



**Understanding and overcoming baseline isotopic variability
in running waters**

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3 25 **Abstract**
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5 26 Natural abundances of stable isotopes in lotic food webs yield valuable information
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7 27 about sources of organic matter for consumers and trophic structure. However,
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9 28 interpretation of isotopic information can be challenging in the face of variability in
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11 29 organisms at the base of food webs. Unionid and dreissenid mussels, commonly used
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13 30 as baseline organisms in lakes, are uncommon in many river settings and can have
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15 31 variable diets, thus making them unsuitable as a universal baseline for many river
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17 32 food web studies and often forcing reliance on more common benthic insects for this
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19 33 purpose. Turnover rates of body carbon and nitrogen in insects are relatively rapid (1
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21 34 to 50 days half life). These rapid turnover rates in primary consumers can result in
22
23 35 considerable temporal variability in $\delta^{13}\text{C}$ that rivals that of algae (>10‰ range within
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25 36 a site). This suggests that using primary consumers as a surrogate baseline for algae
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27 37 may not circumvent the problem of temporal variability and the resultant mismatch of
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29 38 sources with longer-lived, slow-growing secondary and tertiary consumers. There are
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31 39 several strategies for reducing the influence of these confounding factors when
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33 40 bivalves with a known diet are not present. These include sampling over large spatial
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35 41 scales and correlating $\delta^{13}\text{C}$ of consumers with the source of interest (e.g. benthic
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37 42 algae), sampling baseline organisms multiple times in the weeks preceding sampling
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39 43 of larger consumers (particularly in response to large changes in discharge), and using
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41 44 algal-detrital separation methods and multiple tracers as much as possible.
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43 45 Incorporating some of these recommendations and further exploring variability at the
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45 46 base of the food web will potentially provide greater insights into consumer–resource
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47 47 coupling in running waters and more robust conclusions about food web structure and
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49 48 energy flow in these dynamic systems.
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3 49 **Key words:** bivalves, insects, turnover, temporal variability, food web pathways,
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5 50 organic matter sources
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10 52 **Introduction**

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12 53 The natural abundance of stable isotopes in consumers reveals information
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14 54 about energy provenance, food web structure and habitat use (Fry, 2006). Several
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16 55 assumptions underpin the interpretation of isotopic information, most notably variable
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18 56 diet-tissue fractionation within and among consumers (Post, 2002; Jardine *et al.*,
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20 57 2006). While some of these assumptions are often explicitly stated and addressed,
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22 58 others are rarely discussed. Critical examination and communication of these
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24 59 assumptions can help avoid misinterpretation of isotopic data (Wolf *et al.*, 2009).
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27 60 Potential pitfalls in interpretation of stable isotope patterns may be particularly
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29 61 acute in food web studies of running waters because of the dynamic nature of these
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31 62 systems (e.g. hydrological variability, organic matter inputs, floodplain connectivity).
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34 63 Approaches that account for within- and among-system variation in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ of
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36 64 the organic matter sources that support food webs (Vander Zanden and Rasmussen,
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38 65 1999; Post, 2002) have been successfully employed in lacustrine and marine
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40 66 environments (Post *et al.*, 2000; Jennings *et al.*, 2002), yet studies in running waters
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42 67 continue to be beset by uncertainty in the isotopic composition of basal sources that
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44 68 can inhibit the effective use of isotopes (France, 1995; Finlay, 2001). Despite these
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46 69 challenges, stable isotope analysis (SIA) of carbon, nitrogen, and more recently
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48 70 hydrogen and sulphur, increasingly provide new insights into dietary habits and
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50 71 sources of organic matter for metazoan consumers, food chain lengths, and
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52 72 biogeochemical cycling in streams and rivers (Perry *et al.*, 2003; Reid *et al.*, 2008;
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54 73 Walters and Post, 2008; Walters *et al.*, 2008) and their use will undoubtedly continue
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3 74 to grow. Hidden behind these successful applications of SIA in running waters are
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5 75 numerous unpublished studies where data are difficult to interpret because of
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7 76 insufficient sampling of sources or key consumer groups.
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10 77 One of the most notable challenges facing stream ecologists using isotopes is
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12 78 the spatiotemporal variability in isotope ratios of in-stream primary producers (i.e.
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14 79 periphyton) (Finlay, 2004; Hadwen *et al.*, 2010) and its contamination by detritus
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16 80 (Hamilton *et al.* 2005). Much has been written about the spatial component of
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18 81 isotopic variability, with variability in $\delta^{13}\text{C}$ occurring among streams, reaches within a
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20 82 stream, or even within a reach (e.g. France, 1995) and current methods attempt to take
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22 83 into account some of the $\delta^{13}\text{C}$ “patchiness” in running waters by either measuring
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24 84 water velocity to standardize across sites (Rasmussen and Trudeau, 2010), limiting
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26 85 sampling to either pools or riffles (Finlay *et al.* 2002), or amalgamating samples from
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28 86 representative habitats (pools and riffles) to produce an average value for a given
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30 87 reach (e.g. Jardine *et al.*, 2008). However, temporal isotopic variability is both less
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32 88 well understood and acknowledged (Hadwen *et al.*, 2010; Hladyz *et al.*, 2010).
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36 89 One of the proposed solutions to the challenges posed by primary producers
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38 90 (spatiotemporal variability, impure samples of microalgae, and herbivore N isotope
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40 91 fractionation) is to select representative primary consumers that are known to feed on
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42 92 the sources of interest (Anderson and Cabana, 2007) and employ them as indicators of
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44 93 algal isotope ratios (Finlay *et al.*, 1999; Walters and Post, 2008; Olsson *et al.*, 2009).
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46 94 This technique makes the assumption, among others, that primary consumer isotope
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48 95 ratios are less temporally variable than those of algae (Cabana and Rasmussen, 1996),
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50 96 yet few data are available to support this. Given that many stable isotope studies in
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52 97 streams employ single-event sampling (e.g. Jardine *et al.*, 2008; McHugh *et al.*,
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54 98 2010), the growing appreciation of seasonal variability in different food web
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3 99 components suggests that it is important to characterize temporal isotopic variability
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5 100 (Hadwen *et al.*, 2010; Hladyz *et al.*, 2010).
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7 101 In this paper we address the relative advantages and disadvantages of using
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9 102 primary producers or primary consumers as isotopic baselines in food web studies of
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11 103 running waters. First we present empirical presence/absence data for bivalves (the
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13 104 most frequently nominated primary consumer to indicate baseline isotopic ratios) in
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15 105 rivers. We then combine published and unpublished data on turnover rates and
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17 106 resultant temporal isotopic variability in algae and primary consumers from divergent
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19 107 riverine systems (temperate and subtropical, hydrologically stable and dynamic) to
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21 108 compare their utility. We finish by offering some recommendations to help account
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23 109 for the confounding factors associated with the use of stable isotopes in studies of
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25 110 river food webs.
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31 112 **Methods**

32 113 ***Bivalve occurrence***

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35 114 To determine the frequency of occurrence of bivalve molluscs in standard
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37 115 benthological samples, we analyzed presence/absence data for freshwater mussels
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39 116 from 63 sites in New Brunswick (Canada) that were sampled as part of a broader food
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41 117 web stable isotope study (Jardine *et al.*, 2008). These samples were collected with a
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43 118 D-frame kick net in riffles and runs by disturbing the stream bed. A comparable
44
45 119 macroinvertebrate dataset from Australia, derived from surveys using identical
46
47 120 sampling methods, was also examined to establish the relative frequency of
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49 121 occurrence for bivalves in streams in sub-tropical Australia. These data, from the
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51 122 Ecosystem Health Monitoring Program (EHMP) in southeast Queensland, include
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3 123 macroinvertebrate collections from up to 132 sites, sampled twice yearly between
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5 124 2002 and 2007 (Bunn et al., 2010).
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10 126 ***Turnover rates***

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12 127 To assess the time scales of isotopic change in food webs, half-lives of carbon
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14 128 and nitrogen in primary producers and consumers in streams were estimated from the
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16 129 literature (Table 1). Half-lives are calculated by fitting an exponential model to
17
18 130 isotope data plotted through time following a diet switch (Hobson and Clark, 1992).
19
20 131 The equations take the form: $y = b + a * e^{ct}$ where t is the time in days since the diet
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22 132 switch (or the addition of tracer) and c is the derived constant. This constant can then
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24 133 be entered in the formula: half-life = $\ln(0.5)/c$ to yield a half-life estimate (Hobson
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26 134 and Clark, 1992). When data were not amenable to calculations of this type, half-
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28 135 lives were roughly estimated from figures provided (e.g. Doi *et al.*, 2007). Most of
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30 136 the available information on elemental turnover comes from ^{15}N addition experiments
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32 137 and thus represents nitrogen, which may turn over at different rates than carbon or
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34 138 other elements (Jardine *et al.*, 2008). However, differences between elements are
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36 139 likely to be small compared with differences among organisms or tissues; thus, there
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38 140 is reason to suspect that similar trends would emerge for C.
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43 141 To simulate the response of biota with different turnover rates to a
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45 142 hypothetical change in algal isotope ratios, we adapted a compartment model from
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47 143 Hamilton *et al.* (2004). A $\delta^{15}\text{N}$ increase of 5‰ in the dissolved N source was
48
49 144 introduced and the resultant changes in $\delta^{15}\text{N}$ of algae (turnover rate = 0.07 d^{-1}), two
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51 145 benthic insects (mayflies, turnover rate = 0.26 d^{-1} , and beetle larvae, turnover rate =
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53 146 0.06 d^{-1} , representative of selected primary consumers), and unionid mussels
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55 147 (*Pleurobema sintoxia*, turnover rate in muscle = 0.003 d^{-1}) were simulated. These
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3 148 turnover rates are estimates based on empirical data, they include a trophic
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5 149 fractionation for the three consumers of 3.3‰ (Hamilton *et al.*, 2001, 2004), and the
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7 150 model assumes that 100% of the diet is derived from algae. The increase was then
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10 151 removed after 25 days, thereby simulating a change in algal $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ (McCutchan
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12 152 and Lewis, 2002; Hadwen *et al.*, 2010) that can occur as a result of changes in flow
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14 153 rates, CO_2 supply or nutrient inputs (Ostrom *et al.* 1998, Finlay 2004)
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18 19 155 ***Temporal variability***

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21 156 To examine seasonal variation in isotope ratios of primary producers and
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23 157 consumers, we collated data from published and unpublished stream and river studies
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25 158 in diverse settings (Table 2). Data sources are derived from collections in eastern
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27 159 Canada and eastern Australia, consisting of sites sampled at varying temporal
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29 160 intervals (weekly to bi-monthly) and covering periods ranging from two to 12 months
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31 161 (Table 2). These sites encompass the range of hydrological conditions seen in rivers,
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33 162 from highly stable to unpredictable (Puckridge *et al.*, 1998). The Canadian streams
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35 163 are temperate, forested headwaters with spring and autumn peaks in discharge
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37 164 associated with snowmelt and precipitation, respectively. During summer, baseflow
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39 165 is relatively stable with a slow decline from May to October when sampling was
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41 166 conducted. The Australian systems include subtropical streams in forested and
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43 167 urbanized catchments in southeast Queensland and temperate rivers in the northern
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45 168 and southern parts of the Murray-Darling Basin, Australia's largest catchment. Most
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47 169 of these lotic sites are far more hydrologically variable than their Canadian
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49 170 counterparts, with large flows typically (but not always) occurring in either the
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51 171 summer months (December to February, SE Queensland) or winter/spring (June to
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53 172 November, southern Murray Darling system). They also have somewhat
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3 173 unpredictable flows that occur throughout the year depending on sporadic rainfall and
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5 174 releases from dams in regulated systems (e.g. Murrumbidgee River). Sampling
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7 175 occurred over a range of flow conditions in these rivers throughout the year, but
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9 176 system-specific discharge data was unavailable.

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11 Biofilm scrubbed from rocks and other surfaces (wood, mud) is a complex
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13 178 mixture of attached algae (periphyton), bacteria, fungi, small invertebrates and non-
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15 179 living organic matter (Lock, 1981). In this study we restricted our analyses to the
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17 180 evaluation of variability in biofilm and attached filamentous algae because they are
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19 181 the primary producers most commonly sampled in streams and small rivers. Though
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21 182 they may be important in some river settings, we did not focus on aquatic vascular
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23 183 plants or phytoplankton because they are less commonly sampled in running waters
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25 184 and in our data sets they were not analysed often enough to permit temporal analyses.
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27 185 Sampling procedures differed among studies; those reported here are examples of how
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29 186 primary producers and consumers are typically collected for SIA in riverine studies.
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31 187 In the Canadian streams, a minimum of three biofilm samples per site was collected
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33 188 and values averaged for a given site and time. Each of these three samples consisted
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35 189 of material from the non-embedded surface of three rocks, and the three samples were
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37 190 taken across representative areas of the stream reach (typically ~100 m long)
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39 191 including both pools and riffles. Given the known heterogeneity of algal $\delta^{13}\text{C}$ in
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41 192 pools and riffles (Finlay *et al.*, 2002), this approach attempted to capture the mean
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43 193 value for algae growing in the reach. Primary consumers include all taxa collected on
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45 194 a given date that are typically considered scrapers or grazers (Merritt and Cummins,
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47 195 1996; Gooderham and Tsyrlin, 2002). This includes mayflies (e.g. Heptageniidae),
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49 196 water pennies (Psephenidae), shrimps (Atyidae), and others used previously in food
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51 197 web studies (Anderson and Cabana, 2007), but the composition of the primary
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3 198 consumer community differed among sites and times. Consumers were collected
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5 199 from pools and riffles and, similar to algae, multiple samples were analysed and
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7 200 averaged within a site. Guts were cleared for a brief period of several hours prior to
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9 201 freezing.

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11 202 For the Australian systems in southeast Queensland and northern New South
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13 203 Wales and northern Victoria, at least three replicate samples of food web components
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15 204 were collected at each site on each date. The length of study reaches ranged from 20
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17 205 to 35 m. Triplicate benthic algal samples (biofilm and filamentous algae categories
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19 206 described above) were collected from pools, runs and riffles, and from a range of
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21 207 different substrata (typically cobbles and submerged wood). As in the Canadian
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23 208 studies, means across all habitats were calculated to characterize reach-scale isotopic
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25 209 values, and primary consumers belonging to grazer and scraper feeding guilds were
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27 210 included in the analyses and collected in a similar manner.

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29 211 All data presented here were generated by combusting samples in a Carlo Erba
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31 212 NC2500 elemental analyser coupled to a Thermo Finnigan Delta Plus mass
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33 213 spectrometer (University of New Brunswick, Fredericton, Canada) or a Eurovector
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35 214 EA 3000 coupled to an Isoprime mass spectrometer (Griffith University, Brisbane,
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37 215 Australia). Internal standards run repeatedly to monitor accuracy and precision
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39 216 yielded $\delta^{13}\text{C}$ S.D. = 0.2‰, $\delta^{15}\text{N}$ S.D. = 0.3‰, %C S.D. = 1.5%, %N S.D. = 0.5% (n =
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41 217 10, dragonfly larvae at University of New Brunswick) and $\delta^{13}\text{C}$ S.D. = 0.2‰, $\delta^{15}\text{N}$
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43 218 S.D. = 0.4‰, %C S.D. = 2.6%, %N S.D. = 0.9% (n = 29, fish muscle at Griffith
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45 219 University).

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47 220 Variability in time in algae or primary consumers at a given site is presented
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49 221 both as one standard deviation around the overall mean for the sampling period, and
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51 222 the range of mean values observed over the sampling period. Replicate samples of
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3 223 algae or consumers on a given sampling day were averaged and the range and
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5 224 standard deviations of these averages within a site were used as the measure of
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7 225 variability. Ranges therefore represent the range of mean values for a given site
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10 226 sampled through time, rather than the range in individual replicates collected over the
11
12 227 entire study period. For context, extremes in temporal variability were compared with
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14 228 spatial variability within and among sites (France, 1995). For those locations where
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16 229 we had data for both primary producers and primary consumers, we ran paired t-tests
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18 230 to determine if temporal variability was higher in one of these groups (using the range
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20 231 in values as the dependent variable). To determine if sites with high variability in
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22 232 algal isotope ratios also had high variability in primary consumer isotope ratios, we
23
24 233 regressed ranges in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of consumers against that of algae, with site as the
25
26 234 unit of replication. Finally, we assessed whether, in the face of such high temporal
27
28 235 variability, there remained strong links between algal isotope ratios and primary
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30 236 consumer isotope ratios by regressing site-specific overall means of consumers
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32 237 against that of algae for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$.
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39 **Results**

40 *Bivalve occurrence*

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43 241 Bivalve molluscs appeared in only six of 63 sites (9.5%) in the Canadian
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45 242 survey over three years of sampling. Although the incidence of occurrence for
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47 243 bivalves in the southeast Queensland dataset was much higher, less than half of the
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49 244 sites (48%) had bivalves.
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53 54 *Turnover Rates*

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3 247 Turnover rates, based mainly on ^{15}N , were relatively fast in benthic organisms
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5 248 of streams (Table 1). Short-lived animals such as blackflies, mayflies, caddisflies and
6
7 249 chironomids had fast elemental turnover with half-lives ranging between one and six
8
9 250 days, while snails showed slower turnover rates (half-lives > 20 days). Amphipods
10
11 251 and beetle larvae exhibited intermediate turnover rates (11 to 21 days). Large mussels
12
13 252 can have very slow turnover rates (e.g. muscle tissue half-life = 231 days), similar to
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15 253 fish and other vertebrates, but sampling faster-turnover tissues (e.g. digestive glands,
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17 254 half-life = four days, Table 1) can make them more comparable to whole-body
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19 255 samples of smaller organisms (Raikow and Hamilton, 2001).
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23 256 The model predicted that an increase in $\delta^{15}\text{N}$ of 5‰ lasting for 25 days would
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25 257 result in almost immediate changes in $\delta^{15}\text{N}$ of algae, followed soon after by increases
26
27 258 in the $\delta^{15}\text{N}$ of insect larvae (Fig. 1). The algae came closest to steady-state
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29 259 equilibrium with their N source, but values for insects remained lower than the new
30
31 260 steady state $\delta^{15}\text{N}$ (i.e. 13.3‰) by the end of day 30. The bivalve muscle tissue,
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33 261 however, showed almost no response (~0.3‰ increase) to the increase in $\delta^{15}\text{N}$
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35 262 because of the very slow turnover rate of this tissue. The mean value for algae for the
36
37 263 entire 115 experimental period was $6.1 \pm 1.4\%$ S.D. but rapid turnover and isotopic
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39 264 change meant that an algal sample collected on a single day (as is typical in an isotope
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41 265 field study) from day 5 to day 120 would almost always have a $\delta^{15}\text{N}$ value that was
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43 266 higher (e.g. 9.2‰ on day 30) or lower (e.g. 5.0‰ from day 90 onwards) than the
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45 267 mean for the period. . Only on days 7, 8 and 44 to 52 would the sampled value be
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47 268 within 0.3‰ of the mean for the period (0.3‰ being the typical analytical error for
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49 269 $\delta^{15}\text{N}$). Likewise, for mayflies with rapid turnover rates, the value measured on a
50
51 270 given day would be within 0.3‰ of the mean value for the period ($9.4 \pm 1.3\%$ S.D.)
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53 271 only on days 9, 10, 11 and 48 to 56. For beetle larvae with intermediate turnover
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3 272 rates, only on days 14 to 19, and 59 to 73 would the sampled value match the mean
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5 273 value for the period ($9.4 \pm 1.2\%$ S.D.). However, for a sample with a slower
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7 274 turnover rate such as the muscle tissue of a bivalve, the measured value on any day in
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9 275 the 115 day period would be within 0.3‰ of the mean value for the period ($8.6 \pm$
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11 276 0.1‰ S.D., Fig. 1).

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15 278 *Temporal Variability*

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18 279 Temporal variability in isotope ratios of algae and primary consumers was
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20 280 high at some sites but relatively low at others (Table 2, two examples in Fig. 2).
21
22 281 Ranges in $\delta^{13}\text{C}$ of up to 15‰ across sampling periods occurred in some of the
23
24 282 Australian rivers (both subtropical and temperate), corresponding to standard
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26 283 deviations around the overall mean of almost 5%. This variation was in many cases
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28 284 marked over short time periods, with shifts of up to 5‰ occurring over a period as
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30 285 short as two weeks (Fig. 2).

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34 286 There was no difference in the temporal variability of primary producer and
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36 287 consumer isotope ratios. The site-specific ranges in $\delta^{13}\text{C}$ of algae were similar
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38 288 compared with that of primary consumers ($t = 1.23$, $p = 0.234$, Fig. 3a), and the site-
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40 289 specific ranges in $\delta^{15}\text{N}$ also did not differ between the two groups ($t = -0.23$, $p =$
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42 290 0.590, Fig. 3b). Surprisingly, the $\delta^{13}\text{C}$ variability (range) in algae did not predict $\delta^{13}\text{C}$
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44 291 variability in primary consumers ($r^2 = 0.12$, $p = 0.130$, Fig. 3a). However, there was a
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46 292 significant association between the range in $\delta^{15}\text{N}$ of primary producers and that of
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48 293 consumers ($r^2 = 0.47$, $p = 0.001$, Fig. 3b). Despite the high temporal variability in
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50 294 algal $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, site-specific mean algal $\delta^{13}\text{C}$ predicted site-specific mean
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52 295 primary consumer $\delta^{13}\text{C}$ ($r^2 = 0.51$, $p < 0.001$, Fig. 4a), and the relationship between
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54 296 these variables was even stronger for $\delta^{15}\text{N}$ ($r^2 = 0.78$, $p < 0.001$, Fig. 4b).

297

298 **Discussion**

299 The use of SIA to resolve food web patterns in streams has become a standard
300 tool for aquatic ecologists but many of these isotopic studies produce results that are
301 difficult to interpret. To improve this outcome here we discuss issues and make
302 recommendations that will lead to more robust conclusions about sources of organic
303 matter (assessed using carbon stable isotopes) and food chain length (assessed using
304 nitrogen stable isotopes) in running waters.

305 A possible solution to the problems associated with spatial and temporal
306 variability in lower trophic levels is to collect long-lived primary consumers such as
307 suspension-feeding bivalve molluscs to provide an indicator of the isotopic ratios of
308 the microalgal food resource (Howard *et al.*, 2005; Gustafson *et al.*, 2007). Turnover
309 modelling in the current study supports this choice because these longer-lived
310 organisms provide a long-term baseline average for $\delta^{15}\text{N}$ that can be better related to
311 top predators that themselves exhibit slow turnover (Cabana and Rasmussen 1996).
312 This approach has been used successfully in lakes (Post, 2002) and is an ideal option
313 for baseline isotopic assessment in streams and rivers when bivalves are present and
314 their diet is well known. Mussels can be uncommon and/or patchy in many streams
315 and rivers (Bogan, 1993; Anderson and Cabana, 2007), and even in areas where they
316 are common (e.g. southeast United States, Atkinson *et al.*, 2010) their dietary
317 preferences are highly variable and in many cases unknown. They often consume and
318 assimilate fine particulate organic matter that is itself a mix of terrestrial and algal
319 material (Raikow and Hamilton, 2001; Atkinson *et al.*, 2009). As such, they are
320 useful representatives as baseline organisms for food chain length studies because
321 they represent the “average” baseline $\delta^{15}\text{N}$ for the food web, but they are less suitable

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3 322 as an end-member to discriminate amongst organic matter sources (e.g. algae versus
4
5 323 leaf litter) with $\delta^{13}\text{C}$, the latter being a more common question answered with isotopes
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7 324 in streams and rivers (Hamilton *et al.* 1992, France 1995, Finlay *et al.* 2002,
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9 325 McCutchan and Lewis 2002, Bunn *et al.* 2003, Perry *et al.* 2003, Doucett *et al.* 2007,
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11 326 Jardine *et al.* 2008, Reid *et al.* 2008, Hadwen *et al.* 2010, Hladysz *et al.* 2010). When
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13 327 lacking an obvious long-lived consumer to serve as a baseline in streams and rivers,
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15 328 we are faced with two options – 1) measuring algae as the base of the food web, or 2)
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17 329 measuring benthic insects as the base of the food web. While both of these options
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19 330 present their own unique challenges, the common feature of both is a high degree of
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21 331 temporal variability related to rapid turnover of C and N in tissues.
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25 332 Previous studies have shown a rapid response of primary producers and
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27 333 consumers to ^{15}N tracer additions (Mullholland *et al.*, 2000; Hamilton *et al.*, 2004;
28
29 334 Hadwen and Bunn, 2005), suggesting fast turnover of body N (and associated C) in
30
31 335 short-lived aquatic biota (Cabana and Rasmussen, 1996). Consumers with short life
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33 336 spans (e.g. blackflies and mayflies, Table 1) are likely to respond quickly to isotopic
34
35 337 changes in primary producers because their relative growth rates are rapid (Fig. 1).
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37 338 Furthermore, because of the relative synchrony in the turnover rates (Table 1) and
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39 339 resultant isotope ratios of algae and short-lived primary consumers (Finlay 2001;
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41 340 McCutchan and Lewis 2002, Fig. 1), neither group may be adequate at representing
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43 341 the long-term average for these resources; yet the long-term average is more
44
45 342 appropriate to match with isotope ratios of higher-order consumers with slow turnover
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47 343 rates (O'Reilly *et al.* 2002). An empirical example of this is shown in Fig. 5
48
49 344 (modified from Jardine *et al.*, 2009a). Benthic feeding sculpin (*Cottus* sp.) with an
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51 345 invertebrate diet and limited mobility (Rasmussen *et al.*, 2009) are isotopically out-of-
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53 346 phase with their equally-immobile invertebrate prey, likely due to rapid changes in
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3 347 invertebrate $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in response to a nutrient pulse (in this case from a trout
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5 348 farm located upstream of the site). A more appropriate match in this case would have
6
7 349 been the mean value for multiple temporal samples of short-lived invertebrates at this
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9 350 site with the muscle tissue of the sculpin, or the comparison of a rapid turnover tissue
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11 351 such as liver and blood plasma in the sculpin (Dalerum and Angerbjorn 2005) with
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13 352 the one-time sampling of short-lived invertebrates.
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16 353 Some primary consumer taxa with strict diets have relatively long
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18 354 development times and would therefore be more suited as isotopic baselines for top
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20 355 predators. These taxa include Pteronarcyidae stoneflies that shred leaf litter and water
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22 356 pennies (Psephenidae) that graze periphyton. Other primary consumers such as snails
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24 357 and algivorous crustaceans (e.g. Atyidae and *Macrobrachium* spp.) are relatively
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26 358 long-lived and can be common, particularly in tropical streams, but more information
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28 359 is needed about their dietary flexibility prior to their adoption as baseline organisms
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30 360 for food web studies. Clearly more research is also required to define the range of
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32 361 turnover rates in aquatic consumers, particularly from tropical and subtropical systems
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34 362 where data are particularly sparse.
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38 363 Following from the above observations regarding elemental turnover in lower-
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40 364 trophic level organisms, it is not surprising that these taxa can exhibit high temporal
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42 365 variability. In many food web studies using isotopes in streams and rivers, single-
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44 366 event sampling is conducted (e.g. Jardine *et al.*, 2008; McHugh *et al.*, 2010). Based
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46 367 on the results of the current study, whenever it is feasible organisms at the base of the
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48 368 food web should be sampled on multiple dates to provide an adequate representation
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50 369 of the potential isotopic variability at a given site (Walters and Post, 2008, Sabo *et al.*
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52 370 2010). Budgetary considerations will largely dictate the use of this approach. If
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54 371 sampling locations are remote and field costs high, it may be more cost-effective to
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3 372 collect more samples and/or employ a second tracer (e.g. δD , $\delta^{34}\text{S}$) during a single
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5 373 visit rather than make multiple visits to sites.
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7 374 Our model predicts that organisms with slow turnover rates (e.g. mussels, but
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9 375 any equivalent taxa including benthic insects with long generation times and slow
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11 376 turnover rates) will show less variable responses to changes in isotope ratios at the
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13 377 base of the food web. Our empirical data suggest that wide fluctuations in $\delta^{13}\text{C}$ of
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15 378 benthic algae can occur over a period as short as two weeks, most likely in response to
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17 379 flow events and associated changes in turbulence, dissolved CO_2 concentrations
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19 380 (Singer *et al.*, 2005), and productivity (Rasmussen and Trudeau, 2007). As a result,
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21 381 more dynamic river systems with large seasonal changes in flow (such as those from
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23 382 Australia shown here) are most likely to exhibit large temporal variations in algal C
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25 383 isotope ratios, particularly if flow ceases during prolonged dry spells, leading to high
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27 384 productivity and CO_2 limitation in isolated pools or waterholes (Bunn *et al.* 2003).
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29 385 Conversely, more hydrologically stable systems such as those in eastern Canada may
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31 386 not exhibit such vast ranges in algal $\delta^{13}\text{C}$ over time.
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35 387 An additional drawback in using algae as an end-member in mixing models
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37 388 when determining the diets of consumers in streams is the possibility that the sample
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39 389 collected is contaminated by organic detritus. In running waters this detritus is often
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41 390 composed primarily of terrestrial or macrophytic material, thus shifting the $\delta^{13}\text{C}$ of
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43 391 biofilm away from pure algae towards the $\delta^{13}\text{C}$ of C_3 plants (-28‰, France, 1995).
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45 392 Furthermore, the biofilm community growing on organic substrates (e.g. wood) tends
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47 393 to be more heterotrophic than that growing on inorganic substrates (e.g. rocks)
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49 394 (Sabater *et al.*, 1998). These heterotrophs may use the substrate directly as a carbon
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51 395 source, leading to a $\delta^{13}\text{C}$ value that resembles the substrate rather than the algae
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53 396 (Walters *et al.*, 2007; Hladysz *et al.*, 2011). One solution to this problem is a technique
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3 397 for purifying algae that employs colloidal silica to create a density gradient (Hamilton
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5 398 *et al.*, 1992, 2005), allowing the denser detritus to settle to the bottom of a collection
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7 399 tube during centrifugation. Alternative approaches exist to either characterize or
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10 400 discriminate between algal sources within biofilm matrices, particularly in those
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12 401 situations where algal purifications are logistically difficult (e.g. remote sampling).
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14 402 Specifically, determining the chlorophyll-*a*:C ratio of biofilm samples, coupled with
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16 403 C:N ratios, can provide some indication of the degree to which biofilm samples are
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18 404 dominated by algal vs. other forms of carbon (Hamilton and Lewis, 1992).

20 405 Filamentous or colonial algae are often easily collected from submerged
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22 406 surfaces in streams and rivers with little contamination by detritus, and it is tempting
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24 407 to use such samples as surrogates for microalgae. However, this conspicuous material
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26 408 may be present largely because it is poorly digestible and thus rarely enters aquatic
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28 409 food webs (Bunn *et al.*, 1999; Delong *et al.*, 2001). Despite this limitation,
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30 410 filamentous algae may be worth sampling if they are consistently present and have
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32 411 isotope ratios that are equivalent to or correlated with those of benthic microalgae
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34 412 (e.g. diatoms) that are more likely to be consumed by grazers. For example,
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36 413 Rasmussen (2010) found that biofilm (rock scrapings) had $\delta^{13}\text{C}$ that was strongly
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38 414 correlated ($r^2 = 0.77$) with that of pure attached filamentous algae (*Cladophora* sp.,
39
40 415 etc) in temperate streams and rivers. Rasmussen (2010) also estimated that up to 33%
41
42 416 of the carbon in biofilm was of terrestrial origin (i.e. detritus). As such, purification
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44 417 of biofilm using density separation techniques (Hamilton *et al.*, 2005) may be useful
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46 418 in combination with sampling of filamentous algae to ascertain the true aquatic end-
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48 419 member for mixing models.

50 420 Given the high variability observed in algal $\delta^{13}\text{C}$ through space and time at a
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52 421 given site, the error that this produces in mixing models, and the logistical difficulties
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3 422 in resampling baseline organisms in remote locations, an alternative method to
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5 423 estimate dietary source proportions (i.e. leaf litter vs. algae) is to use a gradient
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7 424 approach (Bunn *et al.*, 2003; Rasmussen, 2010). To do this, collections of source and
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9 425 consumer material are made at many sites at a single time. The mean values at a
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11 426 given site for the consumer of interest are then regressed against the mean values for
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13 427 the source of interest (typically benthic algae). A slope close to 1 and good fit (high
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15 428 r^2) denote a strong reliance on that particular food source, and spatial and temporal
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17 429 variability of the source is reflected by the scatter around the line (Rasmussen, 2010).
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19 430 An example of this can be seen in Figure 4. Even though source and consumer $\delta^{13}\text{C}$
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21 431 variability was high within the data set presented here (Table 2), the average values
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23 432 were relatively well correlated ($r^2 = 0.51$) with a slope of 0.61, suggesting
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25 433 approximately 60% contribution from benthic algae to primary consumer diet
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27 434 (Rasmussen, 2010). One complication in the application of this technique, however,
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29 435 is consumer movement. A highly mobile consumer will integrate variability among
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31 436 sites and thus not track site to site changes in algal $\delta^{13}\text{C}$ even if benthic algae is
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33 437 important in the diet, resulting in a slope of zero in these plots (Rasmussen *et al.*,
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35 438 2009). The gradient approach is therefore best used when combined with some
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37 439 knowledge of consumer movement patterns from natural history or tagging studies.
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43 At the site level, in many situations $\delta^{13}\text{C}$ does not differ enough between food
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45 441 sources to allow calculation of consumer diets using mixing models. For example, in
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47 442 New Brunswick Canada streams, 43 of 88 sites had $\delta^{13}\text{C}$ in non-purified algae that
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49 443 was within 2‰ of the value (-28‰) for terrestrial vegetation (Jardine *et al.*, 2008).
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51 444 The addition of other source tracers such as nitrogen (e.g. Udy and Bunn, 2001; Bunn
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53 445 *et al.*, 2003; Reid *et al.*, 2008), hydrogen (Doucett *et al.*, 2007; Jardine *et al.*, 2009b)
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55 446 or sulphur (Croisetièrè *et al.*, 2009) or artificial enrichment (Hamilton *et al.*, 2001,
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3 447 2004; Pace *et al.*, 2004; Hadwen and Bunn, 2005) may help in these situations to
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5 448 discern food sources for consumers. In addition, newly emerging compound-specific
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7 449 isotope ratio analysis can reveal both sources and transfers of nitrogen and carbon in
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9 450 food webs (Chikaraishi *et al.* 2009; Lorraine *et al.* 2009). However, the use of these
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11 451 tools comes with its own analytical, methodological and financial challenges;; hence,
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13 452 more research is needed to fully understand sources of variability in their application.
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16 453 With all of the challenges listed herein it is perhaps surprising that SIA of
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18 454 stream and river food webs even works at all. Yet broad scale patterns do suggest
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20 455 links between consumers and their algal diet (Finlay, 2001; Rasmussen, 2010, Fig. 4)
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22 456 and the processes driving food web structure in streams are emerging with the aid of
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24 457 stable isotopes (Walters and Post, 2008; McHugh *et al.*, 2010, Sabo *et al.* 2010).
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26
27 458 What is perhaps poorly represented to new users of SIA in this field is the large
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29 459 number of unpublished data held by numerous users of this technique (including the
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31 460 authors of this paper) that was deemed too difficult to interpret because of the
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33 461 confounding influence of the myriad of factors described above (e.g. Fig. 5). A
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35 462 healthy dose of realism is needed in the isotope community to convey that isotope
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37 463 analysis is not a silver bullet that will answer all questions about food webs in streams
38
39 464 and rivers (and other ecosystems), but rather carries with it many equally challenging
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41 465 assumptions as other traditional techniques. However, by incorporating some of the
42
43 466 recommendations listed here we anticipate improved studies that draw more accurate
44
45 467 and robust conclusions about pure and applied issues in river and stream ecology.
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50 468

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685 **Table 1.** Half-lives (the time to 50% change in isotope ratio following a switch in
 686 nutrient or food source) for various food web components in streams. Consumer data
 687 represent whole-body samples except in the case of mussels *Pleurobema sintoxia* and
 688 crayfish *Oronectes propinquus* where data are for muscle and digestive gland.

Common name	Scientific name	Isotope	Turnover rate (day ⁻¹)	Half-life (days)	Reference
<u>Primary producers</u>					
Algae	algae in epilithon	$\delta^{15}\text{N}$	0.07	8.9	Hamilton <i>et al.</i> , 2001
biofilm	epilithon	$\delta^{15}\text{N}$		11.8	Mulholland <i>et al.</i> , 2000
<u>Heterotrophic bacteria</u>					
microbes	microbes in leaves	$\delta^{15}\text{N}$	0.22	2.9	Hamilton <i>et al.</i> , 2001
microbes	microbes in FBOM	$\delta^{15}\text{N}$	0.14	4.6	Hamilton <i>et al.</i> , 2001
<u>Primary consumers/Omnivores</u>					
blackflies	Simuliidae	$\delta^{15}\text{N}$		1.5 ± 0.2	Overmyer <i>et al.</i> , 2008
blackflies	Simuliidae	$\delta^{15}\text{N}$	0.26	2.3	Hamilton <i>et al.</i> , 2004
mayflies	<i>Baetis</i>	$\delta^{15}\text{N}$	0.22	2.9	Hamilton <i>et al.</i> , 2004
mussels ^a	<i>Pleurobema sintoxia</i>	$\delta^{15}\text{N}$	0.16	4.1	Hamilton <i>et al.</i> , 2004
caddisflies	Hydropsychidae	$\delta^{15}\text{N}$	0.15	4.3	Hamilton <i>et al.</i> , 2004
crayfish ^b	<i>Oronectes propinquus</i>	$\delta^{15}\text{N}$	0.15	4.6	Hamilton <i>et al.</i> , 2004
crayfish ^{c,d}	<i>Oronectes propinquus</i>	$\delta^{15}\text{N}$	0.13	5.3	Hamilton <i>et al.</i> , 2004
mayflies	<i>Stenonema</i> + <i>Stenacron</i>	$\delta^{15}\text{N}$	0.12	5.3	Hamilton <i>et al.</i> , 2004
midges	Chironomidae	$\delta^{13}\text{C}$		~6	Doi <i>et al.</i> , 2007
midges	Chironomidae	$\delta^{15}\text{N}$		~6	Doi <i>et al.</i> , 2007
Beetle larvae	<i>Psephenus</i>	$\delta^{15}\text{N}$	0.06	11.0	Hamilton <i>et al.</i> , 2004
amphipods	<i>Gammarus</i>	$\delta^{15}\text{N}$	0.06	11.6	Hamilton <i>et al.</i> , 2004
Beetle larvae	Elmidae	$\delta^{15}\text{N}$	0.03	21.0	Hamilton <i>et al.</i> , 2004
Snails	<i>Tarebia granifera</i>	$\delta^{15}\text{N}$	0.03, 0.01	20.2, 49.5	McIntyre and Flecker, 2006
mussels ^e	<i>Elliptio complanata</i>	$\delta^{15}\text{N}$		113.0	Gustafson <i>et al.</i> , 2007
mussels ^d	<i>Pleurobema sintoxia</i>	$\delta^{15}\text{N}$	0.003	231.1	Hamilton <i>et al.</i> , 2004

689 ^adigestive gland, ^bjuveniles, ^cadults, ^dmuscle, ^ehaemolymph

Table 2. Annual mean \pm S.D. and range in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ for aquatic primary producers (algae, biofilm) and primary consumers (benthic invertebrates) from streams and rivers sampled seasonally in Australia and Canada. All Canadian sites (references a and b) are in a temperate climate, while the Australian sites are located in temperate (refs c and f) and subtropical climates (refs d and e).

Site	No. of sample dates	Frequency (duration)	$\delta^{13}\text{C}$				$\delta^{15}\text{N}$				Ref.
			1° producers		1° consumers		1° producers		1° consumers		
			Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	Range	
<u>Canadian sites</u>											
Corbett Brook	8	bi-weekly (May-Oct '07)	-28.3 (1.3)	1.6	-29.7 (2.4)	5.4	3.0 (0.9)	2.2	4.4 (1.1)	2.5	a
English Brook	10	bi-weekly (May-Oct '07)	-27.9 (1.6)	3.9	-28.5 (1.6)	3.8	3.1 (0.6)	1.6	5.2 (1.1)	3	a
McKenzie Brook	10	bi-weekly (May-Oct '07)	-26.7 (2.7)	6.7	-27.7 (1.4)	3.2	2.1 (0.6)	1.2	3.7 (1.1)	2.2	a
Parks Brook	10	bi-weekly (May-Oct '07)	-32.9 (2.0)	3.6	-34.6 (2.8)	3.9	4.1 (1.0)	2.1	5.1 (0.9)	1.1	a
Doctor's Brook 1	9	weekly/bi-weekly (May-Sept '07)	-28.4 (1.1)	3.1	-30.4 (2.0)	5.3	4.0 (1.0)	3.2	3.7 (0.9)	2.5	b
Doctor's Brook 2	6	tri-weekly (May-Sept '07)	-28.2 (0.7)	1.8	-28.5 (1.6)	3.1	2.6 (1.4)	3.9	1.6 (1.2)	2.4	b
<u>Australian sites</u>											
Murrumbidgee River 1	5	variable (Sept '00-Nov '01)	-28.3 (2.2)	5.8	-29.5 (1.8)	4.7	11.2 (0.7)	1.9	11.9 (1.7)	4.2	c
Murrumbidgee River 2	7	variable (Feb '00-Nov '01)	-26.8 (0.9)	2.8	-28.8 (1.1)	3.3	10.9 (2.0)	5.9	12.2 (2.4)	5.2	c
Tumut River 1	3	variable (Oct '00-Aug '01)	-27.5 (2.2)	4.4	-29.8 (4.4)	8.6	5.3 (1.1)	2.2	4.9 (1.1)	2.0	c
Tumut River 2	4	variable (May '00-Aug '01)	-28.0 (1.5)	3.4	-29.3 (3.2)	7.2	6.1 (0.9)	2.1	5.3 (1.7)	3.7	c
Goobarragandra River 1	7	variable (Feb '00-Nov '01)	-25.9 (0.8)	2.4	-25.7 (1.6)	4.1	3.0 (0.9)	1.9	3.0 (0.8)	2.6	c

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7	Goobarragandra River 2	5	variable (Aug '00-Nov '01)	-26.0 (1.0)	2.5	-26.3 (0.8)	1.9	1.5 (1.5)	3.8	0.6 (0.2)	0.5	c
8	Goodradigbee River 1	7	variable (Feb '00-Nov '01)	-26.6 (0.9)	2.9	-26.3 (1.1)	3.3	1.8 (0.4)	1.0	0.9 (0.4)	0.9	c
9	Goodradigbee River 2	5	variable (Sept '00-Nov '01)	-26.0 (1.2)	3.4	-26.5 (1.5)	3.8	1.9 (0.6)	1.3	1.6 (0.7)	1.8	c
10	Stockyard Creek	8	weekly (Feb-April '07)	-24.8 (2.7)	8.1	N/A	N/A	0.0 (0.8)	2.7	N/A	N/A	d
11	Left Hand Branch	8	weekly (Feb-April '07)	-24.7 (1.0)	3.2	N/A	N/A	1.4 (0.6)	1.8	N/A	N/A	d
12	Lost World	8	weekly (Feb-April '07)	-25.3 (0.9)	2.9	N/A	N/A	0.6 (0.4)	1.0	N/A	N/A	d
13	Widgee Creek	8	weekly (Feb-April '07)	-23.6 (1.8)	6.0	N/A	N/A	4.2 (0.6)	2.2	N/A	N/A	d
14	Christmas Creek	8	weekly (Feb-April '07)	-27.0 (1.5)	4.5	N/A	N/A	2.7 (0.6)	1.9	N/A	N/A	d
15	Blunder Creek	8	weekly (Jan-March '08)	-34.3 (3.8)	11.7	-35.8 (1.9)	8.2	5.0 (2.1)	7.4	4.8 (2.3)	8.7	e
16	Moolabin Creek	8	weekly (Jan-March '08)	-27.2 (1.5)	5.6	-27.1 (1.8)	6.0	8.1 (0.8)	3.3	9.4 (1.3)	4.0	e
17	Stable Swamp Creek	8	weekly (Jan-March '08)	-36.5 (4.2)	14.3	-29.0 (1.3)	4.5	8.6 (0.9)	3.4	8.2 (0.4)	1.4	e
18	Sheep Station Gully	8	weekly (Jan-March '08)	-31.5 (1.6)	13.6	-29.4 (1.6)	4.7	5.6 (1.6)	5.4	9.4 (1.4)	4.3	e
19			bi-monthly (May 07 - May									
20	Ovens River 1	7	08)	-25.4 (4.9)	14.5	-27.7 (1.1)	3.2	4.7 (1.3)	4.1	9.9 (1.4)	3.9	f
21			bi-monthly (May 07 - May									
22	Ovens River 2	7	08)	-26.4 (3.2)	14.8	-26.9 (2.5)	10.1	3.8 (0.7)	3.3	6.2 (1.6)	5.8	f

^athis study, ^bJardine *et al.*, 2009a, ^cChessman *et al.*, 2009, ^dSpears 2007, ^eTsoi 2008, ^fHladyz *et al.*, 2010

1 **Figure legends**

2 Figure 1. Dynamics of $\delta^{15}\text{N}$ in stream algae and primary consumers during and after a
3 hypothetical step change in the $\delta^{15}\text{N}$ of the dissolved N source that lasts for 30 days.
4 Responses are based on a compartment model and typical consumer N turnover rates
5 for a woodland stream in Michigan (USA), as observed during a summer ^{15}N addition
6 experiment (Hamilton et al. 2001, 2004; Raikow and Hamilton 2001).

7
8 Figure 2. Illustrative examples of streams with low (English Brook, New Brunswick
9 Canada, solid circles) and high (Stockyard Creek, Queensland Australia, open
10 triangles) algal isotopic variability in space and time for $\delta^{13}\text{C}$ (A) and $\delta^{15}\text{N}$ (B).
11 Means for the period of study (\pm standard deviation) are shown for the two streams.

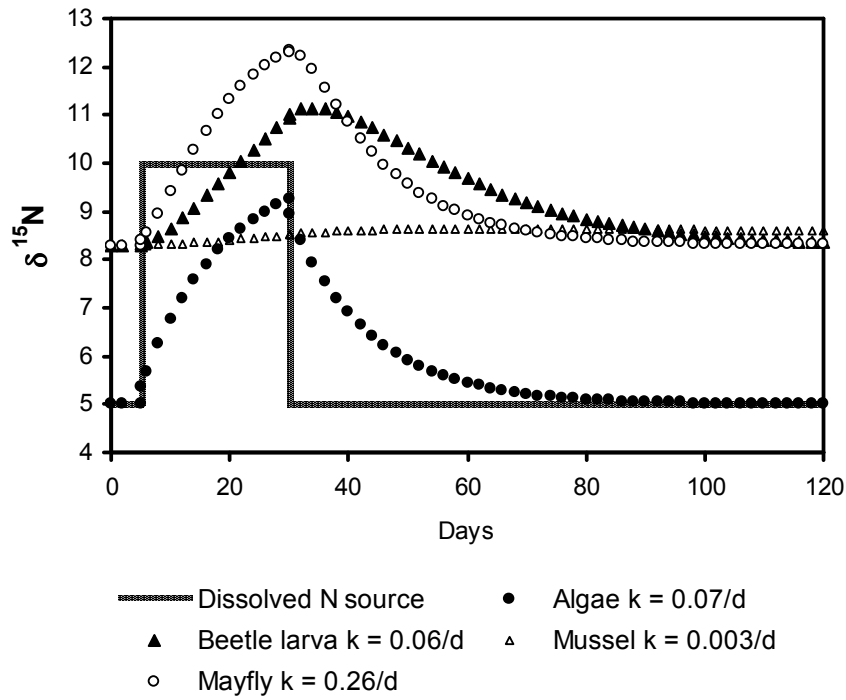
12
13 Figure 3. Relationship between the site-specific range in $\delta^{13}\text{C}$ (A) and $\delta^{15}\text{N}$ (B) of
14 primary producers versus that of primary consumers in Australia and Canada (data
15 from Table 2). Each point represents the maximum minus the minimum value within
16 a site that was sampled multiple times.

17
18 Figure 4. Relationship between the overall site-specific mean $\delta^{13}\text{C}$ (A) and $\delta^{15}\text{N}$ (B)
19 of primary producers versus that of primary consumers. Each point represents a site
20 sampled multiple times in Australia and Canada (data from Table 2).

21
22 Figure 5. An example of a poor isotopic “match” between a consumer (the benthic
23 invertivore sculpin) and its invertebrate prey in a French river (Kerlegan Brook).
24 Modified from Jardine et al. 2009.

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26 Figure 1.



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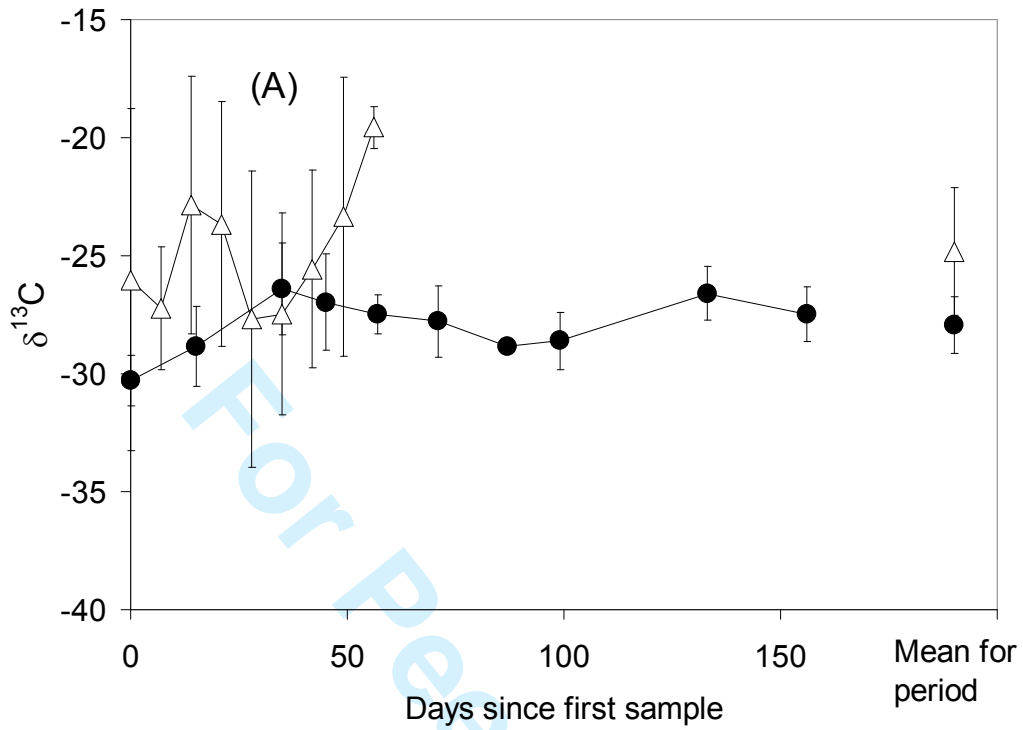
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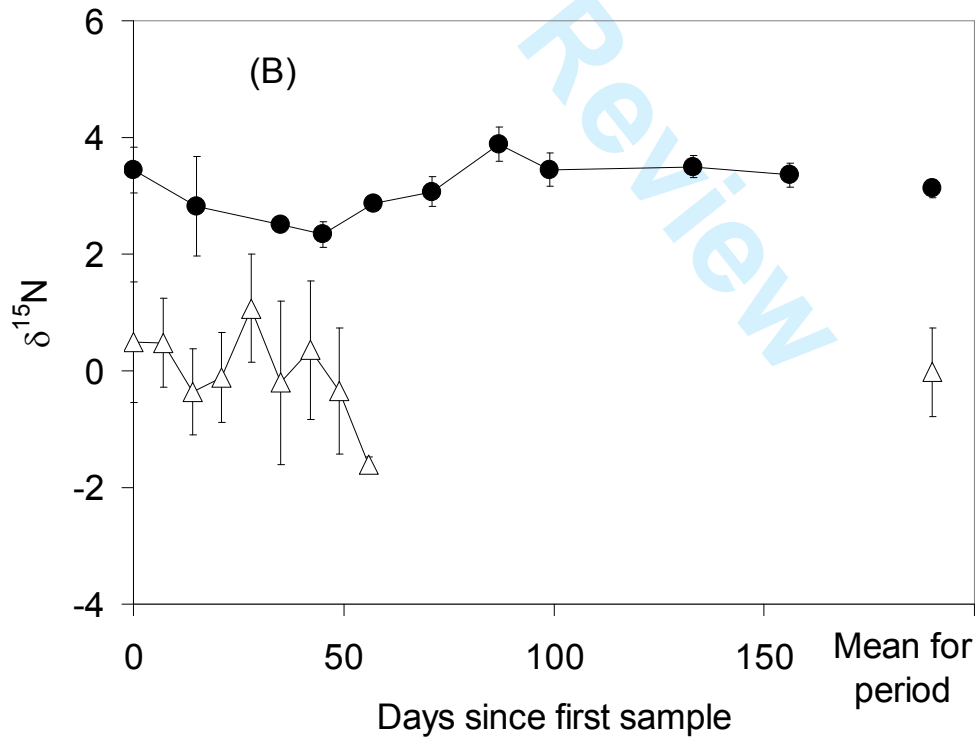
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41 Figure 2.



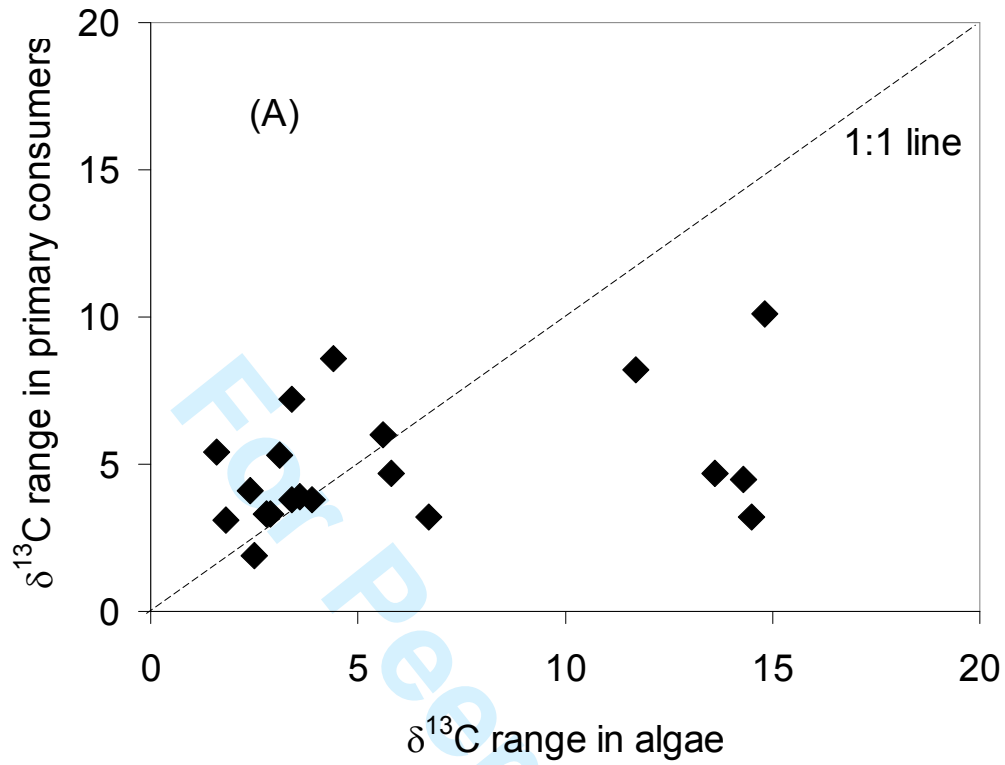
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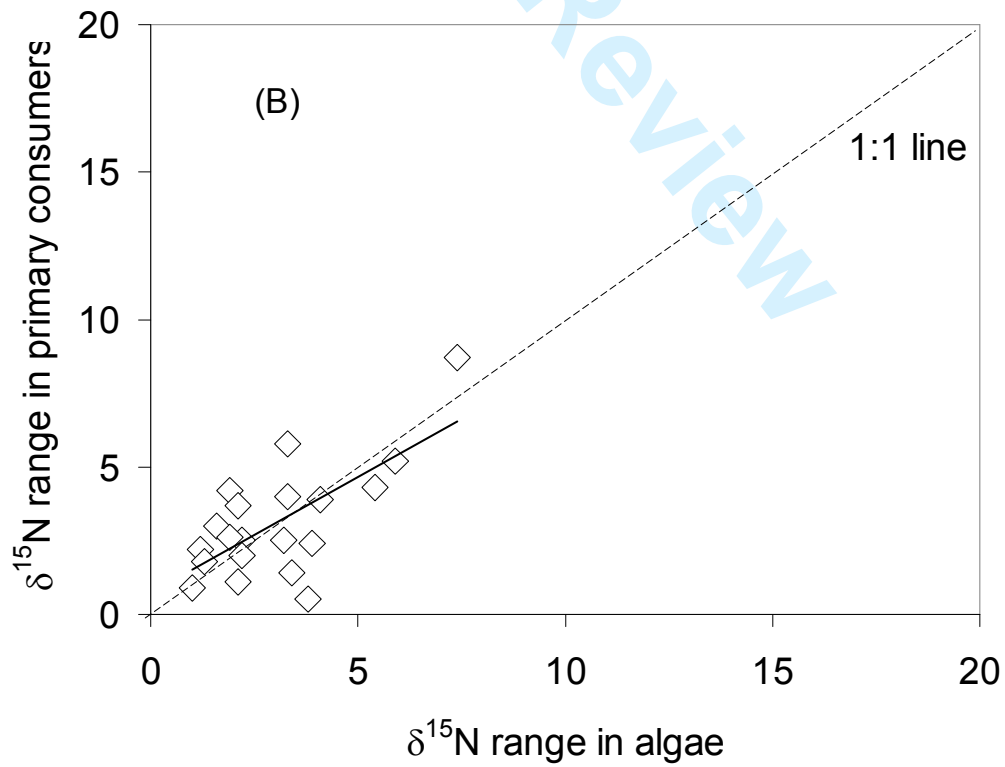
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45 Figure 3

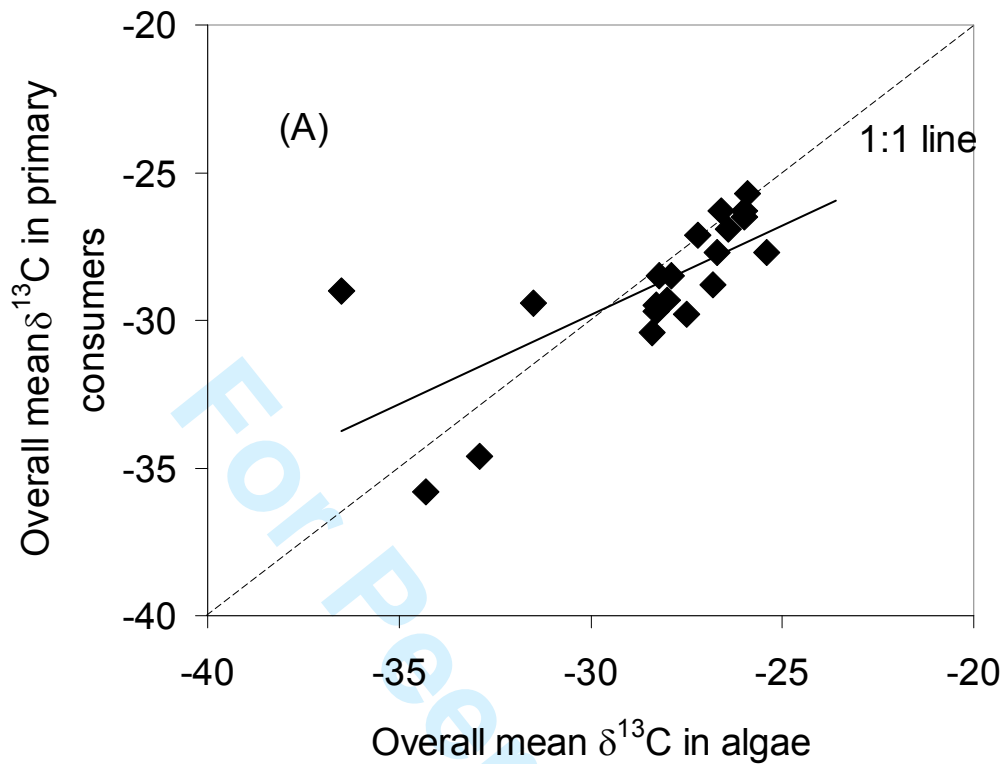


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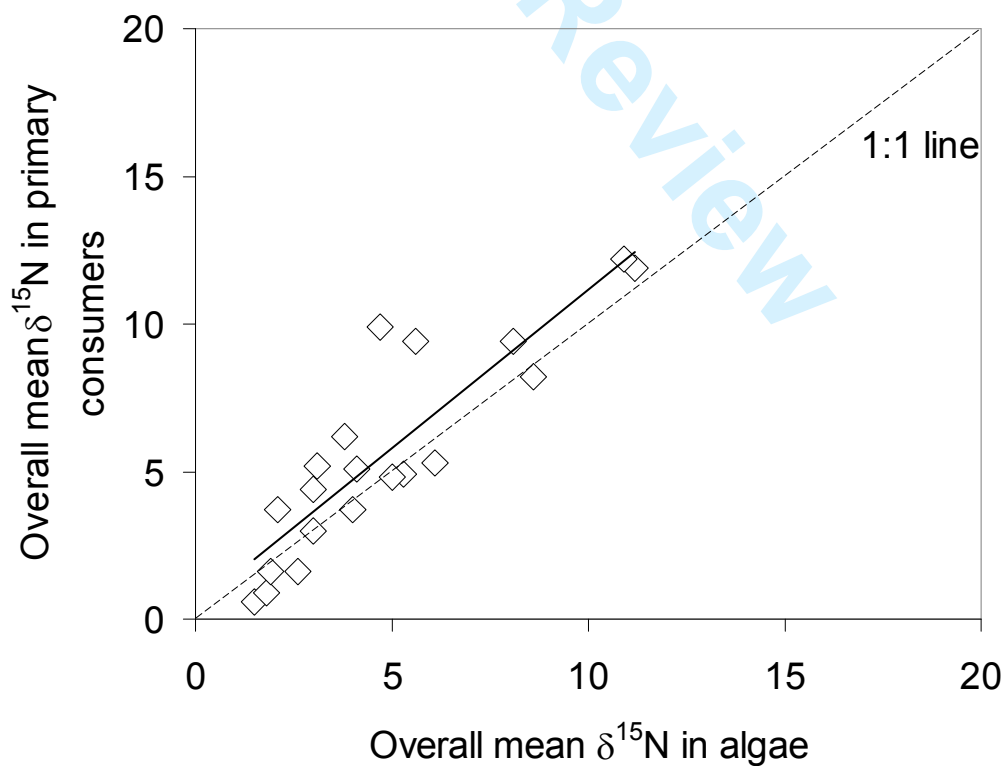


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48 Figure 4.

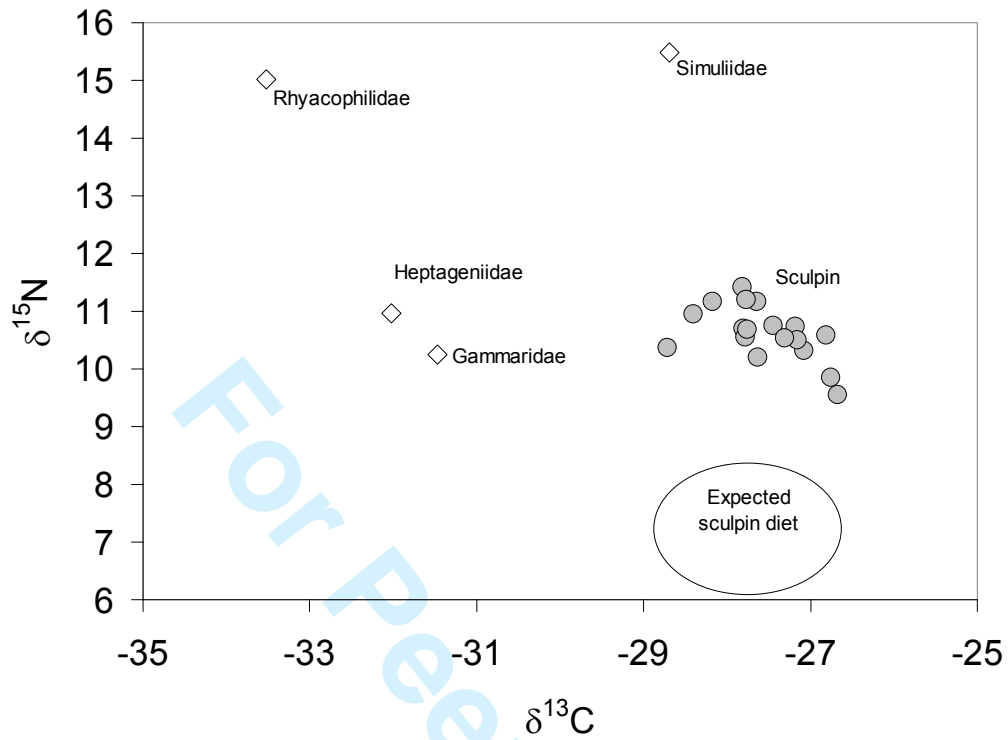


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51 Figure 5.



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