

Stable isotope patterns of benthic organisms from the Great Lakes region indicate variable dietary overlap of *Diporeia* spp. and dreissenid mussels

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Abstract: Competition between native and invasive species may bring about a suite of ecological and evolutionary outcomes, including local extirpations. In the Laurentian Great Lakes, competition for food may explain the dramatic decline of *Diporeia* spp. amphipods following the introduction of dreissenid mussels. This hypothesis has not been confirmed, in part because dreissenids and *Diporeia* appear to co-exist and flourish in other systems, including the Finger Lakes of New York. We used carbon, nitrogen, hydrogen, and oxygen stable isotope ratios to examine resource use by *Diporeia* from three spatially distinct populations (Lake Michigan, Lake Superior, and Cayuga Lake), dreissenids from areas where they co-occur with *Diporeia* (Lake Michigan and Cayuga Lake), and *Diporeia* from Lake Michigan collected before and after dreissenid invasion (1986–2009). Our results suggest that dreissenids may affect resource use by *Diporeia* in areas of co-occurrence, but the extent to which those effects are positive or negative is unclear. Terrestrial inputs may provide an important subsidy for *Diporeia* populations in small systems but may not be substantial enough in the Great Lakes to ensure that both taxa thrive.

Résumé : La concurrence entre espèces indigènes et envahissantes peut entraîner une série de changements écologiques et évolutifs, dont la disparition locale de certaines espèces. Dans les Grands Lacs laurentiens, la concurrence pour la nourriture pourrait expliquer l'importante diminution de l'abondance des amphipodes *Diporeia* spp. après l'introduction de moules de la famille des dreissenidés. Cette hypothèse n'a toutefois pas été confirmée, notamment parce que les dreissenidés et les *Diporeia* semblent bien coexister dans d'autres systèmes, dont les Finger Lakes de l'État de New York. Nous avons utilisé les rapports d'isotopes stables du carbone, de l'azote, de l'hydrogène et de l'oxygène pour examiner l'utilisation des ressources par les *Diporeia* de trois populations distinctes dans l'espace (les lacs Michigan, Supérieur et Cayuga), les dreissenidés de régions où des *Diporeia* sont également présents (les lacs Michigan et Cayuga) et des *Diporeia* du lac Michigan prélevés avant et après l'invasion de dreissenidés (1986–2009). Nos résultats donnent à penser que les dreissenidés pourraient avoir une incidence sur l'utilisation des ressources par les *Diporeia* dans les régions où les deux groupes coexistent, bien que la mesure dans laquelle ses effets sont positifs ou négatifs demeure incertaine. Des apports de source terrestre pourraient fournir un important subside aux populations de *Diporeia* dans de petits systèmes, mais ne pas être suffisamment importants pour assurer le succès des deux taxons dans les Grands Lacs. [Traduit par la Rédaction]

Introduction

Accidental or intentional introductions of non-native species can have dire ecological and economic consequences to freshwater ecosystems. In North America, the offshore benthic macroinvertebrate communities of deep, glaciated lakes were historically dominated by the native amphipod *Diporeia* spp. (hereafter *Diporeia*; Dermott et al. 2005a; Nalepa et al. 2005). *Diporeia* feed on diatoms, bacteria, and organic matter in the upper few centimetres of sediment at the lake bottom, serve as a major food source for fish in offshore areas, and play an important role in the transfer of energy up the food web (Nalepa et al. 2005, 2009). In the late 1980s, two non-native mussels, zebra mussel (*Dreissena polymorpha*) and quagga mussel (*Dreissena rostriformis bugensis*), were unintentionally introduced into North America and spread into many of the

same lakes where *Diporeia* was found. Dreissenid mussels are filter feeders that consume small particles from the water column. Even in large, deep lakes, dreissenids can reach abundances where populations filter up to 30% of the water column per day (Vanderploeg et al. 2010). Zebra mussels typically colonize hard substrates in shallower water (<50 m) and were the first of the two species to spread to all five of the Laurentian Great Lakes. Quagga mussels can colonize both hard and soft substrates in both shallow and deep areas (>90 m) and spread more slowly than zebra mussels, but are now the dominant taxon in Lakes Huron, Michigan, and Ontario and in the eastern basin of Lake Erie (Karatayev et al. 2013).

Often times when dreissenids establish themselves in an area, local populations of *Diporeia* quickly decline (Dermott et al. 2005a;

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Nalepa et al. 2009; Barbiero et al. 2011). In the Great Lakes, *Diporeia* completely disappeared from the shallowest lake (Erie) and all but disappeared from shallower regions (<90 m) in Lakes Huron, Michigan, and Ontario following the establishment of dreissenids (Nalepa et al. 2009; Barbiero et al. 2011). Dreissenids are confined to shallow regions in the western portion of Lake Superior, and *Diporeia* populations in this lake have remained stable thus far (Nalepa et al. 2006b; Barbiero et al. 2011). Mechanism(s) that lead to declines of *Diporeia* are unclear but may include toxins associated with dreissenid pseudofeces or competition with dreissenids for food (Dermott et al. 2005b; Nalepa et al. 2006a). This last hypothesis has received much attention but has been difficult to prove. Since dreissenids filter food from the water column, researchers have hypothesized that where dreissenids and *Diporeia* co-occur and *Diporeia* populations are declining, not enough food reaches the sediment for both taxa to persist (Nalepa et al. 2006a). In contrast, populations of *Diporeia* in some of the New York Finger Lakes have remained steady or even increased as dreissenids established, leading researchers to hypothesize that the taxa exploit different food resources in these systems (Dermott et al. 2005a; Watkins et al. 2012). These small, mesotrophic lakes with very high perimeter to surface area ratios have a primarily organic substrate, as coarse allochthonous inputs (e.g., leaf litter and other large organic materials) can rapidly settle to the bottom (Dermott et al. 2005a). *Diporeia* can scavenge on bacteria or diatoms associated with the larger allochthonous material while dreissenids filter smaller particles of food from the water column (Watkins et al. 2012). Though physical characteristics of the Finger Lakes may limit the likelihood of exploitative competition between the two taxa, *Diporeia* in large, oligotrophic Great Lakes may have limited access to external inputs to the system and thus be more vulnerable to competition. While this simple explanation seems plausible, studies have shown that older *Diporeia* collected from areas where both taxa co-occur, including areas where *Diporeia* are in decline, have healthy lipid levels and off-spring production and thus do not appear to be starving (Nalepa et al. 2006a).

Stable isotope ratios of organic tissues and biologically produced mineral material have been used extensively to examine aquatic food webs and potential dietary overlap. Nitrogen isotope ratios ($\delta^{15}\text{N}$) are most commonly used to determine trophic position within the food chain, where a greater $\delta^{15}\text{N}$ value denotes a higher trophic position (Vander Zanden and Rasmussen 2001). Carbon isotope ratios ($\delta^{13}\text{C}$) have been used to determine the source of an animal's nutrients, where lower $\delta^{13}\text{C}$ values reflect a diet derived from pelagic sources of primary production, such as phytoplankton, rather than benthic ones, such as benthic algae (Finlay and Kendall 2007). Hydrogen ($\delta^2\text{H}$) and oxygen ($\delta^{18}\text{O}$) ratios have been used at the land, estuary, and ocean interface to evaluate the relative importance of resources derived from each habitat (Fry 2002), and there is some evidence that hydrogen ratios could be similarly used to assess the relative importance of terrestrial or riverine inputs to freshwater systems (Doucett et al. 2007). Oxygen, and to a lesser degree hydrogen, isotope ratios can also reflect variation in the water in which aquatic organisms live (Soto et al. 2013) and reveal movement of individuals among habitats (e.g., Rooper et al. 2008).

In this study, we examined carbon, nitrogen, hydrogen, and oxygen stable isotope ratios of *Diporeia* from stable populations in eastern Lake Superior and Cayuga Lake (one of the Finger Lakes) and a declining population in southern Lake Michigan (Fig. 1). We compared isotope ratios for *Diporeia* and dreissenids collected from the same locations in Lake Michigan and Cayuga Lake (Fig. 1). We also compared *Diporeia* collected from Lake Michigan with *Diporeia* collected before dreissenids became abundant in southern Lake Michigan. Taken together, values for these four stable isotope ratios should define the source of food ($\delta^{13}\text{C}$, $\delta^2\text{H}$, $\delta^{18}\text{O}$), the relative trophic position ($\delta^{15}\text{N}$), and the ambient water condi-

tions ($\delta^2\text{H}$, $\delta^{18}\text{O}$) for the two taxa. This information can provide insights into dietary overlap between *Diporeia* and dreissenids for food resources, including potential mechanisms for *Diporeia* decline. We expect that location-corrected stable isotope ratios of the three *Diporeia* populations will differ. If the two taxa co-occur in Cayuga Lake because they exploit different resources, we expect that the magnitude of differences in stable isotope values for *Diporeia* and dreissenids in this lake will be greater than that for *Diporeia* and dreissenids in Lake Michigan. If dreissenids have influenced the availability of potential prey for *Diporeia* in Lake Michigan, we expect to observe that stable isotope values for contemporary *Diporeia* specimens differ from specimens collected predreissenid invasion.

Methods

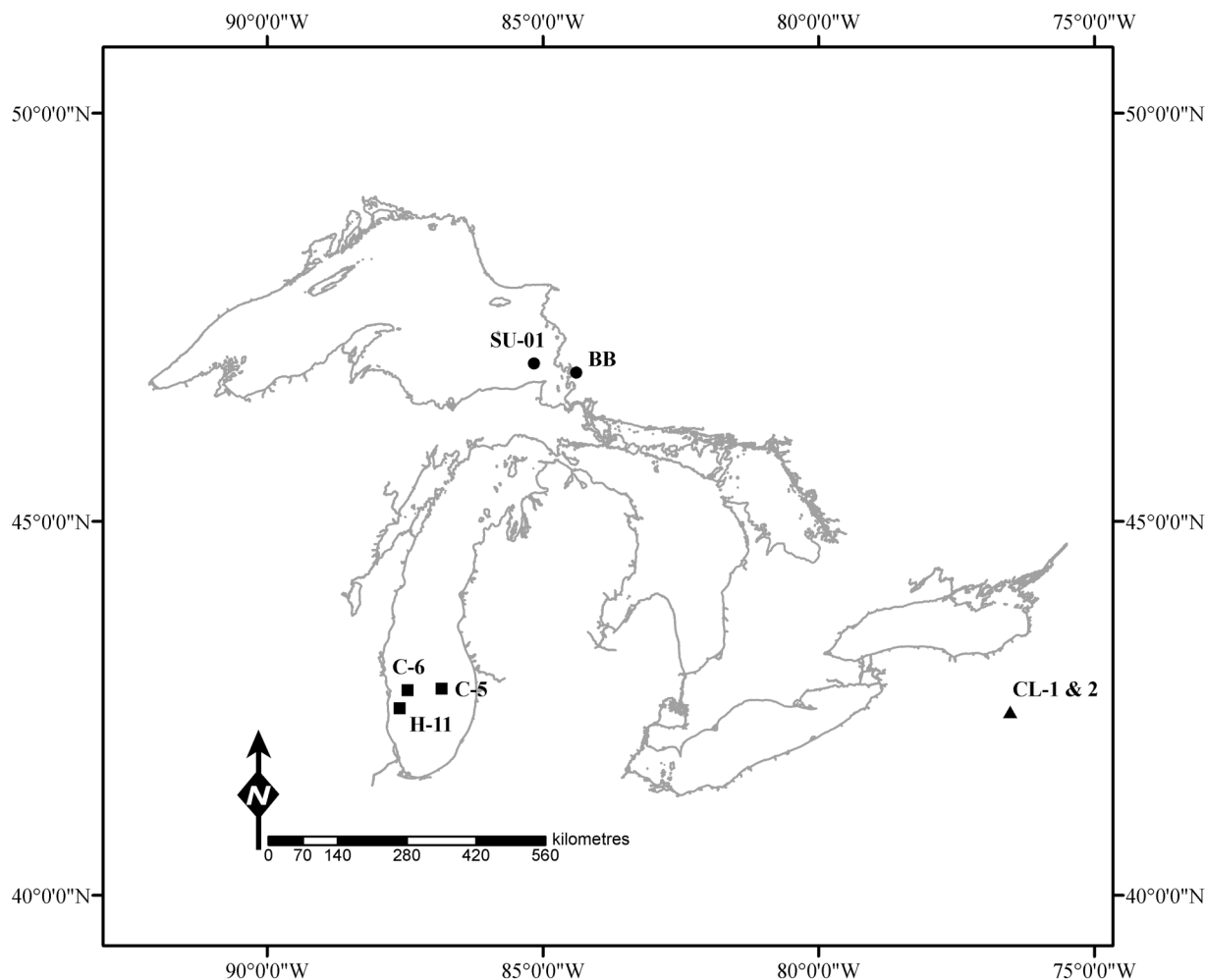
Field collections and processing

We collected *Diporeia* and dreissenids from two sites each in Lake Superior, Lake Michigan, and Cayuga Lake during spring, summer, and fall of 2008 and 2009 (Fig. 1; Table 1) using Ponar grabs (0.023 and 0.052 m² sampling area). Immediately after collection, we placed live individual animals rinsed with lake water in 2 mL cryovials to be flash-frozen in liquid nitrogen and stored at -80 °C until processing. At each site, we collected three additional grab samples, which were stored in 10% formalin stained with Rose Bengal until processing. Upon return to the laboratory, we picked organisms from these samples under a dissecting microscope and placed them in 70% ethanol for long-term storage (see Ryan et al. 2012 for details). Historic specimens of *Diporeia* were obtained from samples collected in fall from sites C-5 and H-11 in Lake Michigan in 1986, 1992, and 2002 (Fig. 1). These specimens were preserved in 10% formalin both immediately upon collection and also for long-term storage (see Nalepa et al. 2009 for details).

Stable isotope analyses

Owing to the small amount of material required for analyses and the potential for individual variation (Lancaster and Waldron 2001; Post 2002), we created three replicates for each sampling event (site-season-year) to be analyzed for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ and for $\delta^2\text{H}$ and $\delta^{18}\text{O}$. Each replicate contained five to seven individually frozen animals. Most *Diporeia* replicates contained a mix of adult (3–9 mm) and juvenile (<3 mm) specimens. We analyzed shell-free dreissenids (all quagga mussels) and *Diporeia* separately. For some sites where few animals were collected, we supplemented frozen sample replicates with additional replicates that had been stored in formalin and ethanol. To ensure that results for all preservatives were comparable, we determined stable isotope ratios for a subset of samples collected during the same sampling event (site-season-year) but preserved by different methods (frozen, formalin then ethanol, and formalin only). We calculated mean differences between sample pairs and used these as correction factors (see Results).

To account for any biases in stable isotope ratios associated with lipid content of the animal and (or) preservation in organic solvents (Soto et al. 2013), we washed all replicates with a 2:1 mixture of chloroform:methanol then allowed them to air-dry for at least 24 h before homogenizing by hand with a mortar and pestle. Wherever possible, we analyzed the same homogenate for all four stable isotope ratios. We report stable isotope data as δ values ($\delta X = (R_{\text{sample}}/R_{\text{reference}} - 1)$) in units of per mil (‰), where $R_{\text{reference}}$ is an international standard included within each run (see below for details). We analyzed 0.65 mg of homogenate for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ via combustion and chromatographic separation using a Carlo Erba elemental analyzer coupled to a SerCon 20-22 isotope ratio mass spectrometer (SerCon, Ltd., Cheshire, UK), corrected raw $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ data for sample size dependence, and normalized to the Vienna Pee Dee Belemnite (C) and air (N) reference scales using

Fig. 1. Collection sites in Lake Superior (circles), Lake Michigan (squares), and Cayuga Lake (triangle).**Table 1.** Characteristics of sampling sites, including status of *Diporeia* amphipod and dreissenid mussel populations in the spring of 2009 (mean \pm SE).

Site name	Lake	Coordinates	Depth (m)	Substrate	Sampling years	Animals collected	Spring density, 2009	
							<i>Diporeia</i> ^a	Dreissenids (quagga)
CL-1	Cayuga	42.5382°N, 76.5534°W	40	Organic	2008, 2009	<i>Diporeia</i> , dreissenids	3 477 \pm 1 562	11 580 \pm 1 011
CL-2	Cayuga	42.5053°N, 76.5230°W	80	Organic	2008, 2009	<i>Diporeia</i> , dreissenids	—	—
C-5	Michigan	42.8167°N, 86.8333°W	130	Silt–mud	1986, 1992, 2002, 2008, 2009	<i>Diporeia</i>	149 \pm 33	0
C-6	Michigan	42.7945°N, 87.4472°W	97	Silt–mud	2008, 2009	<i>Diporeia</i> , dreissenids	1 007 \pm 63	343 \pm 180
H-11	Michigan	42.5542°N, 87.5972°W	69	Silt–mud	1986, 1992, 2002, 2008, 2009	<i>Diporeia</i> , dreissenids	816 \pm 156	457 \pm 318
BB	Superior	46.8802°N, 84.3904°W	46	Silt–mud	2008, 2009	<i>Diporeia</i>	206 \pm 58	0
SU-01 ^b	Superior	46.9927°N, 85.1605°W	96	Silt–mud	2008, 2009	<i>Diporeia</i>	628 \pm 11	0

^aFrom Ryan et al. (2012).^bOnly sampled in spring and fall.

measured values of National Institute of Standards and Technology peach leaf ($\delta^{13}\text{C} = -26.1\text{‰}$ and $\delta^{15}\text{N} = +1.9\text{‰}$). We analyzed another 0.15 mg of homogenate for $\delta^2\text{H}$ and $\delta^{18}\text{O}$ via pyrolysis and chromatographic separation with a Thermo Fisher Temperature Conversion Elemental Analyzer coupled to a Delta V isotope ratio mass spectrometer (Thermo Scientific, Inc., Massachusetts, USA). Following the recommendations of Bowen et al. (2005), we included three keratin materials with known nonexchangeable H isotope ratios as reference materials within each run: one kudu horn (KHS) and one caribou hoof (CBS) keratin standard provided by L. Wassenaar (Environment Canada, Saskatoon, Saskatchewan, Canada) plus one commercial powdered keratin used for internal checks (Spectrum Chemical, New Brunswick, New Jersey, USA).

We placed samples and reference materials in silver capsules, stored them in a 96-well culture tray, and allowed them to equilibrate with ambient water vapor in the laboratory atmosphere for a period of at least 7 days. We then placed the tray in a vacuum desiccator for another 7+ days before rapidly transferring the tray to the instrument's autosampler (Zero Blank Autosampler, Costech, California, USA) for analysis. We corrected raw $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values to the Vienna Standard Mean Ocean Water reference scale using a two-point linear fit between average measured values and known values for the KHS and CBS reference materials (KHS: $\delta^2\text{H} = -54.1\text{‰}$, $\delta^{18}\text{O} = +20.3\text{‰}$; CBS: $\delta^2\text{H} = -197.0\text{‰}$, $\delta^{18}\text{O} = +3.8\text{‰}$). All stable isotope analyses were performed at the Purdue Stable Isotope Facility in West Lafayette, Indiana, USA.

Hydrogen and oxygen isotopes vary considerably among different freshwater environments and are correlated in part with distance from the ocean (Dansgaard 1954; Craig 1961). We report our $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values corrected to baseline water values -68% and -8.7% for Lake Superior (Longstaffe et al. 2011), -46% and -6.1% for Lake Michigan (G. Bowen, unpublished data), and -61% and -8.6% for Cayuga Lake (Michel and Kraemer 1995) and refer to these corrected values as $\delta^2\text{H}_{\text{LC}}$ and $\delta^{18}\text{O}_{\text{LC}}$. Carbon and nitrogen isotopes also have the potential to vary through time and space, where carbon isotope patterns can be described by the Suess effect and nitrogen isotopes depend on the baseline food web signature of a given location. Given the relatively short temporal and narrow spatial extents of our analyses, we did not believe that our carbon isotope results would be substantially biased and therefore retained measured carbon isotope ratios. We adjusted for potentially different baseline $\delta^{15}\text{N}$ values in each lake by converting the $\delta^{15}\text{N}$ signature for a given sample to trophic factor using the equation $\text{TF} = \lambda + (\delta^{15}\text{N}_{\text{Animal}} - \delta^{15}\text{N}_{1^\circ\text{Consumer}})/3.4$, where λ is the baseline trophic level, $\delta^{15}\text{N}_{\text{Animal}}$ is the preservative-corrected value for either *Diporeia* or dreissenids, and $\delta^{15}\text{N}_{1^\circ\text{Consumer}}$ is the mean $\delta^{15}\text{N}$ value for primary consumers in the lake of sampling (Vander Zanden and Rasmussen 2001). We used nonpredatory zooplankton as our primary consumer (after Matthews and Mazumder 2003) and set $\delta^{15}\text{N}_{1^\circ\text{Consumer}}$ to 2.7‰ for Lake Superior (Harvey and Kitchell 2000), 5.5‰ for Lake Michigan (H. Bootsma, unpublished data), and 6.5‰ for Cayuga Lake (Post 2002). We set λ to 2 for all lakes.

Statistical tests

We made three sets of statistical comparisons following methods summarized in Turner et al. (2010) to determine (i) resource use of *Diporeia* from spatially distinct populations; (ii) resource overlap between *Diporeia* and dreissenids from the same location; and (iii) temporal changes in *Diporeia* resource use from a location where the population is now in decline. Briefly, for each comparison we graphed stable isotope ratios (e.g., $\delta^2\text{H}_{\text{LC}}$ versus $\delta^{18}\text{O}_{\text{LC}}$) for each sample in a group, computed a variety of statistics to describe emerging within- and among-group patterns, and then used nested linear models and residual permutation procedures to assess the statistical likelihood of observed differences. Stable isotope data are often not normally distributed, and transformations make meaningful interpretation of results difficult. Use of residual permutation procedures eliminates the need for transforming data and is particularly useful when combined with means of quantifying distances between centroids of groups and geometric morphometries of linear relationships between points (Turner et al. 2010; Layman et al. 2012).

For all three sets of statistical comparisons, we analyzed two bivariate plots: $\delta^{13}\text{C}$ versus TF and $\delta^2\text{H}_{\text{LC}}$ versus $\delta^{18}\text{O}_{\text{LC}}$. We determined statistical significance of each test by permuting residuals 999 times and then calculating the percentile rank of the true value versus permuted values. Given that we were making multiple comparisons between groups within each set, we set our α significance level to 0.01. All analyses were performed in R (R Development Core Team 2012) using custom scripts adapted from those described in Turner et al. (2010). To examine resource use in different *Diporeia* populations, we compared *Diporeia* collected from Lakes Superior (SU), Michigan (MI), and Cayuga (CL) in 2008 and 2009. To examine the potential for resource overlap between taxa, we compared *Diporeia* and dreissenids collected in MI and CL in 2008 and 2009. For all spatial analyses, we assessed overall differences by adjusting sample stable isotope values to baseline values for each lake and then calculating the Euclidean distance between group centroids (D). We evaluated the effect of season by calculating mean straight-line paths between seasonal centroids (spring, summer, fall) for all taxa and populations

(SU *Diporeia*, MI *Diporeia* and dreissenids, CL *Diporeia* and dreissenids) and then comparing differences between the total path length (Length), the direction the path takes (Direction), and the overall shape of the path (Shape). The Length gives an indication of the absolute difference between seasonal pattern means, while the Direction and Shape of the relationship between means helps identify subtle differences (e.g., if one population tends to rely on allochthonous inputs or feed at a higher trophic level in summer only). If resource use for each group being compared (i.e., *Diporeia* from the three lakes; *Diporeia* and dreissenids in the same lake) is similar throughout the year, we expect to observe no differences among seasonal patterns. We also examined the relationship between *Diporeia* and dreissenids by calculating the Length, Direction, and Shape of a line connecting the two taxa in MI to the Length, Direction, and Shape of a line connecting the two taxa in CL. To examine the possibility of a shift in *Diporeia* resource use after the establishment of dreissenids, we calculated *D* for *Diporeia* collected from MI in the fall of 1986, 1992, 2002, 2008, and 2009. We adjusted all $\delta^{15}\text{N}$ isotope values for historic specimens to present-day baseline values for MI.

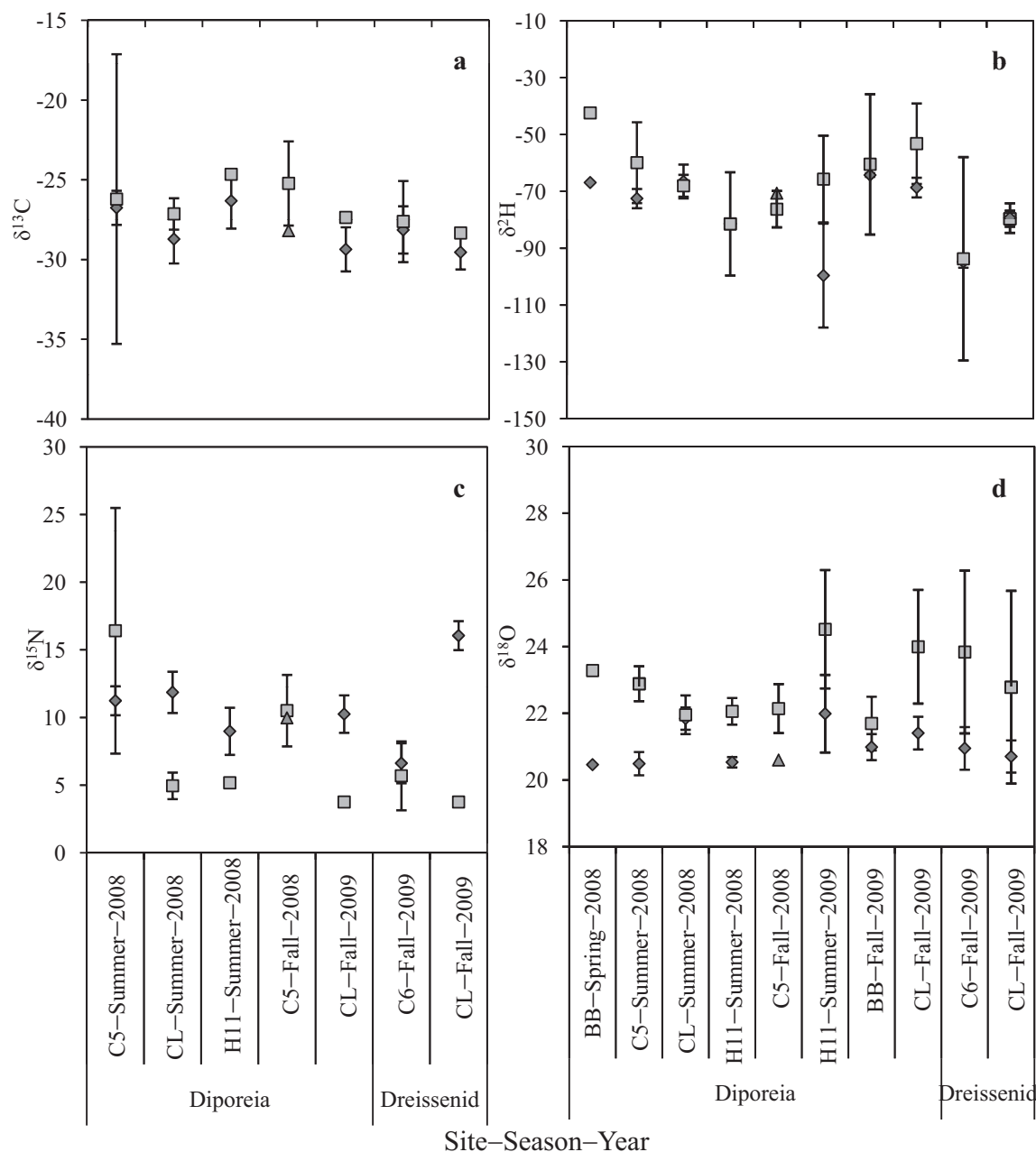
Results

Adjustment for preservative

We determined $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures for 87 *Diporeia* replicates (61 frozen, 11 formalin–ethanol-preserved and 15 formalin-preserved) and 43 dreissenid replicates (20 frozen and 23 formalin–ethanol-preserved). We determined $\delta^2\text{H}$ and $\delta^{18}\text{O}$ signatures for 104 *Diporeia* replicates (68 frozen, 21 formalin–ethanol-preserved and 15 formalin-preserved) and 50 dreissenid replicates (21 frozen and 29 formalin–ethanol-preserved). Within these, 10 site–season–year pairs were used to develop correction factors for preservation differences in $\delta^2\text{H}$ and $\delta^{18}\text{O}$, while seven site–season–year pairs were similarly used to develop correction factors for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. These paired samples span taxa, years, and sites (Fig. 2). Samples preserved in formalin–ethanol consistently had lower $\delta^{13}\text{C}$, $\delta^2\text{H}$, and $\delta^{18}\text{O}$ values and higher $\delta^{15}\text{N}$ values compared with frozen samples collected at the same time and location (Fig. 2). Similarly, samples preserved in formalin only displayed lower $\delta^{13}\text{C}$, $\delta^2\text{H}$, and $\delta^{18}\text{O}$ values as compared with frozen samples (Fig. 2). We generated correction factors by calculating mean differences between paired samples. Based on the mean observed differences between preservation groups, we applied correction factors of +1.5‰ to the $\delta^{13}\text{C}$ value (range of absolute value of differences: 0.52‰–2.97‰), +10‰ to the $\delta^2\text{H}$ value (range: 0.05‰–33.96‰), and +1.9‰ to the $\delta^{18}\text{O}$ value (range: 0.11‰–2.89‰) for all replicates preserved in either formalin–ethanol or formalin only, and -4.1% to the $\delta^{15}\text{N}$ value (range: 0.54‰–5.69‰) for replicates preserved in formalin–ethanol. We did not adjust the $\delta^{15}\text{N}$ value of replicates preserved only in formalin. We applied preservative correction factors before calculating TF, $\delta^2\text{H}_{\text{LC}}$, and $\delta^{18}\text{O}_{\text{LC}}$.

The carbon and nitrogen preservative correction factors are similar to those reported in previous studies of the effect of formalin preservation on stable isotope values of aquatic invertebrates (e.g., Bosley and Wainright 1999; Sarakinos et al. 2002). However, to ensure that these correction factors did not substantially affect the results of subsequent statistical analyses, we performed all analyses on location-corrected values ($\delta^{13}\text{C}$ and TF, $\delta^2\text{H}_{\text{LC}}$ and $\delta^{18}\text{O}_{\text{LC}}$) in triplicate whenever possible, including (i) all replicates corrected for preservative effects, (ii) only frozen replicates, and (iii) only replicates preserved in formalin–ethanol or formalin only. Results were similar among all analyses regardless of the preservation method used. For brevity, we present only results with all replicates corrected for preservative effects herein. Results of analyses with frozen or formalin–ethanol-preserved

Fig. 2. Effect of preservation method on isotopic values. Comparisons were made between samples collected at the same site–season–year but preserved frozen (squares), in formalin followed by ethanol (diamonds), or in formalin only (triangles). Mean differences between values for frozen and nonfrozen samples were used to correct values for samples preserved in formalin–ethanol and for those in formalin only. Error bars represent ± 1 SD of the mean.



and formalin-preserved animals can be found in online Supplementary material¹.

Resource use in spatially distinct *Diporeia* populations

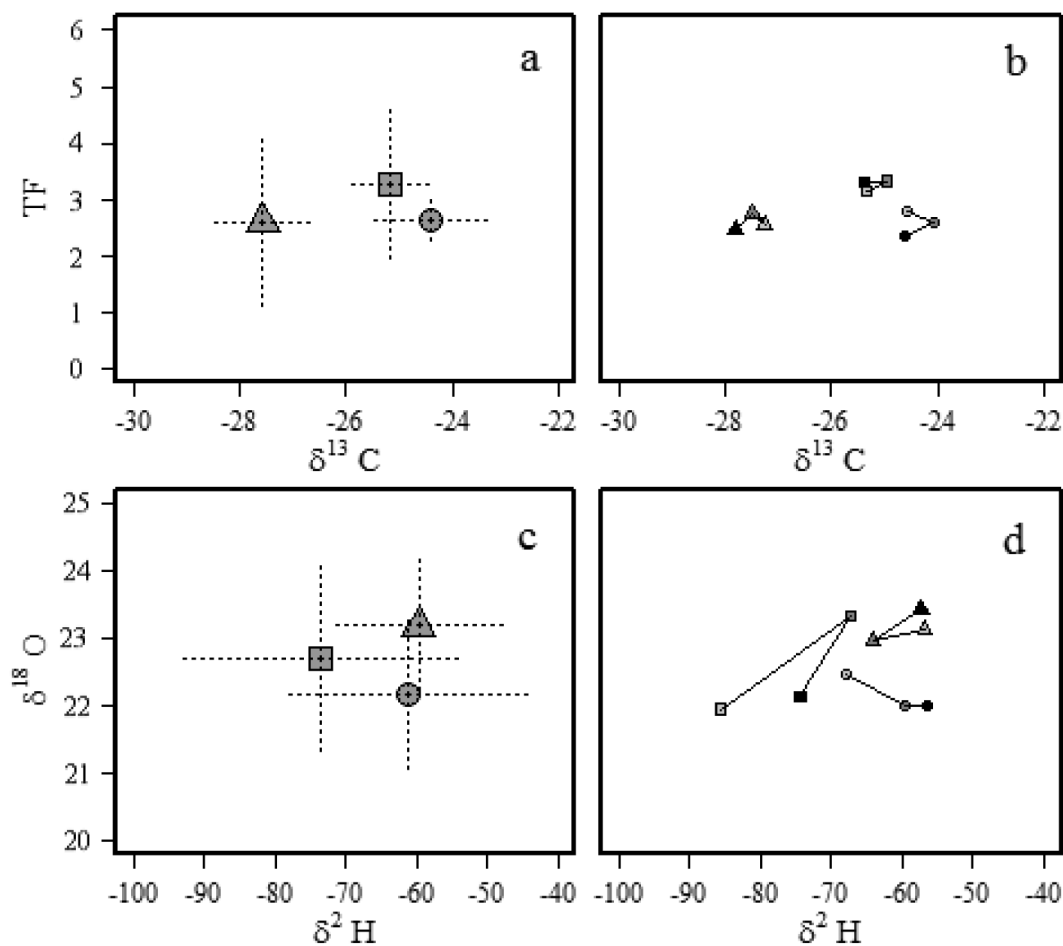
Isotopic signatures of *Diporeia* differed across lakes. For $\delta^{13}\text{C}$ and TF, the centroid values from all three lakes were significantly distant from each other (Fig. 3a; $D_{\text{SU-MI}} = 1.02$, $p = 0.0045$; $D_{\text{SU-CL}} = 3.16$, $p < 0.0001$; $D_{\text{MI-CL}} = 2.48$, $p < 0.0001$). The shape of seasonal resource use within a lake was significantly different between MI and CL, seemingly driven by differences in springtime values (Fig. 3b; $\text{Shape}_{\text{MI-CL}} = 1.8$, $p = 0.0085$). Animals from MI exhibited

enriched $\delta^{13}\text{C}$ in summer as compared with spring or fall, while animals from CL became progressively less enriched in $\delta^{13}\text{C}$ as the year went on (Fig. 3b). There were no other significantly different seasonal patterns in $\delta^{13}\text{C}$ and TF of *Diporeia* between lakes (Fig. 3b; Length, Direction, Shape for all other comparisons, $p > 0.05$). In general, *Diporeia* from CL exhibited lower $\delta^{13}\text{C}$ values than their counterparts in MI and SU, while animals from MI potentially feed at a slightly higher trophic level than those in other lakes (Fig. 3a).

The centroid values of $\delta^2\text{H}_{\text{LC}}$ and $\delta^{18}\text{O}_{\text{LC}}$ for *Diporeia* from MI differed significantly from the centroid values for both other

¹Supplementary data are available with the article through the journal Web site at <http://nrcresearchpress.com/doi/suppl/10.1139/cjfas-2013-0620>.

Fig. 3. Isotopic signatures of *Diporeia* from Lake Superior (circles), Lake Michigan (squares), and Cayuga Lake (triangles). Centroid values were calculated over 2 years and three seasons for each lake (a, c). Error bars represent ± 1 SD of the centroid. Centroid values for each season (b, d) were calculated over 2 years and are joined with straight line paths to demonstrate seasonal shifts in resource use (spring: light grey; summer: dark grey; fall: black). TF, trophic factor.



lakes (Fig. 3c; $D_{MI-SU} = 12.2$, $p = 0.0025$; $D_{MI-CL} = 13.9$, $p < 0.0001$). However, the centroid values of δ^2H_{LC} and $\delta^{18}O_{LC}$ for *Diporeia* from SU and CL were not significantly different from each other (Fig. 3c; $D_{CL-SU} = 1.97$, $p > 0.05$). While MI δ^2H_{LC} values were lower than in the other lakes, there were few differences in $\delta^{18}O_{LC}$ values (Fig. 3c). Though patterns of seasonal use of δ^2H_{LC} and $\delta^{18}O_{LC}$ by *Diporeia* as demonstrated by the shapes of the relationships appeared to differ among lakes, these differences were not statistically significant (Fig. 3d; Length, Direction, Shape for all comparisons, $p > 0.05$). For all stable isotope signatures, isotopic differences among lake populations do not appear to be related to site, depth of site, or spatial location in terms of east-west or north-south gradients within a lake (Fig. 4).

Resource overlap between *Diporeia* and dreissenids

Isotopic signatures of *Diporeia* and dreissenids varied between lakes (Fig. 5). While δ^2H_{LC} and $\delta^{18}O_{LC}$ centroids for each taxon were significantly distant from each other within both MI (Fig. 5e; $D_{TaxaMI} = 32.1$, $p < 0.0001$) and CL (Fig. 5g; $D_{TaxaCL} = 16.0$, $p < 0.0001$), $\delta^{13}C$ and TF centroids were only significantly different in MI (Fig. 5a; $D_{TaxaMI} = 3.18$, $p < 0.0001$; Fig. 5c; $D_{TaxaCL} = 0.43$, $p > 0.47$). *Diporeia* always exhibited higher $\delta^{13}C$ and δ^2H_{LC} values than dreissenids, appeared to be feeding at a higher trophic level than dreissenids in MI, and had slightly higher $\delta^{18}O_{LC}$ values than dreissenids in CL (Fig. 5g). In MI, dreissenids showed significantly greater shifts in resource use throughout the year than *Diporeia* (Fig. 5b; Length_{TaxaMI} = 2.17, $p = 0.0075$), demon-

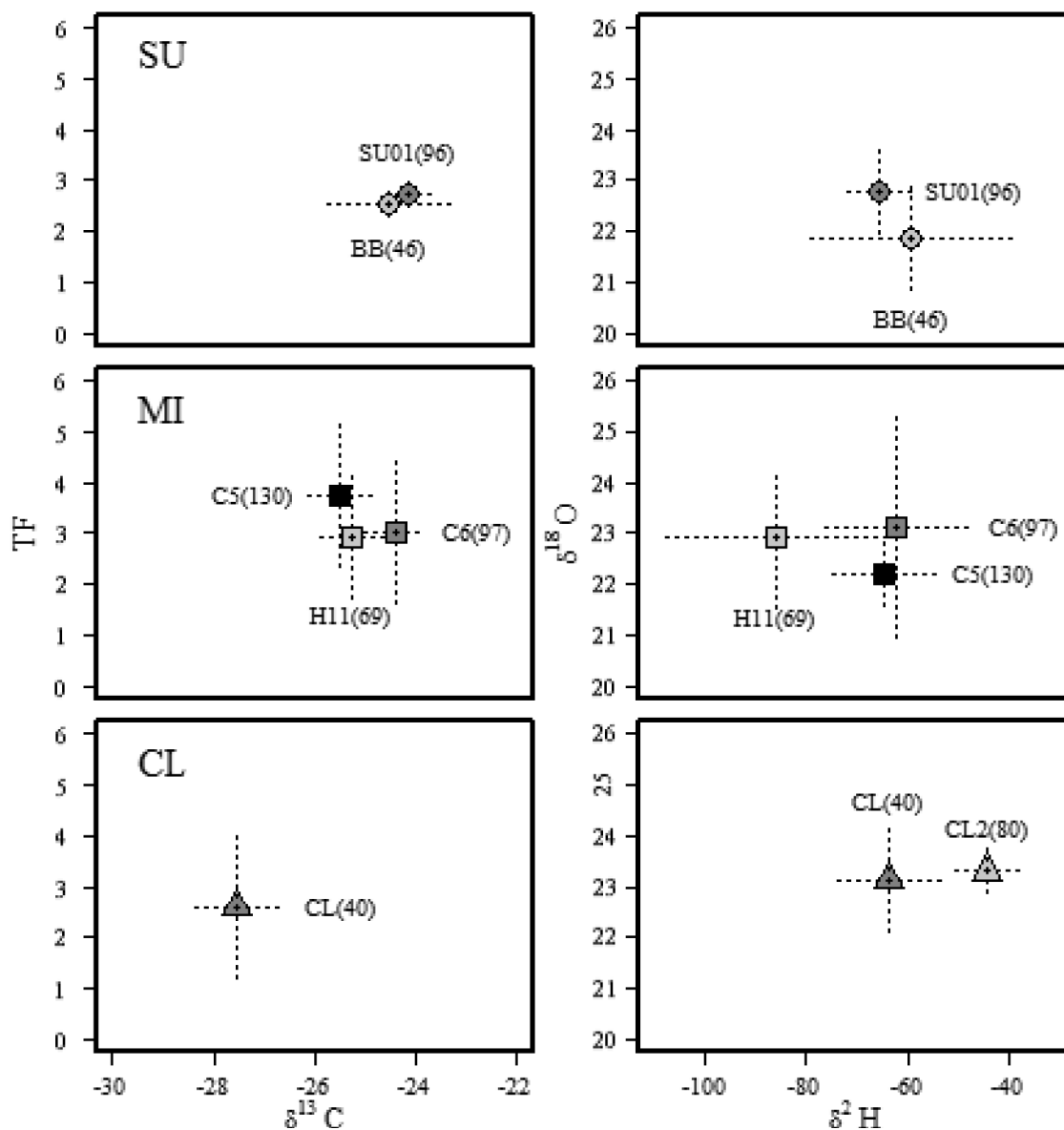
strating enriched $\delta^{13}C$ values in spring and fall. All other seasonal patterns were not significantly different (Figs. 5d, 5f, 5h; $p > 0.05$).

The relationship between *Diporeia* and dreissenids also varied with lake. The distance between centroids for both sets of isotope values were significantly longer in MI than in CL (Figs. 5a, 5c; Length_{TaxaCTF} = 2.75, $p < 0.0001$; Figs. 5e, 5g; Length_{TaxaHO} = 16.1, $p = 0.0075$), and the δ^2H_{LC} - $\delta^{18}O_{LC}$ relationship in CL had a different slope than that in MI, where specimens from CL demonstrated greater absolute differences in $\delta^{18}O_{LC}$ values (Figs. 5e, 5g; Direction_{TaxaHO} = 4.15, $p = 0.0025$; Shape_{TaxaHO} = 0.072, $p = 0.0025$).

Temporal changes in isotopic composition of *Diporeia* from MI

For $\delta^{13}C$ and TF, centroids of *Diporeia* specimens collected in 1986, 2008, and 2009 were significantly distant from centroids of *Diporeia* specimens collected in 1992 and 2002 (Fig. 6a; $D_{1986-1992} = 1.95$, $p < 0.0001$; $D_{1986-2002} = 2.2$, $p < 0.0001$; $D_{1992-2008} = 2.22$, $p < 0.0001$; $D_{1992-2009} = 2.48$, $p < 0.0001$; $D_{2002-2008} = 2.67$, $p < 0.0001$; $D_{2002-2009} = 2.89$, $p < 0.0001$). While centroids generally shifted through time toward higher $\delta^{13}C$ values and trophic levels, the directions of shifts were not uniform (Fig. 6b). For δ^2H_{LC} and $\delta^{18}O_{LC}$, centroids of *Diporeia* specimens collected in 2002 were significantly distant from centroids of specimens collected in 1992, 2008, and 2009 (Fig. 6c; $D_{1992-2002} = 18.1$, $p < 0.0001$; $D_{2002-2008} = 18.1$, $p < 0.0001$; $D_{2002-2009} = 14.3$, $p < 0.0001$). Differences were driven primarily by shifts toward higher δ^2H_{LC} ratios, as there was

Fig. 4. Centroid values (± 1 SD) of stable isotope signatures for *Diporeia* broken down by site (SU, Lake Superior; MI, Lake Michigan; CL, Cayuga Lake; see Fig. 1 for specific site locations). Values were calculated over 2 years and three seasons (spring: light grey; summer: dark grey; fall: black) for each site except site SU-01 in Lake Superior. Site names are noted next to the centroid, while depth of site (m) is noted in parentheses.



little change in $\delta^{18}\text{O}_{\text{LC}}$ values over time (Fig. 6d). The centroid of specimens collected in 1986 was marginally nonsignificant when compared with the centroids for specimens from 1992 and 2008 (Fig. 6d; $D_{1986-1992} = 13.1$, $p = 0.042$; $D_{1986-2008} = 13.1$, $p = 0.037$).

Discussion

Though the dreissenid invasion may have initiated or exacerbated the decline of native *Diporeia* amphipods in the Great Lakes region (Nalepa et al. 2006b), causes of the decline, such as direct competition for food, remain unclear. When we compared stable isotope signatures of *Diporeia* and dreissenids collected from the same location, *Diporeia* seemed to derive more of their food from benthic sources (higher $\delta^{13}\text{C}$) and external inputs (higher $\delta^2\text{H}_{\text{LC}}$) than dreissenids regardless of the location or long-term stability of the *Diporeia* population. This is consistent with differences in their feeding ecology, as dreissenids are filter feeders and *Diporeia* are detritivores, and is not in itself strong evidence that dreissenids caused *Diporeia* to alter their feeding patterns. More striking are

the high degree of isotopic overlap between the two taxa in Cayuga Lake, where both co-exist, and the temporal changes in Lake Michigan, where *Diporeia* populations have declined while dreissenid populations have expanded. The *Diporeia* population in Lake Superior that is stable and does not spatially co-occur with dreissenids shares isotopic characteristics with populations in each of the other two lakes. While interpretation of these patterns is not straightforward, we suggest that dreissenids likely affect resource use by *Diporeia*, though whether *Diporeia* population growth is positively or negatively affected is unclear. We also suggest that terrestrial subsidies are important to the feeding regime of *Diporeia* populations.

Centroid values of $\delta^{13}\text{C}$ and TF for dreissenids and *Diporeia* were virtually identical in Cayuga Lake yet differed greatly in Lake Michigan, and the distance between the taxa centroids for $\delta^2\text{H}_{\text{LC}}$ and $\delta^{18}\text{O}_{\text{LC}}$ was significantly shorter in Cayuga Lake than in Lake Michigan. This suggests that *Diporeia* and dreissenids in Cayuga Lake are more likely to be deriving their food from the same

Fig. 5. Isotopic signatures of *Diporeia* and dreissenids from Lake Michigan (MI) and Cayuga Lake (CL). Left panels (a, c, e, g) show overall differences between *Diporeia* (grey centroids) and dreissenids (black centroids) calculated over 2 years and three seasons. Error bars represent ± 1 SD of the centroid. Right panels (b, d, f, h) show seasonal differences in stable isotope values for each taxon, where centroids for each season (spring: light grey; summer: dark grey; fall: black) were calculated over 2 years and are joined with straight line paths to demonstrate seasonal shifts (solid line = *Diporeia*; dashed line = dreissenids).

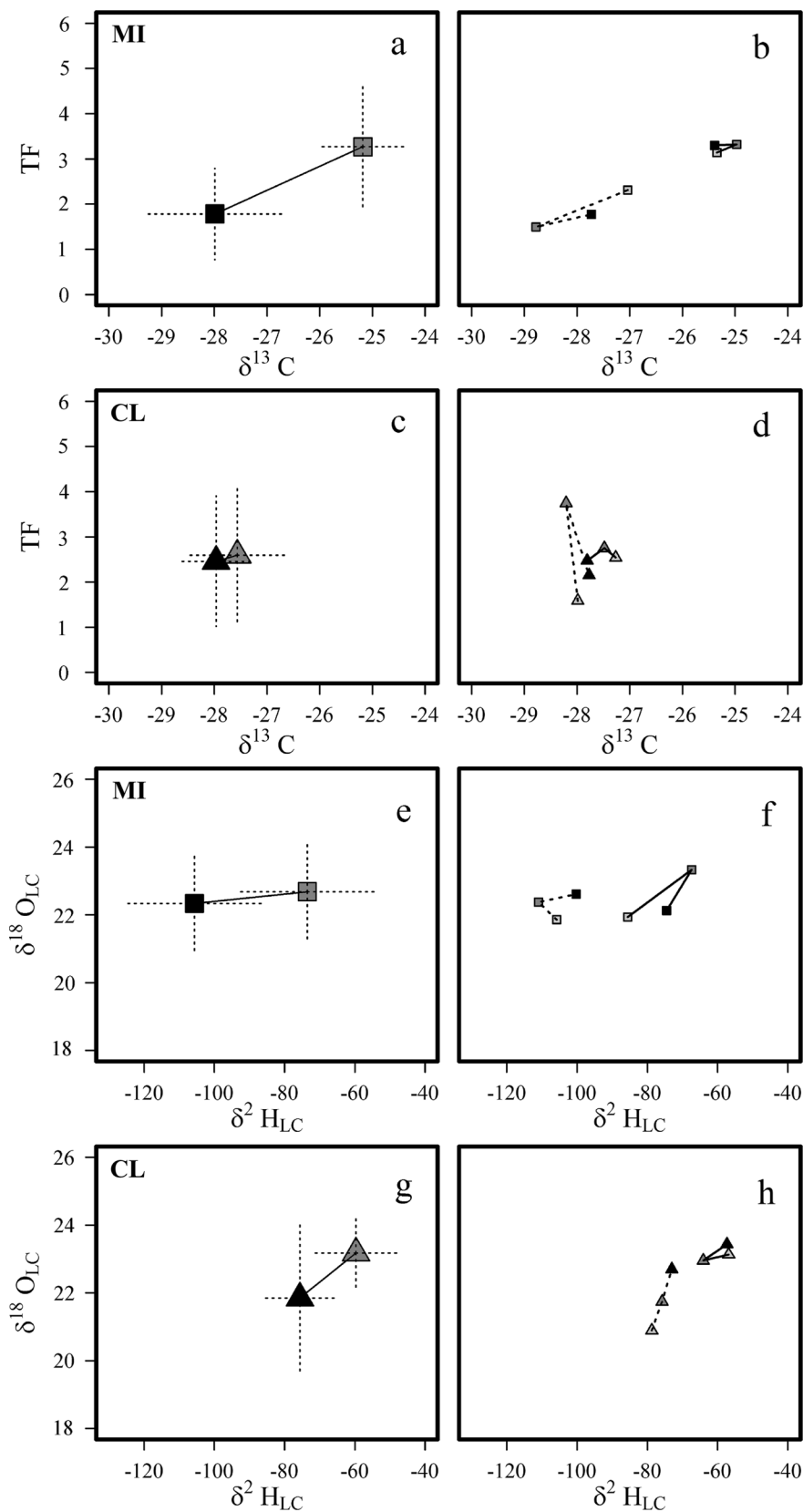
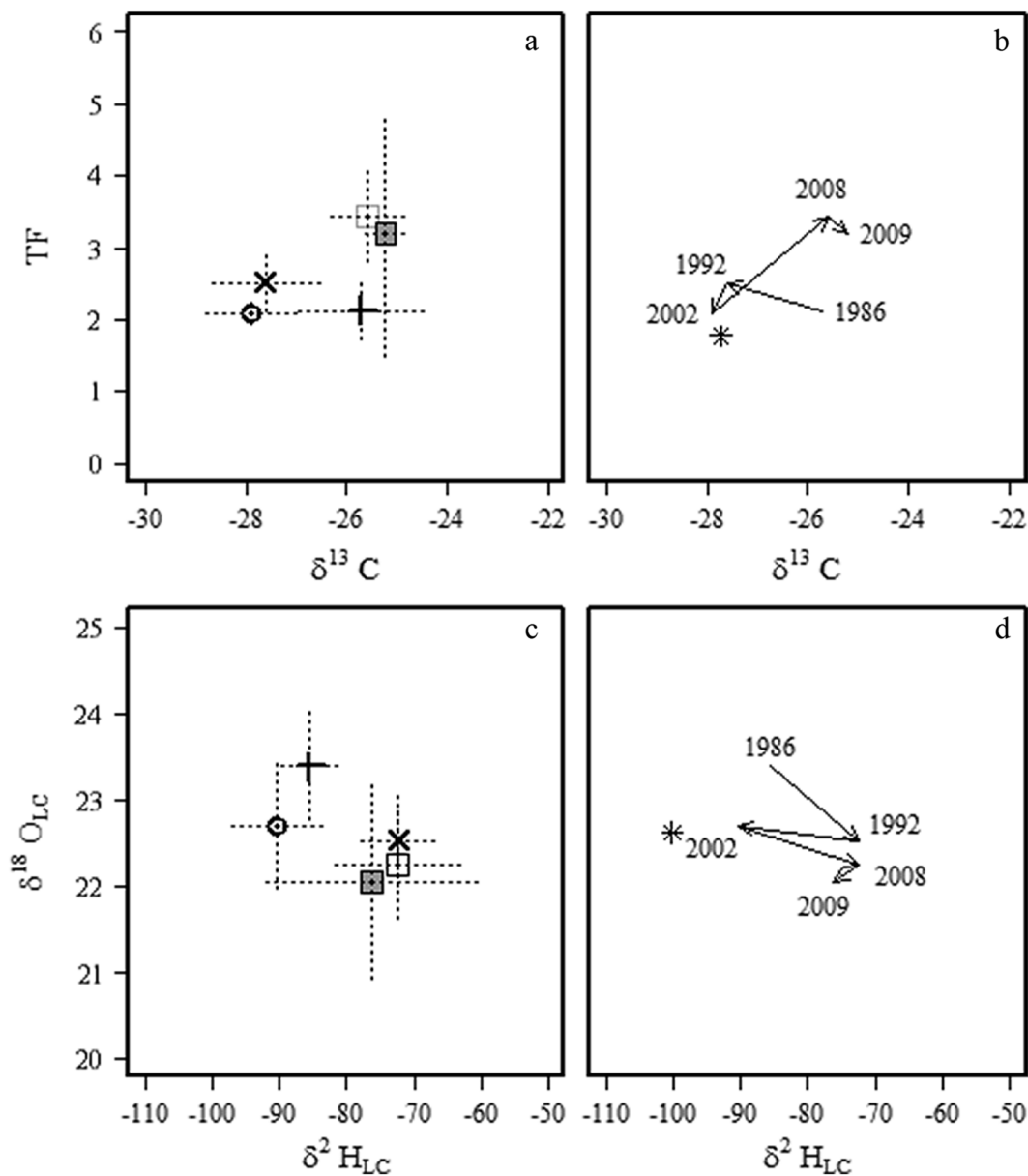


Fig. 6. Isotopic signatures of Lake Michigan *Diporeia* through time. Left panels show centroids ± 1 SD for specimens collected in the fall of 1986 (+ symbol), 1992 (\times symbol), 2002 (open circle), 2008 (open square), and 2009 (shaded square). Right panels demonstrate change in centroid position over time. Mean fall 2009 dreissenid values (indicated by an asterisk, *) are included for reference.



sources than their counterparts in Lake Michigan. The land cover types around each lake are similar. Both are made up of a mixture of agricultural and forested land, where agriculture is dominant in the regions near our sampling sites (Genesee/Finger Lakes Regional Planning Council 2001; Michigan Department of Natural Resources 2013). The most abundant crops surrounding Cayuga Lake are forage (hay and haylage, grass silage, and greenchop), corn, and soybean (United States Department of Agriculture National Agricultural Statistics Service 2007). The most abundant crop in counties surrounding the southern basin of Lake Michigan is overwhelmingly corn followed by soybean (United States Department of Agriculture National Agricultural Statistics Service 2007). Despite the similarities in surrounding land cover, the centroid values for *Diporeia* from Lake Michigan and Cayuga Lake were very different from each other for all isotope ratios but $\delta^{18}\text{O}$. While surrounding land-use and agricultural practices can influence stable isotope ratios of consumers in aquatic systems (e.g.,

Vander Zanden et al. 2005), we suggest that surrounding land use is not the main force driving isotopic differences between *Diporeia* populations.

Carbon stable isotope signatures tend to be higher in more productive systems (Gu et al. 1996; Post 2002; Vander Zanden et al. 2006) and decrease with increasing depth (Guiguer and Barton 2002; Vander Zanden et al. 2006). Based on the characteristics of each lake and our sample sites, we would expect the Cayuga Lake *Diporeia* to have the highest $\delta^{13}\text{C}$ values of all of the animals we studied, followed by animals from Lake Michigan and then Lake Superior. We see the opposite pattern, where Cayuga Lake animals have the lowest $\delta^{13}\text{C}$ values, Lake Superior *Diporeia* have the highest $\delta^{13}\text{C}$ values, and Lake Michigan animals fall in between. The $\delta^{13}\text{C}$ for Lake Michigan animals could potentially reflect ^{13}C -enrichment of external carbon sources, as the surrounding land cover is mostly corn (United States Department of Agriculture National Agricultural Statistics Service 2007), a C_4 crop that will

impart higher $\delta^{13}\text{C}$ values to invertebrate consumers (Gratton and Forbes 2006). Given the isotopic similarities between Lake Michigan and Lake Superior *Diporeia* and the lack of agricultural land around Lake Superior, we feel that this explanation is unlikely. The substrate in Cayuga Lake is highly organic, and it is possible that the lower $\delta^{13}\text{C}$ values in this system are due to activity of methanotrophic bacteria during low oxygen conditions late in summer (Callinan 2001; Guiguer and Barton 2002; Grey et al. 2004), but we feel this is also unlikely. Both *Diporeia* and dreissenids are generally intolerant to low dissolved oxygen (Marzolf 1965; Nalepa et al. 2005; Karatayev et al. 2007), *Diporeia* can actively avoid unsuitable habitat (Marzolf 1965), and $\delta^{13}\text{C}$ values for methanotrophic bacteria tend to be far more negative than those observed in the current study (e.g., Sanseverino et al. 2012).

We suggest that the most likely explanation for our suite of results is that animals in Cayuga Lake rely on terrestrial inputs much more so than animals in Lakes Michigan and Superior. In lakes along the Michigan–Wisconsin border that are similar to the Finger Lakes in surface area and surrounding land cover, animals that derived carbon from terrestrial sources exhibited decreased $\delta^{13}\text{C}$ values compared with counterparts that relied primarily on in-lake sources of food (Cole et al. 2011). The lower $\delta^{13}\text{C}$ signature that we observed in Cayuga Lake may be the result of a similar mechanism, and this is further supported by the $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values. Higher $\delta^2\text{H}$ values generally indicate a greater reliance on external inputs (Doucett et al. 2007), and Cayuga Lake animals have some of the highest $\delta^2\text{H}_{\text{LC}}$ values in our study. The slightly higher $\delta^{18}\text{O}_{\text{LC}}$ value in the Cayuga Lake *Diporeia* versus other populations may also suggest incorporation of relatively ^{18}O -enriched oxygen from allochthonous sources into diet (Nielson and Bowen 2010). Fatty acid analyses suggest that terrestrial inputs subsidize *Diporeia* diets in Cayuga Lake (Watkins et al. 2012). Our results corroborate that conclusion, but contrary to previous studies that assume dreissenids and *Diporeia* exploit different food sources, the proximity of the signatures seen in our study indicate that dreissenids in Cayuga Lake may also derive their food from the same external inputs.

The isotopic similarities of current day Lake Michigan and Lake Superior *Diporeia* populations and the changes in isotopic signature of Lake Michigan *Diporeia* over time could reflect the diminution of the spring diatom bloom in Lake Michigan (Fahnenstiel et al. 2010; Mida et al. 2010). Previous studies have suggested that *Diporeia* populations benefit from the spring diatom bloom (Gardner et al. 1985) and that decreased availability of food in spring can negatively affect the storage of lipids for use later in the year (Kainz et al. 2010; Barbiero et al. 2011). Seasonal patterns in Lake Michigan indicated that dreissenid and *Diporeia* resource use in 2008 and 2009 was most similar in the spring, the season that reflected the greatest influence of benthic resources on mussel diets (higher $\delta^{13}\text{C}$ values) and the least influence of external inputs on *Diporeia* diets (lower $\delta^2\text{H}_{\text{LC}}$ values). If animals do indeed rely on the same, limited resources during springtime, there may be the potential for competition between *Diporeia* and dreissenids in Lake Michigan. The shifting hydrogen signature of Lake Michigan *Diporeia* over time suggests increased reliance on external inputs (higher $\delta^2\text{H}_{\text{LC}}$ values; Fig. 6), though the values of recent collections indicate that Lake Michigan animals still rely more on internal resources than either of the other two populations (lower $\delta^2\text{H}_{\text{LC}}$ values; Fig. 3). *Diporeia* from Great Lakes populations exhibit different seasonal patterns of resource use than those from Cayuga Lake, relying more on pelagic sources of food in the summertime. Periphyton signatures in other systems tend to be depleted in $\delta^{13}\text{C}$ in autumn versus summer (e.g., MacLeod and Barton 1998), and the pattern in Cayuga Lake animal signatures (especially *Diporeia*) may indicate incorporation of periphyton dislodged from shallower waters into *Diporeia* diets later in the year. However, the differences in $\delta^{13}\text{C}$ signatures for Cayuga Lake animals from season to season are relatively small (<1‰), and animal tis-

sue turnover rates may not be quick enough to reflect any such changes in diet.

Lake Michigan *Diporeia* exhibit increased trophic factor values and decreased $\delta^{18}\text{O}$ values after dreissenid invasion. A recent study demonstrated a 2.1‰ increase in $\delta^{15}\text{N}$ of the Lake Michigan offshore region between 2002 and 2010 (Turschak et al. 2014), and increased $\delta^{15}\text{N}$ signatures of a food web after dreissenid invasion has been demonstrated in other systems (e.g., Ozersky et al. 2012). Turschak et al. (2014) also demonstrated changes of approximately 1‰ in $\delta^{13}\text{C}$ values in the Lake Michigan offshore region between 2002 and 2010 and suggest that this may indicate increased importance of nearshore sources of carbon to the offshore region. We see changes in $\delta^{13}\text{C}$ closer to 4‰ over the course of our study, though they are not in a uniform direction. Lake Michigan *Diporeia* signatures shift back and forth along the two resource gradients ($\delta^{13}\text{C}$ and $\delta^2\text{H}$), and this may reflect either reliance on, or interference by, dreissenids with respect to *Diporeia* feeding. In the early stages of invasion, dreissenids rapidly proliferating in the nearshore regions of Lake Michigan may have been intercepting the transfer of nutrients to the offshore region (Watkins et al. 2007; Pothoven and Fahnenstiel 2013), causing *Diporeia* to rely on pelagic sources of food. More recently, the composition of pelagic food resources has been dominated by smaller, faster growing species that are not as available to *Diporeia* (M. Edlund, Science Museum of Minnesota, St. Paul, Minnesota, personal communication, 2012); thus, *Diporeia* may have switched back to benthic resources.

Alternatively, *Diporeia* in Lake Michigan may have fed on dreissenid pseudofeces soon after the invasion and recently changed to other food sources. In laboratory experiments using *Diporeia* from several populations, there were few negative effects directly related to feeding on pseudofeces (Dermott et al. 2005b). Pseudofeces have also been shown to lower the $\delta^{13}\text{C}$ values of amphipods feeding on fine benthic organic matter in the Ohio River while improving the quality of their food (Greenwood et al. 2001). This hypothesis could help explain the high degree of isotopic overlap between dreissenids and *Diporeia* in Cayuga Lake, particularly since Cayuga Lake *Diporeia* had the lowest $\delta^{13}\text{C}$ signatures of all populations studied despite being the most productive system; however, we cannot confirm that Cayuga Lake *Diporeia* feed on pseudofeces nor can we assert that pseudofeces contribute to their survival. At the same time, if Lake Michigan *Diporeia* were able to exploit dreissenid pseudofeces, we might not expect their populations to be in decline.

Although we have demonstrated robust patterns of isotopic difference between lakes and taxa that support first-order interpretation of dietary effects associated with co-occurrence of *Diporeia* and dreissenids in different systems, several sources of uncertainty affect our study. These could be addressed in future work to improve our understanding of the observed patterns. Corrections for preservative, including extracting lipids, had potential to bias our results. Lipid extraction can lead to more positive $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (Logan et al. 2008; Mateo et al. 2008), though nitrogen values for freshwater invertebrates may not be significantly affected and carbon values change by roughly +1‰ (Logan et al. 2008). Lipid analyses of *Diporeia* specimens from these same sites indicate that Cayuga Lake specimens have approximately 6%–10% (by dry mass) higher lipid content than Lake Superior or Lake Michigan specimens (J. Rinchard, State University of New York, Brockport, New York, personal communication, 2012). Had we determined stable isotope ratios of specimens in our study before extracting lipids, the differences between lake populations of *Diporeia* may have been even more pronounced. In addition, the differences in measured stable isotope values between animals and populations in our study spanned a broader range than 1‰. Repetition of statistical analyses with subsets of samples preserved the same way led to similar conclusions, confirming the appropriateness of using preservative-based correc-

tion factors (see online Supplemental material¹), and inclusion of additional samples increased the statistical power of our tests.

Values for historic samples may be affected by length of time spent in preservative, though in that case we would not expect to see a shift over time but rather a grouping of all formalin-preserved samples versus all non-formalin-preserved ones (Bosley and Wainright 1999; Sarakinos et al. 2002) for carbon, hydrogen, and oxygen values (i.e., the components of formalin that could have exchanged with the specimens). It is difficult to draw clear conclusions about the implications of our results without knowing the signatures of potential food items in each system, and our study would have benefited from additional sample collections of dietary material. However, the range of measured values found in our study is large, the observed ranges of carbon and nitrogen isotopic ratios are similar to those from studies that included animals at several trophic levels (e.g., Post 2002; Keough et al. 1996; Vander Zanden et al. 2006), and we are confident that the measured values reflect real differences in food sources.

Our results provide additional insight to a long-standing question in the Laurentian Great Lakes: what has caused the decline of *Diporeia*, arguably the most important benthic invertebrate historically present in offshore regions? Contrary to our expectations, *Diporeia* and dreissenids that co-occur in Cayuga Lake have more similar stable isotope values than the two taxa in Lake Michigan. This leads us to believe that the taxa utilize food derived from the same sources in Cayuga Lake. The large difference in stable isotope values for *Diporeia* and dreissenids in Lake Michigan combined with the shifts in *Diporeia* stable isotope values after the dreissenid invasion suggest that dreissenids have affected *Diporeia* feeding in that lake, though the effect may not be negative. Steady, but spatially distinct, *Diporeia* populations in Lake Superior and Cayuga Lake do not always display similar isotopic signatures, making it difficult for us to point to a mechanism for survival. Our results do, however, indicate that terrestrial subsidies to the system that increase the availability of food throughout the year likely play a role in the persistence of *Diporeia* populations. Future studies should further examine the relative importance of terrestrial subsidies to *Diporeia* nutrition, including an examination of gut contents and explicit breakdown of the relative components of *Diporeia* diets.

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References

Barbiero, R.P., Schmude, K., Lesht, B.M., Riseng, C.M., Warren, G.J., and Tuchman, M.L. 2011. Trends in *Diporeia* populations across the Laurentian Great Lakes, 1997–2009. *J. Gt. Lakes Res.* **37**: 9–17. doi:10.1016/j.jglr.2010.11.009.

Bosley, K.L., and Wainright, S.C. 1999. Effects of preservatives and acidification on the stable isotope ratios (¹⁵N:¹⁴N, ¹³C:¹²C) of two species of marine animals. *Can. J. Fish. Aquat. Sci.* **56**(11): 2181–2185. doi:10.1139/f99-153.

Bowen, G.J., Wassenaar, L.L., and Hobson, K.A. 2005. Global application of stable hydrogen and oxygen isotopes to wildlife forensics. *Oecologia*, **143**: 337–348. doi:10.1007/s00442-004-1813-y. PMID:15726429.

Callinan, C.W. 2001. Water quality study of the Finger Lakes [online]. New York State Department of Environmental Conservation. Available from <http://www.dec.ny.gov/lands/25576.html>.

Cole, J.J., Carpenter, S.R., Kitchell, J., Pace, M.L., Solomon, C.T., and Weidel, B. 2011. Strong evidence for terrestrial support of zooplankton in small lakes based on stable isotopes of carbon, nitrogen, and hydrogen. *Proc. Natl. Acad. Sci.* **108**: 1975–1980. doi:10.1073/pnas.1012807108. PMID:21245299.

Craig, H. 1961. Isotopic variations in meteoric waters. *Science*, **133**: 1702–1703. doi:10.1126/science.133.3465.1702. PMID:17814749.

Dansgaard, W. 1954. The O¹⁸-abundance in fresh water. *Geochim. Cosmochim. Acta*, **6**: 241–260. doi:10.1016/0016-7037(54)90003-4.

Dermott, R., Munawar, M., Bonnell, R., Carou, S., Niblock, H., Nalepa, T.F., and Messick, G. 2005a. Preliminary investigations for causes of the disappearance of *Diporeia* spp. from Lake Ontario [online]. In Proceedings of a Workshop on the Dynamics of Lake Whitefish. Edited by L.C. Mohr and T.F. Nalepa. Available from <http://www.glf.org/pubs/TechReports/Tr66.pdf> [accessed 12 June 2012]. pp. 203–232.

Dermott, R., Munawar, M., Carou, S., Bonnell, R., and Niblock, H. 2005b. Is sediment or pseudofaeces toxicity responsible for the decline of the amphipod *Diporeia hoyi* in Lakes Erie and Ontario? *Aquat. Ecosyst. Health*, **8**: 11–20. doi:10.1080/14634980590927373.

Doucett, R.R., Marks, J.C., Blinn, D.W., Caron, M., and Hungate, B.A. 2007. Measuring terrestrial subsidies to aquatic food webs using stable isotopes of hydrogen. *Ecology*, **88**: 1587–1592. doi:10.1890/06-1184. PMID:17601150.

Fahnenstiel, G., Nalepa, T.F., Pothoven, S., Carrick, H., and Scavia, D. 2010. Lake Michigan lower food web: long-term observations and *Dreissena* impact. *J. Gt. Lakes Res.* **36**: 1–4. doi:10.1016/j.jglr.2010.05.009.

Finlay, J.C., and Kendall, C. 2007. Stable isotope tracing of organic matter sources and food web interactions in watersheds. In *Stable isotopes in ecology and environmental science*. Edited by K. Lajtha and R. Michener. Blackwell, pp. 283–333.

Fry, B. 2002. Conservative mixing of stable isotopes across estuarine salinity gradients: a conceptual framework for monitoring watershed influences on downstream fisheries production. *Estuaries*, **25**: 264–271. doi:10.1007/BF02691313.

Gardner, W.S., Nalepa, T.F., Frez, W.A., Cichocki, E.A., and Landrum, P.F. 1985. Seasonal patterns in lipid content of Lake Michigan macroinvertebrates. *Can. J. Fish. Aquat. Sci.* **42**(11): 1827–1832. doi:10.1139/f85-229.

Genesee/Finger Lakes Regional Planning Council. 2001. Cayuga Lake Watershed Restoration Plan [online]. Available from http://www.gflrpc.org/Publications/CayugaLake/WRAP/Full/CayugaLake_WRAP.pdf [accessed 8 August 2013].

Gratton, C., and Forbes, A.E. 2006. Changes in $\delta^{13}\text{C}$ stable isotopes in multiple tissues of insect predators fed isotopically distinct prey. *Oecologia*, **147**: 615–624. doi:10.1007/s00442-005-0322-y. PMID:16341886.

Greenwood, K.S., Thorp, J.H., Summers, R.B., and Guelida, D.L. 2001. Effects of an exotic bivalve mollusc on benthic invertebrates and food quality in the Ohio River. *Hydrobiologia*, **462**: 169–172. doi:10.1023/A:1013190301967.

Grey, J., Kelly, A., Ward, S., Sommerwerck, N., and Jones, R.I. 2004. Seasonal changes in the stable isotope values of lake-dwelling chironomid larvae in relation to feeding and life cycle variability. *Freshw. Biol.* **49**: 681–689. doi:10.1111/j.1365-2427.2004.01217.x.

Gu, B., Schell, D.M., Huang, X., and Yie, F. 1996. Stable isotope evidence for dietary overlap between two planktivorous fish in aquaculture ponds. *Can. J. Fish. Aquat. Sci.* **53**(12): 2814–2818. doi:10.1139/f96-242.

Guiguer, K.R., and Barton, D.R. 2002. The trophic role of *Diporeia* (Amphipoda) in Colpoys Bay (Georgian Bay) benthic food web: a stable isotope approach. *J. Gt. Lakes Res.* **28**: 228–239. doi:10.1016/S0380-1330(02)70579-0.

Harvey, C.J., and Kitchell, J.F. 2000. A stable isotope evaluation of the structure and spatial heterogeneity of a Lake Superior food web. *Can. J. Fish. Aquat. Sci.* **57**(7): 1395–1403. doi:10.1139/f00-072.

Kainz, M.J., Johannsson, O.E., and Arts, M.T. 2010. Diet effects on lipid composition, somatic growth potential, and survival of the benthic amphipod *Diporeia* spp. *J. Gt. Lakes Res.* **36**: 351–356. doi:10.1016/j.jglr.2010.02.004.

Karatayev, A.Y., Padilla, D.K., Minchin, D., Boltovskoy, D., and Burlakova, L.E. 2007. Changes in global economies and trade: the potential spread of exotic freshwater bivalves. *Biol. Invasions*, **9**: 161–180. doi:10.1007/s10530-006-9013-9.

Karatayev, V.A., Karatayev, A.Y., Burlakova, L.E., and Padilla, D.K. 2013. Lakewide dominance does not predict the potential for spread of dreissenids. *J. Gt. Lakes Res.* **39**: 622–629. doi:10.1016/j.jglr.2013.09.007.

Keough, J.R., Sierszen, M.E., and Hagley, C.A. 1996. Analysis of a Lake Superior coastal food web with stable isotope techniques. *Limnol. Oceanogr.* **41**: 136–146. doi:10.4319/lo.1996.41.1.0136.

Lancaster, J., and Waldron, S. 2001. Stable isotopes of lotic macroinvertebrates: sources of variation, experimental design and statistical interpretation. *Limnol. Oceanogr.* **46**: 723–730. doi:10.4319/lo.2001.46.3.0723.

Layman, C.A., Araujo, M.S., Bouček, R., Hammerschlag-Peyer, C.M., Harrison, E., Jud, Z.R., Matich, P., Rosenblatt, A.E., Vaudo, J.J., Yeager, L.A., Post, D.M., and Bearhop, S. 2012. Applying stable isotopes to examine food-web structure: an overview of analytical tools. *Biol. Rev.* **87**: 545–562. doi:10.1111/j.1469-185X.2011.00208.x. PMID:22051097.

Logan, J.M., Jardine, T.D., Miller, T.J., Bunn, S.E., Cunjak, R.A., and Lutcavage, M.E. 2008. Lipid corrections in carbon and nitrogen stable isotope analyses: comparison of chemical extraction and modelling meth-

- ods. *J. Anim. Ecol.* **77**: 838–846. doi:10.1111/j.1365-2656.2008.01394.x. PMID: 18489570.
- Longstaffe, F.J., Ayalon, A., Hladyniuk, R., Hyodo, A., Macdonald, R., St. Amour, N., Crowe, A., and Huddart, P. 2011. The oxygen and hydrogen isotope evolution of the Great Lakes [online]. Presentation at the International Symposium on Isotopes in Hydrology, Marine Ecosystems, and Climate Change Studies. Available from [http://www-naweb.iaea.org/napc/ih/documents/other/INV002%20LONGSTAFFE%20\(Presentation\).pdf](http://www-naweb.iaea.org/napc/ih/documents/other/INV002%20LONGSTAFFE%20(Presentation).pdf).
- MacLeod, N.A., and Barton, D.R. 1998. Effects of light intensity, water velocity, and species composition on carbon and nitrogen stable isotope ratios in periphyton. *Can. J. Fish. Aquat. Sci.* **55**(8): 1919–1925. doi:10.1139/f98-075.
- Marzolf, G.R. 1965. Substrate relations of the burrowing amphipod *Diporeia affinis* in Lake Michigan. *Ecology*, **46**: 579–592. doi:10.2307/1934998.
- Mateo, M.A., Serrano, O., Serrano, L., and Michener, R.H. 2008. Effects of sample preparation on stable isotope ratios of carbon and nitrogen in marine invertebrates: implications for food web studies using stable isotopes. *Oecologia*, **157**: 105–115. doi:10.1007/s00442-008-1052-8. PMID:18465146.
- Matthews, B., and Mazumder, A. 2003. Compositional and interlake variability of zooplankton affect baseline stable isotope signatures. *Limnol. Oceanogr.* **48**: 1977–1987. doi:10.4319/lo.2003.48.5.1977.
- Michel, R.L., and Kraemer, T.F. 1995. Use of isotopic data to estimate water residence times of the Finger Lakes, New York. *J. Hydrol.* **164**: 1–18. doi:10.1016/0022-1694(94)02586-2.
- Michigan Department of Natural Resources. 2013. Lake Michigan Basin [online]. Available from http://www.michigan.gov/dnr/0,4570,7-153-10370_30909_31053-153460--,00.html.
- Mida, J.L., Scavia, D., Fahnenstiel, G.L., Pothoven, S.A., Vanderploeg, H.A., and Dolan, D.M. 2010. Long-term and recent changes in southern Lake Michigan water quality with implications for present trophic status. *J. Gt. Lakes Res.* **36**(Suppl. 3): 42–49. doi:10.1016/j.jglr.2010.03.010.
- Nalepa, T.F., Fanslow, D.L., and Messick, G. 2005. Characteristics and potential causes of declining *Diporeia* spp. populations in southern Lake Michigan and Saginaw Bay, Lake Huron [online]. In Proceedings of a Workshop on the Dynamics of Lake Whitefish. Edited by L.C. Mohr and T.F. Nalepa. Available from <http://www.glfrc.org/pubs/TechReports/Tr66.pdf> [accessed 12 June 2012]. pp. 157–179.
- Nalepa, T.F., Fanslow, D.L., Foley, A.J., III, Lang, G.A., Eadie, B.J., and Quigley, M.A. 2006a. Continued disappearance of the benthic amphipod *Diporeia* spp. in Lake Michigan: is there evidence for food limitation? *Can. J. Fish. Aquat. Sci.* **63**(4): 872–890. doi:10.1139/f05-262.
- Nalepa, T.F., Rockwell, D.C., and Schloesser, D.W. 2006b. Disappearance of the amphipod *Diporeia* spp. in the Great Lakes: workshop summary, discussion, and recommendations. National Oceanic Atmospheric Administration Technical Memorandum GLERL-136. Great Lakes Environmental Research Laboratory, Ann Arbor, Mich.
- Nalepa, T.F., Fanslow, D.L., and Lang, G.A. 2009. Transformation of the offshore benthic community in Lake Michigan: recent shift from the native amphipod *Diporeia* spp. to the invasive mussel *Dreissena rostriformis bugensis*. *Freshw. Biol.* **54**: 466–479. doi:10.1111/j.1365-2427.2008.02123.x.
- Nielson, K.E., and Bowen, G.J. 2010. Hydrogen and oxygen in brine shrimp chitin reflect environmental water and dietary isotopic composition. *Geochim. Cosmochim. Acta*, **74**: 1812–1822. doi:10.1016/j.gca.2009.12.025.
- Ozersky, T., Evans, D.O., and Barton, D.R. 2012. Invasive mussels alter the littoral food web of a large lake: stable isotopes reveal drastic shifts in sources and flow of energy. *PLoS ONE*, **7**: e51249. doi:10.1371/journal.pone.0051249.
- Post, D.M. 2002. Using stable isotopes to estimate trophic position: models, methods, and assumptions. *Ecology*, **83**: 703–718. doi:10.1890/0012-9658(2002)083[0703:USITET]2.0.CO;2.
- Pothoven, S.A., and Fahnenstiel, G.L. 2013. Recent change in summer chlorophyll *a* dynamics of southeastern Lake Michigan. *J. Gt. Lakes Res.* **39**: 287–294. doi:10.1016/j.jglr.2013.02.005.
- R Development Core Team. 2012. R: a language and environment for statistical computing [online]. R Foundation for Statistical Computing, Vienna, Austria. Available from <http://www.R-project.org/>. ISBN 3-900051-07-0.
- Rooker, J.R., Secor, D.H., DeMetrio, G., Kaufman, A.J., Ríos, A.B., and Ticina, V. 2008. Evidence of trans-Atlantic movement and natal homing of bluefin tuna from stable isotopes in otoliths. *Mar. Ecol. Prog. Ser.* **368**: 231–239. doi:10.3354/meps07602.
- Ryan, D.J., Sepúlveda, M.S., Nalepa, T.F., and Höök, T.O. 2012. Spatial variation in RNA:DNA ratios of *Diporeia* spp. in the Great Lakes region. *J. Gt. Lakes Res.* **38**: 187–195. doi:10.1016/j.jglr.2012.01.007.
- Sanseverino, A.M., Bastviken, D., Sundh, I., Pickova, J., and Enrich-Prast, A. 2012. Methane carbon supports aquatic food webs to the fish level. *PLoS ONE*, **7**(8): e42723. doi:10.1371/journal.pone.0042723.
- Sarakinos, H.C., Johnson, M.L., and Vander Zanden, M.J. 2002. A synthesis of tissue-preservation effects on carbon and nitrogen stable isotope signatures. *Can. J. Zool.* **80**(2): 381–387. doi:10.1139/z02-007.
- Soto, D.X., Wassenaar, L.L., and Hobson, K.A. 2013. Stable hydrogen and oxygen isotopes in aquatic food webs are tracers of diet and provenance. *Funct. Ecol.* **27**: 535–543. doi:10.1111/1365-2435.12054.
- Turner, T.F., Collyer, M.L., and Krabbenhoft, T.J. 2010. A general hypothesis-testing framework for stable isotope ratios in ecological studies. *Ecology*, **91**: 2227–2233. doi:10.1890/09-1454.1. PMID:20836444.
- Turschak, B.A., Bunnell, D., Czesny, S., Höök, T.O., Janssen, J., Warner, D., and Bootsma, H.A. 2014. Nearshore energy subsidies support Lake Michigan fishes and invertebrates following major changes in food web structure. *Ecology*, **95**: 1243–1252. doi:10.1890/13-0329.1. PMID:25000756.
- United States Department of Agriculture National Agricultural Statistics Service. 2007. State and County Census Reports [online]. Available from http://www.agcensus.usda.gov/Publications/2007/Full_Report/.
- Vander Zanden, M.J., and Rasmussen, J.B. 2001. Variation in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ trophic fractionation: implications for aquatic food web studies. *Limnol. Oceanogr.* **46**: 2061–2066. doi:10.4319/lo.2001.46.8.2061.
- Vander Zanden, M.J., Vadeboncoeur, Y., Diebel, M.W., and Jeppesen, E. 2005. Primary consumer stable nitrogen isotopes as indicators of nutrient source. *Environ. Sci. Technol.* **39**: 7509–7515. doi:10.1021/es050606t. PMID:16245822.
- Vander Zanden, M.J., Chandra, S., Park, S.-K., Vadeboncoeur, Y., and Goldman, C.R. 2006. Efficiencies of benthic and pelagic trophic pathways in a subalpine lake. *Can. J. Fish. Aquat. Sci.* **63**(12): 2608–2620. doi:10.1139/f06-148.
- Vanderploeg, H.A., Liebig, J.R., Nalepa, T.F., Fahnenstiel, G.L., and Pothoven, S.A. 2010. *Dreissena* and the disappearance of the spring phytoplankton bloom in Lake Michigan. *J. Gt. Lakes Res.* **36**: 50–59. doi:10.1016/j.jglr.2010.04.005.
- Watkins, J.M., Dermott, R., Lozano, S.J., Mills, E.L., Rudstam, L.G., and Scharold, J.V. 2007. Evidence for remote effects of dreissenid mussels on the amphipod *Diporeia*: analysis of Lake Ontario benthic surveys, 1972–2003. *J. Gt. Lakes Res.* **33**: 642–657. doi:10.3394/0380-1330(2007)33[642:EFREOD]2.0.CO;2.
- Watkins, J.M., Rudstam, L.G., Mills, E.L., and Teece, M.A. 2012. Coexistence of the native benthic amphipod *Diporeia* spp. and exotic dreissenid mussels in the New York Finger Lakes. *J. Gt. Lakes Res.* **38**: 226–235. doi:10.1016/j.jglr.2012.02.001.