An evaluation of deuterium as a food source tracer in temperate streams of eastern Canada

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Abstract. Stable H isotope ratios (D/H, expressed as δD) hold promise as an additional tool for elucidating food sources for consumers in aquatic ecosystems. We tested the applicability of δD as a food source tracer in streams of New Brunswick, Canada. First, we analyzed δD and δ13C in biofilm and terrestrial leaves and compared signal-to-noise ratios (variability within sources vs variability between sources) between the 2 elements. Signal-to-noise ratios were roughly similar, and 23 of the 31 sites had isotopically distinct food sources based on δD compared to 20 of 31 based on δ13C. Second, we used mixing models to estimate % aquatic H and % aquatic C for benthic invertebrates at a subset of sites. Of 16 samples, only 1 had δ13C that was outside the range of the 2 food sources (yielding % aquatic C > 100%), but 12 of the 16 samples had δD outside the range of the food sources, a result suggesting confounding effects of water and lipids on total body H content. Last, we analyzed δD in laboratory-reared consumers (brook trout and water striders) and in their diet before and after lipid extraction to estimate diet–tissue fractionation. Large differences between consumer and diet were apparent before lipid extraction, but no significant differences were found after lipid extraction. All of these measures indicate that δD could serve as a complementary, but not alternative, isotopic method for estimating food sources for consumers in streams. Further laboratory trials are needed to explore the influence of lipids on δD values.

Key words: hydrogen, diet–tissue fractionation, signal-to-noise, mixing models, lipids, exchangeable H, biofilm, alder.

The provision of energy to streams controls consumer productivity (Wallace et al. 1997) and a variety of functional interactions (Thompson and Townsend 2005) and is essential in maintaining biodiversity and other ecosystem attributes. A full understanding of predominant food sources at various scales in lotic ecosystems has long been a challenge (Vannote et al. 1980, Junk et al. 1989, Thorp and Delong 1994). Analysis of stable isotope ratios of C (13C/12C) of consumers and the 2 main food sources in streams—benthic algae and terrestrial leaves—has advanced our understanding of stream organic matter processing by consumers by showing the importance of terrestrial inputs in temperate headwaters and the dominance of algae in tropical ecosystems (e.g., Doucett et al. 1996, Lau et al. 2009). This approach allows estimation of the contribution of the 2 food sources to animal diets because of limited fractionation of 13C/12C during trophic transfer (~0.4‰; Post 2002). However, the technique is limited because of considerable variability in 13C/12C of periphytic algae that often overlaps the 13C/12C of terrestrial organic matter (France 1995) and makes assigning source proportions (i.e., relative importance of sources) to consumers difficult except in situations where the 2 sources are extremely distinct (Rosenfeld and Roff 1992, Jardine et al. 2008a).

Stable isotope ratios of H (2H/1H or D/H) show some promise as an alternative or complementary means to trace organic matter originating from

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autochthonous or allochthonous pathways through stream food webs. Theoretically, transpiration by land plants should enrich leaves in D because the lighter isotope is preferentially lost to the atmosphere (e.g., Walker and Brunel 1990), a phenomenon not observed in aquatic autotrophs. Given the broad range in D/H observed on a global scale (Bowen et al. 2005) and recent improvements in analytical capabilities for this element in organic tissues (Wassenaar and Hobson 2000), δD might provide a stronger signal-to-noise ratio (variability among sources relative to variability within sources) than other more traditionally measured isotopes, such as δ13C (Becker 2005, Jardine and Cunjak 2005). Thus, measures of δD in stream organisms might allow estimation of dietary source proportions at more sites, and with potentially better resolution, than has been achieved with δ13C (Phillips and Gregg 2001). Doucett et al. (2007) reported wide separation in δD between terrestrial and aquatic food sources in the Colorado River, USA, at relatively dry (xeric) sites. What remains to be determined is the applicability of the technique in more mesic environments where evapotranspiration losses in land plants might not be as great. Also, little is known about the isotopic relationship between an animal and its diet (diet–tissue fractionation) for D. Given the variability observed in diet–tissue fractionation for δ13C (±0.9 SD; Post 2002) and its effect on mixing-model outcomes (Phillips and Gregg 2001), a low and consistent δD diet–tissue fractionation would be a positive attribute in its use as a source tracer in aquatic systems.

The purpose of our study was to evaluate the use of D as a food source tracer in streams in a temperate mesic environment. We sought to determine if D could provide additional information (i.e., greater resolution, more sites where food sources were distinct) to that obtained by using δ13C. We sampled instream primary producers (biofilm) and riparian vegetation (alder [Alnus spp.] leaves) from 31 sites in New Brunswick, Canada, and measured δD and δ13C on the samples. We also analyzed δD and δ13C of benthic invertebrates at a subset of sites to determine if values fell within the range delineated by the 2 food sources. Last, in the laboratory, we analyzed the δD of 2 consumers common in streams (brook trout [Salvelinus fontinalis] and water striders [Aquarius remigis]) and their diets to determine diet–tissue δD fractionation.

**Methods**

We sampled 31 sites in New Brunswick, Canada, in August and September 2005 and 2006 (Table 1). Sites were located in 6 major drainages: Restigouche River (lat 47.59°N, long 66.46°W), Renous River (lat 46.49°N, long 65.47°W), Little Southwest Miramichi River (lat 46.57°N, long 65.50°W), Newcastle Creek (lat 46.04°N, long 65.59°W), Cains River (lat 46.40°N, long 65.47°W), and Salmon River (lat 46.06°N, long 65.55°W).

At each site, we collected samples of biofilm and terrestrial leaves (the 2 main sources of organic matter for consumers). Phytoplankton was unlikely to be present in any of these sites because they were shallow and fast-flowing. Macrophytes were rare, and moss was unlikely to be important in the diet of aquatic consumers because sufficient biofilm was present. Thus, we considered biofilm the best representative of instream production. We collected 3 biofilm samples at each site by scraping ≥3 rocks/sample with a toothbrush. We removed 3 samples of fresh leaves from the dominant species (alder) growing in the riparian zone of the stream. Alder are not the only species of tree in the riparian zone of these streams, but they are most likely to contribute to aquatic food webs because they fix N and have correspondingly low C/N (19.5 ± 2.7 SD). We stored samples on ice until we returned to the laboratory, where we freeze-dried the samples and ground them to a homogenous powder for isotope analysis.

We collected benthic invertebrates with D-frame kick nets from a subset of the sites (n = 6 sites in the Cains River, Little Southwest Miramichi River, and Restigouche River). Isotope ratios of guts and body tissues of stream invertebrates are highly correlated (Jardine et al. 2005). Therefore, we held invertebrates for only ~6 h to clear their guts before we freeze-dried and ground them to a homogeneous powder for isotope analysis. Invertebrates included dragonflies (Aeshnidae, n = 2 pooled samples; Gomphidae, n = 7 pooled samples), stoneflies (Perlidae, n = 3 pooled samples), and mayflies (Heptageniidae, n = 4 pooled samples). We did not extract lipids from these samples.

We reared samples of consumers on a consistent laboratory diet and analyzed them for D to measure diet–tissue fractionation. We sampled white muscle tissue from brook trout (n = 6, average fork length = 70 mm) that were raised from the alevin stage on a consistent diet of pellets (n = 3, representing 1 each of 3 pellet sizes) (Jardine et al. 2008a). Trout mass increased by several orders of magnitude after we began feeding them pellets, so we assumed they were in isotopic equilibrium with their diet. We collected water striders from a stream and reared them on crickets (Gryllidae); a subset of these striders and crickets were sampled (n = 3 each) after 72 d of rearing. These striders also were in isotopic equilib-
rium with the cricket diet because their C and N isotope ratios reached a steady state after an increase in mass by striders of ~400% over the course of the experiment (Jardine et al. 2008b). We assumed similar elemental incorporation rates for H as for C and N. These laboratory samples were processed as described above for stable isotope analysis. After initial analysis for δD, we suspended tissue in chloroform-methanol solution to extract lipids (Logan et al. 2008). We redried the samples and analyzed them for δD.

We used a comparative equilibration technique with standards of known H isotope ratios to account for the potential exchange of H between air and tissues and its effects on δD analysis (Wassenaar and Hobson 2000, 2003). We put ~0.3 mg of each of 3 standards (Bowhead Whale Baleen [BWB], Chicken Feather [CFS], and Cow Hoof [CHS]) in silver capsules, placed them beside samples on a bench top in the laboratory, and allowed them to equilibrate with local water vapor for ~72 h. We loaded samples and standards into a zero-blank autosampler. After pyrolysis in a thermal conversion/elemental analyzer (TC/EA; Thermo Finnigan, Bremen, Germany) and analysis in a Delta XP mass spectrometer (Thermo Finnigan), we adjusted all values to bring BWB, CFS, and CHS to δD values of −108‰, −147‰, and −187‰, respectively (Wassenaar and Hobson 2000, 2003). This technique standardizes all values relative to the Vienna Standard Mean Ocean Water (VSMOW) scale. To monitor precision, we analyzed a single sample (homogenized aquatic moss grown in laboratory conditions) every time samples were run. These samples yielded δD = −91.7 ± 3.3‰ (SD; n = 6). BWB, CFS, and CHS had values of −108.4 ± 1.5‰ (n = 63), −146.5 ± 1.8‰ (n = 84), and −187.1 ± 2.1‰ (n = 62), and a commercially available keratin standard yielded δD = −116.2 ± 2.6‰ (n = 31).

For analysis of C isotope ratios, we weighed ~1.0 mg (plants) or ~0.2 mg (animals) of dried powdered samples into tin capsules and combusted them in a NC2500 elemental analyzer (Carlo Erba, Milan, Italy). Resultant gases were delivered via continuous flow to a Thermo Finnigan Delta XP mass spectrometer. We used International Atomic Energy

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**Table 1.** Locations and sizes of stream sites in New Brunswick, Canada, and mean (± SD) stable H isotope ratios of biofilm and alder at those sites. δD = stable H isotope ratio (D/H).

<table>
<thead>
<tr>
<th>Site</th>
<th>Latitude (°N)</th>
<th>Longitude (°W)</th>
<th>Order</th>
<th>Alder δD (%)</th>
<th>Biofilm δD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boston Brook</td>
<td>47.52</td>
<td>67.62</td>
<td>3</td>
<td>−167.7 ± 7.1</td>
<td>−212.1 ± 59.2</td>
</tr>
<tr>
<td>Blue Rock Brook</td>
<td>46.38</td>
<td>66.07</td>
<td>2</td>
<td>−149.9 ± 3.0</td>
<td>−180.7 ± 36.4</td>
</tr>
<tr>
<td>Cains River</td>
<td>46.43</td>
<td>66.02</td>
<td>4</td>
<td>−145.9 ± 2.1</td>
<td>−213.1 ± 10.3</td>
</tr>
<tr>
<td>Middle Branch Catamaran Brook</td>
<td>46.86</td>
<td>66.19</td>
<td>3</td>
<td>−159.2 ± 15.6</td>
<td>−173.5 ± 12.0</td>
</tr>
<tr>
<td>Culvert crossing</td>
<td>46.82</td>
<td>66.21</td>
<td>1</td>
<td>−157.2 ± 3.1</td>
<td>−141.3 ± 5.6</td>
</tr>
<tr>
<td>Cedar Brook</td>
<td>47.42</td>
<td>67.80</td>
<td>3</td>
<td>−165.6 ± 7.0</td>
<td>−253.9 ± 30.7</td>
</tr>
<tr>
<td>Cochrane Brook</td>
<td>46.28</td>
<td>65.85</td>
<td>2</td>
<td>−140.4 ± 14.0</td>
<td>−139.7 ± 9.0</td>
</tr>
<tr>
<td>Cains River Downstream</td>
<td>46.54</td>
<td>65.84</td>
<td>5</td>
<td>−146.8 ± 1.6</td>
<td>−208.4 ± 1.0</td>
</tr>
<tr>
<td>Dungarvon River</td>
<td>46.82</td>
<td>65.92</td>
<td>4</td>
<td>−142.3 ± 4.3</td>
<td>−208.7 ± 1.7</td>
</tr>
<tr>
<td>Flewelling Brook</td>
<td>46.32</td>
<td>66.02</td>
<td>3</td>
<td>−153.5 ± 16.0</td>
<td>−135.3 ± 10.0</td>
</tr>
<tr>
<td>Gaspereau River</td>
<td>46.37</td>
<td>65.94</td>
<td>4</td>
<td>−158.1 ± 15.4</td>
<td>−169.2 ± 11.0</td>
</tr>
<tr>
<td>Gounamitz River washout</td>
<td>47.53</td>
<td>67.65</td>
<td>4</td>
<td>−158.8 ± 6.2</td>
<td>−247.2 ± 28.7</td>
</tr>
<tr>
<td>Little Southwest Miramichi</td>
<td>46.88</td>
<td>66.09</td>
<td>6</td>
<td>−150.7 ± 5.0</td>
<td>−215.5 ± 2.3</td>
</tr>
<tr>
<td>Muzroll Brook tributary</td>
<td>46.50</td>
<td>66.07</td>
<td>2</td>
<td>−146.6 ± 9.1</td>
<td>−193.1 ± 2.1</td>
</tr>
<tr>
<td>Newcastle Creek</td>
<td>46.08</td>
<td>66.05</td>
<td>4</td>
<td>−150.3 ± 8.7</td>
<td>−158.9 ± 2.0</td>
</tr>
<tr>
<td>Newcastle Creek tributary</td>
<td>46.14</td>
<td>66.12</td>
<td>2</td>
<td>−144.9 ± 2.4</td>
<td>−188.8 ± 30.0</td>
</tr>
<tr>
<td>Newcastle Creek upstream</td>
<td>46.14</td>
<td>66.09</td>
<td>3</td>
<td>−153.4 ± 8.2</td>
<td>−188.7 ± 1.5</td>
</tr>
<tr>
<td>North Renous headwaters</td>
<td>46.89</td>
<td>66.65</td>
<td>3</td>
<td>−161.4 ± 3.7</td>
<td>−182.3 ± 11.0</td>
</tr>
<tr>
<td>North Renous Lake</td>
<td>46.89</td>
<td>66.62</td>
<td>4</td>
<td>−168.4 ± 0.8</td>
<td>−196.5 ± 1.5</td>
</tr>
<tr>
<td>North Renous 108</td>
<td>46.79</td>
<td>66.19</td>
<td>4</td>
<td>−165.5 ± 1.2</td>
<td>−177.5 ± 3.9</td>
</tr>
<tr>
<td>North Renous washout</td>
<td>46.87</td>
<td>66.43</td>
<td>4</td>
<td>−155.0 ± 6.2</td>
<td>−206.3 ± 12.2</td>
</tr>
<tr>
<td>Otter Brook</td>
<td>46.88</td>
<td>66.04</td>
<td>2</td>
<td>−156.7 ± 4.6</td>
<td>−193.1 ± 16.0</td>
</tr>
<tr>
<td>Restigouche at Boston Brook</td>
<td>47.53</td>
<td>67.63</td>
<td>5</td>
<td>−156.4 ± 10.2</td>
<td>−265.4 ± 47.0</td>
</tr>
<tr>
<td>Renous River at Red Bridge Road</td>
<td>46.81</td>
<td>65.87</td>
<td>6</td>
<td>−145.4 ± 3.8</td>
<td>−192.7 ± 10.6</td>
</tr>
<tr>
<td>Restigouche straight reach</td>
<td>47.46</td>
<td>67.75</td>
<td>4</td>
<td>−158.4 ± 6.4</td>
<td>−236.1 ± 44.4</td>
</tr>
<tr>
<td>Smith Forks</td>
<td>46.96</td>
<td>66.58</td>
<td>6</td>
<td>−170.2 ± 3.1</td>
<td>−239.5 ± 28.6</td>
</tr>
<tr>
<td>Salmon River downstream</td>
<td>46.23</td>
<td>65.85</td>
<td>5</td>
<td>−128.9 ± 6.1</td>
<td>−172.1 ± 5.3</td>
</tr>
<tr>
<td>Salmon River upstream</td>
<td>46.43</td>
<td>65.83</td>
<td>4</td>
<td>−155.8 ± 5.1</td>
<td>−183.6 ± 11.7</td>
</tr>
<tr>
<td>Upper Libbie’s Brook</td>
<td>46.89</td>
<td>66.39</td>
<td>2</td>
<td>−141.9 ± 7.5</td>
<td>−161.7 ± 6.2</td>
</tr>
<tr>
<td>Unamed stream</td>
<td>46.87</td>
<td>66.39</td>
<td>2</td>
<td>−131.3 ± 11.9</td>
<td>−126.3 ± 3.1</td>
</tr>
<tr>
<td>Wasson Creek</td>
<td>46.14</td>
<td>66.08</td>
<td>2</td>
<td>−134.7 ± 2.1</td>
<td>−173.8 ± 12.1</td>
</tr>
</tbody>
</table>
Agencies (IAEA) standards CH6, CH7, N1, and N2 to calibrate data to the Vienna Peedee Belemnite (VPDB) scale. We monitored accuracy and precision with a commercially available standard (acetanilide; δ13C = -33.60 ± 0.12‰, n = 59; Elemental Microanalysis Ltd., Okehampton, UK; Jardine et al. 2008b).

We use the term signal to describe the average difference in δD or δ13C between the 2 food sources (biofilm and alder) within sites. We use the term noise to describe the variation of each food source within each site. Noise encompasses variability from individual organisms, sample heterogeneity, and analytical error (Jardine and Cunjak 2005). We calculated signal-to-noise ratio as:

\[
\text{Signal-to-noise ratio} = \frac{\left( \frac{1}{n} \sum (\delta_{\text{biofilm}} - \delta_{\text{alder}}) \right)}{\frac{1}{n} \sum \text{SD}_{\text{biofilm}} \text{ or SD}_{\text{alder}}}
\]

where X is 13C or D and SD is the standard deviation for the respective isotope analysis. We considered food sources to be isotopically distinct within a site if there was no overlap of mean ±1 SD between sources. This criterion is similar to that used by Jardine et al. (2008b), where mixing models for water striders were used only if food sources differed by >2%. The δ13C data used in that study are the same data presented here.

We used a general linear model analysis of variance (GLM-ANOVA) with source (alder and biofilm; fixed factor) and site (random factor) to test for differences in δD between sources. We used regression analyses to test for an increase in the difference between biofilm and alder isotope ratios with increasing stream size (order).

For benthic invertebrates analyzed for δD and δ13C, we used simple mixing models to calculate % aquatic H and % aquatic C in the diet as:

\[
\% \text{ aquatic} = \left( \frac{\delta_{\text{consumer}} - \delta_{\text{terrestrial}}}{\delta_{\text{aquatic}} - \delta_{\text{terrestrial}}} \right) \times 100
\]

where X is 13C or D and terrestrial and aquatic end-members are site-specific alder and biofilm isotope ratios, respectively. We assumed no trophic fractionation for either δ13C or δD in these models. We present values >100% and <0% as raw values (rather than setting them to 100 and 0, respectively) to illustrate the variation in source proportions for both δ13C and δD. These values simply reflect consumer δ13C or δD that was outside the range of sources.

We tested for differences in δD between laboratory-reared consumers and their diet with a 1-way ANOVA followed by the Bonferroni test to discriminate among types (consumer and diet) and treatments (lipid-extracted and nonextracted). We analyzed the 2 consumer–diet pairings separately.

**Results**

Biofilm had a much larger range in δD and was generally depleted in D relative to alder, whereas δ13C in biofilm also had a broader range than alder but with a similar mean value (Fig. 1). The site and source interaction term for δD was statistically significant (F = 5.33, df = 30, p < 0.001), largely because biofilm δD was similar or elevated relative to alder δD at 4 sites (Table 1). Variation within and between food sources was much higher for δD than for δ13C, but the signal-to-noise ratios for the 2 elements were similar (Table 2). At 16 sites, both δ13C and δD differed between food sources. At 11 sites, only 1 of the 2 elements differed between food sources. Only at 4

**Table 2.** Comparison of the efficacy of δD and δ13C in discriminating between food sources in 31 streams in New Brunswick, Canada. Signal-to-noise ratio is variability (represented by standard deviation [SD]) within sources vs variability between sources. δD = stable H isotope ratio (D/H).

<table>
<thead>
<tr>
<th>Measure of discrimination</th>
<th>δ13C</th>
<th>δD</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of sites where food sources could be distinguished from each other</td>
<td>20</td>
<td>23</td>
</tr>
<tr>
<td>Average difference between food sources (%)</td>
<td>4.0</td>
<td>41.7</td>
</tr>
<tr>
<td>Average SD within sites for biofilm</td>
<td>1.6</td>
<td>15.1</td>
</tr>
<tr>
<td>Average standard deviation within sites for alder</td>
<td>0.7</td>
<td>6.5</td>
</tr>
<tr>
<td>Signal-to-noise ratio for biofilm</td>
<td>2.5</td>
<td>2.8</td>
</tr>
<tr>
<td>Signal-to-noise ratio for alder</td>
<td>5.7</td>
<td>6.4</td>
</tr>
</tbody>
</table>

**Fig. 1.** H (δD) and C (δ13C) stable isotope ratios of biofilm and alder leaves from streams in New Brunswick, Canada.
sites did neither $\delta^{13}$C nor $\delta^D$ differ between food sources. Food sources were less isotopically distinct in small headwater streams than in larger rivers. Regressions of the difference between biofilm and alder isotope ratios vs stream order were significant for both $\delta^{13}$C ($r^2 = 0.42, n = 31, p < 0.001$) and $\delta^D$ ($r^2 = 0.27, n = 31, p = 0.003$) (Fig. 2).

Mixing models suggested that $\delta^D$ can be confounded by nondietary influences or diet–tissue fractionation. $\delta^{13}$C of invertebrate consumers fell within the bounds of the 2 food sources (with 1 exception) and yielded estimates of % aquatic C between 0 and 100%, but $\delta^D$ of the same consumers was often outside the bounds of the 2 sources (Fig. 3). Consumers were often enriched in D relative to the 2 food sources. At Otter Brook, consumers were isotopically out of phase with their food sources. $\delta^{13}$C of heptageniid mayflies ($-39.0\%_o$) was far lower than $\delta^{13}$C of biofilm ($-33.9\%_o$) at the site and yielded an estimate of % aquatic C = 241%, and $\delta^D$ of aeshnid dragonflies ($-108.5\%_o$) was much higher than $\delta^D$ of alder ($-156.7\%_o$) or biofilm ($-193.1\%_o$).

$\delta^D$ differed significantly between laboratory-reared consumers and their diets (trout vs pellets: $F = 121.7, df = 3, p < 0.001$; striders vs crickets: $F = 204.6, df = 3, p < 0.001$; Fig. 4). $\delta^D$ of trout was higher than that of their pellet diet, but this difference disappeared after lipid extraction (Fig. 4). $\delta^D$ of water striders was lower than that of their cricket diet, but this difference disappeared after lipid extraction (Fig. 4). Lipid extraction caused large changes in $\delta^D$ of the pellets (average change = 46.1 ± 4.3\%_o SD) and water striders (average change = 56.0 ± 2.4\%_o SD). Pellets and water striders were rich in lipids (indicated by high C/N of nonextracted samples; pellet C/N = 6.0 ± 0.2 SD, strider C/N = 5.4 ± 0.5 SD; Jardine et al. 2008b) (Post et al. 2007, Logan et al. 2008). Lipid extraction had less effect on the $\delta^D$ of trout muscle (average change = 12.7 ± 3.5\%_o SD) and crickets (average change = 26.2 ± 3.5\%_o SD). Both of these tissues had low lipid levels (indicated by low C/N prior to extraction; trout C/N = 3.5 ± 0.1 SD, cricket C/N = 3.3 ± 0.1 SD).

![Fig. 2.](image1.png) **Fig. 2.** The difference between alder and biofilm mean $\delta^D$ (stable H isotope ratio; D/H) and alder and biofilm mean $\delta^{13}$C as functions of stream order. Best-fit equations are shown for both isotopes.

![Fig. 3.](image2.png) **Fig. 3.** Estimates of % aquatic H and % aquatic C (calculated from mixing models for $\delta^D$ [stable H isotope ratio; D/H] and $\delta^{13}$C respectively) for benthic invertebrates in New Brunswick, Canada, streams before and after accounting for the potential contribution of water to tissue H. Values <0 and >100 occur when consumer ratios fall outside the range of the 2 food sources.

![Fig. 4.](image3.png) **Fig. 4.** Mean ($\pm 1$ SD) stable H isotope ratios of laboratory-reared consumers and their diets before and after lipid extraction (LE). Trout (*Salvelinus fontinalis*) were fed a formulated pellet diet and water striders (*Aquarius remigis*) were fed crickets (Gryllidae) sold commercially as a pet food. Type refers to consumer vs diet, and treatment refers to before or after LE. Different letters indicate statistically significant differences among type–treatment pairs.
Discussion

Our results suggest that δD might be useful as a complementary, but not an alternative, method of tracing organic matter sources to consumers in temperate streams. δD differed significantly between the 2 food sources in our streams, but these differences were not as large as those reported previously for arid environments (Doucett et al. 2007). Furthermore, mixing models with δD provided values for consumers that were often outside the bounds of the 2 food sources, a result that suggests unaccounted variability either in sources or in consumers. Moreover, laboratory trials indicated that lipids could strongly interfere with interpretation of consumer–diet relationships on the basis of δD.

Signal-to-noise ratios indicate the potential resolution of an isotope of interest. D has higher within-site variability in a food source (average SD within sites for biofilm was ~15%) and poorer precision on modern mass spectrometers (typically ~3‰ SD for a given organic sample measured repeatedly; Tuross et al. 2008) than does 13C. However, the wide separation of δD values between food sources helps overcome these limitations and places δD on par with δ13C as a tool with which to measure reliance on aquatic vs terrestrial organic matter in streams (Doucett et al. 2007). Furthermore, the weak correlation between δ13C and δD in source material (biofilm r = −0.24, alder r = −0.24; Fig. 1) suggests that biogeochemical controls on the 2 elements are different. Thus, δ13C and δD provide 2 independent tracers when used together. This feature could reduce the need to eliminate sites because of poor separation between sources, as in Jardine et al. (2008b), where 43 of 88 stream sites were excluded because aquatic and terrestrial δ13C were not sufficiently distinct. However, the broader utility of δD is questionable, particularly if δD of aquatic vegetation varies systematically with stream physicochemical variables, as Finlay (2004) showed for δ13C and water velocity and CO2 availability.

Theory suggests that evapotranspiration should control terrestrial δD (Pendall et al. 2005), but evapotranspiration might not be the key mechanism underlying differences in δD between terrestrial and aquatic plants. We expected that terrestrial vegetation in our moderately mesic study region (mean annual precipitation = 1000–1500 mm) would be far less enriched in D than was terrestrial vegetation sampled by Doucett et al. (2007) near the arid Colorado River (mean annual precipitation <500 mm). Instead, δD values for terrestrial material in our study (mean for all data = −153.8 ± 1.3‰ SE) were similar to those of riparian plants sampled by Doucett et al. (2007) (mean for all data = −147.2 ± 4.8‰ SE). However, δD of biofilm in our study (mean for all data = −193.7 ± 3.9‰ SE) differed from δD of aquatic food sources reported by Doucett et al. (2007) (mean for all data = −245.5 ± 8.4‰ SE). These results suggest that characteristics of the water body being analyzed (e.g., chemistry and δD of water), the effects of those variables on δD of aquatic vegetation, and the type of aquatic vegetation present (species and growth forms), rather than climatic characteristics that probably influence terrestrial δD, will determine whether δD of aquatic and terrestrial plants differ.

Differences in leaf water retention among species in the 2 regions might have obscured differences in water loss and subsequent isotopic enrichment during transpiration. Doucett et al. (2007) sampled willow (Salix spp.), mesquite (Prosopis spp.), and tamarisk (Tamarix spp.). Willow and mesquite have evapotranspiration rates similar to alder (0.4–0.9 m/y; Herbst et al. 1999, Shafroth et al. 2005). Evapotranspiration rates as high as 3 m/y have been reported for tamarisk, but other studies have found lower values (Shafroth et al. 2005). Assuming equal evapotranspiration rates, δD of terrestrial plants should reflect δD of annual precipitation in the region where the plants grew. δD of annual precipitation in New Brunswick (Canada) (our study) and Nevada/Arizona (USA) (Doucett et al. 2007) is relatively similar (www.waters isotopes.org; Bowen et al. 2005). Ultimately, the δD value of riparian plants will be determined by their proportional use of deep groundwater reserves (Dawson and Ehleringer 1991) vs river water, which could include water from distal locations with different δD. This effect, coupled with the effects of evapotranspiration, could cause riparian plant δD to deviate from the δD of local rainfall.

Analyses of laboratory-reared consumers suggested that lipids might interfere with interpretation of δD values from wild animal populations. Diet–tissue fractionation before lipid extraction was ~30‰ for laboratory-reared trout and ~−25‰ for laboratory-reared striders, but after lipid extraction, we observed no discernable difference in δD between these consumers and their diet, a result similar to recent findings by Solomon et al. (2009). Other laboratory studies of animals reared on constant diets have found large variation (>100‰) in δD among tissues that was caused, at least in part, by differences in lipid content (Hobson et al. 1999, Tuross et al. 2008). Much of the observed variation in δ13C diet–tissue fractionation (Post 2002) also might be a consequence of the confounding influence of lipids, and models have been developed to correct for this variability.
Lipids also interfere with analytical capacity for δD. Lipids largely contain C–H bonds that are theoretically nonexchangeable (Schimmelmann et al. 1999), so lipid-rich tissues have far less H available to exchange with ambient water vapor in the laboratory than does lipid-poor tissue. This difference would confound analyses in which keratin standards are used to calibrate data to the VSMOW scale because these standards are designed for organic samples with ~20% exchangeable H (Wassenaar and Hobson 2000, 2003). Equilibration of lipid-rich tissues (with far lower exchangeable H) with the keratin standards would over-correct the data and would introduce error into δD measurements. Benthic invertebrates synthesize lipids, and some taxa have high % lipid (Meier et al. 2000). Thus, much of the observed variation in % aquatic H in our study could have been caused by the variable lipid content of the organisms themselves.

In addition to analytical challenges presented by lipids, lipids also can affect δD values directly. Similar to discrimination against 13C during de novo lipid synthesis (DeNiro and Epstein 1977), fractionation against D also occurs during this process (Sessions et al. 1999, Chikaraishi et al. 2004). This fractionation could influence δD in invertebrates and other taxa that synthesize lipids. Controlled studies also suggest that a strong linear relationship exists between algal lipid δD and δD of surrounding water (Zhang and Sachs 2007), so the observed differences in biofilm δD between our study and that of Doucett et al. (2007) probably were the result of the unique biogeochemistry and resultant δD values of the waters in which the biofilm was growing. However, variation in δD also exists among lipid classes (Zhang and Sachs 2007), so the bulk δD signature of algae will represent an amalgamation of several factors, including analytical error introduced by reduced H exchange relative to keratin standards.

Direct uptake of water to the body pool also might have contributed to incorrect estimates of % aquatic H. Hobson et al. (1999) reported that ~20% of the H in quail blood, muscle, and liver was from ingested water, not from diet. The average value for ω (the contribution of water to tissue H) is 0.17 ± 0.12 SD for aquatic consumers (Solomon et al. 2009). We do not have data for water for our rivers, but we can estimate δD of river water as being between −100 and −60‰ based on continental maps by Kendall and Coplen (2001). We used the maximum value for water (−60‰) and the average value of ω (0.17; Solomon et al. 2009), to recalculate source proportions using the formula

$$\delta D_{\text{consumer}} = \omega \times \delta D_{\text{water}} + (1 - \omega) \times (t \times \delta D_{\text{terrestrial}}) + (1 - t) \times \delta D_{\text{biofilm}}$$

where t is the proportion of terrestrial H in the diet (Solomon et al. 2009), and solved with least-squares iterations. These calculations yielded source H proportions that were far more constrained than our original values, with only 3 values outside the range of isotope values for food sources (vs 11 when H uptake from water is ignored; Fig. 3). We applied the same calculations to our laboratory data and found a slight negative diet–tissue fractionation for trout (trout δD corrected for water contribution = −118.9 ± 3.6‰ SD compared to pellet diet δD = −105.5 ± 4.9‰ SD, F = 21.9, df = 1, p = 0.002), but statistical equivalence between δD of water strider and their cricket diet (F = 2.5, df = 1, p = 0.188).

Heptageniids and aeshnids living in Otter Brook had $\delta^{13}C$ and δD values that corresponded poorly with available food sources. Otter Brook is a small, groundwater-fed stream that remains ice-free during the winter. It has biofilm and invertebrates that are depleted in 13C (<−32‰; Cunjak et al. 2005), and the δ13C value for heptageniids observed in our study (−39‰) was only 2‰ below the lowest observed value for biofilm from the system. Therefore, trophic fractionation could account for the high % aquatic H estimate for this taxon because consumers can be depleted in 13C by as much as 3‰ relative to their diet (Post 2002). However, aeshnids require a different explanation. Their δD values were ~50‰ higher than the most D-enriched food source (terrestrial matter) at the site. This taxon might have a high contribution to tissue H from water. If we use the maximum value of ω (0.39; Solomon et al. 2009) and determine source proportions for aeshnids from Otter Brook, we find that % aquatic C = −11%, a far more reasonable value than values calculated with no contribution from water (−135% aquatic H) and with the average (0.17) contribution from water (−50% aquatic H).

At 4 sites where alder and biofilm δD were not distinct, deposition of terrestrially derived particulate organic matter might have caused contamination of biofilm samples. The difference in isotope ratios (for both δD and δ13C) between food sources approached 0 in the smallest streams (orders 1–2), possibly reflecting the larger input of terrestrial material into these small streams (Wallace et al. 1997). This problem...
could be overcome by using colloidal silica to separate algae from terrestrial detritus (Hamilton et al. 2005) to provide better end-members in mixing models when using δD and δ13C.

A final consideration when evaluating the occasional mismatch between δD of consumers and food sources is conditioning of terrestrial leaves. Bacterial breakdown of leaf matter once it reaches the stream might result in isotopic fractionation of δD. A similar phenomenon has been observed for δ13C (Finlay 2001). We sampled terrestrial material as fresh leaves from the riparian zone, and the source of H for bacteria colonizing leaf matter in the stream is unknown. Thus, our δD values for this food source could have been slightly different from that available to consumers as detritus. However, changes in δ13C with conditioning are relatively small (~2‰) change from fresh material to fine particulate organic matter; Finlay (2001), so changes in δD duringconditioning are likely to have introduced a small amount of error compared to other factors discussed above.

Our data expand the potential applicability of δD as a source tracer in aquatic systems, but they also highlight some of the challenges of using this isotope in a relatively new manner. The contribution of ambient water to total body H of aquatic consumers is now better understood (Solomon et al. 2009), but our study illustrates that more work is needed to understand the effects of lipid content on δD, both from a physiological (fractionation) and technological (exchangeable H) perspective. If these and other unknowns continue to generate uncertainty, the use of models that incorporate this uncertainty into source proportion estimates (e.g., Moore and Semmens 2008) might ultimately aid in the further expansion of the technique.

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Deuterium in Temperate Streams

893