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

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21 organic matter

1 **Abstract**

2 1. Despite the ubiquity and abundance of water striders (Hemiptera: Gerridae) in
3 temperate streams and rivers and their potential usefulness as sentinels in contaminant
4 studies, little is known about their feeding ecology and lipid dynamics.

5 2. In this study we used stable isotopes of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) and
6 elemental carbon to nitrogen ratios (C/N) to assess dietary habits and lipid content,
7 respectively, for water striders.

8 3. To determine diet-tissue fractionation factors, nymphs of the most common species in
9 New Brunswick, Canada, *Aquarius remigis* Say, were reared in the laboratory for 73 days
10 and exhibited rapid isotopic turnover in response to a switch in diet (C half-life = 1.5
11 days, N half-life = 7.8 days). Their lipid content increased towards the end of the growing
12 season and resulted in lower $\delta^{13}\text{C}$ values. Diet-tissue fractionation factors were
13 established after correction of $\delta^{13}\text{C}$ data for the confounding effect of *de novo* lipid
14 synthesis (strider $\delta^{13}\text{C}_{\text{adj}}$ – diet $\delta^{13}\text{C}_{\text{adj}}$ = 0.1‰, strider $\delta^{15}\text{N}$ – diet $\delta^{15}\text{N}$ = 2.7‰).

15 4. Water striders from the majority of 45 stream sites (83%) in New Brunswick had less
16 than 50% contribution of aquatic carbon to their diets but showed a gradual increase in
17 the contribution of this carbon source to their diet with increasing stream size.

18 5. These data indicate that striders exhibit a strong connection to terrestrial carbon
19 sources, making them important users of energy subsidies to streams from the
20 surrounding catchment. However, this dependence on terrestrial organic matter may
21 limit their utility as indicators of contamination of aquatic systems by heavy metals and
22 other pollutants.

23

1 **Introduction**

2 The ability to study the ecology of wild animal populations has been expanded
3 greatly in the last 25 years through the use of stable isotope analysis (SIA). SIA has
4 improved our understanding of energy pathways (e.g. Hecky and Hesslein 1995), animal
5 movement patterns (Hobson 1999), effects of invasive species (e.g. Vander Zanden et al.
6 1999) and contaminant biomagnification (e.g. Cabana and Rasmussen 1994). Accurate
7 measurements of food source pathways are important in conservation and management
8 (Araujo-Lima et al. 1986) and in understanding larger patterns in food webs (Post et al.
9 2000). Stable carbon isotope ratios ($^{13}\text{C}/^{12}\text{C}$ or $\delta^{13}\text{C}$) typically reveal carbon sources at
10 the base of food webs (Hecky and Hesslein 1995, Bastviken et al. 2004) because they
11 show little change during food web transfer and sources of primary production often
12 differ in their $\delta^{13}\text{C}$, while stable nitrogen isotope ratios ($^{15}\text{N}/^{14}\text{N}$ or $\delta^{15}\text{N}$) are indicators of
13 trophic position, as ^{15}N becomes enriched approximately 3.4‰ with each step up a food
14 chain (Post 2002).

15 Over time, our ability to use SIA to answer ecological questions has improved
16 through the use of rearing experiments that have established diet-tissue fractionation
17 (Post 2002) and elemental turnover rates (Dalerum and Angerbjorn 2005) for the target
18 species and tissues of interest (Hobson and Clark 1992), the advent of mathematically-
19 sound mixing models to assess diet (Phillips and Gregg 2001), and the means to account
20 for the effects of endogenous lipid synthesis on tissue $\delta^{13}\text{C}$ values (Kiljunen et al. 2006).
21 However, many of the laboratory rearing experiments were aimed at understanding
22 isotopic fractionation and turnover in birds (e.g. Hobson and Clark 1992) and fishes (e.g.
23 Suzuki et al. 2005), with fewer studies on insects (e.g. Ostrom et al. 1997). Furthermore,

1 the uncertainty surrounding literature-based estimates of diet-tissue fractionation can
2 often impair precise estimates of diet for field populations for which no diet-tissue
3 fractionation estimate is available (Jardine et al. 2006). This necessitates the use of
4 rearing experiments to establish diet-tissue fractionation in cases where there is an
5 interest in inferring field diets using stable isotopes.

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

15 Water striders (Hemiptera: Gerridae) are predaceous insects that are ubiquitous
16 and abundant on lakes and slow-moving sections of streams and rivers of temperate and
17 tropical regions (Spence and Andersen 1994). In North America, adults of one common
18 species (*Aquarius remigis* Say) overwinter and return to streams following ice-out
19 (Fairbairn 1985). They breed and then senesce in the spring, producing a new generation
20 of nymphs by mid-summer that store lipids in late summer after their development to
21 adulthood (Lee et al. 1975). While it is generally known that water striders feed mainly
22 upon terrestrial and aquatic insects trapped in the surface film of the streams (Matthey
23 1974, MacLean 1990), little is known about the relative contributions of these two food

1 source pathways. Striders make up a large proportion of the biomass of insects in many
2 stretches of small rivers and streams (Svensson et al. 2002). Consequently, striders could
3 serve as general indicators of the overall flow of energy to predatory insects and fishes,
4 and may also potentially be used for determining if changes occur in the importance of
5 different carbon sources in an upstream-downstream direction in rivers (Vannote et al.
6 1980, Thorp and Delong 1994). Due to their ubiquity and abundance and potential
7 importance as conduits of energy into small streams, water striders therefore offer a good
8 model to study feeding ecology and nutritional status in animal populations. Striders are
9 also recently gaining attention as environmental sentinels for heavy metal contamination
10 (Jardine et al. 2005, Nummelin et al. 2007). Because much of the contaminant burden in
11 an animal comes from its diet (Hall et al. 1997) and because contaminant concentrations
12 can be affected by growth, sex, and season, it is important to understand strider dietary
13 and life-history patterns.

14 In order to investigate feeding ecology in the most common water strider species
15 in New Brunswick streams (*A. remigis*) and the relative importance of terrestrial and
16 aquatic carbon to its diet in time and space, we began by conducting a laboratory diet-
17 switch experiment to measure diet-tissue isotope fractionation and turnover rates. We
18 then repeatedly sampled striders and potential food sources from four NB stream sites
19 over the growing season to determine the temporal changes in isotope ratios and C/N in
20 field populations. Adult gerrids in temperate climates typically have one to three
21 generations per year (Galbraith and Fernando 1977, Fairbairn 1985), and they accrue
22 lipids over the course of the growing season prior to overwintering (Lee et al. 1975). We
23 therefore predicted that C/N, a proxy for lipid content (McConnaughey and McRoy

1979), would increase from spring to fall. 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.

9 **Methods**

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

22 In the field, water striders were collected using hand nets from 81 stream sites in
23 New Brunswick, Canada in the months of September and October from 2004-2007.

1 Thirty-four sites were sampled in 2004, nine in 2005, 24 in 2006 (with a return visit to
2 one site), and 26 in 2007 (including return visits to 11 sites). When sites were sampled in
3 more than one year, data were combined to yield one point for each site assuming no
4 difference between years. Streams were assigned orders manually with maps at the
5 1:150,000 scale. Sites ranged from 1st to 6th order systems, varied in width from one to
6 >50 m, and had limited or no catchment disturbance from forestry, agriculture or
7 urbanization. An additional four sites (Corbett Brook, N 45.92 W 66.64, English Brook
8 N 46.43 W 66.60, McKenzie Brook N 46.22 W 66.53, and Parks Brook N 45.46 W
9 66.35) were sampled approximately bi-weekly from May to October 2007.

10 *A. remigis* was the most commonly captured water strider, appearing at 73 of the
11 81 sites (including the four index sites). At the index sites, in two instances (July 3rd at
12 Parks Brook, July 17th at Corbett Brook) only nymphs were in sufficient abundance at the
13 time of collections. The other, smaller-bodied, gerrid species (*M. hesperius*) occurred at
14 16 sites (including Parks Brook, one of the index sites). The two species were found
15 together at only six sites. At each site where striders were captured, multiple samples of
16 two individuals each were submitted for SIA. In 2004 and 2005 sexes were not
17 identified, while in 2006 and 2007 sexes were analyzed separately.

18 To characterize the different carbon sources in these streams, we collected aquatic
19 mosses (*Fontinalis* sp.), macrophytes (e.g. *Potamogeton* sp.), and filamentous algae (e.g.
20 *Cladophora* sp.) by hand from each site in 2004. In the years 2005 to 2007 aquatic
21 vegetation was sampled by scrubbing rocks with a toothbrush to collect biofilm (n = 3
22 samples of a minimum of three rocks per sample). Biofilm is an amalgamation of
23 different types of aquatic vegetation (diatoms, moss, filamentous algae) as well as fungi

1 and bacteria (Battin et al. 2003), and thus is representative of in-stream primary
2 production. Any carbon fixed within the stream was therefore considered aquatic carbon.
3 The pH of the streams ranged from 4.7 to 8.1, with the majority of values between 6.5
4 and 7.5. Most streams were therefore circumneutral or acidic and calcareous soils are
5 uncommon in New Brunswick, hence we did not acidify our biofilm samples to remove
6 carbonates that can confound $\delta^{13}\text{C}$.

7 All samples for SIA were oven-dried @ 60°C for 48h and ground to a powder
8 using a mortar and pestle, or ball-mill grinder. Samples were analyzed for stable carbon
9 and nitrogen isotope ratios by combustion in a Carlo Erba NC2500 Elemental Analyzer
10 and delivery to a Finnigan Mat Delta Plus mass spectrometer (Thermo Finnigan, Bremen,
11 Germany) via continuous flow. Data are reported as delta values relative to international
12 standards Pee Dee Belemnite Carbonate (PDB) and atmospheric nitrogen (AIR) and
13 calibrated using International Atomic Energy Agency standards CH6 (-10.4‰), CH7 (-
14 31.8‰), N1 (0.4‰) and N2 (20.3‰). A single water strider sample (working laboratory
15 standard) was analyzed every time samples were run to assess precision across analytical
16 runs, and yielded values of $\delta^{13}\text{C} = -27.53 \pm 0.20\text{‰}$ (unless otherwise indicated, data are
17 shown as mean $\pm$ S.D.) and $\delta^{15}\text{N} = 4.21 \pm 0.65\text{‰}$ (n = 11). The poorer precision in $\delta^{15}\text{N}$
18 was due to a single analysis of this standard, and several samples analyzed in that
19 particular run were repeated to ensure values were comparable with other analytical runs.
20 A commercially available standard (acetanilide, Elemental Microanalysis, Ltd.) analyzed
21 alongside the samples yielded values of $\delta^{13}\text{C} = -33.60 \pm 0.12\text{‰}$ and $\delta^{15}\text{N} = -3.06 \pm$
22 0.25‰ (n = 59).

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

$$\delta^{13}\text{C}_{\text{adj}} = \delta^{13}\text{C} - (2.08 - 1.92 * \ln(\text{C/N}))$$

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

We used one-isotope, two source mixing models (Phillips and 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 $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ value of $-28.2 \pm 0.9\text{‰}$ S.D. (Finlay 2001) for terrestrial detritus (coarse particulate organic matter) that agreed with an earlier estimate reported by France (1995), and a site-specific $\delta^{13}\text{C}$ for aquatic vegetation from our own measurements. The value for terrestrial detritus also approximates that reported for riparian arthropods by Collier et al. (2002) and Paetzold et al. (2005). To avoid drawing false conclusions about food source pathways where the two sources potentially overlapped, we only quantitatively assessed

1 proportions of the different dietary sources using data from sites where aquatic vegetation
2 had $-30\text{‰} > \delta^{13}\text{C} > -26\text{‰}$ (i.e. values outside the likely range of terrestrial vegetation
3 $\delta^{13}\text{C}$; France 1995, Finlay 2001). This narrowed the number of sites from the 88 sampled
4 down to a total of 45 sites. It also eliminated three of our four index sites, with only
5 Parks Brook consistently having aquatic vegetation that was distinct from terrestrial
6 organic matter. Striders with $\delta^{13}\text{C}$ outside the $\delta^{13}\text{C}$ range delimited by terrestrial and
7 aquatic vegetation (after correcting for slight diet-tissue fractionation from our rearing
8 experiment) were constrained to values of 0 or 100% (Vander Zanden and Vadeboncoeur
9 2002).

10 Data were analyzed using NCSS (Kaysville, UT) and SYSTAT (version 9, SPSS,
11 Inc., Chicago, IL) software. For the laboratory experiment, we used exponential models
12 ($y = b + a \cdot e^{ct}$) to fit the isotope data ($\delta^{13}\text{C}$, $\delta^{13}\text{C}_{\text{adj}}$ and $\delta^{15}\text{N}$) vs. time, following Hobson
13 and Clark (1992), where t is the time (in days) since the diet switch and c is the derived
14 constant. Half-life was calculated by the formula: $\text{half-life} = \ln(0.5)/c$. Diet tissue
15 fractionation was calculated by subtracting isotope ratios of crickets ($\delta^{13}\text{C} = -24.1 \pm$
16 0.5‰ , $\delta^{13}\text{C}_{\text{adj}} = -23.5 \pm 0.2\text{‰}$, $\delta^{15}\text{N} = 3.8 \pm 0.2\text{‰}$, $n = 6$) from that of striders.

17 For the field data, strider $\delta^{13}\text{C}$ was adjusted for lipids (using C/N) and diet-tissue
18 fractionation (multiplying by two to represent the two trophic levels from primary
19 producers to striders) and then used to estimate percent aquatic carbon in the diet.
20 Comparisons of percent aquatic carbon in the diet of striders to stream size (order) at the
21 45 sites sampled in the fall were made using linear regressions. To examine whether
22 dietary habits or lipids varied significantly over time or between sexes at the four streams
23 that were regularly sampled, we grouped our data into two generations, the first that

1 returned to stream surfaces after overwintering (“post-winter”) and the new generation
2 that hatched in early summer (“pre-winter”). Adults sampled in the period after ice-out
3 in the spring up to and including the first day when a new generation of nymphs was
4 present (typically early July) were considered as the post-winter sample. Adults sampled
5 for the remainder of the growing season (July – October) were made up of a new
6 generation and considered pre-winter. The timing of the arrival of the new generation,
7 and hence our delineation of the two samples, varied slightly from one stream to another
8 (see Table 1 for details). Comparisons of adult water strider isotope ratios ($\delta^{13}\text{C}_{\text{adj}}$ and
9 $\delta^{15}\text{N}$) and C/N across sampling periods were made using general linear model analysis of
10 variance (GLM-ANOVA) with three factors – site (random factor: Corbett Brook,
11 English Brook, McKenzie Brook, and Parks Brook), sex (fixed factor: male and female),
12 and generation (fixed factor: post- and pre-winter). Paired comparisons of differences
13 among levels of fixed factors were made using the Bonferroni test. Finally, average
14 values for males and females were compared to those of nymphs for those times that they
15 co-occurred (mainly summer) with two factors (stage – fixed, and site - random) in a
16 GLM-ANOVA. Interactions and main effects were considered significant at $\alpha = 0.05$ for
17 all tests.

18

19 **Results**

20 In the laboratory, *A. remigis* nymphs grew rapidly on the cricket diet from a wet
21 weight of 10.8 ± 5.2 mg (3.0 ± 1.5 mg dry weight (d.w.)) to a final wet weight of $49.3 \pm$
22 2.7 mg (21.2 ± 1.9 mg d.w.) as adults on day 73 of the experiment (Fig. 1a). As is
23 common in water striders (Fairbairn 2005), females reached greater body sizes than

1 males and had 37% higher wet weights (asymptotic values) (Fig. 1a). Striders showed an
 2 abrupt increase in C/N between days 31 and 41 (Day 31 C/N = 3.84 ± 0.03 ; Day 41 C/N
 3 = 5.17 ± 0.53 , $p = 0.002$, Fig. 1b), corresponding to Julian Days of 217 and 227 (late
 4 July/early August). From day 41 onward, C/N remained elevated in both males and
 5 females until the end of the rearing experiment (Fig. 1b). Initial $\delta^{13}\text{C}$, $\delta^{13}\text{C}_{\text{adj}}$ and $\delta^{15}\text{N}$ of
 6 *A. remigis* upon being brought to the laboratory were $-26.9 \pm 0.3\text{‰}$, $-26.5 \pm 0.3\text{‰}$, and
 7 $3.2 \pm 0.2\text{‰}$, respectively. Asymptotic values were -24.3‰ ($\delta^{13}\text{C}$), -23.4‰ ($\delta^{13}\text{C}_{\text{adj}}$) and
 8 6.4‰ ($\delta^{15}\text{N}$) and were achieved (>99% of asymptote) on days four, 16, and 48,
 9 respectively (Fig. 2). The best-fit exponential model for isotopic change with time
 10 obtained when using $\delta^{13}\text{C}_{\text{adj}}$ captured more variation ($r^2 = 0.78$) compared with using
 11 unadjusted $\delta^{13}\text{C}$ ($r^2 = 0.68$) (Fig. 2). By subtracting isotope ratios for crickets from
 12 asymptote values for *A. remigis*, we calculated diet-tissue fractionation for this species as
 13 -0.2‰ for $\delta^{13}\text{C}$, 0.1‰ for $\delta^{13}\text{C}_{\text{adj}}$ and 2.6‰ for $\delta^{15}\text{N}$. *A. remigis* showed a rapid turnover
 14 of carbon (half-life = 1.1 days modeled using $\delta^{13}\text{C}$ and half-life = 1.5 days modeled using
 15 $\delta^{13}\text{C}_{\text{adj}}$, Fig. 2) and a relatively fast turnover of nitrogen (half-life = 7.8 days, Fig. 2).

16 In the field, aquatic vegetation $\delta^{13}\text{C}$ varied widely among sites; mean $\delta^{13}\text{C}$ values
 17 ranged from -39.1‰ to -18.2‰ and were positively related to stream order ($\delta^{13}\text{C} =$
 18 $2.2 \times \text{Order} - 36.0$, $r^2 = 0.39$, $p < 0.001$, data not shown). The range of $\delta^{13}\text{C}$ of aquatic
 19 vegetation enveloped the $\delta^{13}\text{C}$ for terrestrial vegetation estimated from the literature ($\sim -$
 20 28‰ , France 1995, Finlay 2001).

21 The percentage of aquatic carbon in the diet of *A. remigis* increased with
 22 increasing stream size ($p < 0.001$, $n = 41$, Fig. 3). Striders rarely showed any evidence of
 23 even minimal consumption of aquatic carbon in streams of order three and smaller. In 4th

1 order streams, the entire range of aquatic carbon contribution (0% to 100%) was
2 observed, while in the largest systems (5th and 6th order) striders had a mix of aquatic and
3 terrestrial carbon in their diet (range = 25-89% aquatic carbon). *M. hesperius* was only
4 present in larger streams ($\geq 4^{\text{th}}$ order) and appeared to have similar proportions of aquatic
5 carbon as *A. remigis* at those sites (Fig. 3).

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

(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 $\delta^{13}\text{C}_{\text{adj}}$, 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 $\delta^{13}\text{C}_{\text{adj}}$ between adults and nymphs ($p = 0.298$, Table 1). Strider $\delta^{15}\text{N}$ 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 $\delta^{15}\text{N}$. 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 overwintering period.

1 Our estimates of turnover rate for nitrogen (half-life = 7.8 days) and carbon (half-
2 life $\approx$ 2 days) were faster than has been previously observed in rapidly-growing
3 ectotherms (Suzuki et al. 2005) and similar to metabolically active tissues, such as liver,
4 in endotherms with high energy demands (Tieszen et al. 1983). The rapid turnover
5 observed for striders may be due to a fast metabolism and resultant high energy demands
6 characteristic of insects (Altman and Dittmer 1968). The difference in half-life between
7 carbon and nitrogen may be related to differential metabolic needs (e.g. energy vs.
8 growth) during nymph development. Ostrom et al. (1997) found rapid carbon turnover in
9 beetle tissues (*Harmonia variegata* Goeze), with 75% change occurring in six days
10 following a diet switch. In contrast, nitrogen turnover in the same organism was slower,
11 with 75% change occurring in 21 days. Faster turnover of carbon than nitrogen has been
12 shown in other diet-switch experiments with stable isotopes (Hobson and Bairlein 2003,
13 Suzuki et al. 2005), but in most cases turnover rates of these two elements have been
14 similar (Bearhop et al. 2002, Haramis et al. 2003, Pearson et al. 2003, Evans-Ogden et al.
15 2004, Doi et al. 2007). A faster turnover of carbon than nitrogen may be due to the rapid
16 uptake and breakdown of labile energy sources (e.g. sugars and lipids) during growth, as
17 well as whole-body changes in biochemical composition that occur during moulting (Doi
18 et al. 2007). Striders grew from 2nd and 3rd instars to adults during the course of the
19 rearing experiment and hence would have moulted more than once during this period.

20 We observed a better fit of the isotopic change model to our carbon-13 data when
21 using $\delta^{13}\text{C}_{\text{adj}}$ as opposed to $\delta^{13}\text{C}$, suggesting that lipids could potentially interfere with
22 interpretation of $\delta^{13}\text{C}$ of animal populations in the field. The decline in $\delta^{13}\text{C}$ towards the
23 end of the experiment as striders began synthesizing lipids (Fig. 2) may be similar to that

1 observed by Gratton and Forbes (2006) for tissues of female ladybeetles (*H. axyridis*
2 Pallas). In that study, ladybeetles were switched to a corn based diet that was enriched in
3 ^{13}C , and after an initial increase in $\delta^{13}\text{C}$, their tissues became more ^{13}C -depleted near the
4 end of the study. However, because no elemental % data (i.e. C/N) were available, $\delta^{13}\text{C}$
5 remained uncorrected for lipids and the interpretation of the late decline in $\delta^{13}\text{C}$ was left
6 to speculation (Gratton and Forbes 2006). The abrupt increase in C/N in both our
7 laboratory-reared striders and in field populations suggests a switch to lipid synthesis
8 prior to the overwintering period. Given that striders effectively hibernate for ~six
9 months (November to April) in northern North America (Fairbairn 1985), the storage of
10 sufficient lipid reserves may be critical to survival. Similar suggestions of the importance
11 of lipids have been made for other ectotherms in northern climates (e.g. fishes, Biro et al.
12 2004, Hurst and Conover 2003).

13 Our estimates of diet-tissue ^{13}C and ^{15}N fractionation for striders are consistent
14 with ranges reported previously for a variety of species (Post 2002, McCutchan et al.
15 2003). McCutchan et al. (2003) suggested that fluid feeders may exhibit lower ^{15}N
16 fractionation than other taxa, however our value for fluid-feeding water striders (2.6‰) is
17 well within the range reported for most animals (Post 2002, McCutchan et al. 2003,
18 Vanderklift and Ponsard 2003). By obtaining a diet-tissue fractionation ^{13}C estimate
19 ($\Delta\delta^{13}\text{C}$) specific to *A. remigis*, we were able to avoid the use of literature fractionation
20 estimates that, because they summarize data for a large number of species, contain a large
21 amount of uncertainty in their application and error (e.g. $\Delta\delta^{13}\text{C} = 0.4 \pm 1.3\text{‰}$ S.D., Post
22 2002, McCutchan et al. 2003). This improved confidence in our mixing model estimates
23 for % aquatic energy in the diet of striders.

1 Changes occurred in the isotope ratios of aquatic vegetation and their importance
2 to the diet of striders from small headwater streams to larger, wider stretches of river.
3 Our ability to detect changes in the diet of striders, however, was limited by the necessity
4 of eliminating ~half of our sites because $\delta^{13}\text{C}$ of aquatic vegetation was similar to that of
5 terrestrial organic matter. Conclusions, therefore, apply only to those sites (45 of 88)
6 where aquatic and terrestrial vegetation had different $\delta^{13}\text{C}$ values. Aquatic vegetation
7 $\delta^{13}\text{C}$ generally increased with increasing stream order, consistent with observations by
8 Finlay (2001) who found a significant relationship between aquatic vegetation $\delta^{13}\text{C}$ and
9 drainage basin size. This observed change is likely a consequence of increased CO_2
10 availability in CO_2 -saturated headwater streams, which leads to greater discrimination
11 against ^{13}C during photosynthesis and lower $\delta^{13}\text{C}$ in aquatic algae in smaller, more
12 turbulent systems (Finlay 2004). *A. remigis* showed a limited increase in % aquatic
13 carbon in a downstream direction, suggesting a continued reliance on terrestrial carbon
14 even in medium sized systems (stream orders 3 and 4). Only at seven of 42 sites did *A.*
15 *remigis* show a dominant contribution (>50%) from aquatic food sources (e.g. emerging
16 insects) in the diet.

17 A high proportion of terrestrial carbon in the diet of *A. remigis* estimated from
18 mixing models supports field observations of the species' habitat associations. *A. remigis*
19 is often found in riparian edges, even in larger systems (T.D.J., pers. obs.), which may
20 result in considerable consumption of terrestrial insects that fall onto the water surface.
21 This would be another example of a reciprocal subsidy (Baxter et al. 2005), wherein
22 seemingly distinct environments are connected by energy and material flow across
23 boundaries. Given the ubiquity and abundance of *A. remigis* in small streams (1st to 4th

1 order, Svensson et al. 2002), the species may be a conduit for the incorporation and
2 possible recycling of terrestrial energy in these types of systems (Vannote et al. 1980).

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

13 Determination of the relative importance of the two possible terrestrial subsidies
14 in the diet of striders (consumption of emerging insects that had consumed leaf litter vs.
15 direct consumption of terrestrial insects) is made possible by considering the timing of
16 fluxes of different prey types to the stream surface (Nakano and Murakami 2001). *A.*
17 *remigis* nymphs typically appear on stream surfaces in July and grow rapidly. Early in
18 their development, these nymphs may feed on various microbes from the surface film of
19 the water as well as catching the end of the peak in insect emergence. The trophic level of
20 nymphs is low relative to adults as indicated by their lower $\delta^{15}\text{N}$. Importantly, the
21 majority of *A. remigis* growth occurs later in the summer, when terrestrial insect supply
22 to streams is typically maximized relative to aquatic insect emergence in temperate
23 systems such as those studied here (Nakano and Murakami 2001). This means that the

1 likely pathway for terrestrial carbon entering the diet of striders is via direct consumption
2 of terrestrial insects. Our estimates of turnover rates suggest that isotope ratios in field
3 populations should respond rapidly to changes in availability of different prey types
4 provided those prey types have distinct isotope ratios. At Parks Brook where we were
5 able to estimate % aquatic carbon over the course of the growing season, the peak in %
6 aquatic carbon (~60% for males, females, and nymphs) was observed in mid-July and the
7 importance of this carbon source declined thereafter. Striders are therefore likely to be
8 opportunistic, feeding on emerging aquatic insects (both grazers that consumed aquatic
9 carbon and shredders that processed leaf material) for a brief period while they are
10 available in late spring and switching to terrestrial insects for their major growth phase in
11 the summer.

12 *M. hesperius* was only present in lower reaches of rivers (order ≥ 4) suggesting
13 that this species may use more energy derived from aquatic food sources. However it
14 was difficult to estimate source proportions using mixing models for this species; *M.*
15 *hesperius* is far less common than *A. remigis* in New Brunswick streams, we lacked
16 species-specific diet-tissue fractionation or lipid correction estimates, and aquatic
17 vegetation $\delta^{13}\text{C}$ was similar to terrestrial vegetation at many sites where *M. hesperius*
18 occurred. Only at eight sites were we able to estimate source proportions for this species
19 and values of % aquatic carbon in their diets ranged from 25% to 100% (4% to 78% at
20 our index site Parks Brook). *M. hesperius* rarely occurred in sympatry with *A. remigis*
21 and was absent from all streams that were $< \sim 8$ m in width. Kittle (1977) similarly
22 reported that *M. hesperius* occurred in large and medium-sized streams and was not
23 typically found in association with other gerrids; where present, it was most commonly

1 located above and below riffles (i.e. in pools). Given that algae growing in pools
2 typically exhibit enriched $\delta^{13}\text{C}$ (Finlay et al. 2002), consumption of emerging insects
3 from these habitats may explain the relatively enriched $\delta^{13}\text{C}$ in *M. hesperius* at some of
4 the sites where the species did occur. At Parks Brook, *A. remigis* and *M. hesperius*
5 appeared to feed on similar resources suggesting that the lack of occurrence of *M.*
6 *hesperius* in smaller streams may not be driven by diet limitations but other factors such
7 as stream turbulence or predation.

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

20 Seasonal variation in strider dietary habits could have implications for our
21 understanding of their long-term diet as indicated by SIA. The seasonal variation in %
22 aquatic carbon use by *A. remigis* at Parks Brook indicates that we may be
23 underestimating short-term consumption of aquatic insects by this species at sites where

1 collections were made outside of the brief period (the month of July) of increased use of
2 aquatic carbon. The majority of our sites were sampled in August and September, a time
3 that corresponds to when striders at Parks Brook were back to feeding on terrestrial
4 carbon after short term reliance ($> 50\%$) on aquatic sources. However, because the bulk
5 of the diet is made up of terrestrial sources, as at Parks Brook where % terrestrial carbon
6 averaged 73% when all sexes and dates were considered, sampling in late summer will
7 indeed be appropriate as an indicator of long-term diet.

8 The large amount of error surrounding our estimates of % aquatic carbon in the
9 diet of *A. remigis* is largely due to the variability in aquatic vegetation $\delta^{13}\text{C}$ within sites.
10 Due to heterogeneity in parameters such as water velocity and light availability which
11 ultimately affect CO_2 delivery (Finlay 2004) within stream reaches, aquatic vegetation
12 $\delta^{13}\text{C}$ can vary widely (France 1995). Our sampling rigor was likely not appropriate to
13 fully capture all variability within a site given our small sample sizes of aquatic
14 vegetation ($n = 1\text{-}3$ pooled samples per site). However, our use of a laboratory diet-
15 switch experiment allowed us to obtain a better estimate for diet-tissue fractionation for
16 the species of interest, and hence derive more valid estimates of proportions of different
17 dietary sources in our field populations. We recommend that future studies with stable
18 isotopes perform simple laboratory rearing trials to gain this important information and
19 better assess diet in wild animal populations. We also recommend close attention to the
20 information provided by elemental C/N as both an indicator of the confounding influence
21 of lipids on $\delta^{13}\text{C}$ (McConnaughey and McRoy 1979, Logan et al, in press) and, perhaps
22 more critically, as an index of the lipid content of the organism (Cherel et al. 2005).

23

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- 23

1 **Figure captions:**

2 Figure 1. Wet weight (A, in mg, mean of multiple individuals) and C/N (B, individuals)
3 of *Aquarius remigis* nymphs (circles), adult females (squares) and adult males
4 (diamonds) vs. time in a laboratory diet-switch experiment. Animals were captured from
5 a stream on day 0 and placed on a diet of crickets. Derived growth equations for female
6 (F, $\text{Weight} = 49.3 - 41.6e^{(-0.035t)}$, $r^2 = 0.96$, $n = 14$) and male (M, $\text{Weight} = 35.9 - 27.9e^{(-$
7 $0.052t)}$, $r^2 = 0.96$, $n = 13$) striders are shown in (A).

8
9 Figure 2. $\delta^{13}\text{C}$ (A, in ‰), $\delta^{13}\text{C}_{\text{adj}}$ (B, in ‰), and $\delta^{15}\text{N}$ (C, in ‰) in individual *Aquarius*
10 *remigis* nymphs (circles), adult females (squares) and adult males (diamonds) vs. time in
11 a laboratory diet-switch experiment. Animals were captured from a stream on day 0 and
12 placed on a diet of crickets (dashed line indicates isotope ratio of the diet). Open
13 symbols = tank 1 ($n = 2/\text{date}$), solid symbols = tank 2 ($n = 2/\text{date}$), solid triangle = mean
14 value ($\pm$ S.D., $n = 2$ pooled samples) for a sample collected from the source stream on
15 day 51. Best-fit lines ($n = 52$) for $\delta^{13}\text{C}$ ($\delta^{13}\text{C} = -24.23 - 2.71e^{(-0.653t)}$, $r^2 = 0.68$), $\delta^{13}\text{C}_{\text{adj}}$
16 ($\delta^{13}\text{C}_{\text{adj}} = -23.44 - 2.95e^{(-0.472t)}$, $r^2 = 0.78$) and $\delta^{15}\text{N}$ ($\delta^{15}\text{N} = 6.39 - 2.86e^{(-0.089t)}$, $r^2 = 0.93$)
17 are shown in (A), (B), and (C) respectively.

18
19 Figure 3. Percent aquatic carbon (as estimated from mixing models using stable carbon
20 isotopes) in the diet of *Aquarius remigis* (solid symbols) and *Metrobates hesperius* (open
21 symbols) vs. stream order in New Brunswick, Canada streams. The best fit line for A.
22 *remigis* ($\% \text{ Aquatic} = 14.2 * \text{Order} - 23.1$, $r^2 = 0.33$, $n = 41$) is shown.

23

1 Figure 4. Percent aquatic carbon (as estimated from mixing models using stable carbon
2 isotopes) in the diet of *Aquarius remigis* (solid symbols) and *Metrobates hesperius* (open
3 symbols) vs. sampling date in Parks Brook, New Brunswick, Canada. Squares = females,
4 diamonds = males, circles = nymphs.
5

1 Table 1. Stable isotope ratios ($\delta^{13}\text{C}_{\text{adj}}$ and $\delta^{15}\text{N}$, in ‰) and C:N for *A. remigis* females (F), males (M) and nymphs (N) collected over
2 the course of the growing season in 2007 in four streams in New Brunswick, Canada. Each point represents a single pooled sample.
3 Superscripts on dates refer to the grouping of generations (1 = adults that were hatched the previous year and overwintered, 2 = adults
4 that were hatched in the current year) for statistical analysis.

Site	Date	$\delta^{13}\text{C}_{\text{adj}}$ (‰)			$\delta^{15}\text{N}$ (‰)			C:N		
		F	M	N	F	M	N	F	M	N
Corbett Brook	7-May ¹	-26.7	-26.7		5.9	5.3		4.2	4.2	
	22-May ¹	-27.6	-27.1		4.5	5.3		4.3	4.2	
	11-Jun ¹	-27.3	-26.7		5.2	6.0		4.2	4.1	
	21-Jun ¹	-27.2	-26.8		5.2	5.3		4.2	4.2	
	4-Jul ¹	-26.7	-26.8	-26.6	5.7	6.0	3.7	3.9	4.0	3.7
	17-Jul			-26.3			4.6			3.6
	2-Aug ²	-25.7	-26.0	-25.8	4.7	4.7	4.7	4.4	4.2	4.1
	14-Aug ²	-26.6	-26.8	-26.0	5.2	4.9	3.2	4.1	4.1	3.7
	28-Aug ²	-26.1	-26.6		4.6	5.0		4.8	4.6	
	17-Sep ²	-27.4	-27.1	-26.3	5.5	5.6	4.3	4.9	4.7	4.0
	10-Oct ²	-26.2	-26.8		4.7	5.3		5.5	5.0	
Average (S.D.)		-26.8 (0.6)	-26.7 (0.3)	-26.2 (0.3)	5.1 (0.5)	5.3 (0.4)	4.1 (0.7)	4.4 (0.5)	4.3 (0.3)	3.8 (0.2)
English Brook	7-May ¹	-26.6	-26.6		5.2	4.9		4.6	4.4	
	22-May ¹	-27.4	-26.5		4.8	4.6		4.3	4.2	
	11-Jun ¹	-26.2	-26.3		5.0	5.3		4.2	4.1	
	21-Jun ¹	-26.6	-26.1		4.9	5.8		4.0	4.2	

	4-Jul ¹	-25.8	-25.4		5.5	7.2		4.5	3.9	
	17-Jul ¹	-25.8	-25.9	-25.9	6.0	5.8	5.3	4.0	3.8	3.4
	2-Aug ²	-26.3	-26.6	-26.2	5.7	5.9	4.4	3.7	3.8	3.8
	14-Aug ²	-26.9	-26.0	-26.5	4.4	4.9	4.7	4.2	4.0	4.1
	14-Sep ²	-26.3	-26.5		5.3	4.8		5.4	5.4	
	10-Oct ²	-25.8	-26.1		5.9	5.6		5.8	5.4	
Average (S.D.)		-26.4 (0.5)	-26.2 (0.4)	-26.2 (0.3)	5.3 (0.5)	5.5 (0.8)	4.8 (0.5)	4.5 (0.7)	4.3 (0.6)	3.8 (0.3)
McKenzie Brook	7-May ¹	-26.8	-26.2		5.2	5.1		4.5	4.1	
	22-May ¹	-26.7	-26.8		4.8	5.4		4.1	4.3	
	11-Jun ¹	-26.9	-26.1		5.2	5.5		4.2	4.2	
	21-Jun ¹	-26.0	-25.8		5.9	7.1		4.1	3.8	
	4-Jul ¹	-25.9	-26.5		7.2	5.3		4.0	4.1	
	17-Jul ¹	-26.1	-26.6	-25.9	6.8	6.3	5.8	4.0	3.8	4.0
	2-Aug ²	-25.7	-25.1	-25.8	5.7	6.1	5.4	4.2	3.7	4.3
	14-Aug ²	-25.9	-25.6	-25.8	5.6	6.2	5.7	4.3	4.0	4.1
	30-Aug ²	-26.2	-26.4	-26.2	6.1	5.2	4.1	5.3	4.3	3.9
	17-Sep ²		-26.4	-25.8		6.7	5.3		5.3	3.8
Average (S.D.)		-26.2 (0.4)	-26.1 (0.5)	-25.9 (0.2)	5.8 (0.8)	5.9 (0.7)	5.3 (0.7)	4.3 (0.4)	4.2 (0.5)	4.0 (0.2)
Parks Brook	7-May ¹	-28.6	-28.4		6.2	6.3		4.3	4.2	
	22-May ¹	-29.1	-28.1		6.5	6.0		3.9	4.2	
	11-Jun ¹	-27.5	-27.5		6.7	6.6		4.2	4.1	
	21-Jun ¹	-28.9	-27.9		6.3	6.8		4.0	4.1	
	3-Jul			-28.4			5.9			4.1
	17-Jul ²	-30.7	-30.7	-31.0	6.9	6.9	6.2	4.1	3.9	4.0
	2-Aug ²	-30.0	-31.9	-28.4	6.6	6.1	6.3	4.4	5.1	3.5

	14-Aug ²	-27.5	-28.1		7.0	7.0		5.2	5.2	
	30-Aug ²		-27.9	-26.5		6.6	6.2		5.8	4.2
	17-Sep ²	-27.3	-26.6		6.3	7.2		5.7	5.1	
Average (S.D.)		-28.7 (1.2)	-28.4 (1.7)	-29.5 (1.9)	6.6 (0.3)	6.6 (0.4)	6.1 (0.2)	4.5 (0.6)	4.6 (0.7)	3.9 (0.3)

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1 Figure 1.

1 Figure 2.

1 Figure 3.

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1 Figure 4.

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