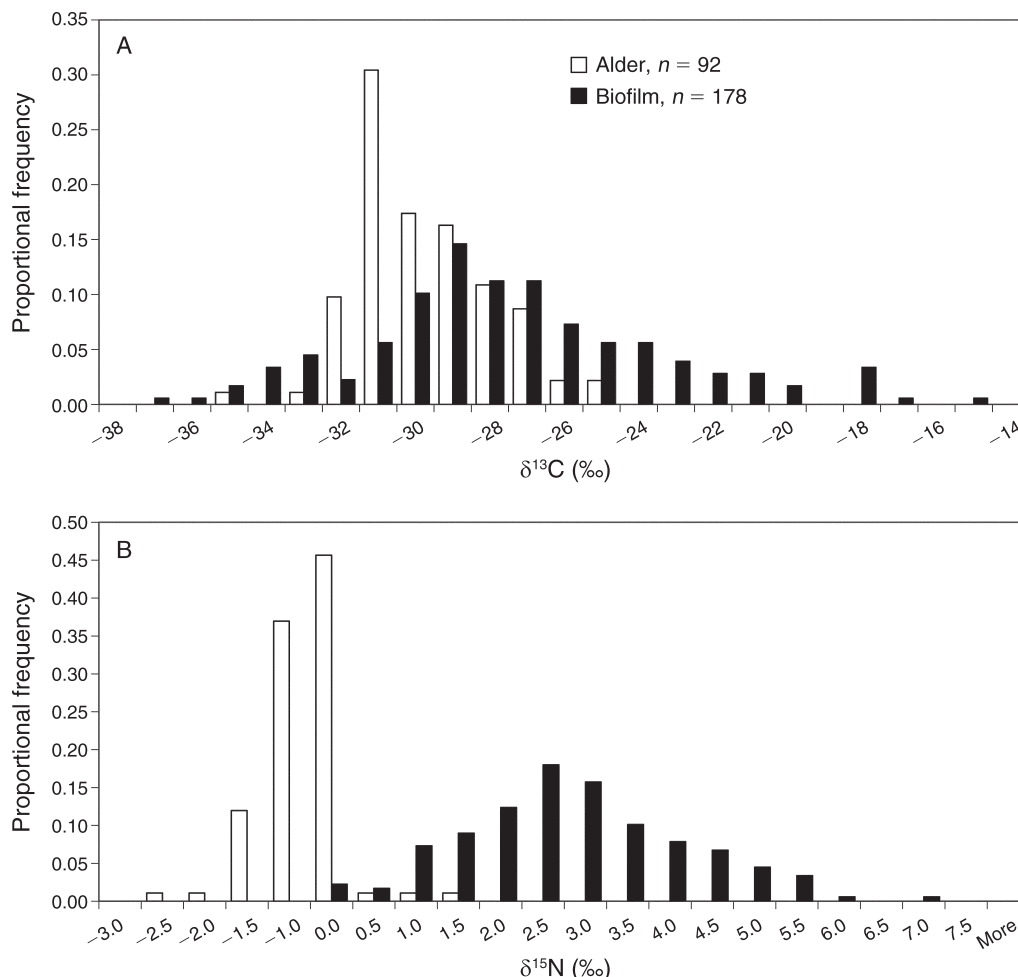
FIG. 1. Frequency histogram for (A) $\delta^{13}C$ and (B) $\delta^{15}N$ of terrestrial (alder) and aquatic (periphyton, biofilm) organic matter sources in New Brunswick, Canada, streams. Proportional frequency is the number of samples that had an isotope value within the range as given in the x-axis, divided by the total number of samples.

for those consumers with a strong connection to in-stream organic matter sources. Our analyses of lower-trophic-level biota are limited to Pteronarcyidae stone-flies and Hydropsychidae caddisflies because other herbivorous and omnivorous taxa (Ephemeroptera, Psephenidae, freshwater mussels, Heptageniidae, and Philopotamidae) were not collected in sufficient quantities at enough sites to generate Hg–pH regressions. For hydropsychid caddisflies we chose to analyze methyl Hg instead of total Hg in the small samples we had available, while for Pteronarcyidae there was sufficient biomass to analyze both methyl and total Hg. This was also true for all predators except the small-bodied water strider *M. hesperius*, for which we only had methyl Hg data from three sites. For fish, a subset of samples (brook trout, $n = 3$; blacknose dace, $n = 29$) analyzed for both methyl and total Hg revealed that all of the Hg was in the methylated form (methyl Hg = 122% ± 4% [mean ± SD] for trout and 108% ± 17% for dace; T. D. Jardine, *unpublished data*), consistent with earlier studies

on Hg speciation in fish (Bloom 1992), so all remaining samples were analyzed for total Hg only.

We used 95% confidence intervals around slopes of best-fit regressions to determine if log Hg–pH relationships differed among predatory invertebrate taxa and between the two fish species, and assessed these slopes in relation to their use of organic matter sources. We then used a one-way analysis of variance (ANOVA) followed by Fisher's LSD multiple comparison test to determine if taxa differed in their mean Hg concentrations (both methyl and total). Because this ANOVA was run to indirectly determine if terrestrial and aquatic sources differ inherently in their Hg concentrations, to remove the confounding effect of pH we only used data from sites with near-neutral pH (7.0–8.0).

RESULTS

Sources of organic matter for consumers

Invertebrate primary consumers in these streams varied in their use of the periphyton organic matter

pathway from taxa that were clearly connected to aquatic sources to those with a partial connection to in-stream production as measured by $\delta^{13}C$ or $\delta^{15}N$ (Table 1). Grazing mayflies (Heptageniidae and other ephemeropterans) were well linked to aquatic organic matter (C, Fig. 2; N, Fig. 3) as evidenced by slopes >0.35 and $r^2 > 0.40$. Surprisingly, both grazing water pennies and filtering freshwater mussels exhibited weak links to aquatic N sources (Fig. 3), despite having strong links to aquatic C sources (Fig. 2). In contrast, shredding pteronarcyid stoneflies had an expectedly weak link to aquatic C (Fig. 2), because they are known to specialize on decaying leaf material of terrestrial origin (Merritt and Cummins 1996), but had a strong connection to aquatic production using $\delta^{15}N$ (Fig. 3). Heptageniidae mayflies had 95% confidence intervals around $\delta^{13}C$ slopes that did not overlap with those of all other herbivorous taxa except Psephenidae. For $\delta^{15}N$, all taxa had overlapping 95% confidence intervals.

In contrast to the primary consumers, there was generally good agreement between $\delta^{13}C$ and $\delta^{15}N$ when assessing organic matter sources for all predatory invertebrates. Stoneflies (Perlidae) were strongly connected to aquatic organic matter, as evidenced by high slopes (0.54 ± 0.11 and 0.77 ± 0.14, respectively) and r^2 (0.53 and 0.60, respectively) for both $\delta^{13}C$ (Fig. 4) and $\delta^{15}N$ (Fig. 5) (Table 1). Intermediate reliance on aquatic production was found for the dragonflies (Odonata) and the water strider *M. hesperius*, with $\delta^{13}C$ slopes and r^2 between 0.44 and 0.63 and $\delta^{15}N$ slopes and r^2 between 0.19 and 0.40 (Table 1). Taxa with the weakest links to aquatic organic matter were *A. remigis* water striders ($\delta^{13}C$ slope = 0.19 ± 0.04, r^2 = 0.29, $\delta^{15}N$ slope = 0.24 ± 0.09, r^2 = 0.12) and dobsonflies (Megaloptera), $\delta^{13}C$ slope = 0.29 ± 0.12, r^2 = 0.31, $\delta^{15}N$ slope = 0.27 ± 0.16, r^2 = 0.17) (Table 1). The strongest contrast between taxa was for Perlidae and *A. remigis*, as there was no overlap in 95% confidence intervals around slope estimates for both $\delta^{13}C$ and $\delta^{15}N$ for these two taxa. All other taxa-by-taxa comparisons had overlapping 95% confidence intervals around the slope estimates.

The two fish species from these streams differed in their use of aquatic organic matter. C isotopes suggested that trout were disconnected from local aquatic C sources, with low slope and r^2, while dace showed strong links to aquatic organic matter (Table 1, Fig. 4). There was no overlap in 95% confidence intervals around $\delta^{13}C$ or $\delta^{15}N$ slopes between the species. These observations were consistent with mixing-model estimates of trout and dace calculated at sites where periphyton and terrestrial $\delta^{13}C$ were distinct. At those sites, P(aquatic) for trout averaged 4.7% ± 13.0% (mean ± SD) and was significantly lower than for dace at 55.6% ± 36.9% (F = 26.2, df = 1, $P < 0.001$). N isotope data were consistent with the $\delta^{13}C$, in that blacknose dace had a stronger link to aquatic sources than did brook trout (Fig. 5).

Hg–pH relationships

Hg concentrations in periphyton were related to pH of the stream. There was a significant negative relationship between periphyton methyl Hg and pH ($F_{1,21}$ = 8.21, $r^2 = 0.28$, $P = 0.009$) and the percentage of methyl Hg and pH ($F_{1,21}$ = 6.95, $r^2 = 0.25$, $P = 0.016$), but no significant relationship between total Hg and pH ($F_{1,21}$ = 0.64, $r^2 = 0.03$, $P = 0.43$) (Fig. 6). Methyl Hg in periphyton ranged from 3% to 27%.

Hg concentrations in some predatory invertebrate taxa were strongly predicted by pH, while others were not (Table 1, Fig. 7). Overall pH was a significant predictor of Hg ($F_{1,104}$ = 13.86, $P < 0.001$) and, although concentrations were not significantly different among taxa ($F_{4,404}$ = 2.12, $P = 0.083$) and there was overlap in 95% confidence intervals around Hg–pH slopes, their individual response to changes in pH was driven by their use of organic matter sources ($F_{1,3}$ = 22.19, $r^2 = 0.88$, $P = 0.018$; Fig. 8). Perlidae, with a high degree of aquatic organic matter use, had total Hg concentrations that increased the most strongly with decreasing pH (slope = −0.45 ± 0.16, $P = 0.012$). This was followed by Odonata (Hg–pH slope = −0.33 ± 0.09, $P = 0.005$) and *M. hesperius* (Hg–pH slope = −0.27 ± 0.12, $P = 0.047$), which both had intermediate use of aquatic organic matter (Table 1). *A. remigis* (slope = −0.09 ± 0.05, $P = 0.077$) and Megaloptera (slope = −0.07 ± 0.15, $P = 0.62$) had Hg–pH slopes that were not significantly different than zero (Table 1), and these two taxa had the lowest use of aquatic organic matter. Patterns for methyl Hg were the same as those for total Hg (Table 1), with pH significantly predicting Hg for all taxa ($F_{1,41}$ = 6.327, $P = 0.016$), but no differences among taxa ($F_{3,41}$ = 1.000, $P = 0.403$).

When data were pooled across all neutral pH streams, total Hg concentrations differed significantly across the predatory invertebrate taxa ($F_{4,84}$ = 2.60, $P = 0.042$, Table 2). For example, total Hg in the water strider *M. hesperius* and in Perlidae were greater than concentrations measured in the other strider species (*A. remigis*). Methyl Hg concentrations, with smaller sample sizes, were not significantly different among taxa ($F_{4,37}$ = 1.52, $P = 0.22$, Table 2) even though trends were the same.

Despite having very different patterns of organic matter use in these streams, both dace and trout had Hg concentrations that were significantly predicted by pH ($F_{1,62}$ = 26.77, $P < 0.001$; Table 1). Overall, dace that were more connected to the aquatic organic matter pathway had higher Hg concentrations compared with trout ($F_{1,62}$ = 4.81, $P = 0.032$). The Hg–pH slope for dace (−0.31 ± 0.07) appeared higher than that for trout (−0.16 ± 0.06), but 95% confidence intervals around slopes overlapped. In streams with pH between 7.0 and 8.0, dace (total Hg = 1.27 ± 0.66 µg/g, $n = 30$) also had significantly higher Hg than trout (total Hg = 0.55 ± 0.29 µg/g, $n = 14$) ($F_{1,42}$ = 26.6, $P < 0.001$).

Table 1. Slopes and coefficients of determination (r^2) for equations relating consumer $\delta^{13}C$ and $\delta^{15}N$ to periphyton $\delta^{13}C$ and $\delta^{15}N$ and corresponding associations between log(Hg) and pH for invertebrates and fish in New Brunswick, Canada, streams.

| | Consumer isotope ratios vs. periphyton† | | | | | Consumer Hg vs. pH | | | | | |
| | $\delta^{13}C$ | | $\delta^{15}N$ | | | Total Hg | | | Methyl Hg | | |
Taxon	Slope (SE)	r^2	Slope (SE)	r^2	n	Slope (SE)	r^2	n	Slope (SE)	r^2	n
Primary consumers											
Mayflies (Heptageniidae)	**1.03 (0.13)**	0.72	**0.60 (0.15)**	0.40	27						
Mayflies (Ephemeroptera)	**0.36 (0.13)**	0.52	**1.16 (0.27)**	0.73	7						
Water pennies (Psephenidae)	0.85 (0.38)	0.60	−0.17 (0.31)	0.09	5						
Stoneflies (Pteronarcyidae)	0.20 (0.19)	0.12	**0.57 (0.17)**	0.69	6	−0.88 (0.54)	0.40	6	0.03 (0.36)	0.00	5
Mussels (Unionoida)	**0.37 (0.12)**	0.71	0.23 (0.33)	0.11	6						
Omnivores											
Caddisflies (Philopotamidae)	**0.97 (0.40)**	0.41	0.35 (0.27)	0.18	10						
Caddisflies (Hydropsychidae)	**0.63 (0.09)**	0.59	**0.74 (0.12)**	0.52	35				−0.20 (0.11)	0.26	12
Predators											
Stoneflies (Perlidae)	**0.54 (0.11)**	0.53	**0.77 (0.14)**	0.60	23	**−0.45 (0.16)**	0.26	24	−0.39 (0.20)	0.21	16
Dobsonflies (Megaloptera)	**0.29 (0.12)**	0.31	0.27 (0.16)	0.17	15	−0.07 (0.15)	0.02	16	−0.05 (0.17)	0.01	13
Dragonflies (Odonata)	**0.44 (0.10)**	0.63	0.40 (0.26)	0.19	13	**−0.33 (0.09)**	0.52	13	**−0.34 (0.12)**	0.59	8
Water striders (*Aquarius remigis*)	**0.19 (0.04)**	0.29	**0.24 (0.09)**	0.12	52	−0.09 (0.05)	0.07	49	−0.10 (0.17)	0.04	12
Water striders (*Metrobates hesperius*)	**0.45 (0.10)**	0.58	**0.40 (0.15)**	0.35	15	**−0.27 (0.12)**	0.34	12			
Fish											
Blacknose dace	**0.52 (0.08)**	0.52	**0.58 (0.09)**	0.53	39	**−0.31 (0.07)**	0.34	41			
Brook trout	−0.01 (0.10)	0.00	0.18 (0.10)	0.15	23	**−0.16 (0.06)**	0.27	25			

Note: Slopes in boldface type are significantly different from zero ($P < 0.05$).
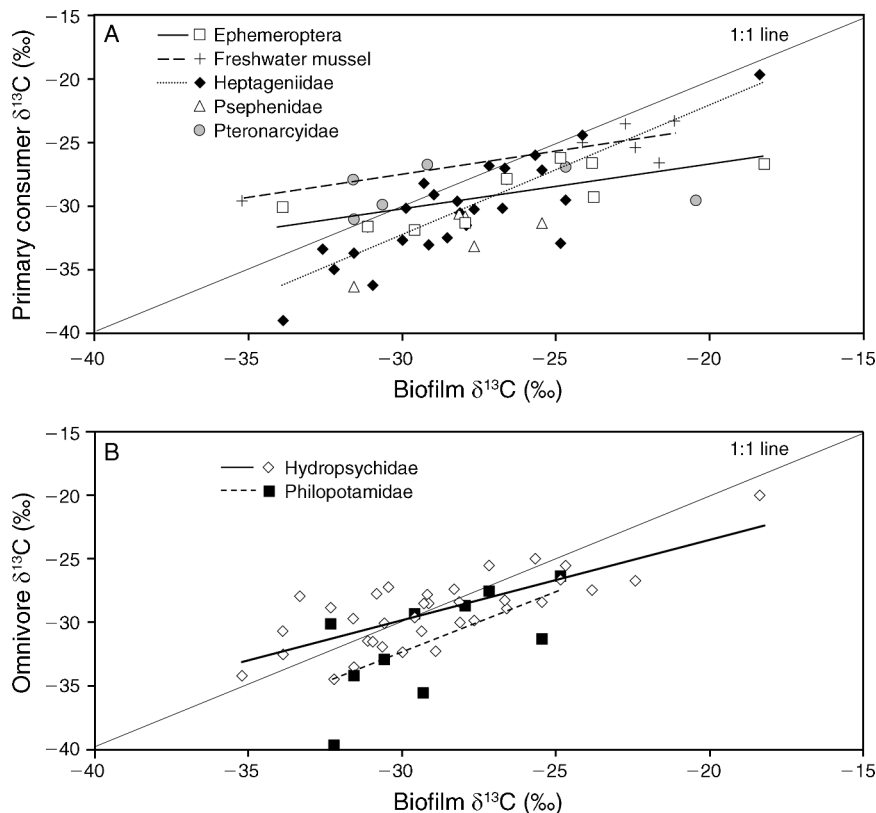† $\delta^{13}C$ and $\delta^{15}N$ are measured simultaneously on a single sample so n is the same for both isotopes.

Fig. 2. Correlations between $\delta^{13}C$ in consumers and $\delta^{13}C$ in periphyton (biofilm) from New Brunswick, Canada, streams. Each point represents a mean or pooled value for a site for (A) primary consumers and (B) omnivores. Slopes and r^2 values are provided in Table 1, and best-fit lines are shown when slopes differ significantly from zero ($P < 0.05$).
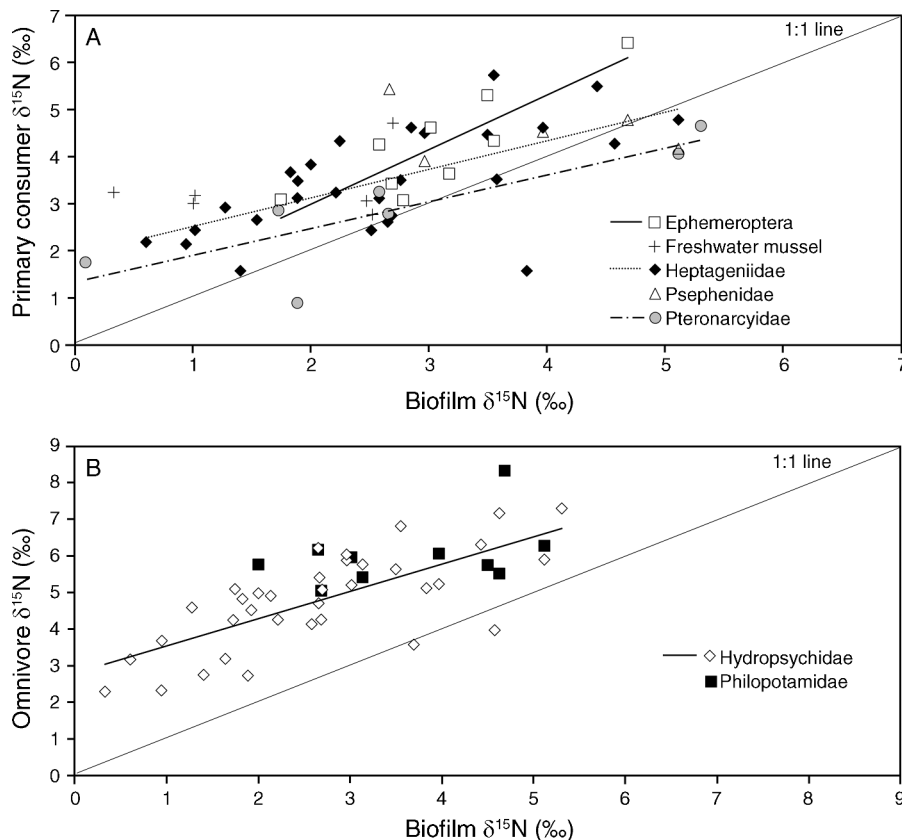
FIG. 3. Correlations between $\delta^{15}N$ in consumers and $\delta^{15}N$ in periphyton from New Brunswick, Canada, streams. Each point represents a mean or pooled value for a site for (A) primary consumers and (B) omnivores. Slopes and r^2 are provided in Table 1, and best-fit lines are shown when slopes differ significantly from zero ($P < 0.05$).

DISCUSSION

Our data provide new insights into the role of varying organic matter sources in stream food webs, and highlight one of the implications of variable dietary sources, a reduced effect of acidic conditions on Hg concentrations in consumers linked to terrestrial material. Predatory invertebrates that were more strongly linked to aquatic C and N sources (Perlidae, Odonata, *M. hesperius*) showed a greater increase in Hg concentrations per unit decrease in pH compared to those predators that had weak links to aquatic organic matter (Megaloptera, *A. remigis*) (Fig. 8). The two fish species also followed the same trend, with dace that were more strongly connected to aquatic sources having much higher Hg concentrations than trout. Increased Hg concentrations in periphyton in low pH waters is the apparent mechanism for the stronger consumer Hg–pH response in taxa dependent on aquatic sources of production. These findings have implications for the choice of appropriate taxa for monitoring studies of aquatic food webs and in understanding fluxes of contaminants between land and water and within aquatic food webs.

Despite being captured in similar habitats, predatory invertebrates obtained varying amounts of C and N from aquatic and terrestrial sources. Perlidae showed the strongest dietary link to aquatic organic matter, which is not surprising given their tendency to consume small mayflies and chironomids (Bo et al. 2008). The preferred prey of megalopterans, meanwhile, has rarely been studied. Their weak link to aquatic C sources indicates feeding on either terrestrial insects that fall into the stream or predation on aquatic insects that themselves consume terrestrial organic matter. The latter is more likely the case, and Pteronarcyidae stoneflies are a possible candidate prey family. Megalopterans are large enough to feed on these shredding stoneflies, and occurred at six of the seven sites where Pteronarcyidae were collected (T. D. Jardine, *unpublished data*).

Using $\delta^{15}N$ in conjunction with $\delta^{13}C$ helped to confirm feeding linkages in these streams for some organisms, but the two sometimes provided conflicting information. For example, Pteronarcyidae stoneflies showed a weak link to aquatic organic matter when using $\delta^{13}C$, but the link with $\delta^{15}N$ was strong. This may support the assumption that shredders use terrestrial detritus (either directly or via bacterial assimilation) as a C source but obtain their N solely from bacteria
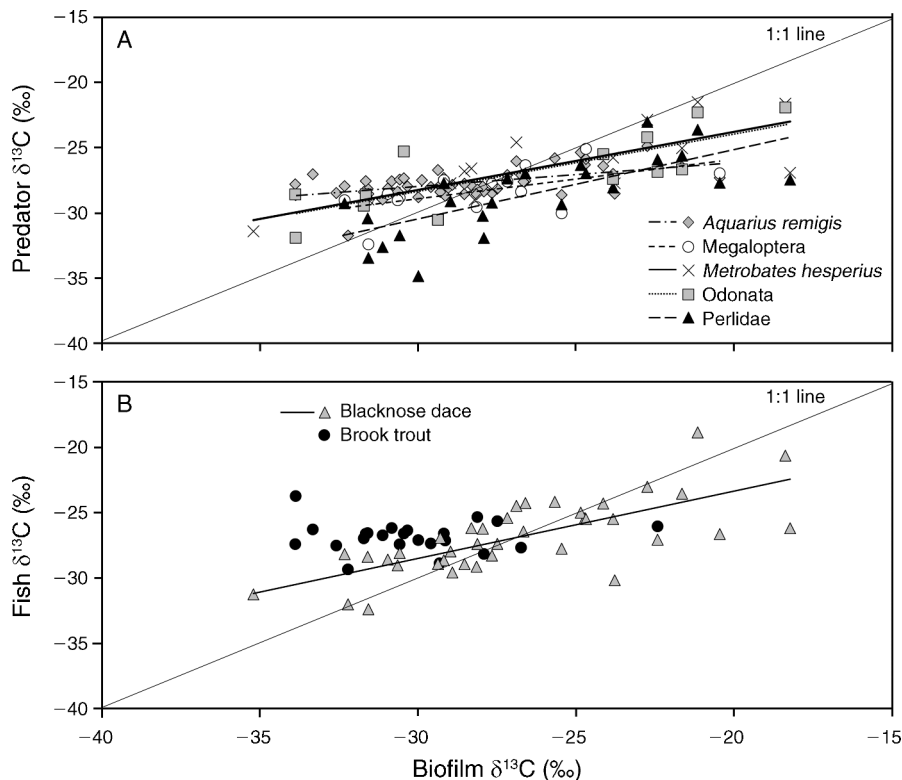
FIG. 4.   Correlations between $\delta^{13}C$ in consumers and $\delta^{13}C$ in periphyton from New Brunswick, Canada, streams. Each point represents a mean or pooled value for a site for (A) predatory invertebrates and (B) fish. Slopes and r^2 values are provided in Table 1, and best-fit lines are shown when slopes differ significantly from zero ($P < 0.05$).

(Anderson and Cabana 2007). These bacteria would likely have $\delta^{15}N$ similar to algae because they can assimilate N directly from the water (Gulis and Suberkropp 2003). However, the $\delta^{15}N$ gradient was less pronounced than that for $\delta^{13}C$ ($\delta^{13}C$ range in periphyton was 17‰; $\delta^{15}N$ range in periphyton was 7.5‰), and there are confounding effects of trophic fractionation and food chain length (Cabana and Rasmussen 1994). This makes $\delta^{15}N$ a less reliable indicator of source organic matter use by consumers and may explain poor relationships between periphyton $\delta^{15}N$ and the $\delta^{15}N$ of known grazers (e.g., Psephenidae). The use of $\delta^{15}N$ in this capacity could be improved by including a broad range of streams and rivers that span a considerable disturbance gradient from forested to agricultural catchments (Anderson and Cabana 2005). Across this type of gradient, owing to N loading and enhanced N cycling in more disturbed sites, a range in $\delta^{15}N$ of >12‰ can be expected (Cabana and Rasmussen 1996, Jardine et al. 2006). Understanding the relative use of N from the two organic-matter sources is important because animals are far more likely to be N-limited than C-limited and, as such, constraints on growth and reproduction are more likely linked to N use (Elser et al. 2000).

The two water strider species differed in their use of terrestrial and aquatic sources, and this translated into

differences in the effect of pH on their Hg concentrations. Differences in source proportions between the two species may be a function of where in the river network they occur, as *A. remigis* is typically found in headwater streams where the input of terrestrial insects is higher, while *M. hesperius* prefers lowland pool habitats with higher aquatic insect emergence; only rarely do they co-occur (Jardine et al. 2008b). Accordingly, in the data available here, *M. hesperius* clearly used more aquatic organic matter than *A. remigis*. These two species also are known to occupy different microhabitats within the stream, with *A. remigis* more associated with the edges of riffle areas and *M. hesperius* occurring on the surfaces of larger pools.

Blacknose dace accumulated far more Hg than brook trout despite the small body sizes of dace and similar trophic position for the two species, and all Hg in dace tissues was methyl Hg, supporting a dietary source of Hg (Watras and Bloom 1992). Dace are more sensitive to acidic conditions in streams than are trout (Simonin et al. 1993). Higher plasma sodium concentrations have been observed in dace from low-alkalinity waters (Dennis and Bulger 1995), suggesting that dace have difficulty with ion regulation in low pH streams. Indeed, no dace were captured in streams that had pH <6 and where trout were collected (T. D. Jardine, *unpublished data*). This suggests that the pH threshold for dace
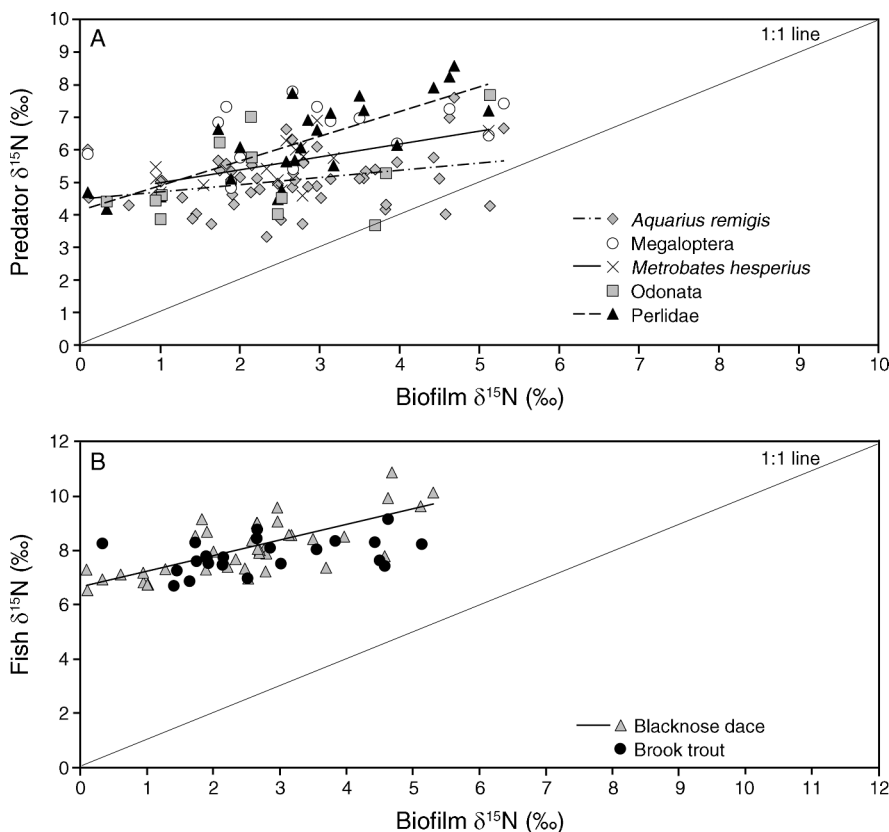
Fig. 5. Correlations between $\delta^{15}N$ in consumers and $\delta^{15}N$ in periphyton from New Brunswick, Canada, streams. Each point represents a mean or pooled value for a site for (A) predatory invertebrates and (B) fish. Slopes and r^2 values are provided in Table 1, and best-fit lines are shown when slopes differ significantly from zero ($P < 0.05$).

survival is higher than that of trout and, when encountering waters with pH near this threshold, Hg concentrations in dace increase markedly, possibly due to an enhanced stress response and the higher proportion of aquatic C in their diet that contains more Hg in low pH waters.

Our data suggest that invertebrates relying on aquatic organic matter sources show an enhanced Hg response to declining stream pH, but that in neutral waters (pH 7.0 to 8.0) there are no clear differences in Hg concentrations in organisms at the same trophic level despite different C sources. *A. remigis* had the lowest Hg concentrations and a weak link to aquatic organic matter, whereas *M. hesperius* had higher concentrations and a stronger link to in-stream production; these data suggest that organic matter fixed within the stream may be higher in Hg (Jardine et al. 2008*b*, 2009). In contrast, there were no differences in Hg concentrations between megalopterans and perlids at near neutral pH despite the latter exhibiting a much stronger connection to in-stream organic matter sources. Furthermore, Hg concentrations in obligate scrapers (Psephenidae, methyl Hg = 0.06 and 0.08 μg/g, $n = 2$ pooled samples) and shredders (Pteronarcyidae, methyl Hg = 0.05 ± 0.03 μg/g, $n = 5$ pooled samples) were similar, providing

evidence for similar baseline Hg concentrations in aquatic and terrestrially derived food chains when pH is near-neutral.

Bioaccumulation of Hg from the two possible source pathways will likely depend on catchment characteristics and proximity to aquatic Hg discharges. The majority of
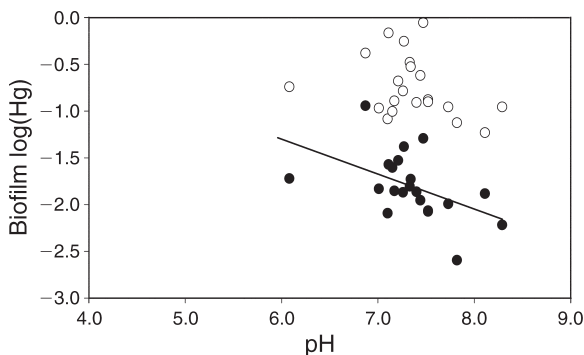

Fig. 6. The relationship between total Hg (open circles) and methyl Hg (solid circles) in periphyton and pH in New Brunswick, Canada, streams, where Hg was measured as μg/g dry mass. Each point represents a mean value from a site. The best-fit line for methyl Hg is shown ($P < 0.05$). The regression with total Hg was not significant.
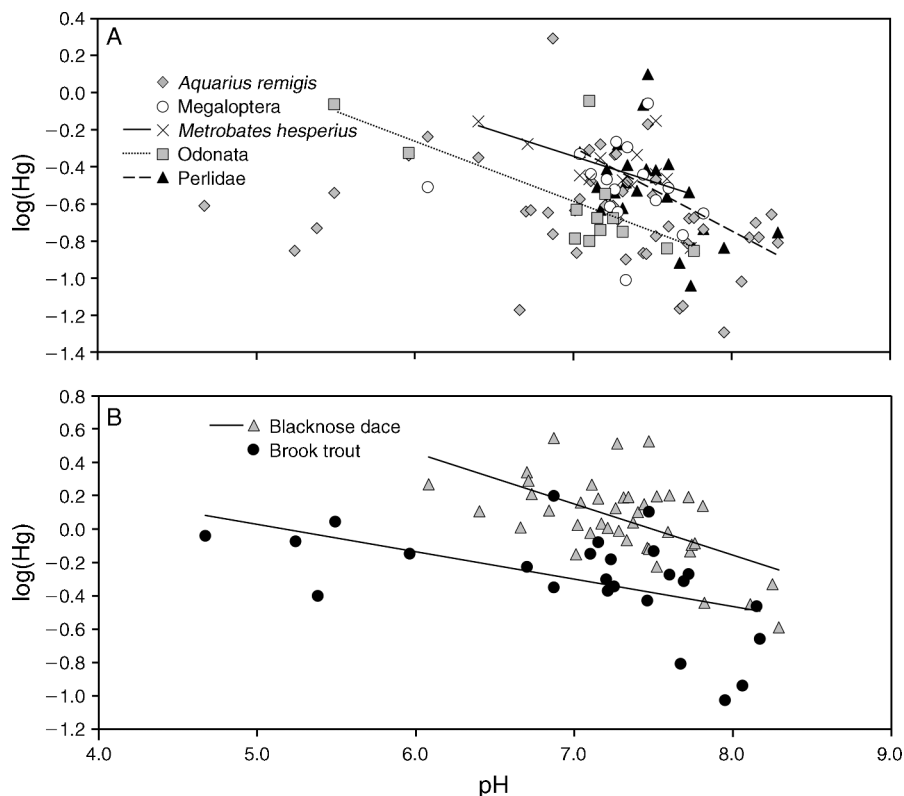
Fig. 7. The relationship between total Hg in consumers (log-transformed, where Hg was measured as µg/g dry mass) and pH from New Brunswick, Canada, streams. Each point represents a mean or pooled value for a site for (A) predatory invertebrates and (B) fish. Slopes and r^2 values are provided in Table 1, and best-fit lines are shown when slopes differ significantly from zero ($P < 0.05$).

Hg deposition from atmospheric sources ultimately ends up on the forest floor (St. Louis et al. 2001, Hintelmann et al. 2002). As such, decomposing leaf litter and other organic material can have appreciable concentrations of Hg that increase as decomposition proceeds (Demers et al. 2007). However, the proportion of the total Hg as methyl Hg in terrestrial vegetation is typically quite low, always being <10% (Hall and St. Louis 2004). Therefore animals consuming the leaf material directly are unlikely to be exposed to high concentrations of methyl Hg. Hg concentrations in aquatic primary producers (periphyton and filamentous algae) are typically similar to those
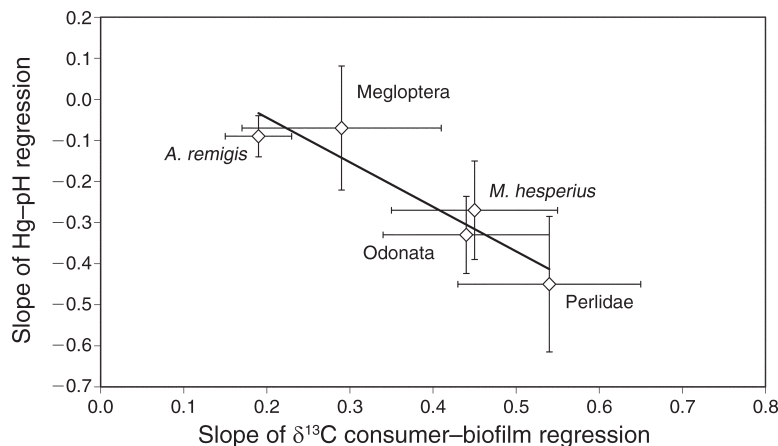

Fig. 8. The relationship between the proportion of aquatic C in the diet (estimated from the slope of consumer δ^{13}C vs. periphyton δ^{13}C) and the response of predatory invertebrate Hg concentrations to changes in pH (Hg–pH slope; mean ± SE). Each point represents a single taxon.

TABLE 2. Total and methyl Hg concentrations of periphyton, predatory invertebrates, and fish in New Brunswick, Canada, streams with pH 7.0–8.0.

Taxon	Total Hg (µg/g dry mass)			Methyl Hg (µg/g dry mass)		
	Mean	SE	n	Mean	SE	n
Periphyton	0.26	0.06	17	0.02	0.01	17
Predators						
Water striders (*A. remigis*)	0.26[a]	0.04	30	0.21[x]	0.07	6
Dragonflies (Odonata)	0.26[acd]	0.06	11	0.20[x]	0.06	6
Dobsonflies (Megaloptera)	0.35[abcd]	0.05	15	0.28[x]	0.04	12
Stoneflies (Perlidae)	0.37[bd]	0.04	23	0.31[x]	0.05	15
Water striders (*M. hesperius*)	0.38[b]	0.06	10	0.34[x]	0.05	3
Fish						
Brook trout (*S. fontinalis*)	0.55[e]	0.08	14			
Blacknose dace (*R. atratulus*)	1.36[f]	0.12	30			

Note: Means followed by the same lowercase letters are not significantly different ($P \geq 0.05$).

found in leaf litter, with values ranging from 10 to 100 ng/g dry mass (Tsui et al. 2009). Yet in certain environments these aquatic primary producers can have values that exceed this range. For example, *Cladophora* spp. in highly productive reaches of the South Fork Eel River had concentrations >200 ng/g, and all of this Hg was in the methyl form (Tsui et al. 2009).

These observations and the results from the current study suggest that aquatic and terrestrial organic matter have similar Hg concentrations at near-neutral sites. However, because consumers generally obtain Hg from their diet and not directly from the water (Hall et al. 1997) at those locations where chemical conditions in the water promote higher Hg in periphyton (e.g., wetlands [Rudd 1995], mining discharges [Hill et al. 1996, Zizek et al. 2007]), this change will be more strongly reflected in those consumers reliant on the aquatic food source pathway (Chasar et al. 2009). Acidified lakes also have higher Hg in phytoplankton compared with neutral lakes (Watras et al. 1998, Watras and Bloom 1992), suggesting that the supply of Hg to lower trophic levels is higher in low pH systems (Mason et al. 1996). This was confirmed in our study streams by the significant negative relationship between stream pH and the methyl Hg concentrations (and percentage of methyl Hg) of periphyton. Although exposure via the gills may be important under certain conditions (Block et al. 1997), leading to significant Hg–pH relationships that are independent of diet (e.g., trout; Fig. 7B), consumers that use organic matter originating in the terrestrial environment, particularly insects that fall into the stream (Mookerji et al. 2004), will be insulated to a degree from the effects of increased Hg availability in the water. This has implications for the choice of taxa in research and monitoring studies that seek to determine the effects of a variety of factors on Hg in aquatic food webs (Greenfield et al. 2001) and those assessing effects of mining discharges (Nevado et al. 2010).

These results may also be extended to our understanding of the dynamics of other contaminants in stream food webs. For example, at sites heavily contaminated with PCBs, riparian predators had PCB concentrations that were directly linked to their consumption of emerging aquatic insects (Walters et al. 2008b). In that study, estimation of the export of PCBs from the stream to the riparian zone was made possible by differences in $\delta^{13}C$ and $\delta^{15}N$ that existed between aquatic and terrestrial sources of organic matter. A broad-scale estimate of these types of fluxes between streams and the riparian zone can be achieved by using the gradient approach presented here accompanied by measurements of contaminant concentrations in biota from the two habitats.

The broad variation in the use of aquatic organic matter by consumers living in close proximity in small-stream ecosystems underscores the importance of considering intertaxa variation in large-scale models of river function (e.g., Vannote et al. 1980). Furthermore, the isotope gradient approach helps to identify sources of organic matter fueling growth of consumers, allowing for calculation of the contribution of these sources to the biomass present at any given location in a river network (Rasmussen 2010). These techniques will help to further refine our understanding of the fluxes of organic matter and contaminants across ecological interfaces.

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SUPPLEMENTAL MATERIAL

Appendix

Location, stream order, pH, and total organic carbon (TOC) of sites sampled in this study (*Ecological Archives* A022-046-A1).