An elemental and stable isotope assessment of water strider feeding ecology and lipid dynamics: synthesis of laboratory and field studies

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SUMMARY
1. Despite the ubiquity and abundance of water striders (Hemiptera: Gerridae) in temperate streams and rivers and their potential usefulness as sentinels in contaminant studies, little is known about their feeding ecology and lipid dynamics.
2. In this study we used stable isotopes of carbon (δ13C) and nitrogen (δ15N) and elemental carbon to nitrogen ratios (C/N) to assess dietary habits and lipid content, respectively, for water striders.
3. To determine diet-tissue fractionation factors, nymphs of the most common species in New Brunswick, Canada, Aquarius remigis were reared in the laboratory for 73 days and exhibited rapid isotopic turnover in response to a switch in diet (C half-life = 1.5 days, N half-life = 7.8 days). Their lipid content increased towards the end of the growing season and resulted in lower δ13C values. Diet-tissue fractionation factors were established after correction of δ13C data for the confounding effect of de novo lipid synthesis (strider δ13Cadj – diet δ13Cadj = 0.1‰, strider δ15N – diet δ15N = 2.7‰).
4. Water striders from the majority of 45 stream sites (83%) in New Brunswick had less than 50% contribution of aquatic carbon to their diets but showed a gradual increase in the contribution of this carbon source to their diet with increasing stream size.
5. These data indicate that striders exhibit a strong connection to terrestrial carbon sources, making them important users of energy subsidies to streams from the surrounding catchment. However, this dependence on terrestrial organic matter may limit their utility as indicators of contamination of aquatic systems by heavy metals and other pollutants.

Keywords: Aquarius remigis, C/N, diet-tissue fractionation, organic matter, stable isotopes, streams

Introduction
The ability to study the ecology of wild animal populations has been expanded greatly in the last 25 years through the use of stable isotope analysis (SIA). SIA has improved our understanding of energy pathways (e.g. Hecky & Hesslein, 1995), animal movement patterns (Hobson, 1999), effects of invasive species (e.g. Vander Zanden, Casselman & Rasmussen, 1999) and contaminant biomagnification (e.g. Cabana & Rasmussen, 1994). Accurate measurements of food source pathways are important in conservation and management (Araujo-Lima et al., 1986) and in understanding larger patterns in food webs (Post, Pace & Hairston, 2000). Stable carbon isotope ratios (13C/12C or δ13C) typically reveal carbon sources at the base of
food webs (Hecky & Hesslein, 1995; Bastviken et al., 2003) because they show little change during food web transfer and sources of primary production often differ in their $\delta^{13}$C, while stable nitrogen isotope ratios ($^{15}$N/$^{14}$N or $\delta^{15}$N) are indicators of trophic position, as $^{15}$N becomes enriched approximately 3.4% with each step up a food chain (Post, 2002).

Over time, our ability to use SIA to answer ecological questions has improved through the use of rearing experiments that have established diet-tissue fractionation (Post, 2002) and elemental turnover rates (Dalerum & Angerbjorn, 2005) for the target species and tissues of interest (Hobson & Clark, 1992), the advent of mathematically sound mixing models to assess diet (Phillips & Gregg, 2001), and the means to account for the effects of endogenous lipid synthesis on tissue $\delta^{13}$C values (Kiljunen et al., 2006). However, many of the laboratory rearing experiments were aimed at understanding isotopic fractionation and turnover in birds (e.g. Hobson & Clark, 1992) and fishes (e.g. Suzuki et al., 2005), with fewer studies on insects (e.g. Ostrom, Colunga-Garcia & Gage, 1997). Furthermore, the uncertainty surrounding literature-based estimates of diet-tissue fractionation can often impair precise estimates of diet for field populations for which no diet-tissue fractionation estimate is available (Jardine, Kidd & Fisk, 2006). This necessitates the use of rearing experiments to establish diet-tissue fractionation in cases where there is an interest in inferring field diets using stable isotopes.

In addition to its traditional application of answering ecological questions, SIA has proven useful as a tool to understand the nutritional state of consumers, including the recycling of proteins during starvation (Hobson, Alisauskas & Clark, 1993) and the synthesis and storage of lipids (Cherel et al., 2005). Elemental carbon to nitrogen ratios (C/N) are indicators of lipid content of an organism (McConnaughey & McRoy, 1979), with higher C/N values associated with higher % lipid due to the presence of long chain fatty acids and a low proportion of N-bearing proteins (Campbell, 1995). Given that C/N data are often provided alongside $\delta^{13}$C and $\delta^{15}$N, a simultaneous assessment of diet and lipid content can be conducted (Cherel et al., 2005).

Water striders (Hemiptera: Gerridae) are predaceous insects that are ubiquitous and abundant on lakes and slow-moving sections of streams and rivers of temperate and tropical regions (Spence & Andersen, 1994). In North America, adults of one common species (Aquarius remigis Say) overwinter and return to streams following ice-out (Fairbairn, 1985). They breed and then senesce in the spring, producing a new generation of nymphs by mid-summer that store lipids in late summer after their development to adulthood (Lee, Polhemus & Cheng, 1975). While it is generally known that water striders feed mainly upon terrestrial and aquatic insects trapped in the surface film of the streams (Matthey, 1974; McLean, 1990), little is known about the relative contributions of these two food source pathways. Striders make up a large proportion of the biomass of insects in many stretches of small rivers and streams (Svensson, Danielsson & Rydell, 2002). Consequently, striders could serve as general indicators of the overall flow of energy to predatory insects and fishes, and may also potentially be used for determining if changes occur in the importance of different carbon sources in an upstream-downstream direction in rivers (Vannote et al., 1980; Thorp & Delong, 1994). Due to their ubiquity and abundance and potential importance as conduits of energy into small streams, water striders therefore offer a good model to study feeding ecology and nutritional status in animal populations. Striders are also recently gaining attention as environmental sentinels for heavy metal contamination (Jardine et al., 2005; Nummelin et al., 2007). Because much of the contaminant burden in an animal comes from its diet (Hall et al., 1997) and because contaminant concentrations can be affected by growth, sex and season, it is important to understand strider dietary and life-history patterns.

In order to investigate feeding ecology in the most common water strider species in New Brunswick streams (A. remigis) and the relative importance of terrestrial and aquatic carbon to its diet in time and space, we began by conducting a laboratory diet-switch experiment to measure diet-tissue isotope fractionation and turnover rates. We then repeatedly sampled striders and potential food sources from four NB stream sites over the growing season to determine the temporal changes in isotope ratios and C/N in field populations. Adult gerrids in temperate climates typically have one to three generations per year (Galbraith & Fernando, 1977; Fairbairn, 1985), and they accrue lipids over the course of the growing season prior to overwintering (Lee et al., 1975). We therefore predicted that C/N, a proxy for lipid content
(McConnaughey & McRoy, 1979), would increase from spring to autumn. Finally, we sampled water striders 
(mainly *A. remigis* but also the less common *Metrobates hesperius* Uhler) and aquatic vegetation from a large number of streams representing a range of sizes and 
used SIA to assess food source pathways for striders in relation to the size of the system in which they were 
living. These studies were conducted to better understand the feeding ecology of the water strider and 
illustrate the value in pairing field and laboratory observations to better understand ecological and 
nutritional processes in a predatory aquatic invertebrate.

**Methods**

We conducted a rearing experiment in 2006 by capturing water strider nymphs from a New Brunsw 
wick stream (Cow Pasture Brook, 45.95N 66.23W), placing them in two plastic tanks (volume = 48 L, 
initial density c. 40 individuals per tank) with standing water and feeding them crickets obtained from a 
single batch at a commercial pet food store. Striders were held on the diet for approximately 72 days, with 
samples (*n* = 2) removed from each of the two tanks on days 0, 1, 2, 4, 8, 16, 23, 31, 41, 48, 54 and 61, and 
from one tank on days 68 and 73. The amount of food added to the tanks was adjusted over the course of the 
experiment in response to decreased strider densities, with approximately one cricket added for every two 
striders present. Striders from the source stream (Cow Pasture Brook) were also re-sampled on day 51 of the 
experiment to compare isotope ratios with changes observed as a result of the diet-switch in the laborato 
y.

In the field, water striders were collected using hand nets from 81 stream sites in New Brunswick, 
Canada in the months of September and October from 2004 to 2007. Thirty-four sites were sampled in 2004, 
nine in 2005, 24 in 2006 (with a return visit to one site), and 26 in 2007 (including return visits to 11 sites). 
When sites were sampled in more than 1 year, data were combined to yield one point for each site 
assuming no difference between years. Streams were assigned orders manually with maps at the 1 : 150 000 
scale. Sites ranged from 1st to 6th order systems, varied in width from one to >50 m, and had limited or 
no catchment disturbance from forestry, agriculture or urbanization. An additional four sites (Corbett Brook, 
45.92N 66.64W, English Brook 46.43N 66.60W, McKenzie Brook 46.22N 66.53W and Parks Brook 
45.46N 66.35W) were sampled approximately bi-weekly from May to October 2007.

*Aquarius remigis* was the most commonly captured water strider, appearing at 73 of the 81 sites 
(including the four index sites). At the index sites, in two instances (July 3rd at Parks Brook, July 17th 
at Corbett Brook) only nymphs were in sufficient abundance at the time of collections. The other, 
smaller-bodied, gerrid species (*M. hesperius*) occurred at 16 sites (including Parks Brook, one of the 
index sites). The two species were found together at only six sites. At each site where striders were 
captured, multiple samples of two individuals each were submitted for SIA. In 2004 and 2005 sexes were 
not identified, while in 2006 and 2007 sexes were analysed separately.

To characterize the different carbon sources in these streams, we collected aquatic mosses (*Fontinalis* sp.), 
macrophytes (e.g. *Potomogen* sp.), and filamentous algae (e.g. *Cladophora* sp.) by hand from each site in 
2004. In the years 2005–07 aquatic vegetation was sampled by scrubbing rocks with a toothbrush to 
collect biofilm (*n* = 3 samples of a minimum of three rocks per sample). Biofilm is an amalgamation of 
different types of aquatic vegetation (diatoms, moss, filamentous algae) as well as fungi and bacteria 
(Battin et al., 2003), and thus is representative of in-stream primary production. Any carbon fixed 
within the stream was therefore considered aquatic carbon. The pH of the streams ranged from 4.7 to 8.1, 
with the majority of values between 6.5 and 7.5. Most streams were therefore circumneutral or acidic and 
calcareous soils are uncommon in New Brunswick, hence we did not acidify our biofilm samples to 
remove carbonates that can confound δ13C.

All samples for SIA were oven-dried at 60 °C for 48 h and ground to a powder using a mortar and pestle, or 
ball-mill grinder. Samples were analysed for stable carbon and nitrogen isotope ratios by combustion in a 
Carlo Erba NC2500 Elemental Analyzer and delivery to a Finnigan Mat Delta Plus mass spectrometer (Thermo 
Finnigan, Bremen, Germany) via continuous flow. Data are reported as delta values relative to international 
standards Peedee Belemnite Carbonate (PDB) and atmospheric nitrogen (AIR) and calibrated using 
International Atomic Energy Agency standards CH6 
(−10.4‰), CH7 (−31.8‰), N1 (0.4‰) and N2 (20.3‰).
A single water strider sample (working laboratory standard) was analysed every time samples were run to assess precision across analytical runs, and yielded values of $\delta^{13}C = -27.53 \pm 0.20\%_o$ (unless otherwise indicated, data are shown as mean ± SD) and $\delta^{15}N = 4.21 \pm 0.65\%_o$ ($n = 11$). The poorer precision in $\delta^{15}N$ was due to a single analysis of this standard, and several samples analysed in that particular run were repeated to ensure values were comparable with other analytical runs. A commercially available standard (acetanilide, Elemental Microanalysis, Ltd., Okehampton, U.K.) analysed alongside the samples yielded values of $\delta^{13}C = -33.60 \pm 0.12\%_o$ and $\delta^{15}N = -3.06 \pm 0.25\%_o$ ($n = 59$).

Given that high lipid levels (as indicated by high C/N) can drive $\delta^{13}C$ in a negative direction (McConnaughey & McRoy, 1979; Matthews & Mazumder, 2005), we expected more negative $\delta^{13}C$ later in the season as gerrids accrued lipids before the onset of winter. To normalize stable carbon data (denoted $\delta^{13}C_{adj}$) and account for varying lipid levels across individuals and sites, we corrected $\delta^{13}C$ using an equation outlined in Logan et al. (2008):

$$\delta^{13}C_{adj} = \delta^{13}C - (2.08 - 1.92 \times \ln(C/N))$$

This equation was developed by lipid extracting a series of pooled $A. remigis$ samples after their initial analysis for $\delta^{13}C$ and $\delta^{15}N$. The samples were then re-analysed following extraction and data compared to initial C/N. Using this equation to correct for lipids reduces within-site variability in $A. remigis$ $\delta^{13}C$ (Logan et al., 2008).

We used one-isotope, two source mixing models (Phillips & Gregg, 2001) to estimate the percentage of carbon from each source (terrestrial and aquatic) in the diet of striders. Mixing models compare the isotope ratio of the consumer with isotope ratios in underlying food sources. Aquatic vegetation has been shown to have highly variable $\delta^{13}C$ both within and across sites (Osmond et al., 1981) and overlaps the range observed for terrestrial vegetation (France, 1995). In our mixing models, we ran our analyses with a $\delta^{13}C$ value of $-28.2 \pm 0.9\%_o$, SD (Finlay, 2001) for terrestrial detritus (coarse particulate organic matter) that agreed with an earlier estimate reported by France (1995), and a site-specific $\delta^{13}C$ for aquatic vegetation from our own measurements. The value for terrestrial detritus also approximates that reported for riparian arthropods by Collier, Bury & Gibbs (2002) and Paetzold, Schubert & Tockner (2005). To avoid drawing false conclusions about food source pathways where the two sources potentially overlapped, we only quantitatively assessed proportions of the different dietary sources using data from sites where aquatic vegetation had $-30\%_o > \delta^{13}C > -26\%_o$ (i.e. values outside the likely range of terrestrial vegetation $\delta^{13}C$; France, 1995; Finlay, 2001). This narrowed the number of sites from the 88 sampled down to a total of 45 sites. It also eliminated three of our four index sites, with only Parks Brook consistently having aquatic vegetation that was distinct from terrestrial organic matter. Striders with $\delta^{13}C$ outside the $\delta^{13}C$ range delimited by terrestrial and aquatic vegetation (after correcting for slight diet-tissue fractionation from our rearing experiment) were constrained to values of 0 or 100% (Vander Zanden & Vadeboncoeur, 2002).

Data were analysed using NCSS (Kaysville, UT, U.S.A.) and SYSTAT (version 9, SPSS, Inc., Chicago, IL, U.S.A.) software. For the laboratory experiment, we used exponential models ($y = b + ae^{ct}$) to fit the isotope data ($\delta^{13}C$, $\delta^{13}C_{adj}$ and $\delta^{15}N$) versus time, following Hobson & Clark (1992), where $t$ is the time (in days) since the diet switch and $c$ is the derived constant. Half-life was calculated by the formula: half-life $= \ln(0.5)/c$. Diet tissue fractionation was calculated by subtracting isotope ratios of crickets ($\delta^{13}C = -24.1 \pm 0.5\%_o$, $\delta^{13}C_{adj} = -23.5 \pm 0.2\%_o$, $\delta^{15}N = 3.8 \pm 0.2\%_o$) from that of striders.

For the field data, strider $\delta^{13}C$ was adjusted for lipids (using C/N) and diet-tissue fractionation (multiplying by two to represent the two trophic levels from primary producers to striders) and then used to estimate percent aquatic carbon in the diet. Comparisons of percent aquatic carbon in the diet of striders to stream size (order) at the 45 sites sampled in the autumn were made using linear regressions. To examine whether dietary habits or lipids varied significantly over time or between sexes at the four streams that were regularly sampled, we grouped our data into two generations, the first that returned to stream surfaces after overwintering (‘post-winter’) and the new generation that hatched in early summer (‘pre-winter’). Adults sampled in the period after ice-out in the spring up to and including the first day when a new generation of nymphs was present (typically early July) were considered as the
post-winter sample. Adults sampled for the remainder of the growing season (July–October) were made up of a new generation and considered pre-winter. The timing of the arrival of the new generation, and hence our delineation of the two samples, varied slightly from one stream to another (see Table 1 for details). Comparisons of adult water strider isotope ratios ($\delta^{13}$C$_{adj}$ and $\delta^{15}$N) and C/N across sampling

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Superscripts on dates refer to the grouping of generations (1 = adults that were hatched the previous year and overwintered, 2 = adults that were hatched in the current year) for statistical analysis.

periods were made using general linear model analysis of variance (GLM-ANOVA) with three factors – site (random factor: Corbett Brook, English Brook, McKenzie Brook, and Parks Brook), sex (fixed factor: male and female) and generation (fixed factor: post- and pre-winter). Paired comparisons of differences among levels of fixed factors were made using the Bonferroni test. Finally, average values for males and females were compared to those of nymphs for those times that they co-occurred (mainly summer) with two factors (stage – fixed, and site - random) in a GLM-ANOVA. Interactions and main effects were considered significant at $\alpha = 0.05$ for all tests.

**Results**

In the laboratory, *A. remigis* nymphs grew rapidly on the cricket diet from a wet weight of 10.8 ± 5.2 mg [3.0 ± 1.5 mg dry weight (d.w.)] to a final wet weight of 49.3 ± 2.7 mg (21.2 ± 1.9 mg d.w.) as adults on day 73 of the experiment (Fig. 1a). As is common in water striders (Fairbairn, 2005), females reached greater body sizes than males and had 37% higher wet weights (asymptotic values) (Fig. 1a). Striders showed an abrupt increase in C/N between days 31 and 41 (Day 31 C/N = 3.84 ± 0.03; Day 41 C/N = 5.17 ± 0.53, $P = 0.002$, Fig. 1b), corresponding to Julian Days of 217 and 227 (late July/early August). From day 41 onward, C/N remained elevated in both males and females until the end of the rearing experiment (Fig. 1b). Initial $\delta^{13}$C, $\delta^{13}$C$_{\text{adj}}$ and $\delta^{15}$N of *A. remigis* upon being brought to the laboratory were $-26.9 \pm 0.3_{\text{oor}}$, $-26.5 \pm 0.3_{\text{oor}}$ and $3.2 \pm 0.2_{\text{oor}}$, respectively. Asymptotic values were $-24.3_{\text{oor}}$ ($\delta^{13}$C), $-23.4_{\text{oor}}$ ($\delta^{13}$C$_{\text{adj}}$) and $6.4_{\text{oor}}$ ($\delta^{15}$N) and were achieved (>99% of asymptote) on days four, 16, and 48, respectively (Fig. 2). The best-fit exponential model for isotopic change with time obtained when using $\delta^{13}$C$_{\text{adj}}$ captured more variation ($r^2 = 0.78$) compared with using unadjusted $\delta^{13}$C ($r^2 = 0.68$) (Fig. 2). By subtracting isotope ratios for crickets from asymptote values for *A. remigis*, we calculated diet-tissue fractionation for this species as −0.2% for $\delta^{13}$C, 0.1% for $\delta^{13}$C$_{\text{adj}}$ and 2.6% for $\delta^{15}$N. *A. remigis* showed a rapid turnover of carbon (half-life = 1.1 days modelled using $\delta^{13}$C and half-life = 1.5 days modelled using $\delta^{13}$C$_{\text{adj}}$, Fig. 2) and a relatively fast turnover of nitrogen (half-life = 7.8 days, Fig. 2).

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**Fig. 1** Wet weight (a, in mg, mean of multiple individuals) and C/N (b, individuals) of *Aquarius remigis* nymphs (circles), adult females (squares) and adult males (diamonds) versus time in a laboratory diet-switch experiment. Animals were captured from a stream on day 0 and placed on a diet of crickets. Derived growth equations for female (solid line, Weight = 49.3–41.6e$^{(0.035)}$, $r^2 = 0.96$, $n = 14$) and male (dashed line, Weight = 35.9–27.9e$^{(-0.052)}$, $r^2 = 0.96$, $n = 13$) striders are shown in (a).
In the field, aquatic vegetation $\delta^{13}C$ varied widely among sites; mean $\delta^{13}C$ values ranged from $-39.1_%{oo}$ to $-18.2_%{oo}$ and were positively related to stream order ($\delta^{13}C = 2.2^*\text{Order} - 36.0, r^2 = 0.39, P < 0.001$, data not shown). The range of $\delta^{13}C$ of aquatic vegetation enveloped the $\delta^{13}C$ for terrestrial vegetation estimated from the literature (c. $-28_%{oo}$, France, 1995; Finlay, 2001).

The percentage of aquatic carbon in the diet of A. remigis increased with increasing stream size ($P < 0.001, n = 41$, Fig. 3). Striders rarely showed any evidence of even minimal consumption of aquatic carbon in streams of order three and smaller. In 4th order streams, the entire range of aquatic carbon contribution (0–100%) was observed, while in the largest systems (5th and 6th order) striders had a mix of aquatic and terrestrial carbon in their diet (range = 25–89% aquatic carbon).

Stable isotope ratios and C/N of striders varied among sexes and generations within the four streams (Table 1). Striders collected from Corbett Brook,
English Brook and McKenzie Brook showed little change in $\delta^{13}C_{\text{adj}}$ over the course of the growing season, with a minimum value of $-27.6_{\text{oo}}$ and a maximum value of $-25.1_{\text{oo}}$. Because there was no difference between $\delta^{13}C$ of aquatic vegetation and the assumed terrestrial end-member at Corbett Brook, English Brook and McKenzie Brook, we were only able to estimate % aquatic carbon in the diet of striders from Parks Brook. At this site, the dietary source of C for striders varied over time with a decrease in $\delta^{13}C_{\text{adj}}$ in males, females and nymphs occurring in mid-summer (Table 1) due to increased reliance on aquatic carbon (Fig. 4). In the spring and late summer, striders at Parks Brook had less than 40% of their carbon coming from aquatic sources (Fig. 4). For $\delta^{15}N$, there were no consistent temporal trends within the four sites, with $\delta^{15}N$ peaks occurring randomly throughout the growing season (e.g. Corbett Brook June 11th and July 17th males, English Brook July 4th males, McKenzie Brook July 4th females, Parks Brook September 17th males). There were no consistent differences in $\delta^{15}N$ between males and females within streams, but nymphs had consistently lower $\delta^{15}N$ than adults at all sites (average difference = $0.8 \pm 0.7_{\text{oo}}$, range = $-0.1$ to $2.1_{\text{oo}}$, $P = 0.023$, Table 1). At all sites, higher C/N were observed in late summer and autumn (C/N < 5) compared with spring (C/N c. 4) (Table 1), and this was similar in timing and magnitude to the increase in C/N observed in our experimental population (Fig. 1b).

Across streams, there were significant differences in stable isotope ratios and C/N. No interactions, including three way interactions (sex x generation x site), were significant ($P > 0.05$). For $\delta^{13}C_{\text{adj}}$, there were significant differences among sites ($P < 0.001$) but no differences among sexes ($P = 0.056$) or generations ($P = 0.913$). There were no differences in $\delta^{13}C_{\text{adj}}$ between adults and nymphs ($P = 0.298$, Table 1). Strider $\delta^{15}N$ values were also significantly different across sites ($P < 0.001$) while sexes ($P = 0.053$) and generations ($P = 0.916$) did not differ in their $\delta^{15}N$. For C/N, only generation was significant ($P = 0.009$), with the 2nd generation (pre-winter) having higher C/N than overwintered adults (post-winter). There were no differences between sexes ($P = 0.273$) or sites ($P = 0.475$) in C/N. Adults did not have significantly higher C/N than nymphs ($P = 0.055$).
Discussion

By combining a laboratory diet-switch experiment with field collections, we were able to use stable isotopes and C/N to gain insights into water strider feeding ecology and lipid dynamics. Our data indicate that striders have a strong connection to terrestrial carbon sources, particularly *A. remigis* inhabiting small streams. They also suggest that strider nymphs exhibit rapid elemental turnover during development, and that a pronounced increase in lipid synthesis occurs in late summer in advance of the over-wintering period.

Our estimates of turnover rate for nitrogen (half-life = 7.8 days) and carbon (half-life c. 2 days) were faster than has been previously observed in rapidly growing ectotherms (Suzuki et al., 2005) and similar to metabolically active tissues, such as liver, in endotherms with high energy demands (Tieszen et al., 1983). The rapid turnover observed for striders may be due to a fast metabolism and resultant high energy demands characteristic of insects (Altman & Dittmer, 1968). The difference in half-life between carbon and nitrogen may be related to differential metabolic needs (e.g. energy versus growth) during nymph development. Ostrom et al. (1997) found rapid carbon turnover in beetle tissues (*Harmonia variegate* Goeze), with 75% change occurring in 6 days following a diet switch. In contrast, nitrogen turnover in the same organism was slower, with 75% change occurring in 21 days. Faster turnover of carbon than nitrogen has been shown in other diet-switch experiments with stable isotopes (Hobson & Bairlein, 2003; Suzuki et al., 2005), but in most cases turnover rates of these two elements have been similar (Haramis et al., 2001; Bearhop et al., 2002, Pearson et al., 2003; Evans-Ogden, Hobson & Lank, 2004; Doi et al., 2007). A faster turnover of carbon than nitrogen may be due to the rapid uptake and breakdown of labile energy sources (e.g. sugars and lipids) during growth, as well as whole-body changes in biochemical composition that occur during moulting (Doi et al., 2007). Striders grew from 2nd and 3rd instars to adults during the course of the rearing experiment and hence would have moulted more than once during this period.

We observed a better fit of the isotopic change model to our carbon-13 data when using Δδ13Cadj as opposed to Δδ13C, suggesting that lipids could potentially interfere with interpretation of Δδ13C of animal populations in the field. The decline in δ13C towards the end of the experiment as striders began synthesizing lipids (Fig. 2) may be similar to that observed by Grattan & Forbes (2006) for tissues of female ladybeetles (*H. axyridis* Pallas). In that study, ladybeetles were switched to a corn based diet that was enriched in 13C, and after an initial increase in δ13C, their tissues became more 13C-depleted near the end of the study. However, because no elemental % data (i.e. C/N) were available, δ13C remained uncorrected for lipids and the interpretation of the late decline in δ13C was left to speculation (Grattan & Forbes, 2006). The abrupt increase in C/N in both our laboratory-reared striders and in field populations suggests a switch to lipid synthesis prior to the overwintering period. Given that striders effectively hibernate for c. 6 months (November–April) in northern North America (Fairbairn, 1985), the storage of sufficient lipid reserves may be critical to survival. Similar suggestions of the importance of lipids have been made for other ectotherms in northern climates (e.g. fishes, Biro et al., 2004; Hurst & Conover, 2003).

Our estimates of diet-tissue 13C and 15N fractionation for striders are consistent with ranges reported previously for a variety of species (Post, 2002; McCutchan et al., 2003). McCutchan et al. (2003) suggested that fluid feeders may exhibit lower 15N fractionation than other taxa, however our value for fluid-feeding water striders (2.6%o) is well within the range reported for most animals (Post, 2002; McCutchan et al., 2003; Vanderklift & Ponsard, 2003). By obtaining a diet-tissue fractionation 13C estimate (Δδ13C) specific to *A. remigis*, we were able to avoid the use of literature fractionation estimates that, because they summarize data for a large number of species, contain a large amount of uncertainty in their application and error (e.g. Δδ13C = 0.4 ± 1.3%o SD, Post, 2002; McCutchan et al., 2003). This improved confidence in our mixing model estimates for % aquatic energy in the diet of striders.

Changes occurred in the isotope ratios of aquatic vegetation and their importance to the diet of striders from small headwater streams to larger, wider stretches of river. Our ability to detect changes in the diet of striders, however, was limited by the necessity of eliminating c. half of our sites because δ13C of aquatic vegetation was similar to that of terrestrial organic matter. Conclusions, therefore, apply only to those sites (45 of 88) where aquatic
and terrestrial vegetation had different $\delta^{13}$C values. Aquatic vegetation $\delta^{13}$C generally increased with increasing stream order, consistent with observations by Finlay (2001) who found a significant relationship between aquatic vegetation $\delta^{13}$C and drainage basin size. This observed change is likely a consequence of increased CO$_2$ availability in CO$_2$-saturated headwater streams, which leads to greater discrimination against $^{13}$C during photosynthesis and lower $\delta^{13}$C in aquatic algae in smaller, more turbulent systems (Finlay, 2004). A. remigis showed a limited increase in % aquatic carbon in a downstream direction, suggesting a continued reliance on terrestrial carbon even in medium sized systems (stream orders 3 and 4). Only at seven of 42 sites did A. remigis show a dominant contribution (>50%) from aquatic food sources (e.g. emerging insects) in the diet.

A high proportion of terrestrial carbon in the diet of A. remigis estimated from mixing models supports field observations of the species’ habitat associations. A. remigis is often found in riparian edges, even in larger systems (T.D.J., pers. obs.), which may result in considerable consumption of terrestrial insects that fall onto the water surface. This would be another example of a reciprocal subsidy (Baxter, Fausch & Saunders, 2005), wherein seemingly distinct environments are connected by energy and material flow across boundaries. Given the ubiquity and abundance of A. remigis in small streams (1st to 4th order, Svensson et al., 2002), the species may be a conduit for the incorporation and possible recycling of terrestrial energy in these types of systems (Vannote et al., 1980).

A second possibility for explaining the strong connection between A. remigis and terrestrial carbon may stem from reliance by striders on aquatic insects that directly process leaf litter and its associated microbes (Wallace et al., 1997). Doucett et al. (1996) found a strong terrestrial $\delta^{13}$C signal in muscle tissue of juvenile Atlantic salmon (Salmo salar Linnaeus) in the upper reach of a New Brunswick stream that likely originated from the consumption of abundant ‘shredder’ taxa such as the stoneflies Pteronarcyidae. Perry, Bradford & Grout (2003) found strong evidence for terrestrial carbon in the diet of juvenile Chinook salmon (Oncorhynchus tshawytscha Walbaum) in five headwater tributaries of the Yukon River that may have resulted from consumption of a shredder (Tipulidae) in those streams.

Determination of the relative importance of the two possible terrestrial subsidies in the diet of striders (consumption of emerging insects that had consumed leaf litter versus direct consumption of terrestrial insects) is made possible by considering the timing of fluxes of different prey types to the stream surface (Nakano & Murakami, 2001). A. remigis nymphs typically appear on stream surfaces in July and grow rapidly. Early in their development, these nymphs may feed on various microbes from the surface film of the water as well as catching the end of the peak in insect emergence. The trophic level of nymphs is low relative to adults as indicated by their lower $\delta^{13}$N. Importantly, the majority of A. remigis growth occurs later in the summer, when terrestrial insect supply to streams is typically maximized relative to aquatic insect emergence in temperate systems such as those studied here (Nakano & Murakami, 2001). This means that the likely pathway for terrestrial carbon entering the diet of striders is via direct consumption of terrestrial insects. Our estimates of turnover rates suggest that isotope ratios in field populations should respond rapidly to changes in availability of different prey types provided those prey types have distinct isotope ratios. At Parks Brook where we were able to estimate % aquatic carbon over the course of the growing season, the peak in % aquatic carbon (c. 60% for males, females, and nymphs) was observed in mid-July and the importance of this carbon source declined thereafter. Striders are therefore likely to be opportunistic, feeding on emerging aquatic insects (both grazers that consumed aquatic carbon and shredders that processed leaf material) for a brief period while they are available in late spring and switching to terrestrial insects for their major growth phase in the summer.

Metrobates hesperius was only present in lower reaches of rivers (order ≥4) suggesting that this species may use more energy derived from aquatic food sources. However it was difficult to estimate source proportions using mixing models for this species; M. hesperius is far less common than A. remigis in New Brunswick streams, we lacked species-specific diet-tissue fractionation or lipid correction estimates, and aquatic vegetation $\delta^{13}$C was similar to terrestrial vegetation at many sites where M. hesperius occurred. Only at eight sites were we able to estimate source proportions for this species and values of % aquatic carbon in their diets ranged from 25% to 100% (4% to
78% at our index site Parks Brook). *M. hesperius* rarely occurred in sympathy with *A. remigis* and was absent from all streams that were < 8 m in width. Kittle (1977) similarly reported that *M. hesperius* occurred in large and medium-sized streams and was not typically found in association with other gerrids; where present, it was most commonly located above and below riffles (i.e. in pools). Given that algae growing in pools typically exhibit enriched \( \delta^{13}C \) (Finlay, Khandwala & Power, 2002), consumption of emerging insects from these habitats may explain the relatively enriched \( \delta^{13}C \) in *M. hesperius* at some of the sites where the species did occur. At Parks Brook, *A. remigis* and *M. hesperius* appeared to feed on similar resources suggesting that the lack of occurrence of *M. hesperius* in smaller streams may not be driven by diet limitations but other factors such as stream turbulence or predation.

The extensive use of terrestrial carbon by *A. remigis* has implications for studies that seek to use this species to identify aquatic systems with high contaminant loads (Jardine et al., 2005; Nummelin et al., 2007). Given the importance of diet as the primary route of exposure of organisms to contaminants such as mercury (Hall et al., 1997), a disconnect between striders and emerging aquatic insects as a diet and contaminant source would limit their usefulness for monitoring pollutants in aquatic systems if the pollutants were originating in the aquatic environment. As air breathers that are not immersed in the aquatic environment, striders also lack a secondary source of exposure to contaminants (e.g. waterborne exposure to fishes) leaving diet as their only contaminant source. If the diet is composed primarily of terrestrial sources as observed here, strider contaminant concentrations will be more representative of processes occurring in the terrestrial environment, such as atmospheric deposition.

Seasonal variation in strider dietary habits could have implications for our understanding of their long-term diet as indicated by SIA. The seasonal variation in % aquatic carbon use by *A. remigis* at Parks Brook indicates that we may be underestimating short-term consumption of aquatic insects by this species at sites where collections were made outside of the brief period (the month of July) of increased use of aquatic carbon. The majority of our sites were sampled in August and September, a time that corresponds to when striders at Parks Brook were back to feeding on terrestrial carbon after short-term reliance (>50%) on aquatic sources. However, because the bulk of the diet is made up of terrestrial sources, as at Parks Brook where % terrestrial carbon averaged 73% when all sexes and dates were considered, sampling in late summer will indeed be appropriate as an indicator of long-term diet.

Any error surrounding our estimates of % aquatic carbon in the diet of *A. remigis* is largely due to the variability in aquatic vegetation \( \delta^{13}C \) within sites. Due to heterogeneity in parameters such as water velocity and light availability which ultimately affect CO\(_2\) delivery (Finlay, 2004) within stream reaches, aquatic vegetation \( \delta^{13}C \) can vary widely (France, 1995). Our sampling rigor was likely not appropriate to fully capture all variability within a site given our small sample sizes of aquatic vegetation (\( n = 1–3 \) pooled samples per site). However, our use of a laboratory diet-switch experiment allowed us to obtain a better estimate for diet-tissue fractionation for the species of interest, and hence derive more valid estimates of proportions of different dietary sources in our field populations. We recommend that future studies with stable isotopes perform simple laboratory rearing trials to gain this important information and better assess diet in wild animal populations. We also recommend close attention to the information provided by elemental C/N as both an indicator of the confounding influence of lipids on \( \delta^{13}C \) (McConnaughey & McRoy, 1979; Logan et al., 2008) and, perhaps more critically, as an index of the lipid content of the organism (Cherel et al., 2005).

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