Spatial variability in trophic offset and food sources of *Hemimysis anomala* in lentic and lotic ecosystems within the Great Lakes basin

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Invasive species are a known stressor on aquatic ecosystems, particularly in the waters of the Great Lakes basin. A recent invader, *Hemimysis anomala*, has had significant impacts on food webs in Europe, where it invaded previous to its spread to North America. This study used carbon and nitrogen stable isotopes to characterize and compare the diet of *Hemimysis* from 13 sites in the Great Lakes basin. Results indicated that: (i) *Hemimysis* relied predominantly on pelagic carbon sources at the majority of sites, and isotopic differences between life-stages existed at two of the 13 sites examined, (ii) the trophic offset and reliance on pelagic food sources did not differ significantly between lotic and lentic sites, and (iii) the isotopic niche width of *Hemimysis* was spatially heterogeneous, varying by an order of magnitude among sites, but was unrelated to the degree of isotopic variation in the basal food web at each site. Observed ranges in trophic offset and the pelagic fraction of dietary carbon indicate that *Hemimysis* derives carbon from both benthic and water column sources, as well as at multiple trophic levels. Our results support the view that *Hemimysis* is an opportunistic omnivore that displays significant dietary flexibility.
INTRODUCTION

Aquatic invasive species are a known and significant stressor on aquatic ecosystems, but the impacts of a particular organism can be very difficult to predict. When a new invader is found, or is predicted to invade a site, investigators often rely on impacts seen at other sites in the invasion history of the organism (Ricciardi, 2003; Ricciardi et al., 2012). This, however, is not always effective, as the impacts of an aquatic invader are a function of not only the biology of the invader, but also the structure of the invaded food web and the physical environment at an invaded site (Ricciardi, 2003; Kulhanek et al., 2011). The bloody red mysid, *Hemimysis anomala* (hereafter *Hemimysis*), is one such invader where invasion history has been used to make predictions about future impacts in newly invaded sites. In the case of *Hemimysis*, which recently invaded the Laurentian Great Lakes basin, predictions were made based on impacts seen in European invaded sites (Ketelaars et al., 1999; Borcherring et al., 2006; Ricciardi et al., 2012), which may differ from sites in the Great Lakes basin in both environmental characteristics and food web dynamics.

Native to the Ponto-Caspian region, *Hemimysis* is one of the most recent invaders in the Great Lakes basin. Intentionally introduced in Eastern Europe in the 1950s and 1960s, *Hemimysis* spread through Eastern Europe in the 1990s (Ketelaars et al., 1999; Wittmann, 2007; Audzijonyte et al., 2008) and eventually to Western Europe (Dumont, 2006; Minchin and Holmes, 2008). Transported via shipping from Europe, *Hemimysis* was first discovered in Lake Ontario and Lake Michigan in 2006 (Pothoven et al., 2007) and has since been found in every Great Lake except Lake Superior (Marty et al., 2010), at numerous sites along the St Lawrence River (Kestrup and Ricciardi, 2008; Marty et al., 2010; de Lafontaine et al., 2012) and in some inland lakes in New York State (Brooking et al., 2010; Brown et al., 2012).

*Hemimysis* undergoes a diel vertical migration, hiding in the substrate or in structural interstices during the day, and moving up into the water column at night (Borcherring et al., 2006; Boscarino et al., 2012). Previous studies have shown that it is able to feed on both benthic and pelagic food sources (Marty et al., 2010, 2012) and will feed on zooplankton, especially cladocerans, algae, detritus, small benthic invertebrates, and is occasionally cannibalistic (Ketelaars et al., 1999; Borcherring et al., 2006; Ricciardi et al., 2012). Significant food web impacts, including dramatic decreases in zooplankton abundance and changes in species composition, have been reported at invaded European sites (Ketelaars et al., 1999; Borcherring et al., 2006; Stich et al., 2009), and the discovery of *Hemimysis* in the Great Lakes has raised much concern about the potential ecological impacts of this new invader (Koops et al., 2010; Marty et al., 2010; de Lafontaine et al., 2012; Marty et al., 2012). Potential *Hemimysis* impacts in North America have been explored using its European invasion history (Ricciardi et al., 2012). However, to date, field studies of *Hemimysis* have mainly focused on reservoirs and lakes, although the species has been recorded in large European rivers (Dumont, 2006; Borcherring et al., 2006). In the Great Lakes basin, *Hemimysis* has been found in high abundances in both lentic and lotic sites (Koops et al., 2010; Marty et al., 2010, 2012; de Lafontaine et al., 2012) yet spatial differences in *Hemimysis* diet and food web impacts in both environments have yet to be determined and explicitly compared.

Previous invaders, including the zebra mussel (*Dreissena polymorpha*) and the quagga mussel (*Dreissena bugensis*), have had a significant impact on the food webs of nearshore environments within the Great Lakes (Strayer, 1999; Vanderploeg et al., 2001; Hecky et al., 2004; Higgins and Vander Zanden, 2010). The establishment of dreissenid mussels led to a fundamental redirection of energy flows, termed the nearshore phosphorus shunt, via the removal of water column nutrients through filter feeding and the benthic deposition of fecal materials (Hecky et al., 2004). The resulting decoupling between the benthic and pelagic food webs has been associated with an increase in benthic versus pelagic production (Hecky et al., 2004). Depending on how *Hemimysis* integrates into nearshore ecosystems, it could exacerbate the decoupling (Ricciardi et al., 2012), or alternatively restore linkages between benthic and pelagic food webs (Marty et al., 2012). Understanding *Hemimysis* diet at different sites throughout the Great Lakes basin is essential to understand the potential impacts of this recent invader.

In light of the documented impacts of previous invaders on Great Lakes food webs and the remaining unknowns about the potential impacts of *Hemimysis*, particularly the lack of data on food web impacts of *Hemimysis* in lotic ecosystems, this study aimed to evaluate the main carbon pathways supporting *Hemimysis* diet and to compare *Hemimysis* trophic niche use between lentic and lotic habitats within the same watershed. Specifically,
our first study objective was to describe the trophic offset and main food sources of *Hemimysis*, including adults and juveniles, from both lentic and lotic sites using stable isotope methods. Trophic offset is a continuous measure of the $\delta^{15}$N isotopic distance between an organism and basal food web organisms, and was used in place of the trophic level concept to avoid strict trophic categorization of *Hemimysis*. Given that evidence for an ontogenetic shift in *Hemimysis* diet from juveniles feeding on phytoplankton to adults feeding on zooplankton was previously observed in Europe (Borcherding et al., 2006), but not in North America (Marty et al., 2010; Marty et al., 2012), we tested the hypothesis that *Hemimysis* would primarily rely on pelagic carbon sources and exhibit no significant differences in trophic offset or carbon sources between life stages. Our second study objective was to compare the trophic offset and food sources of *Hemimysis* between lentic and lotic sites. It was predicted that, due to the lower zooplankton biomass in lotic sites (Pace et al., 1992), *Hemimysis* populations in rivers would rely more strongly on food sources derived from the benthic zone and would occupy a lower trophic level than that in lake populations. As a corollary to this hypothesis, a third study objective was to better describe the variability of *Hemimysis* diet. *Hemimysis* is described as a generalist feeder (Dumont, 2006; Marty et al., 2012), but differences in the food web niche width of different populations have remained unexplored. We predicted that greater inherent variability in the basal food web at a site would afford *Hemimysis* a diet with a larger range of carbon sources, and thus a significant positive relationship would exist between site-specific *Hemimysis* isotopic niche width and baseline food web isotopic width.

**METHOD**

**Sampling**

Between 15 August and 12 September 2011, sampling was conducted at seven lentic sites in lakes Ontario ($n = 3$) and Erie ($n = 4$) and at five lotic sites in the St Lawrence River (Fig. 1; Table I). Sites were located in urbanized areas with permanent manmade structures, such as piers, as a main feature of the site. Lotic sites were located in the vicinity of the Port of Montreal and included two sites (Q8 and Q12) situated in protected port basins with lessened water flows, and three sites (Q45, Q56 and Q57) exposed to higher flow from the main channel of the St Lawrence River (Fig. 1). A detailed description of the Montreal sampling sites is available in de Lafontaine et al. (Lafontaine et al., 2012). One additional site (St Timothée, ST), which was sampled 29 July 2011, was located upstream of the Montreal sites in the regional Parc des Îles (Fig. 1; Table I) and was considered a natural site characterized by clear waters and a shallow bottom covered by boulders.

Sampling was conducted after dusk following standardized methods optimized for the capture of *Hemimysis* (Walsh et al., 2010; de Lafontaine et al., 2012). Briefly, all lentic sampling was conducted directly off available piers or breakwaters, using multiple vertical tows with a 0.75 m diameter, 400 μm mesh plankton net that was dropped to the substrate, allowed to rest for a minimum of 30 s and then raised to the surface at a steady rate of approximately 1 m s$^{-1}$. Samples were transferred to 1 L containers filled with lake water and stored at $\sim 4{^\circ}$C overnight. Lotic sampling was similarly performed using a boat positioned adjacent to the permanent structures of the Port, including cement walls and piers. *Hemimysis* samples were kept alive for 24 h to allow for gut evacuation (Marty et al., 2012). Lower food web samples (e.g. zooplankton, algae, etc.) were collected with horizontal net tows using a 0.5 m diameter, 243 μm mesh plankton net within 50 m of the *Hemimysis* collections. A kick net was also used to scrape biological material from pier walls, from which invertebrates (snails and zebra mussels) and periphyton were sorted. All samples were sorted within 24 h of collection.

An integrated volume of water (0.5 to 1 L, depending on turbidity), incorporating water from the entire water column, was collected in triplicate and filtered in the field onto pre-weighed, pre-combusted quartz filters (Whatman QMA, 47 mm) for analysis of particulate organic carbon (POC).

**Stable isotope analysis**

In the laboratory, zooplankton samples were sorted into main functional groups (e.g. herbivorous cladocerans, predatory cladocerans, calanoid copepods and cyclopoid copepods). For the lentic sites, 10 adult *Hemimysis* were sexed, measured and processed individually for SIA. Gender was determined through examination of the fourth pleopod, which is elongated in males, and through the presence or absence of a marsupium, or brood pouch, in females (Borcherding et al., 2006). Fifteen juveniles from each site were also measured and grouped into three samples of five individuals. When the number of captured *Hemimysis* permitted, additional bulk samples were prepared by gender and analysed. For sites in the St Lawrence, three replicates of each gender were processed with three individuals in each sample. Three samples of juveniles were also processed, with five individuals in each sample. At sites where the number of captured
Hemimysis was low (<10 adults or <15 juveniles), all individuals available were processed for SIA. When available, food web samples (e.g. periphyton, amphipods, mussels, snails and zooplankton) were processed in triplicate. Sorted samples were kept frozen at −20°C until SIA.

Samples for SIA were first dried at 50°C for 24–72 h and then homogenized with a mortar and pestle. Sample material from plant- and animal-based samples were weighed to 600 or 300 mg, respectively, using a microbalance (model CH-8730, Mettler-Toledo, Uznach, Switzerland). All SIA were performed at the University of Waterloo Environmental Isotope Laboratory (Waterloo, Ontario) on a Delta Plus Continuous Flow Stable Isotope Ratio Mass Spectrometer (Thermo Finnigan, Bremen, Germany), coupled to a Carlo Erba elemental analyzer (model CHNS-O EA1108, Carlo Erba, Milan, Italy). Results are expressed in standard δ notation as parts per thousand (‰) where

$$\delta = \left[\frac{R_{\text{sample}}}{R_{\text{reference}}} - 1\right] \times 1000,$$

and

$$R = \frac{^{13}C}{^{12}C} \text{ or } \frac{^{15}N}{^{14}N}.$$ (Verardo et al., 1990) with respect to the standard international reference materials of atmospheric nitrogen for nitrogen (Mariotti, 1983) and Pee Dee Belemnite for carbon (Craig, 1957). Precision of the obtained δ values was calculated as the standard deviation of the values obtained from repeat analysis of a random subset of samples, and was, on average (± SE), 0.14 ± 0.02‰ for $\delta^{13}C$ ($n = 25$) and
The relative importance of pelagic versus benthic food sources in the diet of Hemimysis was calculated using a two source mixing model modified from Vander Zanden and Vadeboncoeur (Vander Zanden and Vadeboncoeur, 2002) as:

$$\delta^{13}C_{HA} - \Delta = \gamma(\delta^{13}C_{PP}) + (1 - \gamma)(\delta^{13}C_{BP})$$  \hspace{1cm} (1)$$

$$\Delta = F_C \left[ \frac{T_O}{F_N} + 1 \right]$$  \hspace{1cm} (2)$$

re-arranged to extract $\gamma$ such that:

$$\gamma = \frac{(\delta^{13}C_{HA} - \Delta - \delta^{13}C_{BP})}{(\delta^{13}C_{PP} - \delta^{13}C_{BP})}$$  \hspace{1cm} (3)$$

where $\Delta$ is the total difference in $\delta^{13}C$ between the Hemimysis and dietary items at the primary consumer level due to trophic fractionation, allowing us to account for differing degrees of omnivory among Hemimysis, $F_C$ and $F_N$ are the discrimination factors for $\delta^{13}C$ and $\delta^{15}N$ between an organism and its diet (accounting for one trophic level and set at 0.4 and 3.4‰, respectively, following Post (Post, 2002)), TO is the trophic offset of an organism, described below, $\gamma$ is the pelagic fraction of dietary carbon, $\delta^{13}C_{PP}$ and $\delta^{13}C_{BP}$ are the end-members for pelagic and benthic primary production, respectively, and $\delta^{13}C_{HA}$ is the $\delta^{13}C$ value of Hemimysis. The pelagic end-member ($\delta^{13}C_{PP}$) was calculated using the mean of the $\delta^{13}C$ values for mussels and herbivorous cladocerans, corrected to the level of primary producer by subtracting the discrimination factor of 0.4‰ from the mean $\delta^{13}C$ of these primary consumers. At one lotic site, Q45, no primary consumers were obtained, and at this site the $\delta^{13}C$ value of POC was used in the pelagic end-member calculation in lieu of primary consumer values. The benthic end-member ($\delta^{13}C_{BP}$) was calculated using snails (corrected to the level of primary producer, as with mussels) when available, and periphyton. The use of long-lived primary consumers such as mussels and snails gives temporally integrated isotopic values for the benthic and pelagic food webs (Post, 2002). Any model results that were not between the bounds of zero and one were labelled as biologically unfeasible. The proportion of biologically unfeasible results computed for individual samples using the mixing model were, respectively, 0.35, 0.47 and 0.10 for Port Burwell, ST and Port Maitland and 0% for all other sites. All unfeasible cases had $\gamma$ values greater than one, because the $\delta^{13}C$ values of Hemimysis were similar to those of the pelagic end-member causing $\delta^{13}C_{HA} - \Delta < \delta^{13}C_{PP}$. The largest calculated pelagic contribution was 1.12 and occurred at ST. All non-feasible cases were set to one for the purposes of subsequent statistical analyses. The sensitivity of our results to this modification was tested in two ways: (i) the unfeasible cases were left unchanged and statistical tests were re-run, and (ii) unfeasible cases were removed completely and statistical tests were re-run. Sensitivity analysis indicated that altering non-feasible cases in the manner noted had no substantive effect on the patterns of statistical results obtained.

To evaluate the trophic offset, site-specific baselines were calculated using the nitrogen isotope values of primary consumers (site-specific combinations of snails, dreissenid mussels and/or herbivorous cladocerans as given in Table II). At sites where primary consumers from both the benthic and pelagic food webs were found $t$-tests were used to compare the $\delta^{15}N$ values between the two groups to determine if they were significantly different. Trophic offset was then calculated as:

$$TO = \delta^{15}N_{ORG} - \delta^{15}N_{BASE}$$  \hspace{1cm} (4)$$

where $\delta^{15}N_{ORG}$ and $\delta^{15}N_{BASE}$ are the $\delta^{15}N$ values of the sample organism and the relevant site-specific baseline.

Isotopic niche width of Hemimysis was determined using the area of the standard ellipse (SEA) of Hemimysis $\delta^{13}C$ and $\delta^{15}N$, corrected to account for small sample sizes (SEA,) as proposed in Jackson et al. (Jackson et al., 2011):

$$SEA = \pi AB$$  \hspace{1cm} (5)$$

$$SEA_C = \frac{SEA(n - 1)}{n - 2}$$  \hspace{1cm} (6)$$

where $A$ and $B$ are the semi-major and semi-minor axes of the standard ellipse, and $n$ is the sample size. The standard ellipse is the bivariate counterpart of the univariate standard deviation and is more robust to differences in sample size than the often used convex hull method of measuring isotopic niche width (Jackson et al., 2011). The isotopic niche of an organism is not the same as the trophic niche, but in general they are closely related (Jackson et al., 2011).

**Statistical analyses**

All statistical analyses were conducted using SPSS Statistics 17.0 statistical package with statistical significance set at $\alpha = 0.05$ except for niche width calculations, which were done using the Stable Isotope Analysis in R (SIAR) package in R version 2.13.2. Hemimysis $\delta^{15}N$ and $\delta^{13}C$ values, and C:N ratios, were compared among sites, using analysis of variance (ANOVA). When
Table II: Site-specific baseline values

<table>
<thead>
<tr>
<th>Site</th>
<th>Total baseline data</th>
<th>Dreissenid mussels</th>
<th>Herbivorous zooplankton</th>
<th>Snails</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (± SE) δ15N (‰)</td>
<td>N</td>
<td>Mean (± SE) δ15N (‰)</td>
<td>N</td>
</tr>
<tr>
<td>Port Dalhousie</td>
<td>9.0 ± 0.2</td>
<td>3</td>
<td>9.0 ± 0.2</td>
<td>0 n/a</td>
</tr>
<tr>
<td>Bronte</td>
<td>9.4 ± 0.3</td>
<td>18</td>
<td>9.4 ± 0.3</td>
<td>0 n/a</td>
</tr>
<tr>
<td>Waupoos</td>
<td>9.4 ± 0.2</td>
<td>3</td>
<td>9.4 ± 0.2</td>
<td>0 n/a</td>
</tr>
<tr>
<td>Port Burwell</td>
<td>10.3 ± 0.3</td>
<td>6</td>
<td>9.8 ± 0.1</td>
<td>3 n/a</td>
</tr>
<tr>
<td>Port Colborne</td>
<td>9.3 ± 0.2</td>
<td>8</td>
<td>9.2 ± 0.4</td>
<td>5 n/a</td>
</tr>
<tr>
<td>Port Dover</td>
<td>9.4 ± 0.4</td>
<td>4</td>
<td>9.0 ± 0.2</td>
<td>3 n/a</td>
</tr>
<tr>
<td>Port Maitland</td>
<td>10.5 ± 0.6</td>
<td>5</td>
<td>10.4 ± 0.7</td>
<td>4 n/a</td>
</tr>
<tr>
<td>St Timotheé</td>
<td>9.3 ± 0.2</td>
<td>15</td>
<td>9.1 ± 0.2</td>
<td>3 n/a</td>
</tr>
<tr>
<td>Q8</td>
<td>7.7 ± 0.1</td>
<td>2</td>
<td>7.7 ± 0.1</td>
<td>0 n/a</td>
</tr>
<tr>
<td>Q12</td>
<td>7.1 ± 0.1</td>
<td>3</td>
<td>7.1 ± 0.1</td>
<td>0 n/a</td>
</tr>
<tr>
<td>Q45</td>
<td>9.0</td>
<td>1</td>
<td>n/a</td>
<td>0 n/a</td>
</tr>
<tr>
<td>Q56</td>
<td>n/a</td>
<td>0</td>
<td>n/a</td>
<td>0 n/a</td>
</tr>
<tr>
<td>Q67</td>
<td>6.3</td>
<td>1</td>
<td>6.3</td>
<td>0 n/a</td>
</tr>
</tbody>
</table>

Sites excluded from trophic offset analyses are denoted with a cross (\(^\ast\)).

necessary, Welch’s ANOVA was used to account for data heteroscedasticity (Field, 2009). When significant differences were identified, post hoc analyses were conducted with a Games–Howell test to locate differences among sites (Field, 2009). At each site, δ15N and δ13C were compared between life stages (adult and juvenile) and the pelagic fraction of dietary carbon and trophic offset were compared between genders (male and female) using one-way ANOVAs. Among site differences in trophic offset and pelagic fraction of dietary carbon were also assessed using one-way ANOVAs. Nested ANOVAs were run on the trophic offset and pelagic fraction of dietary carbon with site nested within location (Lake Ontario, Lake Erie, and the St Lawrence River) to determine if differences existed among the three ecosystems.

The variability of Hemimysis isotopic niche use was examined by comparing the variances of Hemimysis δ13C and δ15N among sites using Levene’s test (Levene, 1960; Zar, 1999). The impact of baseline variability on Hemimysis food web niche space was assessed by regressing each of SEA, site-specific mean trophic offset and site-specific mean pelagic fraction of dietary carbon against the isotopic difference between the mixing model end-members used to compute the pelagic fraction of carbon in Hemimysis diets. The effect of the discrimination value on the relationship between pelagic fraction of dietary carbon and the δ13C isotopic difference between end-members was subject to sensitivity testing. The analysis was implemented by re-calculating the pelagic fraction of dietary carbon values using different carbon discrimination factors (F C = 0 and 0.8‰). We then compared the parameters of each regression using an ANCOVA with the ‘discrimination factor’ as a covariate. Finally, the relationships between the SEA (as a proxy for trophic niche variability) and Hemimysis trophic offset or the pelagic fraction of dietary carbon were determined using simple linear regression.

RESULTS

The length of Hemimysis at the lentic sites ranged from 1.83 to 8.50 mm (mean ± SE: 5.75 ± 0.13 mm) indicating the presence of both juveniles and adults in the samples. Length measurements were not recorded for individuals processed for SIA from the St Lawrence River, but Hemimysis were sorted into size classes (≤4 mm and >4 mm), with samples classed in both size classes similarly indicating the presence of multiple life stages. No differences were found in lengths between males and females (P ≥ 0.124 for all sites).

Stable isotope analyses

The site-specific mean stable isotope values for Hemimysis ranged from −28.67 to −18.32‰ for δ13C and from 9.24 to 14.28‰ for δ15N (Fig. 2) and varied significantly among sites for both δ13C and δ15N (Welch’s ANOVAs: δ13C: F(12, 40.3) = 100.4, P < 0.001, n = 176; δ15N: F(12, 38.7) = 42.6, P < 0.001, n = 176). Games–Howell post hoc tests identified multiple homogeneous groups among site-specific δ13C and δ15N values, denoted by letters in Fig. 2. Levene’s test found significant spatial variability in the within site variances of δ13C and δ15N values of Hemimysis (δ13C: F(12, 177) = 7.629, P < 0.001, n = 190; δ15N: F(12, 177) = 4.092, P < 0.001, n = 190). Males at Bronte had significantly lower (by 0.3) C:N ratios than females (F(1, 21) = 9.008, P = 0.007, n = 23), and sexes were therefore separated at Bronte to examine spatial differences among sites in two separate...
analyses: Bronte males versus Hemimysis at all other sites, and Bronte females versus Hemimysis at all other sites (Fig. 2; ANOVAs: [Hemimysis] $F_{(12, 145)} = 3.403$, $P < 0.001$, $n = 157$; [Hemimysis] $F_{(12, 142)} = 3.088$, $P < 0.001$, $n = 155$).

There were no significant differences among mean C:N ratios when compared among locations (Lake Ontario, Lake Erie and the St Lawrence River) ($P = 0.110$).

ANOVA showed no consistent significant differences in $\delta^{13}C$ and $\delta^{15}N$ between life stages among sites. Adult Hemimysis had statistically higher $\delta^{13}C$ than juveniles at Port Dalhousie (1.12‰), Bronte (0.65‰) and Q57 (0.72‰) ($P \leq 0.048$). Adults had significantly higher $\delta^{13}C$ values than juveniles at Bronte (0.50‰, $P = 0.001$), but juveniles from ST and Q12 had significantly higher $\delta^{13}C$ values than adults (1.21 and 0.72‰, respectively; $P \leq 0.039$). At all other sites, differences between adults and juveniles in $\delta^{13}C$ or $\delta^{15}N$ were not significant. C:N ratios did not differ between juveniles and adults across all sites ($P > 0.05$).

Mixing models

Site-specific values for end-members, as well as the associated standard errors, are given in Table III. Two river sites (Q8 and Q57) were excluded from mixing model analyses due to the overlap in benthic and pelagic $\delta^{13}C$ end-member values at those sites (Table III). One additional river site (Q56) had no trophic offset values (discussed further below), and was also excluded from analyses.

The estimated pelagic fraction of dietary carbon for individual Hemimysis samples ranged from 5 to 100% with an overall mean ($\pm$ SE) of 62.6 ± 2.4%. Adults relied significantly more on pelagic carbon sources than juveniles at Bronte (by 7%; $P = 0.011$) and significantly less on pelagic sources at both ST and Q12 (by 9 and 14% respectively; $P \leq 0.031$) and the juveniles at these sites were removed for among-site comparisons of the pelagic fraction of dietary carbon. At Bronte, the contribution of pelagic carbon was significantly higher for females (by 0.08, $P = 0.009$), and as a result two spatial comparisons were conducted, with one using each gender at Bronte (male and female). Similar significant differences in the fraction of diet supported by pelagic production were found among sites for both comparisons (Fig. 3; ANOVAs: [Hemimysis] $F_{(9, 108)} = 80.847$, $P < 0.001$, $n = 119$; [Hemimysis] $F_{(9, 102)} = 51.920$, $P < 0.001$, $n = 112$). Results from the nested ANOVA revealed no significant differences among the lotic and lentic ecosystems (Lake Ontario, Lake Erie and St Lawrence River) ($P = 0.136$ and $P = 0.117$ using Bronte males and females respectively), although differences between sites nested within each location were significant ($P < 0.001$). A significant positive relationship existed between the mean pelagic fraction of dietary carbon at a site, and the isotopic difference (as indicated in the methods section) between the end-members at each site (Fig. 4; $F_{(1, 8)} = 6.591$, $R^2 = 0.452$, $P = 0.033$).
When the river sites were removed from the analysis, the relationship became much stronger (Fig. 4; $F_{(1, 5)} = 13.647$, $R^2 = 0.732$, $P = 0.014$). Due to the low number of data points considered in a regression consisting of only lotic sites ($n = 3$) this analysis was not conducted. ANCOVA was used to test the sensitivity of the pelagic fraction of dietary carbon and the difference between end-member values relationship to variation in the discrimination factor and showed no significant interaction between the discrimination factor (0, 0.4 or 0.8‰) and the isotopic difference between end-member $\delta^{13}C$. Therefore, variation in discrimination value did not change the slope of individual relationships. When the interaction term was removed from the model, the relationship between mean *Hemimysis* pelagic fraction of dietary carbon and discrimination value was insignificant ($P = 0.470$), indicating that the intercept value of each relationship was similar.

### Trophic offset

Three lotic sites with low numbers of primary consumers (Q56, Q45 and Q57, with $n = 0$, 1 and 1, respectively) were removed from the trophic offset analyses due to the
low number of samples available for establishing site baselines. A common baseline was not calculated for all river sites due to significant differences among sites in primary producer $\delta^{15}$N values ($P < 0.001$ for POC), indicating the possibility of inherent differences in site isotopic baselines. No significant difference was found between benthic and pelagic primary consumers at sites where both were collected (ST, $P = 0.095$).

Trophic offset ranged from $-0.3$ to $5.4\%$ for individual *Hemimysis* with a mean ($\pm$ SE) of $2.3 \pm 0.1\%$. Adults were significantly higher in trophic offset than juveniles at both Bronte (0.65%) and Port Dalhousie (1.12%) ($P \leq 0.008$) and the juveniles at these sites were removed for among site comparisons. Males at Bronte were found to be significantly higher in trophic offset than females (by 0.66%, $P = 0.001$), and as a result two spatial comparisons were done, one using each gender at Bronte (male and female). *Hemimysis* showed significant spatial differences in trophic offset (Fig. 5; ANOVAs: $\varphi F_{9,119} = 39.965$, $P < 0.001$, $n = 129$; $\varphi F_{9,113} = 43.640$, $P < 0.001$, $n = 123$). A nested ANOVA showed no significant differences between lotic and lentic ecosystems ($P = 0.623$ and $P = 0.605$ with Bronte males and females, respectively).

**Standard ellipse area**

Site-specific SEA$_S$s ranged from 0.23 to 6.22%$^2$ (mean $\pm$ SE $= 1.59 \pm 0.50\%$) and did not differ significantly between Lake Ontario, Lake Erie and the St Lawrence River ($P = 0.161$). No relationship existed between SEA$_S$ and distance between the $\delta^{13}$C values of the end-members at a site ($P = 0.536$), mean trophic offset at a site ($P = 0.589$) or mean pelagic fraction of dietary carbon at a site ($P = 0.357$).

**DISCUSSION**

Our results indicate that: (i) *Hemimysis* relied predominantly on pelagic carbon sources at the majority of sites (7 out of 10), with few isotopic differences between adults and juvenile stages at most sites (11 of 13), (ii) the trophic offset of *Hemimysis* and its reliance on pelagic food sources were comparable at lotic and lentic sites, and (iii) the isotopic niche width of *Hemimysis* was spatially heterogeneous, varying by an order of magnitude among sites, but was unrelated to inherent isotopic variability at a site. The observed ranges in trophic offset and pelagic fraction of dietary carbon indicate that *Hemimysis* derives carbon from both benthic and water column sources, as well as at multiple trophic levels. Over all sites individual *Hemimysis* trophic offset values spanned more than 5%, and the reliance on pelagic sourced carbon by individual *Hemimysis* covered nearly the entire spectrum of possibilities. Spatial differences were significant for both trophic offset and the pelagic fraction of dietary carbon, with site-specific mean trophic offset values of *Hemimysis* spanning a >3% range, approximating one trophic level (Post, 2002). Overall, the above results support the categorization of *Hemimysis* as an opportunistic feeder with significant dietary flexibility.

**General diet and life-stage differences**

We hypothesized that *Hemimysis* would rely predominantly on pelagic production, due to its reported preference in European waters for zooplankton and phytoplankton as a food source (Ketelaars et al., 1999; Borcherding et al., 2006). Although results of the mixing model supported this hypothesis at the majority (7 out of 10) of sites included in the analysis, a marked reliance on benthic production (pelagic fraction of dietary carbon $< 0.6$) was evident at three sites (Bronte, Port Dalhousie, and Q45). This clearly showed that while *Hemimysis* may rely more heavily on water column production in most instances, it is able to make significant use of both benthic and pelagic food sources. Pelagic predators have been documented consuming *Hemimysis* in the Great Lakes basin (Lantry et al., 2012; Yuille et al., 2012), implying that at sites where *Hemimysis* is deriving a significant portion of its diet from the benthic zone, the new invader may act...
as a restored link between benthic and pelagic food webs (Ricciardi et al., 2012) possibly counteracting dreissenid-induced decoupling (Hecky et al., 2004).

The observed relationships between Hemimysis $\delta^{15}N$ and life stage at some sites (Bronte, Port Dalhousie, and Q57) suggest an ontogenetic dietary shift at these sites. Previous European studies on Hemimysis diet provided evidence of a shift from primary producers, such as phytoplankton, to zooplankton as Hemimysis grow (Borcherding et al., 2006), while isotopic values of the Great Lakes basin have not supported such a diet shift conclusion (Marty et al., 2010, 2012). The variability in relationships observed in our study may explain this discrepancy in part. Only 3 of the 13 sites examined showed significantly higher $\delta^{15}N$ values for adults compared with juveniles. Two sites (Port Dalhousie and Bronte) were in Lake Ontario, and the third (Q57) was in the St Lawrence River, indicating that ontogenetic shifts were not a product of occupying lentic or lotic environments. In addition, the significantly higher $\delta^{13}C$ values for adults compared with juveniles at Bronte suggests that at this site adults relied more heavily on benthic organisms than juveniles, since benthic food items are generally lower in $\delta^{13}C$ compared with pelagic food sources (France, 1995c). Study results thus support earlier understanding of the behavioural differences between juvenile and adult Hemimysis, with juveniles migrating into the water column earlier in the evening, and staying longer into the dawn (Brown et al., 2012; Boscarino et al., 2012), since with this migratory pattern juveniles should have greater opportunity to consume food items within the water column than adults. Two other lotic sites (ST and Q12) also showed significant differences in $\delta^{13}C$ between life stages but, unlike Bronte, adults at these sites displayed significantly lower (or more pelagic) carbon signatures than juveniles. Overall, the results reported here indicate ontogenetic shifts are not obligatory and do not always favour a shift towards pelagic predation dominated by zooplankton consumption. As such, the site-specific presence or absence of an ontogenetic dietary shift may vary seasonally as food web items shift in abundance and become more or less available to Hemimysis. Knowledge of both the ubiquity and variability of ontogenetic dietary shifts is important in understanding potential impacts of Hemimysis as dietary differences between adults and juveniles would lead to seasonal differences in potential food web impacts as the relative abundances of juveniles and adults change (Nunn and Cowx, 2012; Ricciardi et al., 2012).

Spatial differences in diet

We hypothesized that Hemimysis from lotic sites would display a significantly lower reliance on water column production as well as a lower trophic offset than those inhabiting lentic sites. Our results do not support this hypothesis, although both the pelagic fraction of dietary carbon and the trophic offset showed significant spatial variation. As these differences in trophic offset and reliance on pelagic carbon were not driven by the environment type (lentic versus lotic), we suggest that they may be due to site-specific physical and/or biological characteristics that were not considered in our study. Such factors may include: water temperature, site productivity, presence or absence of potential predators or availability of preferred prey items. The isotopic values of aquatic organisms also often display seasonal differences (Grey et al., 2001; Syvaranta and Rautio, 2010; Rautio et al., 2011) and Hemimysis are known to vary seasonally in both abundance and population structure (Brown et al., 2012; Nunn and Cowx, 2012). To target spatial differences, we attempted to minimize the impact of seasonality in this study by restricting sampling to a 7-week time period in late summer (5 weeks excluding the ST site) to minimize the amount of temporal variation in environmental characteristics, food resources and Hemimysis population structure (sex ratios, fecundity, reproductive state, life stage, length) that might influence differences among sites. Given the variability remaining among sites after controlling for obvious seasonality, further work on characterizing the temporal variability needs to be completed to better understand whether observed spatial differences are, in part, an artefact of temporal or developmental asynchrony among sites.

The positive relationship between the pelagic fraction of dietary carbon and the isotopic difference between end-members at a site may result from differing degrees of connectivity between benthic and pelagic food webs. Sites showing a small difference between benthic and pelagic end-members may experience greater mixing within the water column, and thus both greater re-suspension of benthic carbon (MacIntyre and Melack, 1995), allowing this carbon to be more easily available and consumed by pelagic Hemimysis, and less enrichment in $^{13}C$ in the benthic food web compared with the pelagic food web due to the boundary layer effect (France, 1995a). Such sites included the majority of lotic sites, where isotopic studies are known to be more difficult, including a lack of end-member differentiation (France, 1995b). We did, however, observe that sites with a large isotopic difference between end-members (>10‰) also displayed wide variation in the dietary fraction of carbon derived from the benthic zone (>20%). The observed differences in pelagic fraction of dietary carbon and trophic offset could not be attributed to differing proportions of carbon versus nitrogen within Hemimysis, as patterns seen in the site differences in mean...
Hemimysis C:N did not reflect those seen in either the pelagic fraction of dietary carbon or trophic offset. Among site differences in C:N could be related to differences in nutrient availability and food quality at sites (Hassett et al., 1997). This implies that the drivers behind the determination of dietary niche in Hemimysis are complicated, with all three dietary metrics (C:N, pelagic fraction of dietary carbon and trophic offset) varying differently among sites.

Isotopic niche variability
The SEA, of Hemimysis varied by more than an order of magnitude among sites. We expected SEA, to be positively related to the isotopic variability in the basal food web if the differences in SEA, were due to inherent isotopic variability at a site. However, no such relationship was seen in this study; with the majority of standard ellipse areas falling within a narrow range of values despite the large variation observed in basal isotopic range. Where the isotopic breadth of available resources is wide, results show Hemimysis isotope values overlapping with proportionally less of what is available than where isotopic breadth is narrow. Results thus imply that Hemimysis populations may differ in the extent of dietary specialization among study sites and may be flexible in the degree of specialization shown depending on the food webs in which they are found (Bearhop et al., 2004). Dietary flexibility is one of the key attributes of a successful invader (Weis, 2010) and may explain why Hemimysis has become so widespread outside of its native range.

Impacts and implications
Overall, it appears that Hemimysis populations in the Great Lakes basin are able to take advantage of a wide spectrum of food sources at multiple trophic levels, and derive nutrients from both water column and benthic production to varying degrees. This observed dietary flexibility likely allows Hemimysis to adapt its diet locally, which may result in more diffuse impacts on invaded food webs, with the impact of Hemimysis spread out, or ‘diffused’ over many species. As such, a larger number of species may be affected by these diffuse impacts, but in less dramatic ways (Moen, 1989). Such diffuse impacts will be more subtle and more difficult to detect and interpret than the direct predatory or competitive effects that would be expected from a more specialist organism (Robinson and Valentine, 1979). The ability of Hemimysis to rely on multiple food sources may give it the potential to alter a wider range of food webs than an invader that relies on one food source as it is able to opportunistically feed and support itself in diverse ecosystems (Weis, 2010). Impacts are likely to be especially pronounced in sites of high Hemimysis density where significant amounts of production will be required to support the high numbers of individuals (Parker et al., 1999; de Lafontaine et al., 2012).

The fact that Hemimysis food web niche differs spatially means that Hemimysis impacts on near shore food webs will be difficult to predict. Food web impacts are likely to vary spatially and will be dependent on the niche that Hemimysis occupies at a given site. Additionally, predicting impacts based on the invasion history of Hemimysis will be more complicated as a result of this dietary flexibility. Assuming little seasonal variation, sites where Hemimysis feed predominantly in the water column may undergo significant declines in zooplankton abundance, and a consequent increase in algal biomass as grazing pressure is lessened (Ricciardi et al., 2012). Conversely, in the case where an ontogenetic shift is present in a Hemimysis population, if a significant portion of the population is made up of juveniles a decrease in algal biomass may result (Ricciardi et al., 2012). This type of impact is typical of mysids in general (Ricciardi et al., 2012) and has been previously observed at sites invaded by Hemimysis in the Netherlands, with significant reductions in both zooplankton abundance and chlorophyll-a concentrations (Ketelaars et al., 1999). However, sites where Hemimysis obtains a significant portion of its diet from the benthic food web may not experience such dramatic changes in plankton abundance. At these sites, if Hemimysis is incorporated into the diets of water column consumers, decoupling between benthic and pelagic food webs may decrease as nutrients consumed by Hemimysis in the benthic zone are reintroduced into the water column food web (Marty et al., 2012). Evidence has suggested that fish in the Great Lakes basin (Lake Ontario and Lake Erie) do consume Hemimysis (Lantry et al., 2012; Yuille et al., 2012), although it is unclear to what degree fish will incorporate Hemimysis into their diet in the long term (Lantry et al., 2012). Variations in the feeding niche used by Hemimysis will make it more difficult to predict potential impacts at sites that have not yet been invaded. Indeed, such variation may make the use of invasion history as a method of predicting future impacts largely ineffective until the drivers of the variation are better understood.

CONCLUSION
In summary, this study supports the view of Hemimysis as an opportunistic omnivore with a flexible trophic niche. Hemimysis displayed significant spatial variation, although not driven by environment type (lentic versus lotic), in nearly every value measured in this study, including:
carbon and nitrogen isotope values (δ^{13}C and δ^{15}N), C:N, trophic offset and reliance on pelagic production. *Hemimysis* also showed spatial variability in the presence of an ontogenetic switch in diet between juveniles and adults. Though *Hemimysis* appears to occupy different trophic niches spatially, the variability was not driven by ecosystem type (lentic versus lotic). Variability in trophic niche is likely to result in spatial differences in food web impacts, although, due to dietary flexibility, these food web impacts may be difficult to identify or interpret. Such high levels of spatial variability in *Hemimysis* food web niche use imply that utilizing past impacts of the potential food web impacts of *Hemimysis* to predict future impacts will be difficult and may be largely ineffective. Further work, including relating isotopic values with abundances of food items at a site and an examination of temporal variability of isotopic values, would lead to a greater understanding of the drivers behind the observed trophic and feeding variability, and allow more confidence in predictions regarding the potential food web impacts of *Hemimysis*.

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