Isotopic niche use by the invasive mysid *Hemimysis anomala* in the Laurentian Great Lakes basin

by

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Author's Declaration

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

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Abstract

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 the food webs of Europe, where it invaded previous to its spread to North America. However, despite the fact that *Hemimysis* is now widespread in the Great Lakes basin, no analysis has been done on the trophic position of *Hemimysis* in North America invaded sites. This thesis used carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotopes to examine spatial and temporal patterns in *Hemimysis* trophic niche use in invaded North American sites in an attempt to broaden the knowledge base on this invader and to examine potential impacts this invader may have on the food webs of the Great Lakes.

A spatial comparison of trophic niche use by *Hemimysis* among 13 sites in Lake Erie, Lake Ontario, and the St. Lawrence River was conducted between late July and mid-September of 2011. Main sources of carbon (benthic versus pelagic production) and trophic offset, or trophic distance from basal food web items, of *Hemimysis* were quantified using *Hemimysis* $\delta^{13}C$ and $\delta^{15}N$ values. Results indicated that: 1) *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, 2) the trophic offset and reliance on pelagic food sources did not differ significantly between lotic and lentic sites, and 3) 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. Results support the notion that *Hemimysis* is an opportunistic omnivore that displays significant dietary flexibility.

To test the relative importance of key biotic and abiotic factors, taken from the literature, in driving *Hemimysis* isotopic variation, a temporal analysis was conducted at two North American sites, one in Lake Ontario and one in the St. Lawrence River, which were repeatedly sampled for *Hemimysis* and related food web items between September 2008 and January 2012. Seasonal patterns of winter enrichment – summer depletion were found in *Hemimysis* $\delta^{15}N$ in Lake Ontario, but a similar pattern was not seen in the St. Lawrence River. Multiple regression models were used to determine the importance of water temperature, *Hemimysis* C:N ratios, *Hemimysis* length, and the isotopic values of basal food web components in explaining observed variation in *Hemimysis* $\delta^{13}C$ and $\delta^{15}N$ values. Significant relationships were found between *Hemimysis* isotopic values and water temperature, but relationships with the isotopic signatures of the pelagic basal food web were weak or nonexistent. *Hemimysis* $\delta^{13}C$ values were significantly correlated with C:N ratios. Strong evidence of an ontogenetic dietary shift was found in Lake Ontario, with length showing a significant positive correlation with *Hemimysis* $\delta^{15}N$. All

together the factors included in the models explained little of the observed variation in *Hemimysis* isotopic values, with approximately 20 % of the observed variation in *Hemimysis* $\delta^{13}C$, and just under half of *Hemimysis* $\delta^{15}N$ variation, being explained by the included factors. As such, *Hemimysis* isotopic variation must be explained by factors not included in this study and may include factors such as species composition of the invaded site and availability of prey.

Overall, the results of this thesis highlight the opportunistic and flexible nature of *Hemimysis* diet, and demonstrate the need for future work to determine the main drivers of isotopic variability and trophic niche selection of *Hemimysis.* The degree of trophic flexibility seen in *Hemimysis* implies that potential food web impacts will be site specific and heavily reliant on food web dynamics and environmental characteristics of the invaded site.

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Chapter 1: General Introduction

Study Region: the Laurentian Great Lakes basin

The Laurentian Great Lakes are an area with an extremely high rate of species invasions, with over 180 invasive species reported since the beginning of European colonization (Ricciardi, 2006). The high rate of invasion is due, in part, to the high shipping traffic in the Great Lakes and St. Lawrence River, with 65 % of the invasive species recorded since the opening of the St. Lawrence Seaway likely being introduced through ballast water release (Ricciardi, 2006). Regulations on transoceanic ballast water were introduced in 1993 to minimize the introduction of new species and required ships to exchange ballast water with saline ocean water before entering the Great Lakes basin (MacIsaac et al., 2002). The hope was that high salinity environments would kill off obligate freshwater organisms within the tanks prior to ships entering freshwater (Ricciardi, 2006). As the regulations did not impact ships declaring 'no ballast on board' (NOBOB), potentially invasive organisms could survive in residual ballast tank water and sediment (Ricciardi, 2006). Additionally, due to the location of the pumps, ballast water is not completely exchanged, and salinity within the tanks may not reach oceanic levels, allowing organisms with wide salinity tolerances to survive on ships where ballast water has been exchanged at sea (Ricciardi, 2006).

A number of the invaders in the Great Lakes basin have had notable effects on the ecosystems they have invaded. A large portion of these invaders are native to the Ponto-Caspian region (Ricciardi and MacIsaac, 2000) and include the highly publicized zebra and quagga mussels (*Dreissena polymorpha* and *Dreissena bugensis*, respectively) and the benthic-feeding round goby (*Neogobius melanostomus*). Dreissenid mussels are functionally unique to the North American Great Lakes in that they are able to colonize hard surfaces in extremely high abundances (Higgins and Vander Zanden, 2010), including the surfaces of native unionid mussels (Ricciardi and Atkinson, 2004). Dreissenid mussels are also much more efficient filter feeders than native mussels (Strayer, 1999). The high filtration rate allows the dreissenid mussels to remove large amounts of nutrients from the water column. Nutrients become trapped in the benthic zone, and this has led to a decoupling between the benthic and pelagic food webs in the Great Lakes (Hecky et al., 2004). The round goby has become extremely abundant and widespread in the Great Lakes basin, and has had significant impacts on the species composition of communities in invaded sites (Corkum et al., 2004, Brush et al., 2012, Kipp and Ricciardi, 2012). Overall, food webs in the Great Lakes have been significantly impacted by invasive species, including dreissenid mussels and round gobies, both directly and indirectly, over the years (Ricciardi, 2006, Ricciardi and MacIsaac, 2000, Higgins and Vander Zanden, 2010, Kipp and Ricciardi, 2012, Strecker et al., 2011).

Hemimysis anomala, the bloody red shrimp

The most recent known invader of the Great Lakes basin is *Hemimysis anomala* (Crustacea, Malacostraca, Mysida, Mysidae, hereafter *Hemimysis*), a Ponto-Caspian mysid that is able to tolerate a wide range of salinities, from freshwater to 19 ‰ (bij de Vaate et al., 2002). *Hemimysis* is a widespread invader, found throughout Europe as well as in the North American Great Lakes (bij de Vaate et al., 2002, Dumont, 2006, Holdich et al., 2006, Kestrup and Ricciardi, 2008, Minchin and Holmes, 2008, Pothoven et al., 2007, Salemaa and Hietalahti, 1993). Based on its invasion history in Europe, and the past impacts on the Great Lakes basin by invasive Ponto-Caspian species, *Hemimysis* has the potential to significantly impact food webs in the Great Lakes basin.

Biology of *Hemimysis*

Hemimysis prefers temperatures between 9 and 20 °C, though it is able to survive much lower temperatures for extended periods of time as evidenced by its ability to overwinter in frozen lakes (Borcherding et al., 2006, Marty et al., 2008). *Hemimysis* is generally found in the warmer, shallow (0 – 9 m) nearshore ecosystems of lakes or slow-moving rivers (Borcherding et al., 2006, Brown et al., 2012, de Lafontaine et al., 2012, Marty et al., 2010, Taraborelli et al., 2012, Yuille, 2012), though instances of individuals or swarms being found in deeper waters, up to 50 m in depth, have been recorded (Borcherding et al., 2006, Marty et al., 2008, Yuille, 2012). *Hemimysis* is a photophobic species that undergoes diel migrations between hiding in the substrate or structural interstices during the day, and moving up into the water column at night (Borcherding et al., 2006, Wittmann, 2007, Boscarino et al., 2012).

Morphologically *Hemimysis* can be distinguished from the native North American mysid, *Mysis diluviana*, as well as other mysid species, by its truncated telson with spines along its entire margin and a wide, straight posterior margin (Pothoven et al., 2007). *Hemimysis* is also smaller than *M. diluviana*, ranging in Lake Ontario between 5 – 11 mm for males, and 5 – 13 mm for females (Yuille, 2012). Live *Hemimysis* may also be distinguished by the distinctive bright red colouring that resulted in the ‘bloody red shrimp’ common name. The colouring is the result of chromatophores located throughout the carapace, abdomen, and telson, and individuals are able to change the amount of colouration displayed by contracting the chromatophores (Pothoven et al., 2007). Changes in colouration intensity may be due to changes in water temperature or light intensity (Salemaa and Hietalahti, 1993), and stress appears to induce increased brightness (personal observation). Male *Hemimysis* can be distinguished by an elongated exopod on pleopod IV with more than three segments while females display a marsupium, or brood pouch (Pothoven et al., 2007).

As an opportunistic omnivore, *Hemimysis* is able to consume a wide range of food items including zooplankton, phytoplankton, detritus, small benthic invertebrates, and is occasionally cannibalistic (Borcherding et al., 2006, Dumont and Muller, 2010, Ketelaars et al., 1999, Ricciardi et al., 2012). There is some evidence from European invaded sites that *Hemimysis* undergoes an ontogenetic dietary shift, feeding heavily on phytoplankton as a juvenile and switching to a more zooplankton based diet as an adult (Borcherding et al., 2006, Ketelaars et al., 1999). North American studies using stable isotopes have found no support to date for such a dietary shift in the Great Lakes basin (Borcherding et al., 2006, Marty et al., 2010).

Invasion history of *Hemimysis*

Hemimysis were intentionally introduced from the Ponto-Caspian region, along with other macroinvertebrates, to lakes in the former USSR in the mid-twentieth century to increase food supplies for fish (bij de Vaate et al., 2002, Ketelaars et al., 1999, Arbaciauskas, 2002). By the early 1980s intentional introductions were stopped, as studies of mysid introductions began to find adverse effects on native ecosystems (Wittmann, 2007, Ketelaars et al., 1999). However, the construction of shipping canals and the resulting high levels of interconnection among river basins facilitated continued range expansion by *Hemimysis* (bij de Vaate et al., 2002, Arbaciauskas, 2002). *Hemimysis* rapidly spread throughout the Rhine-Main-Danube river system in 1997 and 1998 (Wittmann, 2007), reaching Belgium in 1999 (Verslycke et al., 2000), the UK in 2004 (Holdich et al., 2006), and France in 2005 (Dumont, 2006). *Hemimysis* was predicted to invade the Great Lakes basin by Ricciardi and Rasmussen (1998) and was first found in Lake Michigan and Lake Ontario in 2006 (Pothoven et al., 2007, Kipp and Ricciardi, 2007). Since that time *Hemimysis* has been reported in all of the Great Lakes, except for Superior, as well as the St. Lawrence River and several inland lakes in New York State (Kestrup and Ricciardi, 2008, Brown et al., 2012, de Lafontaine et al., 2012, Marty et al., 2010, Brooking et al., 2010).

Hemimysis impacts

Few studies on the food web impacts of *Hemimysis* have been conducted, but European studies of *Hemimysis* food web impacts have found some conflicting results. Some studies have shown evidence of significant reductions in the abundance and diversity of zooplankton, particularly cladocerans, following the introduction of *Hemimysis* (Ketelaars et al., 1999, Stich et al., 2009). However, Dumont and Muller (2010) found no evidence of competitive impacts by *Hemimysis* on a native hydra in an Alsatian gravel pit. The hydra fed primarily on cladocerans, and a significant decrease would be expected if cladoceran abundances had been affected by *Hemimysis* (Dumont & Muller, 2010).

Hemimysis is a novel organism in the nearshore ecosystem of the Great Lakes, with the only other mysid (*Mysis diluviana*) being found in the deeper offshore areas (Ricciardi et al., 2012). The distinctiveness of an invader within the recipient ecosystem is linked to the magnitude of its impact (Ricciardi and Atkinson, 2004). Combined with the high densities seen in some invaded sites in the Great Lakes basin (de Lafontaine et al., 2012, Taraborelli et al., 2012), this novelty could result in significant food web impacts by *Hemimysis*, as the degree of impact of an invasive species is also positively related to abundance (Ricciardi and Atkinson, 2004, Ricciardi, 2003) . Furthermore, as it moves between the benthic and pelagic zones *Hemimysis* has the potential to impact nutrient and energy flows between these two food webs (Ricciardi et al., 2012). As *Hemimysis* is reported to feed predominantly in the water column, if populations are not controlled by fish predation, *Hemimysis* could exacerbate existing patterns of food web decoupling seen between the benthic and pelagic food webs as a result of earlier invasions (Ricciardi et al., 2012). Preliminary studies of fish consumption of *Hemimysis* in the Great Lakes have found inconsistent results, with evidence of predation varying by season, site, year, and fish species (Yuille, 2012, Lantry et al., 2012, Lantry et al., 2010, Fitzsimons et al., 2012). Varied findings may be due in part to the nature of gut content analyses used, since soft-bodied organisms like *Hemimysis* may be digested and rendered unrecognizable in fish stomachs in a matter of hours (Yuille et al., 2012).

Stable Isotopes

Stable isotopes are a useful tool in ecological food web studies as they can act as tracers of energy flow within a food web (Post, 2002). In contrast to gut content analyses which provide a detailed 'snapshot' of an organism's final meal, stable isotopes provide an integrated measure of diet over time based on the assimilated nutrients derived from prey consumption (Peterson and Fry, 1987). Food web studies generally make use of two sets of stable isotopes: ^{12}C and ^{13}C, and ^{15}N and ^{14}N. The isotope pairs are expressed in δ-notation ($\delta^{13}C$ and $\delta^{15}N$) as a ratio of ratios as follows:

$$\delta = \left(\frac{R_S}{R_R} - 1\right) * 1000$$

where R_S and R_R refer to the $^{13}C/^{12}C$ or $^{15}N/^{14}N$ ratios of the sample and a reference material respectively. The stable isotope ratios of the standard reference materials used in δ-notation computations are derived from atmospheric nitrogen for nitrogen (Mariotti, 1983) and Pee Dee Belemnite for carbon (Craig, 1957).

Ecological studies often use $\delta^{13}C$ and $\delta^{15}N$ values in conjunction because they can be useful in determining an organism's trophic position and main dietary sources. A consumer's isotopic value is reflective of the isotope values of its diet, plus a trophic fractionation factor that results from the preferential retention of the heavier isotope in the assimilation of material into the consumer tissues (Post,

2002). With $\delta^{15}N$ the trophic fractionation factor is generally between 3 and 4 ‰, and can be used to determine the trophic position, or trophic level, of an organism (Post, 2002, Peterson and Fry, 1987). In comparison to $\delta^{15}N$, $\delta^{13}C$ undergoes little trophic fractionation as it moves up the food chain. Nevertheless, $\delta^{13}C$ can vary significantly among basal food sources in a food web, such as benthic versus pelagic algae (Post, 2002, Peterson and Fry, 1987, France, 1995c). As such $\delta^{13}C$ can be used to trace the main carbon pathways supporting an organism (France, 1995c).

Study objectives

The overall goal of this thesis was to better understand the potential ecological impacts of the *Hemimysis* invasion by exploring its trophic niche use, both spatially and temporally, in the Great Lakes basin of North America. At present a large portion of the scientific literature on *Hemimysis* has focused on new sightings and range expansions (e.g. bij de Vaate et al., 2002, Dumont, 2006, Holdich et al., 2006, Kestrup and Ricciardi, 2008, Pothoven et al., 2007, Salemaa and Hietalahti, 1993, Marty et al., 2010, Verslycke et al., 2000, Brooking et al., 2010). If an improved ecological understanding of the effects of the *Hemimysis* invasion in the Great Lakes basin is to be obtained, it is important to determine how *Hemimysis* invades the various environments in which it is found and how it integrates into local food webs. Specifically, it is important to compare invasions in lentic and lotic environments since such ecosystems have significant abiotic and biotic differences. To address the overall thesis goal four specific objectives were defined: (1) to examine the trophic offset and main energy pathways supporting *Hemimysis* to determine if these varied spatially within and between ecosystem types (lentic and lotic); (2) to evaluate the potential changes in energy pathways in the St. Lawrence and Great Lakes ecosystems as a result of the *Hemimysis* invasion, particularly with respect to dreissenid mussel induced benthic-pelagic decoupling; (3) to describe seasonal patterns of *Hemimysis* isotopic niche use and to identify environmental correlates that might explain any observed pattern of isotopic variation; and, (4) to use *Hemimysis* as a case study to evaluate the effectiveness of short term studies in determining the impacts of an invasive species. The first two objectives were addressed in Chapter 2 of this thesis, which described spatial variation in trophic niche use by *Hemimysis*. In Chapter 3 the final two objectives were examined through temporal analyses of *Hemimysis* isotopic niche use.

Chapter 2: Spatial variability in trophic position and food sources of *Hemimysis anomala* in lentic and lotic ecosystems within the Great Lakes basin

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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 et al., 2012, Ricciardi, 2003). 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 (Borcherding et al., 2006, Ketelaars et al., 1999, 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 60s, *Hemimysis* spread through Eastern Europe in the 1990s (Wittmann, 2007, Ketelaars et al., 1999, 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, de Lafontaine et al., 2012, Marty et al., 2010), and in some inland lakes in New York State (Brown et al., 2012, Brooking et al., 2010).

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 (Borcherding et al., 2006, Boscarino et al., 2012). Previous studies have shown that *Hemimysis* is able to feed on both benthic and pelagic food sources (Marty et al., 2010, Marty et al., 2012) and will feed on zooplankton, especially cladocerans, algae, detritus, small benthic invertebrates, and is occasionally cannibalistic (Borcherding et al., 2006, Ketelaars et al., 1999, Ricciardi et al., 2012). Significant food web impacts, including dramatic decreases in zooplankton abundances and changes in species composition, have been reported at invaded European sites (Borcherding et al., 2006, Ketelaars et al., 1999, 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 (de Lafontaine et al., 2012, Marty et al., 2010, Marty et al., 2012, Koops et al., 2010). 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, Borcherding et al., 2006). In the Great Lakes basin *Hemimysis* has been found in high abundances in both lentic and lotic sites (de Lafontaine et al., 2012, Marty et al., 2012, Koops et al., 2010, Marty et al., 2010) 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 (Higgins and Vander Zanden, 2010, Hecky et al., 2004, Strayer, 1999, Vanderploeg et al., 2001). 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, *Hemimysis* 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, the 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., 2007), but not in North America (Marty et al., 2010, Marty et al., 2012), it was hypothesized that *Hemimysis* would primarily rely on pelagic carbon sources and exhibit no significant differences in trophic offset or carbon sources between life-stages. The 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 of *Hemimysis* have remained unexplored. It was 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.

Methods

Sampling

Between August 15th and September 12th 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. 2.1; Table 2.1). 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. 2.1; de Lafontaine et al., 2012). One additional site (St. Timothée, ST), which was sampled July 29th 2011, was located upstream of the Montreal sites in the regional Parc des Iles (Fig. 2.1; Table 2.1) and was considered as 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* (de Lafontaine et al., 2012, Walsh et al., 2010). 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 seconds, and then raised to the surface at a steady rate of approximately 1 $m \cdot s^{-1}$. Samples were transferred to 1 L containers filled with lake water and stored at ~4 °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 hours 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 picked. All samples were sorted within 24 hours 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) for stable isotope analysis (SIA).

Stable Isotope Analysis

At 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, ten adult *Hemimysis* were sexed, measured (mm), and processed individually for SIA. Gender was determined through examination of the 4th 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 (mm) 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 triplicates. Sorted samples were kept frozen at -20 °C until SIA.

Samples for SIA were first dried at 50 °C for 24 to 72 hours and then homogenized with a mortar and pestle. Sample material from plant and animal based samples were weighed to 600 or 300 µg, respectively, using a Mettler-Toledo micro-balance (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 (‰), with respect to the standard international reference materials of atmospheric nitrogen for nitrogen (Mariotti, 1983) and Pee Dee Belemnite for carbon (Craig, 1957), as follows:

$$\delta = \left(\frac{R_S}{R_R} - 1\right) * 1000$$

where R = $^{13}C/^{12}C$ or $^{15}N/^{14}N$ of the sample (R_S) and reference (R_R) material (Verardo et al., 1990). 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 0.14 ± 0.03 ‰ for $\delta^{15}N$ (n = 24). The C:N ratios were calculated from % C and % N values provided by the mass spectrometer.

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 (2002) as:

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

$$\Delta = F_C[(TO/F_N) + 1]$$

re-arranged to extract γ such that:

$$\gamma = (\delta^{13}C_{HA} - \Delta - \delta^{13}C_{BP})/(\delta^{13}C_{PP} - \delta^{13}C_{BP})$$

where Δ 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 fractionation 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 (2002)), TO is the trophic offset of an organism, described below, γ 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 fractionation 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 γ values greater than one, because the $\delta^{13}C$ values of *Hemimysis* were similar to that 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 the results to this modification was tested in two ways: 1) the unfeasible cases were left unchanged and statistical tests were re-run, and 2) 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 2.2). 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:

$$TO = \delta^{15}N_{ORG} - \delta^{15}N_{BASE}$$

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_C) as proposed in Jackson et al. (2011):

$$SEA = \pi AB$$

$$SEA_C = SEA(n-1)/(n-2)$$

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 $\alpha = .05$ 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 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 $\delta^{15}N$ and $\delta^{13}C$ 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* $\delta^{13}C$ and $\delta^{15}N$ 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_C, 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 fractionation value on the relationship between pelagic fraction of dietary carbon and the $\delta^{13}C$ 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 fractionation factors (F_C = 0 and 0.8 ‰). The parameters of each regression were then compared using an ANCOVA with the 'fractionation factor' as a covariable. Finally, the relationships between the SEA_C (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 1.85 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 length between males and females ($p \geq 0.124$ for all sites).

Stable isotope analysis

The site-specific mean stable isotope values for *Hemimysis* ranged from -28.67 to -18.32 ‰ for $\delta^{13}C$ and from 9.24 to 14.28 ‰ for $\delta^{15}N$ (Fig. 2.2) and varied significantly among sites for both $\delta^{13}C$ and $\delta^{15}N$ (Welch's ANOVAs: $\delta^{13}C$ $F_{(12, 40.3)} = 100.4$, $p < 0.001$, n = 176; $\delta^{15}N$ $F_{(12, 38.7)} = 42.6$, $p < 0.001$, n = 176). Games-Howell post-hoc tests identified multiple homogeneous groups among site-specific $\delta^{13}C$ and $\delta^{15}N$ values, denoted by letters in Fig. 2.2. Levene's test found significant spatial variability in the within site variances of $\delta^{13}C$ and $\delta^{15}N$ values of *Hemimysis* ($\delta^{13}C$: $F_{(12,177)} = 7.629$, $p < 0.001$, n=190; $\delta^{15}N$: $F_{(12,177)} = 4.092$,$p < 0.001$, n=190). *Hemimysis* males at Bronte had significantly lower (by 0.3) C:N values 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. 2.2; ANOVAs: ♂ $F_{(12, 145)} = 3.403$, $p < 0.001$,

n=157; ♀ $F_{(12, 142)} = 3.088$, $p < 0.001$, 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-stage among sites. Adult *Hemimysis* had statistically higher $\delta^{15}N$ than juveniles at Port Dalhousie (1.12 ‰), Bronte (0.65 ‰), and Q57 (0.72 ‰) ($p \leq 0.048$). Adult *Hemimysis* 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 2.3. 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 2.3). 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 (± 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. 2.3; ANOVAs: ♂ $F_{(9, 108)} = 80.847$, $p < 0.001$, n = 119; ♀ $F_{(9, 102)} = 51.920$, $p < 0.001$, 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. 2.4; $F_{(1, 8)} = 6.591$, $R^2 = 0.452$ $p = 0.033$). When the river sites were removed from the analysis the relationship became much stronger (Fig. 2.4; $F_{(1, 5)} = 13.647$, $R^2 = 0.732$, $p = .014$). Due to the low number of data points considered in a regression consisting of only lotic sites (n = 3) this analysis was not

conducted. An 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 fractionation factor and showed no significant interaction between the fractionation factor (0, 0.4, or 0.8 ‰) and the isotopic difference between end-member $\delta^{13}C$. Therefore variation in fractionation 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 fractionation 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 (± SE) of 2.3 ± 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. 2.5; ANOVAs: ♂ $F_{(9, 119)} = 39.965$, $p < .001$, $n = 129$; ♀ $F_{(9,113)} = 43.640$, $p < .001$, $n = 123$). 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_Cs ranged from 0.23 to 6.22 ‰^{2} (mean ± S.E. = 1.59 ± 0.50 ‰^{2}) and did not differ significantly among Lake Ontario, Lake Erie, and the St. Lawrence River ($p = 0.161$). The majority of sites included in this analysis (10 out of 12) had SEA_C values < 2 ‰^{2}. No relationship existed between SEA_C 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: 1) *Hemimysis* relied predominantly on pelagic carbon sources at the majority of sites (7 out of 10) , with few isotopic differences between adult and juvenile stages at most sites (11 of 13), 2) the trophic offset of *Hemimysis* and its reliance on pelagic food sources were comparable at lotic and lentic sites, and 3) 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 for zooplankton and phytoplankton as a food source in European waters (Borcherding et al., 2006, Ketelaars et al., 1999). 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 *Hemimysis* in the Great Lakes basin have not supported such a diet shift conclusion (Marty et al., 2010, Marty et al., 2012). The variability in relationships observed in the study may explain this discrepancy in part. Only three of the 13 sites examined showed significantly higher $\delta^{15}N$ values for adults compared to 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 *Hemimysis* than for 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 to 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 compulsory 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 (Ricciardi et al., 2012, Nunn and Cowx, 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. The 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), it suggests that they may be due to site-specific physical and/or biological characteristics that were not considered in this 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 (Syvaranta and Rautio, 2010, Rautio et al., 2011, Grey et al., 2001) 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 the impact of seasonality was minimized in this study by restricting sampling to a seven week time period in late summer (five 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 artifact 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 to 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 have more difficulties, including a lack of end-member differentiation (France, 1995b). However, it was observed 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_C of *Hemimysis* varied by more than an order of magnitude among sites. It was expected that SEA_C would be positively related to the isotopic variability in the basal food web if the differences in SEA_C 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 flexibility of diet 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 (de Lafontaine et al., 2012, Parker et al., 1999).

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 abundances, 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 mysid species 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 abundances 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 abundances. 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 in 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.

In summary, this study supports the view of *Hemimysis* as an opportunistic omnivore with a flexible trophic niche. *Hemimysis* displayed significant spatial variation, though not driven by environment type (lentic versus lotic), in nearly every value measured in this study, including: carbon and nitrogen isotope values ($\delta^{13}C$ and $\delta^{15}N$), 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 the dietary flexibility of *Hemimysis*, 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 *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*.

Table 2.1: Latitude and longitudes for sampling sites

Location	Site	Latitude, Longitude
St. Lawrence River	Q8	N45 30.328, W73 33.004
	Q12	N45 30.008, W73 33.142
	Q45	N45 32.726, W73 31.895
	Q56	N45 33.521, W73 31.163
	Q57	N45 33.527, W73 31.324
	St Timothée	N45 17.400, W74 2.467
Lake Ontario	Waupoos	N44 0.043, W76 59.508
	Bronte	N43 23.659, W79 42.456
	Port Dalhousie	N43 12.570, W79 15.806
Lake Erie	Port Burwell	N42 38.387, W80 48.429
	Port Dover	N42 46.876, W80 12.099
	Port Maitland	N42 51.292, W79 34.730
	Port Colborne	N42 52.867, W79 14.955

Table 2.2: Site- and taxa-specific data used to calculate baselines. Sites excluded from trophic offset analyses are denoted with a cross ($^{+}$).

Site	Total baseline data		Dreissenid mussels		Herbivorous zooplankton		Snails	
	Mean (± S. E.) $\delta^{15}N$ (‰)	N	Mean (± S. E.) $\delta^{15}N$ (‰)	N	Mean (± S. E.) $\delta^{15}N$ (‰)	N	Mean (± S. E.) $\delta^{15}N$ (‰)	N
Port Dalhousie	9.0 ± 0.2	3	9.0 ± 0.2	3	n/a	0	n/a	0
Bronte	9.4 ± 0.3	18	9.4 ± 0.3	14	9.1 ± 0.5	4	n/a	0
Waupoos	9.4 ± 0.2	3	9.4 ± 0.2	3	n/a	0	n/a	0
Port Burwell	10.3 ± 0.3	6	9.8 ± 0.1	3	10.9 ±0.1	3	n/a	0
Port Colborne	9.3 ± 0.2	8	9.2 ± 0.4	5	9.4 ± 0.1	3	n/a	0
Port Dover	9.4 ± 0.4	4	9.0 ± 0.2	3	10.6	1	n/a	0
Port Maitland	10.5 ± 0.6	5	10.4 ± 0.7	4	10.8	1	n/a	0
St Timothée	9.3 ± 0.2	15	9.1 ± 0.2	3	10.2 ± 0.1	3	9.1 ± 0.2	9
Q8	7.7 ± 0.1	2	7.7 ± 0.1	2	n/a	0	n/a	0
Q12	7.1 ± 0.1	3	7.1 ± 0.1	3	n/a	0	n/a	0
Q45^{+}	9.0	1	n/a	0	n/a	0	9	1
Q56^{+}	n/a	0	n/a	0	n/a	0	n/a	0
Q57^{+}	6.3	1	6.3	1	n/a	0	n/a	0

Table 2.3: Site-specific end-member values. Sites excluded from mixing model analyses are denoted with an asterisk (*). End-member values are presented as a mean value as described in the methods ± standard error . Samples are categorized as PER (periphyton), SN (snail), M (dreissenid mussel), Z (herbivorous zooplankton), or POC (particulate organic carbon).

Site	Benthic end-member			Pelagic end-member			Difference
	$\delta^{13}C$ (‰)	Sample size	Sample type (n)	$\delta^{13}C$ (‰)	Sample size	Sample type (n)	(‰)
Port Dalhousie	-16.4 ± 0.4	4	PER (4)	-23.1 ± 0.2	3	M (3)	6.7
Bronte	-20.7 ± 0.2	2	PER (2)	-26.4 ± 0.5	18	M (14), Z (4)	5.7
Waupoos	-8.2 ± 0.5	3	PER (3)	-22.8 ± 0.5	3	M (3)	14.6
Port Burwell	-14.3 ± 0.8	3	PER (3)	-29.2 ± 1.2	6	M (3), Z (3)	15.0
Port Colborne	-15.7	1	PER (1)	-26.2 ± 0.5	8	M (5), Z (3)	10.5
Port Dover	-12.6	1	PER (1)	-24.0 ± 0.7	4	M (3), Z (1)	11.4
Port Maitland	-18.6 ± 0.6	7	PER (7)	-31.1 ± 1.5	5	M (4), Z (1)	12.5
Q12	-18.8 ± 0.2	6	PER (6)	-24.7 ± 0.9	3	M (3)	5.9
ST	-16.2 ± 1.1	12	SN (9), PER (3)	-24.2 ± 0.4	6	M (3), Z (3)	8.0
Q8 *	-21.0 ± 0.9	6	PER (6)	-21.4 ± 0.5	5	M (2), POC (3)	0.4
Q45	-19.1 ± 0.5	7	SN (1), PER (6)	-22.4 ± 0.1	3	POC (3)	3.3
Q56*	-20.2 ± 0.1	6	PER (6)	-23.9 ± 0.2	3	POC (3)	3.3
Q57 *	-19.6 ± 0.7	5	PER (5)	-22.4 ± 0.1	4	M (1), POC (3)	2.8

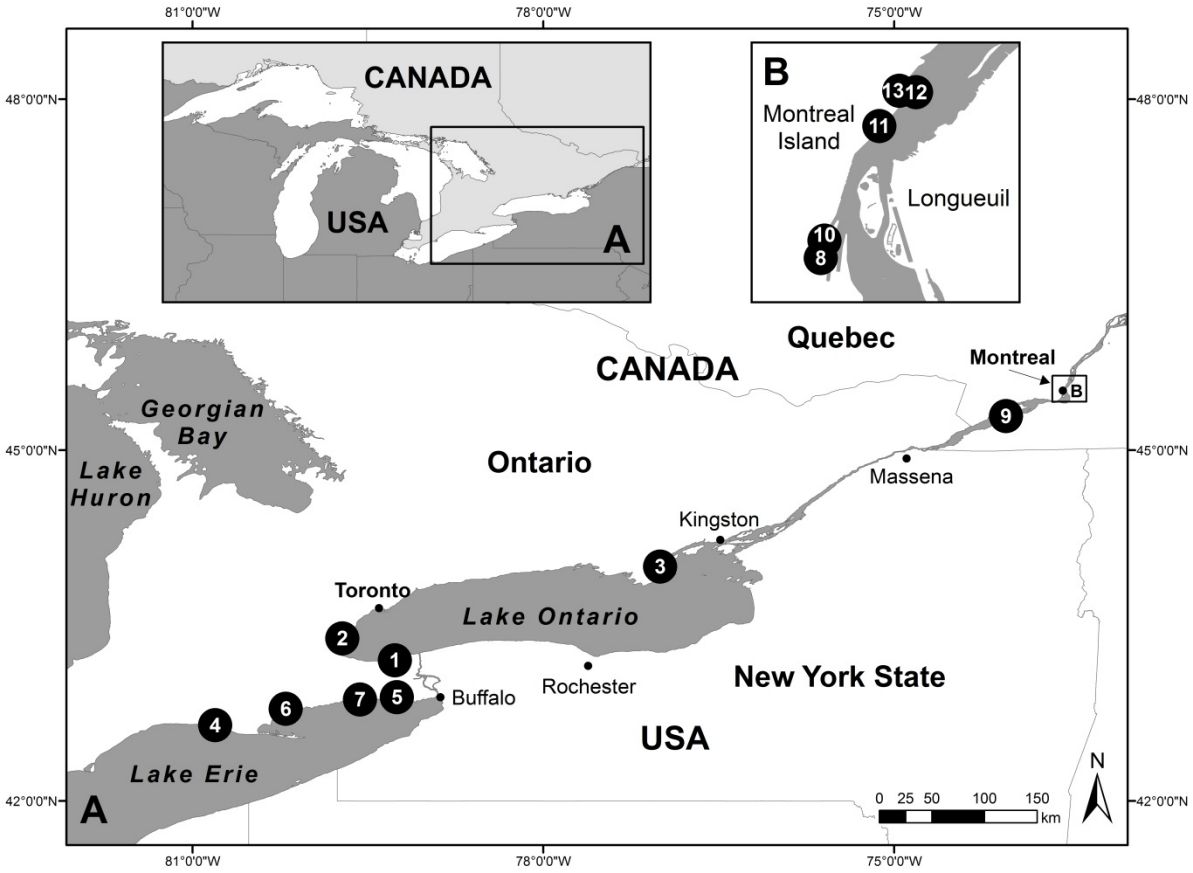

Figure 2.1: Location of sampling sites. Sites are labeled as follows: 1) Port Dalhousie, 2) Bronte, 3) Waupoos, 4) Port Burwell, 5) Port Colborne, 6) Port Dover, 7) Port Maitland, 8) Q12, 9) St Timothée, 10) Q8, 11) Q45, 12) Q56, and 13) Q57.

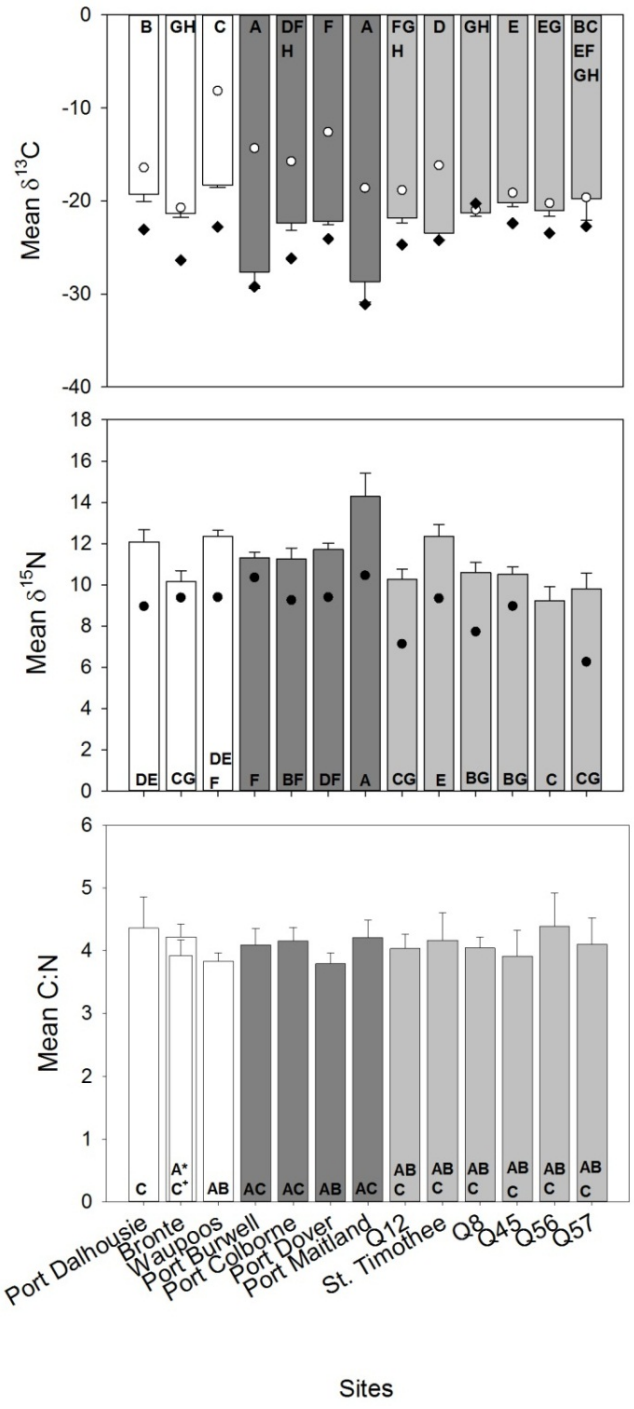

Figure 2.2: Top: Mean (± 1 S.D.) $\delta^{13}C$ of *Hemimysis* by sampling site. Benthic and pelagic end-members are also shown as a ○ or ♦, respectively, for each site. Middle: Mean (± 1 S.D.) $\delta^{15}N$ of *Hemimysis* by sampling site. The baseline used in determining trophic offset is displayed as a ● for each site. Bottom: Mean (± 1 S.D.) C:N ratios by site. Values at Bronte are shown for both female (+) and male (*) *Hemimysis* because significant differences existed between genders. Sites sharing a letter are not significantly different from one another at the 0.05 level of significance. Open bars reflect Lake Ontario sites, dark grey bars Lake Erie sites, and light grey bars St. Lawrence River sites.

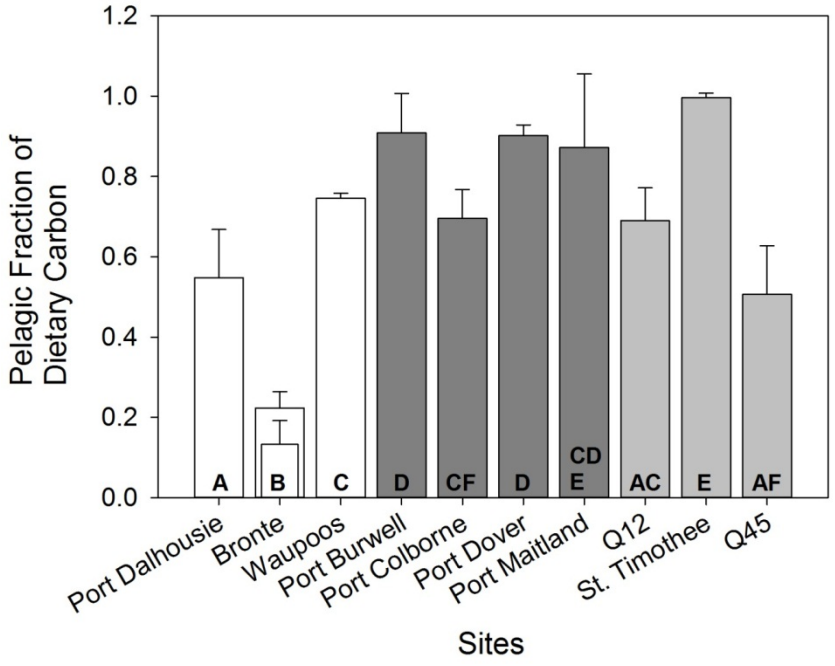

Figure 2.3: The mean fraction of dietary carbon of *Hemimysis* obtained from pelagic sources. Sites with differing letters are significantly different from one another at the 0.05 level of significance. The mean values and standard deviations are shown for both female (higher mean value) and male (lower mean value) *Hemimysis* at Bronte, where significant differences existed between genders. Open bars reflect Lake Ontario sites, dark grey bars Lake Erie sites, and light grey bars St. Lawrence River sites.

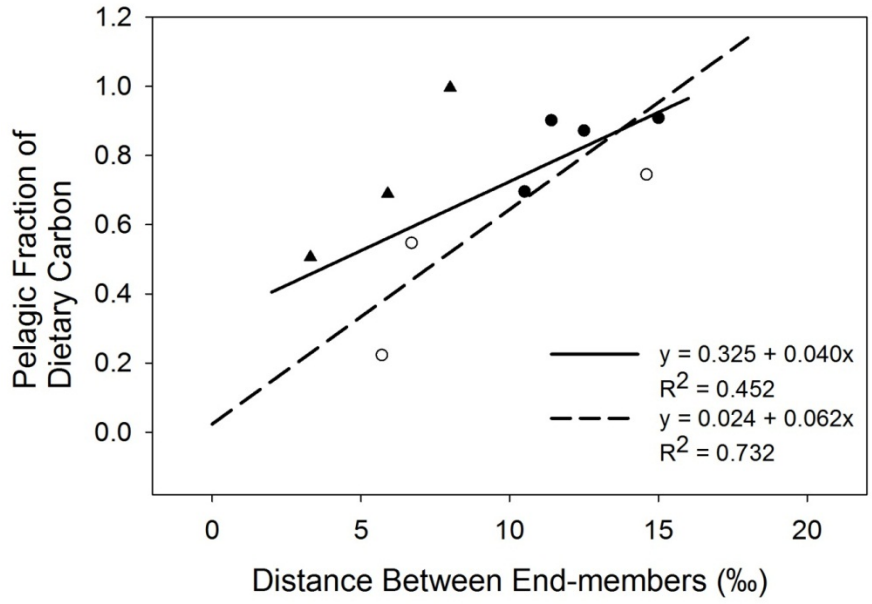

Figure 2.4: Mean pelagic fraction of dietary carbon at a site versus the difference in $\delta^{13}C$ values between end-members (‰). The different locations are denoted by open circles (○) for Lake Ontario, closed circles (●) for Lake Erie, and triangles (▲) for the St. Lawrence River. The regression lines for all sites and for only lentic sites are shown with a solid line and a dashed line respectively.

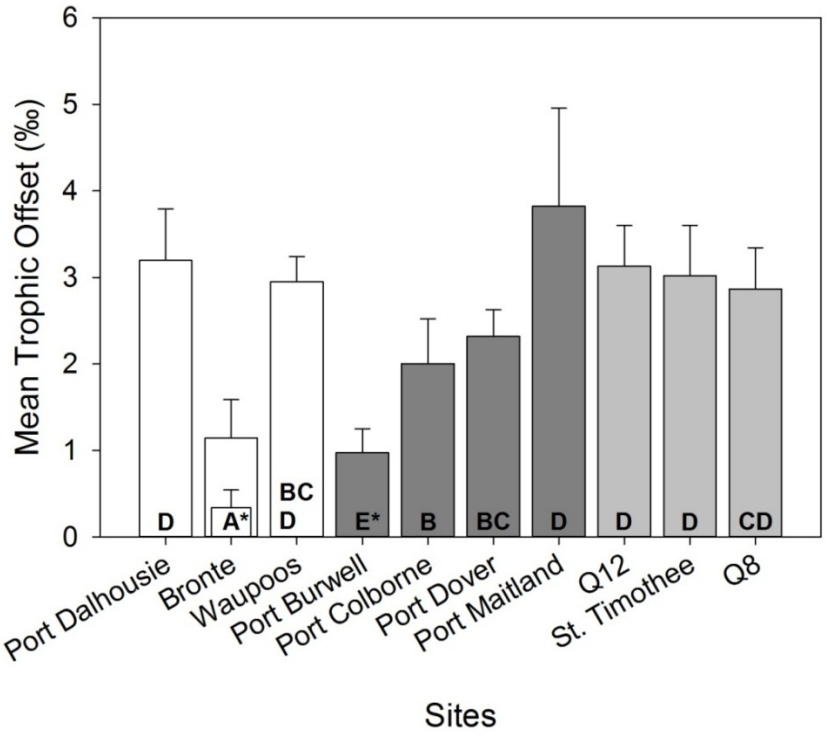

Figure 2.5: Mean (± 1 S.D.) trophic offset of *Hemimysis* by site. Sites sharing a letter are not significantly different at the 0.05 level of significance. Sites connected with an asterisk (*) are significantly different when female *Hemimysis* from Bronte were used, and not significantly different when male *Hemimysis* from Bronte were used. The mean values and standard deviations are shown for both male (higher mean value) and female (lower mean value) *Hemimysis* at Bronte, where significant differences existed between genders. Open bars reflect Lake Ontario sites, dark grey bars Lake Erie sites, and light grey bars St. Lawrence River sites.

Chapter 3: Temporal drivers of isotopic niche variability of *Hemimysis anomala* in the Great Lakes basin

Introduction

Invasive species are a well-known stressor on aquatic ecosystems worldwide, yet predicting invasion success and impacts on native food webs remain difficult tasks (Ricciardi, 2003). One reason for this is that the impact of an invader is a function of not only its biology but also of the environmental characteristics and food web dynamics of the invaded site (Ricciardi, 2003, Kulhanek et al., 2011). Many successful invaders have wide environmental tolerances and broad diets (Ricciardi and Rasmussen, 1998), are able to make use of a large range of environmental conditions and can occupy different trophic niches depending on availability (Weis, 2010). As such, impacts may differ over a range of invaded sites. Furthermore, food web structure at invaded sites is not necessarily static throughout the year (Zhang et al., 2012), and this, coupled with seasonal changes in environmental characteristics, implies that the impact of an invader may also vary seasonally. Such temporal variability points to another difficulty with predicting invader impacts. The majority of studies on invasive species are conducted over a short temporal scale, typically less than a year (Strayer et al., 2006). However, the process of integrating into an invaded food web is a dynamic one which may involve shifts in behaviour or resource use occurring over longer periods of time (Strayer et al., 2006, Kondoh, 2006). Recent invaders often display wider niches when they first invade sites with few interspecific competitors in a phenomenon known as 'ecological release' (Bolnick et al., 2010). Additionally, in the early stages of an invasion the interactions between native species and the invader are often driven by a lack of 'contact experience' between the two sets of organisms (Kondoh, 2006). Over time, and with repeated exposure to an invader, native species often begin to recognize new organisms as predator, prey, or competitor, and adapt their foraging or defensive behaviour as necessary to co-exist with the invader (Kondoh, 2006, Carlsson et al., 2009). As native prey adapt to better avoid the new predator and native predators become better able to control invader abundances, the trophic niche of the invader may become more constrained and its overall functional importance within the food web may change.

After initial reports of an invasion, researchers typically attempt to quickly quantify 'potential impacts' of the recent invader and often within the confines of one season when it is easiest to sample (Strayer et al., 2006). Such studies can give conflicting reports if completed at different times of the year or at different stages of the invasion (Strayer et al., 2006). A prime example of the difficulty associated with impact prediction is provided by the recent invader in the Great Lakes basin *Hemimysis anomala* (hereafter *Hemimysis*). *Hemimysis* is a generalist feeder with wide salinity and temperature tolerances that originated in the Ponto-Caspian region and was first found in the Great Lakes basin in 2006 (Pothoven et al., 2007, Borcherding et al., 2006, Ricciardi et al., 2012). *Hemimysis* is a voracious omnivore (Borcherding et al., 2006), frequently occurring in high abundances at invaded sites (de Lafontaine et al.,

2012, Taraborelli et al., 2012), that has been predicted to have potentially significant impacts on native food webs (Ricciardi et al., 2012, Marty et al., 2012).

Past studies of *Hemimysis* diet have shown differing results in terms of zooplankton prey preferences (Borcherding et al., 2006, Ketelaars et al., 1999). For example, Borcherding et al. (2006) found that copepods were the dominant zooplankton prey of *Hemimysis* in a gravel-pit lake in Germany, whereas Ketelaars et al. (1999) noted that cladocerans made up the bulk of gut contents in *Hemimysis* captured in the Netherlands. Differences in temporal sampling (winter versus autumn) may explain the discrepancy, although as a dietary generalist that consumes phytoplankton, algae, zooplankton, small benthic invertebrates, and detritus, the relative reliance of *Hemimysis* on any single food resource is likely influenced by availability (Borcherding et al., 2006, Marty et al., 2010, Ketelaars et al., 1999). *Hemimysis* has also been reported to undergo ontogenetic dietary shifts that imply differences in sample age and or size composition may further accentuate differences in study conclusions (Borcherding et al., 2006, Ketelaars et al., 1999, Borcherding et al., 2007, Ives et al., in press).

The goal of this study was to examine the temporal patterns in isotopic niche use shown by *Hemimysis* in two sites in Lake Ontario and the St. Lawrence River. Previous studies of *Hemimysis* have found high spatial variability in its isotopic niche use ($\delta^{13}C$ and $\delta^{15}N$) in the Great Lakes basin (Ives et al., in press), but no North American study has looked at the seasonal patterns in *Hemimysis* diet. Gut content analyses have shown potential seasonal variation in *Hemimysis* diet at invaded European sites (Borcherding et al., 2006, Ketelaars et al., 1999), and similar variability might be expected in North American sites, as indicated by results obtained for the native mysid, *Mysis diluviana*, of the Great Lakes basin (Johannsson et al., 2001). Accordingly, the first objective of this study was to quantify the similarity of seasonal isotopic patterns between years and sites in the Great Lakes basin. Specifically, data was collected between September 2008 and January 2012 to test the hypothesis that the seasonal patterns in *Hemimysis* isotopic values are consistent among years but differ among two sites, one lentic and one lotic, as a result of documented spatial variability previously reported in the Great Lakes basin (Marty et al., 2012, Ives et al., in press).

Differences in seasonal patterns among sites raise obvious questions concerning the environmental factors that may contribute to the observed variability in stable isotope values. Water temperature (Power et al., 2003), baseline isotopic values (Matthews and Mazumder, 2007), length-related dietary shifts (Borcherding et al., 2006) and variations in organism C:N ratios (Matthews and Mazumder, 2005) have all been implicated as contributory causes. Accordingly, the second objective of this study was to determine the statistical significance of the above factors as explanations of the observed

within site temporal variability in *Hemimysis* $\delta^{13}C$ and $\delta^{15}N$ stable isotope values. It was hypothesized that the majority of observed variation in *Hemimysis* isotopic values would be explained by water temperature, baseline isotopic values, *Hemimysis* length, and *Hemimysis* C:N.

Finally, given the relatively recent invasion of *Hemimysis* into North America, *Hemimysis* may still be in the process of integrating into the invaded food webs of the Great Lakes basin. Based on the dynamics of invasion and the shifts in behaviour or resource use that occur as an invader integrates into a food web (Strayer et al., 2006, Kondoh, 2006), the time series of sample data was used to test the hypothesis that *Hemimysis* isotopic niche would decrease in width and vary in position in isotopic space over time as native predators and prey became more experienced with *Hemimysis* and constrain and modify its realized trophic niche.

Methods

Site selection

To address the hypotheses outlined above, one site with known high densities of *Hemimysis* (de Lafontaine et al., 2012, Taraborelli et al., 2012) was sampled during the ice-free period in each of Lake Ontario and the St. Lawrence River. The Lake Ontario site (Bronte; 43° 23’ 34.15” N, 79° 42’ 20.95” W) was located on the northern shore of the western end of Lake Ontario and was sampled periodically between September and December of 2008 (n = 3), approximately monthly between May and December in 2009 (n = 10), 2010 (n = 7), and 2011 (n = 9), as well as once in January 2012 (Table 3.1), as the constraints of seasonal (e.g. winter) sampling allowed. The St. Lawrence River site (ECL01; 45° 29’ 56.94” N, 73° 33’ 8.36” W) was located in the old harbour portion of the Port of Montreal and was sampled approximately biweekly from mid-June to mid-November 2010 (n = 8). Both sites were located in highly urbanized areas with permanent manmade structures, such as piers, as a main feature of the site and are described further in Table 3.1.

Sampling

All sampling was conducted after dusk following standardized methods optimized for *Hemimysis* (Walsh 2010, de Lafontaine 2012). Briefly, sampling was conducted directly off available piers (Bronte), or from a boat located next to a cement wall (ECL01), using multiple vertical tows with a 0.75 m diameter, 400 µm mesh plankton net dropped to the substrate, allowed to rest for a minimum of 30 seconds, and then raised to the surface at a steady rate of approximately 1 $m \cdot s^{-1}$. Samples were transferred into 1 L containers filled with lake water and stored at ~4 °C overnight. All samples were maintained alive for approximately 12 hours to allow for gut evacuation (e.g. Marty et al., 2012), after which individuals were removed and sorted for use in stable isotope analysis (SIA).

Temperature profiles were recorded using a YSI™ 6600 multiprobe and an average temperature was calculated as the mean of all temperatures recorded in the water column at the time of sampling. An integrated volume of water (1 L) was collected in triplicate for analysis of particulate organic matter (POM). Water was filtered onto pre-weighed, pre-combusted quartz filters (Whatman QMA, 47 mm) which were kept frozen at -20 °C until prepared for use in SIA.

Stable Isotope Analysis

At Bronte, adult *Hemimysis* were sexed, measured (mm), and processed as individual samples. Juveniles were measured (mm) and grouped into samples of approximately five individuals to ensure enough material was available for SIA. At ECL01, *Hemimysis* were grouped into duplicate samples of small (≤ 4 mm) or large (> 4 mm) individuals, for a total of four samples per sampling day. Samples were kept frozen at -20 °C until prepared for use in SIA.

Samples for SIA were dried at 50 °C for 24 to 72 hours and homogenized with either a mortar and pestle or a Retsch MM 301 ball mill grinder (Retsch GmbH, Haan, Germany). Material was weighed using a Mettler Ultra micro-balance (Mettler-Toledo GmbH, Greifensee, Switzerland) to 600 and 300 µm, for plant and animal based samples respectively, for SIA. 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 (CHNS-O EA1108, Carlo Erba, Milan, Italy), with the exception of POM from 2011, which were processed on an identical mass spectrometer coupled to a Carlo Erba elemental analyzer (NA 1500 NCS, Carlo Erba, Milan, Italy) at Isotope Tracer Technologies Inc., Waterloo, Ontario. Results are expressed in standard δ notation as parts per thousand (‰) where $\delta=[(R_{sample}/R_{reference}) - 1] \times 1000$, and $R=^{13}C/^{12}C$ or $^{15}N/^{14}N$ (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 given sample, and was, on average (± SE), 0.14 ± 0.02 ‰ for $\delta^{13}C$ and 0.20 ± 0.03 ‰ for $\delta^{15}N$ (n = 43).

Isotopic niche width of *Hemimysis* was estimated for each sampling expedition and each year 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_C) as proposed in Jackson et al. (2011):

$$SEA = \pi AB$$

$$SEA_C = SEA(n-1)/(n-2)$$

where A and B are the semi-major and semi-minor axes of the standard ellipse, and n is the sample size in question. The standard ellipse is the bivariate counterpart of the univariate standard deviation and is more robust to outliers and differences in sample size than the often used convex hull method of measuring isotopic niche width (Jackson, et al., 2011).

Statistical analyses

All statistical analyses were conducted using the SPSS Statistics 17.0 statistical package with statistical significance set at $\alpha = 0.05$, except for niche width calculations which were completed using the Stable Isotope Analysis in R (SIAR) package in R version 2.13.2. To examine seasonal patterns *Hemimysis* isotopic values were grouped into seasons (Spring: May, June; Summer: July, August, September; Fall: October, November, December). At ECL01 *Hemimysis* isotopic values were compared among seasons using a one-way analysis of variance (ANOVA), and at Bronte *Hemimysis* isotopic values were compared among years and seasons using a two-way ANOVA. When necessary, Welch's ANOVA was used to account for data heteroscedasticity (Field, 2009). If the interaction between year and season was significant, indicating differing seasonal variation among years, year specific ANOVAs were run to compare *Hemimysis* isotopic values among seasons within a year.

Multiple regression analyses were used to evaluate the importance of key biotic and abiotic factors that may influence *Hemimysis* isotopic values. As a guard against possible spurious correlation between the variables used in the multiple regression analyses, biological rationalizations were used to select an *a priori* set of explanatory variables for possible inclusion in regression models of the form:

$$B_t = a_0 + a_1X_{1t} + a_2X_{2t} + ... + a_kX_{kt} + e_t$$

where B_t defined the dependent data series (e.g., Hemimysis $\delta^{13}C$ and $\delta^{15}N$), a_0, a_1 ... a_k were the estimated regression parameters, X_{1t}, X_{2t} ... X_{kt} were the included explanatory environmental variables and e_t was the normally and independently distributed regression error term. Variables included in the regression models were selected using forward selection step-wise regression techniques (Draper and Smith, 1981, Clark and Dunn, 1987), with coefficient significance judged using the Student's two-tailed *t*-test. A significance threshold of $\alpha = 0.05$ was used to define the required variable *F*-to-enter and *F*-to-remove values (Draper and Smith, 1981) and sensitivity tests on variable selection were completed by lowering the *F*-to-enter and exit criteria in the step-wise regression procedure (Draper and Smith, 1981). A correlation matrix was used to screen for interactions between factors, and interaction terms were only included in the multiple regression models if a significant correlation existed.

As noted above the *a priori* set of possible explanatory variables considered included: water temperature, *Hemimysis* C:N ratios, and POM $\delta^{13}C$ values for *Hemimysis* $\delta^{13}C$ and: water temperature, *Hemimysis* length, and POM $\delta^{15}N$ values for *Hemimysis* $\delta^{15}N$, respectively. Water temperature has been linked to predictable changes in the isotopic fractionation values (Δ_C and Δ_N for $\delta^{13}C$ and $\delta^{15}N$ respectively) used in aquatic ecology as a result of temperature-mediated changes in organism physiological rates, with field and laboratory data showing a general trend toward $\delta^{13}C$ enrichment and $\delta^{15}N$ depletion with increasing temperature in marine and lacustrine zooplankton (Power et al., 2003, Barnes et al., 2007). *Hemimysis* C:N ratios, as an indicator of body composition, which varies as a result of changes in reproductive and/or average nutritional status (Arts, 1999), have been shown to be significantly negatively related to $\delta^{13}C$ values in zooplankton (Matthews and Mazumder, 2005, Barnes et al., 2007). *Hemimysis* length was further considered to account for any ontogenetic dietary shifts. Such shifts would be evidenced by a significant positive relationship between *Hemimysis* $\delta^{15}N$ and length, as has been observed for *Hemimysis* that switch from phytoplankton to zooplankton dominated diets as they transition from juveniles to adults (Borcherding et al., 2006, Marty et al., 2012). POM isotopic values were included in both regressions to account for seasonal variation in the pelagic baseline and have been found to be positively related to both the $\delta^{13}C$ and $\delta^{15}N$ values of lacustrine zooplankton (Matthews and Mazumder, 2007, Matthews and Mazumder, 2005, Marty and Planas, 2008). Where significant statistical trends existed between a variable and calendar day, the residuals from the trend regression were used to form a detrended data series for further analysis following procedures outlined in (Abraham and Ledolter, 1983). Since POM isotopic values, *Hemimysis* length, and water temperature were all missing values for a subsample of the *Hemimysis* data points, if these variables were found to be insignificant in either of the multiple regressions, they were removed as a potential predictor and the regression was rerun to maximize the number of points included in the model. Due to the small number of samples from ECL01, and the large number of factors entered into the regression, river samples were excluded from the regression analysis. Standardized regression coefficients were used to measure the amount by which a unit change in the standard deviation of an independent variable influenced the dependent variable in terms of changes in its standard deviation, when all other independent variable values were held constant, and were computed following Cox (1987). Standardized regression coefficients, therefore, facilitate direct comparisons between variables in terms of their relative importance for explaining variation in the dependent variable (Cox, 1987).

To test the hypothesis that niche width would decrease over time, date-specific SEA_C values for Bronte were compared between years using a two-way ANOVA, with season and year as factors. To test for niche shifts as well as changes in the niche width date-specific mean, *Hemimysis* $\delta^{13}C$ and $\delta^{15}N$ values

were compared among years using ANOVAs. Additionally, SEA_C values were calculated for each year in Bronte, using date-specific mean *Hemimysis* $\delta^{13}C$ and $\delta^{15}N$ values, to compare isotopic niche width and position inter-annually. The area of overlap in isotopic niche was calculated among years as the common area included in compared niches. Mean water temperature and intra-annual temperature variability were compared among years using an ANOVA and Levene's test, respectively, to determine if observed differences were due to inter-annual differences in temperature patterns.

Results

Sample mean isotopic values for *Hemimysis* during the study at Bronte ranged between -24.1 and -20.2 ‰ (mean ± SE = -22.2 ± 0.2 ‰; Coefficient of variation (CV) = 5.1 %) for $\delta^{13}C$ and 8.9 and 13.9 ‰ (mean ± SE = 11.4 ± 0.3 ‰; CV = 11.6 %) for $\delta^{15}N$. Mean isotopic values for *Hemimysis* in the Port of Montreal ranged between -23.8 and -21.2 ‰ (mean ± SE = -22.0 ± 0.3 ‰; CV = 5.9 %) for $\delta^{13}C$ and 9.6 and 10.8 ‰ (mean ± SE = 10.0 ± 0.1 ‰; CV = 5.1 %) for $\delta^{15}N$. No significant differences were found among those years that were sampled over a full year (2009 – 2011) in *Hemimysis* $\delta^{13}C$ ($p = 0.372$) or $\delta^{15}N$ ($p = 0.056$). Annual patterns of *Hemimysis* $\delta^{15}N$ values in Bronte show a consistent winter enrichment-summer depletion cycle (Figure 3.1). Greater intra-annual variability is seen in the lake than in the river (Figure 3.1). Date-specific mean *Hemimysis* $\delta^{13}C$ and $\delta^{15}N$ values showed significant seasonal differences at ECL01 (Table 3.2). Two-way ANOVAs showed that the interaction term between year and season was significant for both date-specific mean *Hemimysis* $\delta^{13}C$ and $\delta^{15}N$ values at Bronte ($p < 0.001$), and so year-specific ANOVAs were run to determine seasonal differences (Table 3.2). In most cases, *Hemimysis* $\delta^{13}C$ values were significantly lower in the spring than in the summer or fall, with a notable difference being Bronte in 2010, where no significant differences existed among seasons. In Bronte, *Hemimysis* $\delta^{15}N$ values were significantly lower in the summer than the fall in all years, as well as the spring in 2009 and 2011. *Hemimysis* $\delta^{15}N$ values in ECL01 did not follow this pattern, with values in the spring being significantly lower than both summer and fall values.

As samples collected in 2008 were the result of opportunistic sampling, during which basal food web items were not collected and measurements of water temperature and individual *Hemimysis* length were not recorded, the samples were not included in multiple regression model analyses. A significantly negative linear trend was found between *Hemimysis* $\delta^{13}C$ and calendar day ($p < 0.001$) and the residuals from the relationship were used to form a detrended *Hemimysis* $\delta^{13}C$ data series. POM $\delta^{13}C$ values were found to be correlated with *Hemimysis* C:N ratios ($p = 0.042$), and so an interaction term between these two factors was initially included in the model, but was found to be non-significant ($p = 0.627$) and was removed. *Hemimysis* $\delta^{13}C$ was found to have a significant negative relationship with *Hemimysis* C:N ratio ($p < 0.001$) and a significant positive relationship with water temperature ($p = 0.003$), though these

relationships together only explained 17 % of the observed variation in detrended *Hemimysis* $\delta^{13}C$. Standardized coefficients showed that *Hemimysis* C:N explained approximately twice as much variation in detrended *Hemimysis* $\delta^{13}C$ as water temperature. No significant linear trend was found between *Hemimysis* $\delta^{15}N$ and calendar day, so *Hemimysis* $\delta^{15}N$ was not detrended. A significant negative correlation was found between *Hemimysis* length and POM $\delta^{15}N$ values ($p = 0.045$). The interaction between length and POM $\delta^{15}N$ was included in the initial model, but was not found to be significant ($p = 0.061$) and was removed. A significant positive relationship was found between *Hemimysis* $\delta^{15}N$ and *Hemimysis* length ($p < 0.001$), and significant negative relationships were found between *Hemimysis* $\delta^{15}N$ and water temperature ($p < 0.001$) and POM $\delta^{15}N$ ($p < 0.001$). Together these three factors explained 55 % of observed variation in *Hemimysis* $\delta^{15}N$ values. Standardized regression coefficients showed that length was the dominant explanatory factor, explaining approximately the same amount of variation explained by water temperature and baseline $\delta^{15}N$ values combined. Model parameters from these regressions are given in Table 3.3, including standardized coefficients. Sensitivity tests varying the *F*-to-enter and exit criteria in the step-wise regression procedure did not change the outcome of the model.

An ANCOVA showed no significant interaction between season and year when examining date-specific SEA_C values ($p = 0.645$) as well as no significant differences in date-specific SEA_C values among seasons ($p = 0.603$). Significant differences in date-specific SEA_C values existed between years (2009, 2010, 2011; $F_{(2, 21)} = 6.32$, $p = 0.007$, $n = 24$), with significantly larger values occurring in 2009 than in 2010 ($p = 0.024$) or 2011 ($p = 0.010$). However no significant difference in date-specific SEA_C values was found between 2010 and 2011 ($p = 0.979$). SEA_C values measured for sampling dates in 2008 fell within the range of values seen in 2010 and 2011 (Figure 3.2). Annual SEA_C values and niche overlap are given in Table 3.4. Annual isotopic niche overlapped considerably among years at Bronte, and the centroid for each year was encompassed by all standard ellipses (Figure 3.3). Years did not differ significantly in water temperature ($p = 0.995$) or in the intra-annual variability of water temperature ($p = 0.952$).

Discussion

In the context of examining temporal patterns of isotopic niche use it was found that: 1) *Hemimysis* $\delta^{15}N$ values show consistent seasonal patterns of winter enrichment and summer depletion among years at Bronte, although the patterns were not similar at the two study sites in 2010; 2) *Hemimysis* $\delta^{13}C$ values showed a general trend toward lower values in the spring than the summer or fall in both sites; 3) variation in *Hemimysis* $\delta^{13}C$ and $\delta^{15}N$ values was only partially explained, with only 17 % of observed variation in *Hemimysis* $\delta^{13}C$ values, and 55 % of observed variation in *Hemimysis* $\delta^{15}N$ being explained by the models; and, 4) *Hemimysis* isotopic niche width was significantly larger on a monthly basis in 2009 than in all other years, but annual isotopic niche use did not decrease over time. Over the

study period, date-specific mean *Hemimysis* isotopic values ranged by almost 4 ‰ for $\delta^{13}C$ and just over 5 ‰ for $\delta^{15}N$. The most important explanatory variable for the variation in *Hemimysis* $\delta^{15}N$ was length, indicating an ontogenetic shift in diet to an increased reliance on carnivory. *Hemimysis* isotopic values were significantly related to water temperature but showed a weak ($\delta^{15}N$) or nonexistent ($\delta^{13}C$) relationship with baseline isotopic values. The results showed that, while *Hemimysis* demonstrate some consistent seasonal patterns of temporal variation in isotopic niche use that were driven by previously suggested influences of temperature, *Hemimysis* length, C:N ratios, and baseline isotopic values, a large portion of the observed variability in *Hemimysis* $\delta^{13}C$ and $\delta^{15}N$ values remained unexplained.

Spatially varying seasonal patterns were evident in the $\delta^{15}N$ values of *Hemimysis* seen in this study, which showed consistent patterns of winter enrichment and summer depletion among years in Bronte, but not in ECL01. Seasonal patterns were less consistent among years in *Hemimysis* $\delta^{13}C$, with no significant seasonal differences found in Bronte in 2010. However, the pattern of depleted *Hemimysis* $\delta^{13}C$ seen in the spring in ECL01 was analogous to that seen in Bronte in both 2009 and 2011. Patterns of winter depletion and summer enrichment for $\delta^{13}C$, and the inverse for $\delta^{15}N$, have been found in previous studies of aquatic invertebrates (Woodland et al., 2012). The patterns potentially correspond with a number of biotic and abiotic intra-annual cycles (water temperature, productivity, stratification, etc.) that may drive variation (Woodland et al., 2012). However, determining which factors drive the cycles of enrichment and depletion is difficult due to interactions among potential drivers (Woodland et al., 2012, Finlay, 2004). This study attempted to address the lack of understanding with respect to *Hemimysis* by examining the importance of a few key factors, previously noted to be of potential importance in *Hemimysis* isotope and diet variation (Borcherding et al., 2006, Matthews and Mazumder, 2005, Matthews and Mazumder, 2007, Power et al., 2003). And while the factors explained some of the variation, much remains to be understood about the determinants of seasonal variability in *Hemimysis* stable isotope values.

Both multiple regression models left a large proportion of observed variation in *Hemimysis* isotopic values unexplained, however, the model for *Hemimysis* $\delta^{15}N$ values explained more than triple the amount of observed variation than the model for *Hemimysis* $\delta^{13}C$ values. Of the variation in *Hemimysis* $\delta^{15}N$ values accounted for by the model, *Hemimysis* length dominated as a factor and explained almost as much variation as temperature and POM $\delta^{15}N$ combined, as evidenced by the standardized regression coefficients. The importance of length in the model strongly supports the presence of a dietary shift between juvenile and adult *Hemimysis* at Bronte. The positive relationship between *Hemimysis* $\delta^{15}N$ and length found at Bronte suggests a shift towards greater reliance on carnivory in the diet of adult *Hemimysis*, as $\delta^{15}N$ values increase with increasing trophic level (Post,

2002). Previous studies have reported mixed findings on the presence and strength of ontogenetic shifts in diet for *Hemimysis* (Borcherding et al., 2006, Ketelaars et al., 1999, Marty et al., 2012, Ives et al., in press). Borcherding et al. (2006) and Ketelaars et al. (1999) found evidence of an ontogenetic shift, from feeding primarily on phytoplankton to zooplankton, using gut content analysis of *Hemimysis* found in invaded sites in Europe. Marty et al. (2012) used stable isotopes to determine if such a shift existed for *Hemimysis* in the St. Lawrence River and found little evidence to support a change in diet with an increase in length. Spatial comparisons of diet using stable isotope analyses have shown patchy support for an ontogenetic shift in the Great Lakes, with the majority of studied sites (three out of 13) showing no evidence of a shift (Ives et al., in press). It is possible that the absence of support for an ontogenetic shift seen in previous studies is a result of temporally limited datasets that do not see the effect of seasonality.

Water temperature was the second most important factor explaining the variation in both *Hemimysis* $\delta^{15}N$ and $\delta^{13}C$ values. Power et al. (2003) found that isotopic fractionation factors varied as a function of water temperature, with $\delta^{15}N$ values of invertebrates showing greater enrichment with respect to their diet in colder temperatures. Barnes et al. (2007) found a similar pattern in fish. The reported temperature-dependent enrichment pattern agrees with the relationship found in this study, with higher temperatures coinciding with lower $\delta^{15}N$ values of *Hemimysis*. The relationship between $\delta^{13}C$ fractionation factors and temperature has been reported to be the reverse, with higher temperatures associated with higher $\delta^{13}C$ fractionation (Power et al., 2003, Barnes et al., 2007). The results for *Hemimysis* $\delta^{13}C$ reflect this relationship, although the relationship is weak. With an increase in Δ_N with decreasing temperatures one would expect to see increased *Hemimysis* $\delta^{15}N$ values during the late fall and early spring even without a change in trophic niche use. However, the results show temporal variation in mean *Hemimysis* $\delta^{15}N$ of approximately 5 ‰ (over a temperature variation of 21.6 °C), which is greater than the amount of variation seen in Δ_N in the literature ($\leq$ 2.5 ‰ over a temperature range of 13.7 °C) (Power et al., 2003, Barnes et al., 2007). Similarly, the range in mean *Hemimysis* $\delta^{13}C$ seen in this study (3.9 ‰) exceeded the range in Δ_C reported in the literature ($\leq$ 1.4 ‰) (Power et al., 2003, Barnes et al., 2007). Though the temperature range was greater in this study than in the cited studies (Power et al., 2003), temperature is thought to have a larger impact on fractionation at temperatures > 21 °C, and temperatures recorded at Bronte did not exceed 22.5 °C. This implies that the relationship seen between *Hemimysis* $\delta^{13}C$ and $\delta^{15}N$ values may not be strictly due to differences in Δ_C and Δ_N.

Along with temperature, it was hypothesized that basal food web isotopic variation would explain a significant amount of *Hemimysis* isotopic variation based on the results of previous studies which have reported a strong positive correlation between the isotopic values of basal food web items and zooplankton (Matthews and Mazumder, 2007, Matthews and Mazumder, 2005). However, it was found

that *Hemimysis* isotopic values showed either no relationship ($\delta^{13}C$) or a weak negative relationship ($\delta^{15}N$) with POM isotopic values. POM isotopic values were chosen as baseline values, representing the pelagic food web, due to the heavy reliance of *Hemimysis* on prey in the water column, namely phytoplankton and zooplankton, reported in the literature (Pothoven et al., 2007, Borcherding et al., 2006). The absence of a strong relationship between *Hemimysis* isotopic values and those of the pelagic basal food web implies a lack of a strong trophic connection between *Hemimysis* and the pelagic food web. Recent work on *Hemimysis* populations has found that at some sites benthic production can support a significant portion of *Hemimysis* diet (Marty et al., 2012, Ives et al., in press). Bronte, in particular, was found to show notably high reliance on benthic production (Ives et al., in press). As such, Bronte may not represent a 'typical' site, and a trophic connection with pelagic basal food web items may exist at other sites within the Great Lakes basin. The observed lack of a strong trophic connection may be explained by two scenarios: *Hemimysis* may consistently derive a portion of its carbon from other sources, such as the benthic food web, throughout the year, or it may vary its relative reliance on pelagic and benthic carbon sources seasonally. Both of these scenarios would theoretically result in the lack of strong connection with the basal components of the pelagic food web (Grey et al., 2001, Matthews and Mazumder, 2007, Matthews and Mazumder, 2005). Another possible explanation for the lack of strong significant relationships is that *Hemimysis* isotopic values may be tracking those in the basal pelagic food web, but they, or their prey, may be preferentially feeding on a subsample of what is included in the POM samples collected in this study. If this is the case, the isotopic values of the POM may not accurately represent the isotopic values of the basal organisms in the food chain directly supporting *Hemimysis* (Matthews and Mazumder, 2005, Marty and Planas, 2008, Pel et al., 2003).

Observed variation in *Hemimysis* $\delta^{13}C$ values was not explained solely by water temperature, as the C:N ratio of *Hemimysis* was found to have a significant negative relationship with *Hemimysis* $\delta^{13}C$, and was approximately twice as important to the multiple regression model as water temperature. Historically, as lipids contain relatively little nitrogen and high amounts of carbon, C:N ratios have been used to quantify the lipid content of an organism under the assumption that increased C:N ratios reflect increased lipid concentrations (e.g. Matthews and Mazumder, 2005, Barnes et al., 2007). Lipids are depleted in ^{13}C compared to proteins and carbohydrates (De Niro and Epstein, 1977, De Niro and Epstein, 1977, Tieszen et al., 1983), and temporal variations in lipid content can thus introduce variation into the $\delta^{13}C$ values of an organism throughout the seasons. As in this study, Matthews and Mazumder (2005) found that C:N ratios of pelagic zooplankton accounted for a significant portion of temporal variation, greater than that explained by the isotopic values of POM, in the $\delta^{13}C$ values of said zooplankton. It has become a common practice to attempt to remove variability related to lipid content by extracting the

lipids from a sample before isotope analysis, or by using the C:N values of organisms to mathematically remove the influence of lipids on $\delta^{13}C$ values (Fagan et al., 2011). If lipids are primarily dietary in origin removing them may remove valuable information about an organism's diet, as has been shown to be the case with pelagic zooplankton (Matthews and Mazumder, 2005, Arts, 1999, Goulden and Place, 1990, Goulden et al., 1999). Dietary items consumed by an organism may not contribute equally to all tissues in the consumer (Cherry et al., 2011, Kelly and del Rio, 2010, Phillips and Koch, 2002), and thus removing lipids from analysis may remove evidence of the consumption of lipid rich items by an organism. Additionally, as noted by Fagan et al. (2011) commonly used C:N ratio – percent lipid models can significantly underestimate organism lipid content and often result in significant error. Syväranta and Rautio (2010) have noted similar zooplankton specific results. While significant relationships do exist between C:N and lipid content (Syvaranta and Rautio, 2010, Matthews and Mazumder, 2005), these relationships are extremely variable and appear to be both species- and site-specific (Syvaranta and Rautio, 2010, Fagan et al., 2011). In the case of *Hemimysis* it appears that body composition (as indicated by C:N) is an important factor in explaining temporal variation in *Hemimysis* $\delta^{13}C$. As such C:N and body composition should be considered when examining or interpreting seasonal isotopic changes in *Hemimysis*, but when dealing with questions of diet lipids should be considered as an important component of the organism and should be included in analyses.

The factors included in the multiple regression analyses were taken from the literature and together were expected to explain a significant portion of *Hemimysis* isotopic variation. However, *Hemimysis* $\delta^{13}C$ values were not strongly related to any of the previously mentioned factors, and the majority of observed variation in $\delta^{13}C$ was left unexplained by the model. Approximately half of the observed variation in *Hemimysis* $\delta^{15}N$ values was also left unexplained. Accordingly, the seasonal patterns observed in *Hemimysis* $\delta^{13}C$ and $\delta^{15}N$ values are the result of seasonal differences in factors not included in this study (e.g. prey availability, abundance of predators). The results show that the use of literature factors cited as driving isotopic variability may not be applicable in some cases, and must be tested for relevance for individual species, particularly if they are to be used in evaluating the potential impacts of an invader.

One possible scenario that may explain additional observed variation is the consumption of non-water column based food items by *Hemimysis*. These may include benthic food web items, as previously mentioned, or detrital resources and the coincidental ingestion of associated bacterial mats, which likely differ in isotopic values from the water column (France, 1995a , France, 1995c). Further work is required to examine the importance of benthic and detrital food sources in the diet of *Hemimysis*. In the case of the consistent depletion in *Hemimysis* $\delta^{13}C$ values seen in spring samples factors may include a shift in diet to

food items with a more depleted $\delta^{13}C$ signature in the spring, which would imply a heavier reliance on pelagically derived carbon (France, 1995c). If *Hemimysis* varies its relative reliance on pelagic versus benthic carbon seasonally, seasonal shifts in the degree of bentho-pelagic coupling in the invaded food webs may be seen (Hecky et al., 2004, Ives et al., in press).

As a relatively new invader, *Hemimysis* may still be in the process of integrating into invaded food webs, and as such have a trophic, and thus isotopic, niche that is more variable than that of a well-established organism. To explore this idea it was hypothesized that *Hemimysis* isotopic niche would vary over time, in both width and isotopic value, as *Hemimysis* integrated into the invaded food webs, and two ideas of niche width were examined. First, date-specific isotopic niche width (SEA_C) values were used to examine the isotopic niche occupied by *Hemimysis* on a given sampling expedition, and how this niche width changed over time. It was hypothesized that SEA_C values would decrease over time as *Hemimysis* integrated into the food web. The results did not support the hypothesis. Although the date-specific SEA_C values calculated for each sampling expedition were significantly larger in 2009 than 2010 and 2011, the SEA_C values for the three sampling dates in 2008 fell well within the range seen in the latter two years. As there were only 3 sampling dates for 2008, it is possible that a complete evaluation of niche width over the ice free season would support the hypothesis. Water temperature did not differ in 2009 in either mean value or degree of variation, so the significantly higher SEA_C values seen in 2009 were not a function of temperature fluctuations. Rather, the differences seen in niche width may be related to population dynamics of *Hemimysis* or other organisms in the food web. Niche width is a function of the balance between intra- and inter-specific competition (Bolnick et al., 2010), and increases in observed niche width may be driven either by increased intra-specific competition, forcing individuals to make use of lower quality resources (Bolnick, 2001, Van Valen, 1965), or lessened inter-specific competition, allowing *Hemimysis* increased access to preferred resources for which they compete with other organisms (Bolnick et al., 2010). Recent invaders often experience a period of rapid population growth, undergoing rapid changes in abundance and overshooting carrying capacity before returning to equilibrium (Crooks and Soulé, 1999, Hengeveld, 1989).

As a second approach to examining temporal integration by *Hemimysis* into invaded food webs the annual SEA_C values and centroids of the standard ellipses of date-specific mean *Hemimysis* $\delta^{13}C$ and $\delta^{15}N$ values were used to compare annual isotopic niche width and position among years. It was hypothesized that niche position would vary and niche width would decrease over the years, indicating that *Hemimysis* was still in the process of integrating into the invaded food web. Contrary to the hypothesis, the results showed no directional reduction in annual SEA_C value and mean *Hemimysis* $\delta^{13}C$ and $\delta^{15}N$ values did not vary significantly among years, implying that while the size of the niche may

change, the central position remains generally unchanged. The results support *Hemimysis* occupying a constant, central niche, and opportunistically making use of niche space outside of this core niche when it is available to them.

The results of this paper reinforce the fact that taking into consideration the time and duration of sampling is important when exploring potential food web impacts of an invader. The fact that *Hemimysis* isotopic values show significant seasonal and inter-annual variability, when coupled with the lack of a spatially consistent seasonal pattern of variation, implies that sampling date will be significant and may impact the conclusions drawn on a site-by site basis. Based on the amount of variation seen in *Hemimysis* $\delta^{15}N$ in Lake Ontario versus the Port of Montreal, investigators sampling in the summer could conclude that *Hemimysis* do not differ spatially in $\delta^{15}N$ values, whereas a study conducted at the same sites in the winter would find a difference of approximately 2 ‰. Thus to obtain a more complete view of an invasion, especially in a temperate region with differing seasons, an invader must be sampled over the long term and with a seasonally structured sampling design. Additionally, the lack of explanatory power seen in the multiple regressions showed that the connections between biotic or abiotic factors taken from literature and organism isotopic niche use must be tested for each species, and, in the case of opportunistic feeders such as *Hemimysis*, each location.

Table 3.1: Study site characteristics.

Parameter	Bronte	ECL01
Latitude, Longitude	43° 23’ 34.15” N, 79° 42’ 20.95” W	45° 29’ 56.94” N, 73° 33’ 8.36” W
Environment type	Lentic	Lotic
Dates sampled	31/10/08 to 10/01/12	02/07/10 to 18/11/10
Number of sampling events	29	7
Substrate	Soft organic material, some larger detritus	Soft organic material
Approximate water depth	5 m	3.5 m
Temperature range	5.0 – 22.6 ^{o}C	6.9 – 25.1 ^{o}C
pH range	7.86 – 8.66	8.04 – 8.45
Conductivity range	214 – 522 µS/cm	205 – 300 µS/cm

Table 3.2: Model results for year- and site-specific ANOVAs testing for differences in *Hemimysis* $\delta^{13}C$ and $\delta^{15}N$ values between seasons. The post-hoc seasonal differences column shows the relationship between seasons where a significant difference existed (< and > denote significantly less than or greater than respectively), and the post-hoc significance column gives the specific significance of the associated relationship. Cases denoted with an asterisk (*) denote models in which the Welch's ANOVA was used to deal with heteroscedasticity of the residuals.

Dependant variable	Site	Year	Test Statistic	N	P	Post-hoc seasonal differences	Post-hoc significance (p)
$\delta^{13}C$	Bronte	2009*	$F_{(2, 53.73)} = 15.35$	91	< 0.001	Fall < Summer	0.022
						Spring < Summer	< 0.001
		2010	$F_{(2, 95)} = 1.06$	98	0.349	n/a	n/a
		2011	$F_{(2, 139)} = 25.24$	142	< 0.001	Spring < Summer	< 0.001
						Spring < Fall	< 0.001
	ECL01	2010*	$F_{(2, 19.04)} = 338.45$	32	< 0.001	Spring < Summer	< 0.001
						Spring < Fall	< 0.001
$\delta^{15}N$	Bronte	2009	$F_{(2, 88)} = 65.52$	91	< 0.001	Summer < Spring	< 0.001
						Summer < Fall	< 0.001
		2010	$F_{(2, 50.99)} = 44.81$	98	< 0.001	Spring < Fall	0.004
						Summer < Fall	< 0.001
		2011	$F_{(2, 139)} = 101.62$	142	< 0.001	Summer < Fall	< 0.001
						Fall < Spring	0.001
	ECL01	2010	$F_{(2, 29)} = 6.44$	32	0.005	Spring < Summer	0.003
						Spring < Fall	0.031

Table 3.3: Multiple regression models for the prediction of *Hemimysis* $\delta^{13}C$ or $\delta^{15}N$ at Bronte. Only significant predictors are included. The variance inflation factor (VIF) is reported to show that factors were not collinear (VIF < 2.5), and the standardized regression coefficients (β) are given as a measure of relative importance of individual factors to the model as described in the methods.

Dependant variable	Predictor	VIF	Estimates (B)	β	P
$\delta^{15}N$	Intercept		10.78		< .001
	Length	1.11	0.39	0.55	< .001
	Temperature	1.04	-0.07	-0.25	< .001
	POM $\delta^{15}N$	1.12	-0.24	-0.22	< .001
	$F_{(3, 159)} = 65.10$, $R^2 = .55$, R^2 adj. = .54, n = 163				
Detrended	Intercept		3.06		< .001
$\delta^{13}C$	C:N	1.08	-0.83	-0.34	< .001
	Temperature	1.08	0.03	0.16	.003
	$F_{(2, 331)} = 33.27$, $R^2 = .17$, R^2 adj. = .16, n = 334				

Table 3.4: Standard ellipse area (corrected for small sample sizes: SEA_C) and overlap of isotopic niche width among years at Bronte.

Site	Year	SEA_C (‰^{2})	Area of overlap (‰^{2})		
			2009	2010	2011
Bronte	2009	6.6	-	1.8	3.6
	2010	1.8		-	1.6
	2011	5.2			-

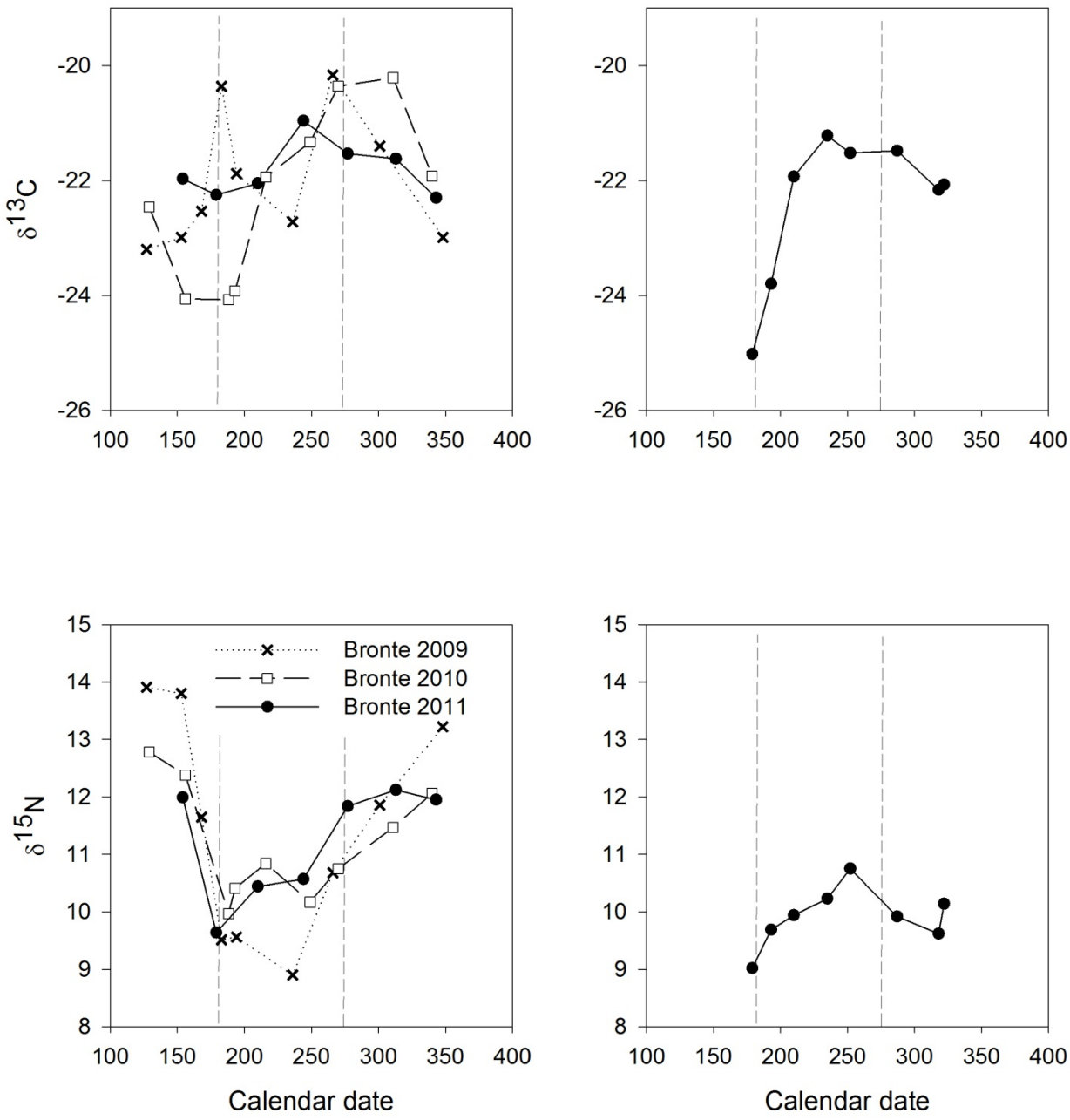

Figure 3.1: Temporal trends in *Hemimysis* isotopic values at Bronte (left plots) and ECL01 (right plots) shown by plotting date-specific mean *Hemimysis* $\delta^{13}C$ and $\delta^{15}N$ values against calendar date, or the number of days from January 1st of the year in question. Vertical dashed lines indicate divisions between seasons, with Spring on the left, Summer in the middle, and Fall on the right.

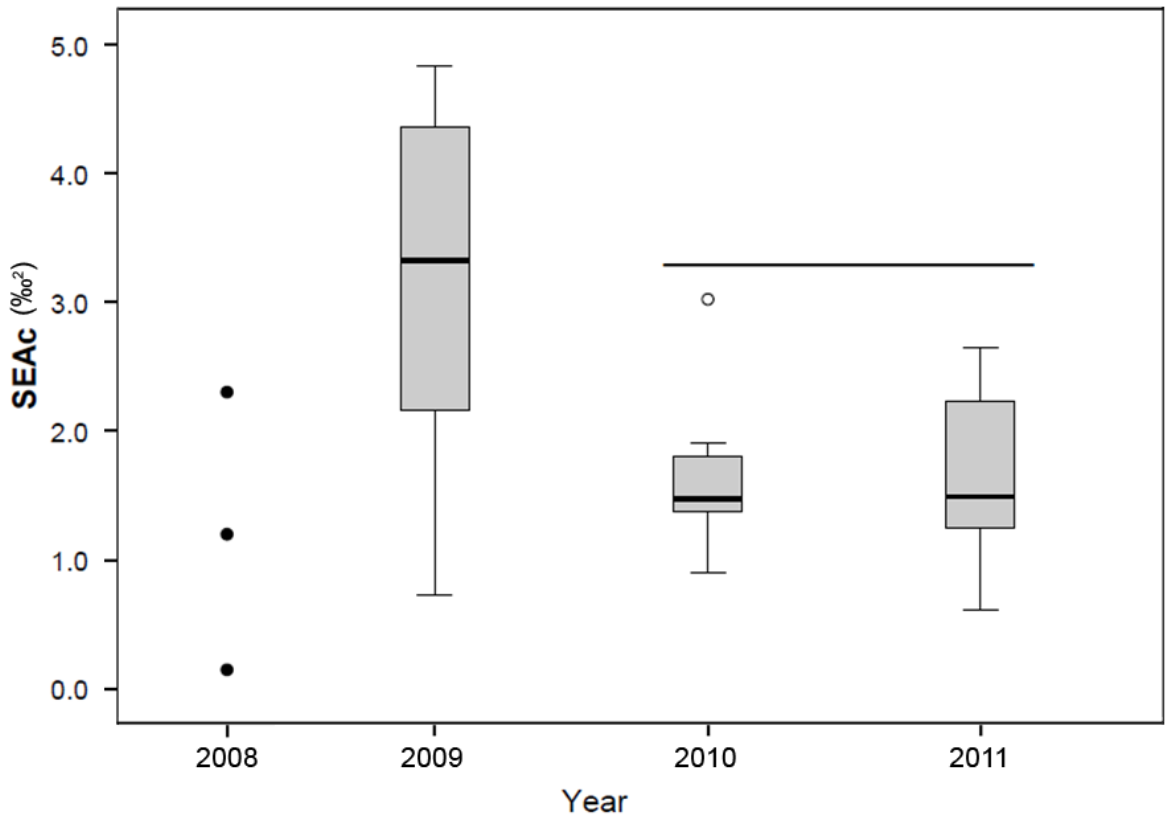

Figure 3.2: Date-specific SEA_C values of *Hemimysis* in Bronte by year. Homogeneous subsets are connected by a bar. Points from 2008 were not tested for significant differences.

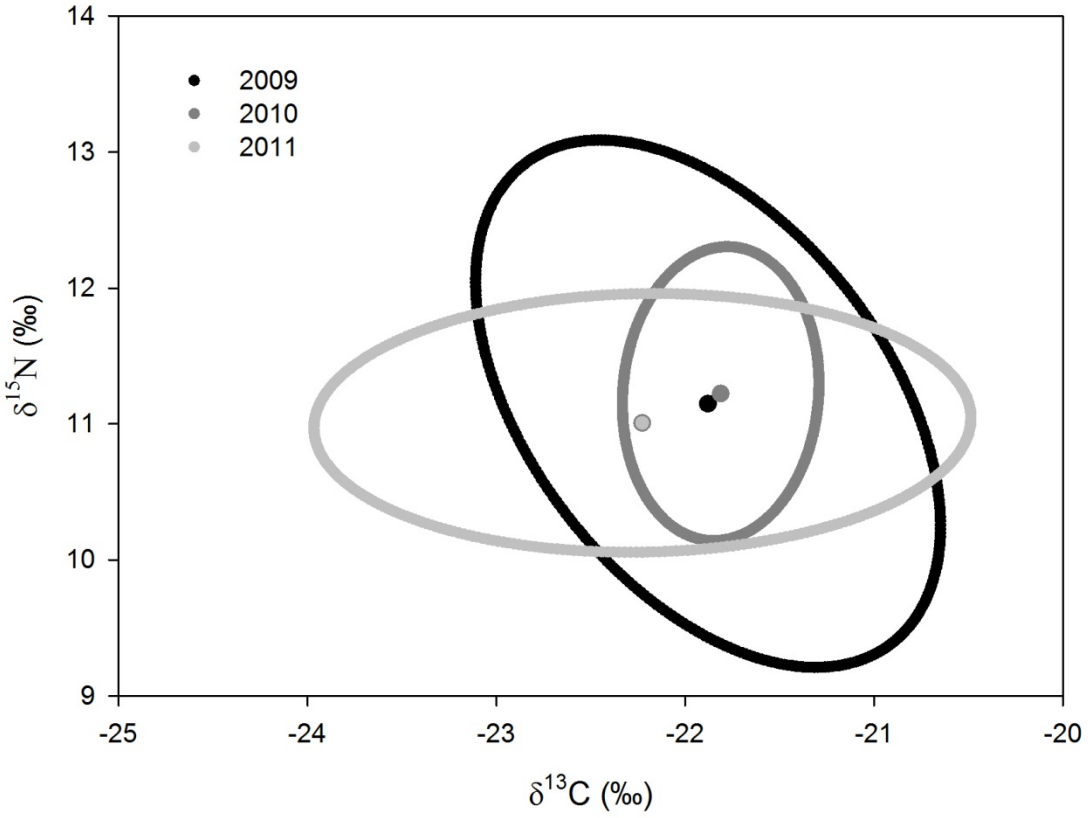

Figure 3.3: Standard ellipses of the date-specific mean $\delta^{13}C$ and $\delta^{15}N$ values of *Hemimysis* for each year at Bronte. Centroids are depicted using a point of the same shade of grey as the ellipse.

Chapter 4: General Conclusions and Future Directions

Summary

The bloody red shrimp *Hemimysis anomala*, hereafter *Hemimysis*, is one of the most recent known invaders in the Laurentian Great Lakes basin of North America. This study addressed knowledge gaps in the trophic niche use of *Hemimysis* in North America using analysis of carbon and nitrogen stable isotopes. Chapter 2 examined the spatial variation in trophic offset and main carbon sources utilized by *Hemimysis* in both lentic and lotic ecosystems within the Great Lakes basin. I found that *Hemimysis* niche use, including niche width, varied considerably among sites, with site-specific mean trophic offset values spanning approximately one trophic level, and site-specific degree of reliance on pelagic production, as opposed to benthic, ranging from under 20 to over 99 %. The variability was not related to environment type (lentic versus lotic), and it was postulated that the variability was related to site specific environmental factors not included in this study.

Chapter 3 aimed to describe seasonal patterns of *Hemimysis* isotopic niche use and to ascertain the relative importance of key biotic and abiotic factors in explaining *Hemimysis* isotopic variability through the analysis of a time series of isotopic data from high density sites in Lake Ontario (Bronte) and the St. Lawrence River (ECL01). Significant seasonal patterns of summer depletion and winter enrichment in *Hemimysis* $\delta^{15}N$ values were found in Bronte, but were not seen in ECL01. Seasonal patterns in *Hemimysis* $\delta^{13}C$ were not as strong, with no significant differences found between seasons in 2010 in Bronte. However, significantly depleted values were found in the spring in 2009 and 2011 in Bronte, as well as 2010 in ECL01. Of the key factors included in the multiple regression model, *Hemimysis* $\delta^{15}N$ values were found to be most strongly correlated with *Hemimysis* length at Bronte, supporting the presence of an ontogenetic shift in diet due to an increased reliance on carnivory. Previous studies have shown mixed results with respect to the presence and strength of an ontogenetic shift, particularly within the Great Lakes (Borcherding et al., 2006, Marty et al., 2012). *Hemimysis* $\delta^{15}N$ were also found to be negatively related to water temperature and baseline $\delta^{15}N$ values, with these two factors, along with *Hemimysis* length, accounting for 55 % of the observed variation in *Hemimysis* $\delta^{15}N$ values. *Hemimysis* $\delta^{13}C$ values were found to be positively related to water temperature and negatively related to *Hemimysis* C:N ratios, but together these two factors explained only 17 % of the observed variation in *Hemimysis* $\delta^{13}C$. The lack of a strong relationship with basal components of the pelagic food web (particulate organic matter, POM) implied that *Hemimysis* does not have a strong trophic link to the pelagic food web at Bronte, and likely makes use of other carbon sources, as was noted by the results of investigations described in Chapter 2.

Study significance

This study is the first study to examine spatial and temporal variation in *Hemimysis* trophic and isotopic niche use in the Great Lakes basin. *Hemimysis* has an extensive invasion history in Europe, including significant impacts on invaded food webs (Borcherding et al., 2006, Ketelaars et al., 1999). Additionally, the functionally similar mysids *Mysis diluviana* and *M. relicta*, which have invaded western North America and Scandinavia respectively, have had notable impacts, generally reported as a significant reduction in macrozooplankton, particularly cladocerans (Ricciardi et al., 2012, Koksvik et al., 2009). Knowledge of the trophic niche of *Hemimysis* and how it integrates into invaded food webs is therefore vital to understanding the potential impacts of *Hemimysis* on the food webs of the Great Lakes basin and other freshwater ecosystems within North America.

Results from both Chapters 2 and 3 supported categorizing *Hemimysis* as an opportunistic omnivore with a flexible trophic niche. Dietary metrics (trophic offset and main carbon source) were spatially variable and likely dependant on site-specific biotic or abiotic characteristics (Chapter 2). The ability to vary its trophic niche dependent on characteristics of the invaded system is likely to result in spatial variability in food web impacts. Thus, predictions of impact will be much more difficult, as generalized descriptions of impacts, including those of *M. diluviana* and *M. relicta*, will likely not be widely applicable. Additionally, while European studies report *Hemimysis* to preferentially feed in the water column on phytoplankton and zooplankton (Borcherding et al., 2006, Ketelaars et al., 1999), I found no significant positive relationship between the isotopic values of basal components of the pelagic food web and *Hemimysis*, implying the lack of a strong trophic connection between *Hemimysis* and the pelagic food web (Chapter 3). However, the mixing models used in Chapter 2 to quantify the relative reliance on pelagic versus benthic production showed a notably low reliance on the pelagic food web in Bronte, which was a site of known high *Hemimysis* density (Taraborelli et al., 2012), so strong relationships with pelagic basal food web components may well exist at other, lower density sites. Annual isotopic niche position of *Hemimysis* did not vary among years at Bronte. Results suggest *Hemimysis* occupy an inter-annually consistent core trophic niche, with annual variations in niche width resulting from variations in environmental characteristics or population dynamics. As Bronte was found to have notably low trophic offset and reliance on pelagic carbon in my spatial analysis (Chapter 2), and niche use at Bronte was found not to vary inter-annually in my temporal analysis (Chapter 3) it implies that *Hemimysis* is able to support itself and be successful on food sources reported to be less preferable long-term.

The spatial inconsistency of seasonal patterns shown in Chapter 3, particularly in $\delta^{15}N$, underlines the importance of considering the temporal domain when investigating potential impacts of invasive

species. Studies conducted in different seasons could draw differing conclusions on niche use and potential impact of an invader. As such, a temporal element should be included in studies examining the potential impacts of invasive species, particularly when results of such a study may drive management decisions.

The flexibility seen in Hemimysis trophic niche use implies that, in many cases, *Hemimysis* may be able to shift trophic niche to avoid excessive competition with, or over consumption of, organisms already present in the invaded food web. While *Hemimysis* is a prolific invader that can occur in high abundances at invaded sites (de Lafontaine et al., 2012, Taraborelli et al., 2012), I believe that its highly flexible diet will result in less severe food web impacts than were initially predicted in such a large and dynamic system as the Great Lakes basin. However, impacts on smaller inland lakes are still uncertain, and may be more severe based on the larger proportion of the total area made up by shallow, nearshore environments in which *Hemimysis* may be successful.

Future research

Results from this project show that *Hemimysis* is an opportunistic consumer with a varied diet, but the main drivers behind the variation were not determined. While analyses in Chapter 3 found some significant factors in isotopic variation of *Hemimysis*, the majority of observed variation remained unexplained. To enable better future predictions of impact to be made, investigators must understand how *Hemimysis* trophic niche will vary based on site-specific environmental and food web characteristics. Thus, further work is required to fully describe the drivers behind trophic niche variation in *Hemimysis*. Some ideas for future work that have come to my attention while writing this thesis are as follows:

1. Field studies linking prey availability and *Hemimysis* trophic niche use could provide interesting insight into *Hemimysis* trophic variability, as an opportunistic consumer would be expected to be strongly influenced by the relative abundances of potential food items. Knowledge of *Hemimysis* abundances would also be useful in this scenario as intra-specific competition may also influence niche width (Bolnick, 2001).
2. Laboratory experiments should be conducted to determine the validity of isotopic fractionation factors reported in the literature with regards to *Hemimysis*, especially over a range of temperatures (Power et al., 2003). As temperature was found to be significantly related to both $\delta^{13}C$ and $\delta^{15}N$ values in *Hemimysis* (Chapter 3), it would be interesting to explore how much of this variation was purely physiological. However, such experimental studies may be difficult due to the opportunistic nature of *Hemimysis* feeding, as *Hemimysis* are known to cannibalise small or weak individuals.

3. Long-term temporal studies with an emphasis on collecting basal food web items from both benthic and pelagic food webs would be interesting to determine potential seasonal variations in the relative reliance on these two food webs. *Hemimysis* reliance on benthic versus pelagic production has implications for the bentho-pelagic decoupling, observed in the Great Lakes after the invasion of dreissenid mussels, due to the diel vertical migrations *Hemimysis* undergoes between the substrate and the water column. The inclusion of fish sampling in this long-term monitoring effort would also be advantageous as the mixed results concerning the impacts on fish seen in previous studies (Yuille et al., 2012, Lantry et al., 2012, Lantry et al., 2010, Fitzsimons et al., 2012) may be partially due to temporal limitations.

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