Temporal and spatial variation in Hg accumulation in zebra mussels (Dreissena polymorpha): Possible influences of DOC and diet

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A B S T R A C T

Zebra mussels (Dreissena polymorpha) are filter feeders located near the base of the food web and these animals are able to utilize a variety of carbon sources that may also vary seasonally. We conducted both a spatial and a temporal study in order to test the hypotheses: (1) dissolved organic carbon (DOC) concentrations influence Hg accumulation in zebra mussels sampled from a series of lakes and (2) seasonal variations in diet influence Hg accumulation. In the spatial study, we found a significant negative relationship between Hg concentrations and DOC concentrations, suggesting an influence of DOC on Hg bioaccumulation. In the temporal study, we used stable isotope ratios of nitrogen (∆15N) and carbon (∆13C) as ecological tools to provide a temporally integrated description of the feeding ecology of zebra mussels. Both ∆15N and ∆13C varied seasonally in a similar manner: more depleted values occurred in the summer and more enriched values occurred in the fall. Mercury concentrations also varied significantly over the year, with highest concentrations occurring in the summer, followed by a progressive decrease in concentrations into the fall. The C/N ratio of zebra mussels also varied significantly over the year with the lowest values occurring mid-summer and then values increased in the fall and winter, suggesting that there was significant variation in lipid stores. These results indicate that in addition to any effect of seasonal dietary changes, seasonal variation in energy stores also appeared to be related to Hg levels in the zebra mussels. Collectively results from this study suggest that DOC concentrations, seasonal variation in diet and seasonal depletion of energy stores are all important variables to consider when understanding Hg accumulation in zebra mussels.

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1. Introduction

Mercury is considered a global contaminant and a serious threat to the health of aquatic ecosystems. In addition to natural sources (e.g. the weathering of Hg-containing rocks), anthropogenic activities including mining and smelting operations, power generation via fossil fuel combustion, and industrial activities can result in the emission and transport of Hg long distances in the atmosphere and the eventual deposition in lakes and rivers. Once in these aquatic environments, Hg enters the base of the food chain where biomagnification results in high concentrations in apex fish species (e.g. (Lavoie et al., 2010)). Due to its high biomagnification potential a solid understanding of factors that control the accumulation of Hg at the base of the food chain will improve our understanding of Hg accumulation in these top predator species.

Dissolved organic material (DOM), as well as its surrogate measure, dissolved organic carbon (DOC), have been shown to reduce Hg accumulation in various biota including algae (Moye et al., 2002; Gorski et al., 2008; Zhong and Wang, 2009), aquatic invertebrates (Guo et al., 2001; Pan and Wang, 2004), bacteria (Barkay et al., 1997) and fish (Choi et al., 1998). The inverse relationship between DOM or DOC and Hg accumulation in these aquatic species is linked to the reduction in bioavailability of Hg due to complexation with the dissolved organic chelators present as constituents of DOM. However a positive relationship between DOC and Hg bioaccumulation was shown in black fly larvae (Diptera, Simuliidae) collected from a series of lakes varying in DOC concentrations ( Harding et al., 2006). This was explained by the fact that black fly larvae collect colloidal material that is included in measurements of DOM or (DOC), resulting in increased intake of complexed Hg. A similar relationship between increased Hg accumulation and DOC concentrations has been reported for other aquatic invertebrates (Rennie et al., 2005). In these examples, the organisms directly relied on DOC as a food source and thus, DOC acted as a delivery system, introducing complexed Hg to the organism via its diet.

The carbon source an animal relies upon may also influence the level to which Hg accumulates (Power et al., 2002). In their study, Power et al., 2002 found that fish species that relied on
benthic-derived carbon had lower Hg levels than fish that relied on pelagic-derived carbon. In contrast, Watanabe et al., 2008 found that although autotrophs in the epilimnion had higher Hg concentrations than adjacent riparian vegetation, the relative contributions of autochthonous and allochthonous carbon in the diet of various macroinvertebrate species did not seem to influence their metal levels. Similarly, Kainz et al. (2002) did not find a relationship between autochthonous and allochthonous carbon sources and methyl mercury concentrations in freshwater zooplankton.

Zebra mussels (*Dreissena polymorpha*) are filter feeders that are able to remove particles ranging in size from <0.2 μm (e.g. low molecular weight DOC) to filamentous algae that are as large as 1.2 mm (Horgan and Mills, 1997; Roditi et al., 2000). Located at the base of the food chain, zebra mussels are important in the diet of some fish species as well as some waterfowl species (Custer and Custer, 1996; Watzin et al., 2008). They encounter a variety of potential carbon sources during the year ranging from low-quality food in the summer (i.e. cyanobacteria and chlorophytes) to high food quality sources (e.g. flagellates and diatoms) in the spring (Åhlgren et al., 1997; Goedkoop et al., 2000).

With the objective of improving our understanding of how Hg accumulates in zebra mussels, we conducted both a spatial and a temporal study in order to test the hypotheses: (1) DOC concentrations influence Hg accumulation in zebra mussels and (2) seasonal variations in diet influence Hg accumulation in zebra mussels. We conducted our study in lakes located in the Kawartha region of eastern Ontario, Canada. As there are no known point sources of Hg to these lakes, the main input will be from atmospheric deposition and therefore Hg loadings will be similar in each lake, while DOC concentrations were variable.

### 2. Materials and methods

#### 2.1. Zebra mussel collection

Zebra mussels used in the spatial study were collected from a series of lakes in the Kawartha lake region (see Fig. 1). The areas surrounding the lakes include forest, farmland and urban areas. These lakes were chosen because they contain zebra mussel populations, and in addition, there are no known point sources of Hg to these lakes; the main input will be from atmospheric deposition and therefore we expect that Hg loadings will be similar in each lake. As a measure of Hg levels in the water column, we measured Hg in seston (33–102 μm), once at the time of zebra mussel collection. In the majority of lakes, Hg concentrations in seston were less than 20 ng g⁻¹ (dry weight) (see Table 1). In order to have enough sample for Hg analysis, seston was pooled into one composite sample. There was no significant relationship observed between Hg concentrations in seston and Hg in zebra mussels (linear regression, \( p > 0.05 \)). Mussels were collected from a 1 m × 1 m area along the shoreline, in less than 1 m of water.
Zebra mussels used in the seasonal study were collected from the same location in Katchewanooka Lake (at Young's Point) monthly for one complete year from June 2008 to May 2009. The sampling site was chosen due to the high density of zebra mussels, even in very shallow water. Mussels were collected from the shoreline in less than 60 cm of water. Zebra mussels of the same size were collected; the average shell length was 17 ± 3 mm.

2.2. Metal analyses

For Hg analyses, whole mussels (n=15) were first lyophilized and the dry weight was recorded. Mussels were not depurated prior to analyses. Total Hg concentrations were measured in the entire dried mussel using atomic absorption spectroscopy on a DMA-80 Direct Mercury Analyzer (Milestone, Shelton, Connecticut, USA). The DMA-80 has a detection limit of 0.1 ng. Prior to each run, several blanks were measured to ensure minimal Hg contamination prior to analyzing the samples. The lakes included in our study all had high dissolved calcium concentrations and lake DOC concentrations. Significant temporal variation in Hg concentrations, δ13C and δ15N of zebra mussels were analyzed with a one-way ANOVA, and a posteriori multiple comparisons were examined with Tukey-Kramer HSD test (p < 0.05).

2.3. Stable isotope analysis

Dried mussels were first ground to a fine particle size using a mortar and pestle that had been cleaned with 100% ethanol prior to each sample. Ground samples were weighed into tin foil capsules (0.2–0.3 mg per sample) in duplicate using a microbalance (Mettler Toledo). The capsules were sealed and rolled into a ball and placed into well caps in a microtiter tray until analysis. Stable isotope analysis was performed using a EuroVector Analyzer and a Micromass IsopPrime Continuous Flow Isotope Ratio Mass Spectrometer (Micromass, Manchester, UK). The ratio of heavy to light isotope is expressed as delta (δ) values in parts per mil deviation from standard reference materials (atmospheric N and PeeDee belemnite C) using the following equation:

\[ \delta X = \left( \frac{R_{\text{sample}}}{R_{\text{std}}} - 1 \right) \times 1000 \]  

(1)

where X represents N or C, the superscript H denotes the heavy isotope mass (15N, 13C) and \( R_{\text{sample}} \) and \( R_{\text{std}} \) are the ratios of the heavy to light isotope in the sample and standard respectively.

2.4. Water analyses

In the seasonal study, dissolved organic carbon (DOC), pH and major cations were measured on four separate occasions: June, August, September, October 2008, at the same time as mussels were sampled. The DOC varied little during this period (ranged from 6.1 mg L\(^{-1}\) to 7.9 mg L\(^{-1}\)). Due to this low seasonal variation in DOC during the summer months, DOC (in addition to major cations and pH) was measured once in the temporal study, at the time of zebra mussel collection. The pH was measured at the sampling location with a portable pH meter (IQ Scientific Instruments). Water samples to be analyzed for DOC and cations were filtered on site through a 0.45 μm filter. Water samples were stored cold until analysis. Cation concentrations measured by ion chromatography ( Dionex DX 500) and organic carbon by a total organic carbon analyzer (Schimazu, TOC-Vcph Total Organic Carbon Analyzer).

2.5. Statistical analysis

All data in figures are presented as mean ± standard error of the mean. The relationship between Hg concentrations and tissue mass was examined initially with a scatterplot, followed by a simple linear regression model. Similarly, a linear regression model was fit to data examining the relationship between Hg concentrations and lake DOC concentrations. Significant temporal variation in Hg concentrations, δ13C and δ15N of zebra mussels were analyzed with a one-way ANOVA, and a posteriori multiple comparisons were examined with the Tukey-Kramer HSD test (p < 0.05).

3. Results

The lakes included in our study all had high dissolved calcium concentrations and high pH values typical of lakes that have large zebra mussel colonies (Table 1) (Ramcharan et al., 1992). Dissolved organic carbon concentrations ranged from approximately 4 to 7.5 mg L\(^{-1}\) (Table 1).

In the spatial study, we were unable to collect zebra mussels of the same size from all sites and consequently zebra mussel samples varied in mass from less than 5 mg to just over 20 mg (dry tissue mass). Due to this size variation, we first had to determine if size influenced Hg concentrations, and indeed a significant negative relationship was found between Hg concentrations and dry mass of the tissue (\( r^2 = 0.35, p = 0.03; \) Fig. 2). Therefore, to avoid the confounding effect of mussel size on Hg concentrations we normalized Hg concentrations to a standard zebra mussel mass. We used an average mass of 12.9 mg, calculated from all the zebra mussels collected from the spatial study.

In the seasonal study, similar sized zebra mussels were collected (average shell length was 17 ± 3 mm (mean ± std dev)). Significant seasonal variation was observed in both the δ13C and δ15N signatures and the isotopic signatures also varied in a similar manner (p < 0.05; Fig. 4). The δ15N ratios showed a trend of becoming progressively more depleted in mid-summer, followed by progressive enrichment in the fall; ranging from approximately 6% in July to 8% in September (Fig. 4a). The δ13C signature varied approximately 3% during the year ranging from the most depleted value being observed in June followed by progressive enrichment of the δ13C value until maximal values being observed in September and October (Fig. 4b).
Hg concentrations were highest during the early summer months, and progressively decreased in late summer and into the fall and winter months. These seasonal changes in Hg concentrations occurred during a time when the $\delta^{13}$C signature was becoming progressively enriched (see arrows, Fig. 4b), suggesting that differences in C source were influencing Hg concentrations. However, in addition to variation in dietary C sources, lipid concentrations can also influence the $\delta^{13}$C signature as lipids produce more depleted $\delta^{13}$C values (DeNiro and Epstein, 1977). Due to the high C content of lipid, the C/N ratio of the sample is linearly correlated to the lipid content and therefore the C/N ratio can be used as a proxy for the lipid content. In our study, we observed an obvious and significant seasonal trend in the C/N ratio of zebra mussels ($p \leq 0.05$; Fig. 5b). The C/N ratio decreased progressively until late summer, after which the C/N ratio started to increase into the fall and winter months.

One way to avoid the confounding effects that lipids have on the $\delta^{13}$C signature is to mathematically correct for lipids. We used the approach proposed by Post et al. (2007) to correct for seasonal variations in the lipid content of the zebra mussels. These corrections produced $\delta^{13}$C values that followed a similar trend to the uncorrected $\delta^{13}$C values (Fig. 6), suggesting that other factors such as diet variability was also influencing the $\delta^{13}$C signature.

4. Discussion

4.1. The relationship between Hg concentrations and body size

We observed a significant negative relationship between Hg concentrations in zebra mussels sampled from the gradient of lakes. It is important to note that although we are comparing Hg accumulation versus size for mussels living in different lakes, Hg loading to these lakes is similar; therefore differences in environmental Hg concentrations in the various study lakes would likely not be the cause of this relationship. In support of this contention, Hg concentrations in seston, an indicator of Hg concentrations in...
the water column, did not vary significantly with Hg concentrations in the zebra mussels sampled from the study lakes. It is important to note that seston collected was in the size range of 33–102 μm, and therefore it was not meant to be a measure of Hg in zebra mussel diet, but rather an indication of Hg in the water column as evidence that the lakes had similar Hg loadings, therefore we did not expect a significant relationship between Hg concentrations in seston and zebra mussels. A negative relationship between Hg concentrations and zebra mussel size has also been previously reported (Wiesner et al., 2001; Carrasco et al., 2008). As Hg concentrations would be expected to generally increase with mussel age and size, we suggest that differences in growth rates between the different lakes might explain, at least in part the relationship we observed between size and Hg concentrations. For example, growth dilution of Hg would occur if mussels were growing at a rate faster than the rate of Hg accumulation.

4.2. The influence of DOC on Hg concentrations in zebra mussels

In order to correct for this potential effect of size on Hg accumulation, we examined the variation in size-standardized Hg concentrations in zebra mussels as a function of DOC. Our results showed that Hg concentrations in zebra mussels were negatively correlated to DOC concentrations in our study lakes. These results contrast with a previous study that showed that zebra mussels demonstrated increased Hg absorption by 3.6 fold in the presence of high molecular weight DOC (Roditi et al., 2000). However, those researchers conducted a controlled laboratory study in which zebra mussels were directly fed DOC. Zebra mussels can filter a range of particle sizes from the water column, and under certain food-limiting conditions, they can even utilize DOC and DOM as a carbon source (Horgan and Mills, 1997; Roditi et al., 2000). In natural waters DOC and DOM could contribute a significant amount of the zebra mussel’s carbon demand, especially where large mussel colonies exist and phytoplankton densities are low. Due to the ability of DOM to complex certain metals such as Hg, this association between DOM and Hg absorption by zebra mussels is the result of the mussels assimilating Hg bound to DOM. A similar positive relationship between DOC/DOM and Hg accumulation has also been reported in several other species including aquatic invertebrates (Carvalho et al., 1999; Rennie et al., 2005; Harding et al., 2006).

An increase in Hg accumulation due to the intake of Hg-bound DOC occurs if the animal directly relies on DOC as a source of energy (i.e., the animal ingests and assimilates DOC–Hg complexes). However, reduced Hg accumulation in the presence of high DOC concentrations has also been reported for different species of algae (Moye et al., 2002; Gorski et al., 2006, 2008), and bivalves (Guo et al., 2001; Pan and Wang, 2004). These results are consistent with the free ion activity model that predicts that the uncomplexed free ion is the form most readily taken up by aquatic biota. Due to the strong binding affinity that mercury has for thiol groups found in DOC, DOC–Hg complexes are the dominant species of mercury found in oxic fresh waters (Hintelmann et al., 1997). It is important to note that pH has also been shown to affect Hg accumulation, where lakes with lower pH have higher levels of Hg bioaccumulation (Rennie et al., 2005). The pH in our study lakes was slightly basic, and did not have an effect on Hg levels in zebra mussels (linear regression, p > 0.05).

When considering the role that DOC plays in controlling Hg availability to zebra mussels, there are two possible scenarios depending on the route of exposure: (1) DOC–Hg complexes are
directly assimilated by zebra mussels (i.e., the DOC–Hg complex is eaten by the zebra mussel) and (2) DOC–Hg complexes are not bioavailable to zebra mussels (i.e., the DOC–Hg complex is not taken up directly via the aqueous route of exposure). There is no evidence that the first scenario occurred in our study lakes as lakes with high DOC concentrations had the lowest Hg levels. The second scenario would result in low Hg levels in mussels from lakes with high DOC, which was indeed what we observed. However, this would mean that zebra mussels would be accumulating large amounts of their total Hg burden from aqueous routes of exposure. It is more likely that zebra mussels are receiving Hg from their diet (i.e., phytoplankton). As the inverse relationship between DOC and Hg levels in algae has been widely reported (Moye et al., 2002; Gorski et al., 2008; Zhong and Wang, 2009). Although we did not see any relationship between seston (33–102 μm) collected from the lakes and Hg concentrations in zebra mussels, microscope examination of the samples revealed that they were composed of detritus and small particles, which are likely not representative of what mussels were eating (note that our objective was to use seston as an indicator of Hg in the water column as a description of Hg concentrations in the lakes, not as an indicator of Hg concentrations in mussel diet). We speculate that the negative correlation observed between DOC and Hg levels in zebra mussels in our study is in fact indirect and was linked to less Hg accumulating in algae in lakes with high DOC.

It is important to note that the DOC concentrations in our study lakes only varied by a factor of 2, and therefore our results should be interpreted within these restrictions. As high DOC lakes tend to have slightly acidic pH values, and zebra mussels are typically found in lakes with an alkaline pH, we could not find lakes that increased our DOC gradient, and also contained zebra mussels.

4.3. Seasonal variation in Hg concentrations

Seasonal variation in total Hg concentrations measured in zebra mussels in this study ranged from approximately 50 ng g\(^{-1}\) dw to 100 ng g\(^{-1}\) dw. These values are similar to THg concentrations in zebra mussels collected from a number of unpolluted sites around the world (Camusso et al., 2001; Wiesner et al., 2001; Lowe and Day, 2002; Kwan et al., 2003; Richman and Somers, 2005). By comparison, average THg measured in zebra mussels collected from industrial sites in the Ebro River in Spain were as high as 6.81 μg g\(^{-1}\) dw, 70–140 times higher than THg concentrations observed in our study (Carrasco et al., 2008).

Higher Hg concentrations were observed in zebra mussels during the summer months than in the fall and winter months. A similar trend in Hg concentrations was observed by Kwan et al. (2003), where Hg concentrations were highest in June to August and then decreased in September and in September to November (Kwan et al., 2003). In a recent study, Zhang et al. (2012) reported that mercury concentrations in several fish and invertebrates species sampled from the littoral zone of eastern Lake Ontario were also highest during the spring and lowest in the summer months. Mercury concentrations in the caddisfly (Hydropsyche morose) found to be highest during the summer months, which these authors attributed to a dietary shifts from algae to pre-dominately seston (Synder and Hendricks, 1995).

4.4. The influence of seasonal dietary changes on Hg levels in zebra mussels

Seasonal changes in phytoplankton biomass and species composition mean that zebra mussels will encounter a variable diet over the year. Studies have shown that zebra mussels are selective grazers, in some cases showing preference for some algal species such as cryptophytes and an avoidance of other species such as cyanobacteria (Naddafi et al., 2007), while other studies have shown that they prefer cyanobacterial species (Pires and Van Donk, 2002). There may also be an effect of season on how selective zebra mussels are. For example, Naddafi et al. (2007) found that there was no food selectivity observed in zebra mussels in months when there were low algal concentrations. In addition to food quantity, the food quality will also vary seasonally, with low food quality cyanobacteria typically occurring in the summer months and high food quality species such as diatoms occurring in the spring and fall months (Ahlgren et al., 1997; Goedkoop et al., 2000). In addition, while phytoplankton are generally preferred as a dietary choice, zebra mussels have also been shown to filter clay particles and detritus (Baker et al., 1998).

As food quality and quantity will vary seasonally, this may also result in variations in dietary Hg exposure. Phytoplankton density was negatively correlated to Hg concentrations in phytoplankton, as well as herbivorous zooplankton (Chen and Folt, 2005). This negative relationship between algal blooms and Hg concentrations in lower trophic species has been reported in other studies (Pickhardt et al., 2002, 2005); however, no effect of phytoplankton biomass on Hg levels in phytoplankton has also been reported (Kirkwood et al., 1999). In addition, while MeHg has been shown to be preferentially accumulated by living particles, inorganic Hg readily complexes to non-living particles; thus, seasonal variation in the filtration of these different carbon sources may also influence Hg exposure. Variation in metal concentrations in pelagic, benthic and riparian zones also suggests that the relative differences of autochthonous and allochthonous carbon sources may influence Hg exposure. Variation in metal concentrations may also influence Hg exposure.

Variations in the abundances of the stable isotopes of nitrogen and carbon can be used as tools to examine the relative importance of autochthonous and allochthonous carbon sources in a consumer’s diet. Carbon isotope (δ\(^{13}\)C) fractionation can be used to distinguish different carbon sources at the base of aquatic food webs as littoral algae are typically enriched in δ\(^{13}\)C relative to planktonic algae (France, 1995; Vander Zanden et al., 1999). The carbon isotope signature of the consumer is similar (within < 1‰) to the carbon signature of its diet. The δ\(^{13}\)C signature has also been shown to vary not only among the different phytoplankton classes, but also the carbon signature of algae may be affected by different abiotic (e.g. salinity) and biotic (e.g. growth phase of the algae) factors (Brutemark et al., 2009). While nitrogen isotope (δ\(^{15}\)N) fractionation is typically used to describe the trophic level at which an animal feeds, there is variation in the nitrogen signature of different algal species (Hamilton and Lewis Jr., 1992). Seasonal variation in both carbon and nitrogen isotope ratios have been reported in particulate organic matter (POM) (Gu et al., 1994; McCusker et al., 1999; Syvaranta et al., 2008; Watanabe et al., 2008).

One of our objectives was to use variation in δ\(^{15}\)N and δ\(^{13}\)C signatures in zebra mussels as an indicator of seasonal variation in diet; however, it is important to note that δ\(^{15}\)N and δ\(^{13}\)C values could be affected by factors other than diet variability. For example, lipid concentrations can influence the δ\(^{13}\)C signature as lipids produce a more depleted δ\(^{13}\)C signature (DeNiro and Epstein, 1977). Due to the high C content of lipid, the C/N ratio of the sample is linearly correlated to the lipid content and therefore the C/N ratio can be used as a proxy for the lipid content. The C/N ratio of zebra mussels in our study shows a distinct seasonal trend with decreasing values occurring up until mid-summer, and then the values start to increase in fall and winter. As the C/N ratio progressively decreased, the δ\(^{13}\)C ratio becomes progressively more enriched (see arrows, Figs. 4b and 5b). Similar seasonal variation in the C/N ratio corresponding to a reduction in lipid stores has been observed in zebra mussels following variations...
spawning in the spring months (Nalepa et al., 1993). Furthermore, lipid content and the C/N ratio of zebra mussels in this previous study showed the same trends, indicating that the C/N ratio is a good proxy for lipids in this species.

Spawning can also influence the δ13C signature due to protein metabolism that occurs during this period (Doucett et al., 1999). Our results for zebra mussels showed that both the δ13N and the δ13C signatures varied significantly over the year in a similar manner with more depleted values occurring mid-summer and more enriched values occurring in the fall. Therefore, there are two possible explanations for these observed seasonal trends in δ15N and δ13C ratios; (1) zebra mussel diet is changing seasonally or (2) seasonal variation in the zebra mussel’s energy stores. Since we did not collect putative food sources for zebra mussels over the year, we cannot rule out the possibility that these seasonal trends in δ15N and δ13C could be explained, at least in part, by factors other than dietary variability, such as variations in the lipid content and energy stores of zebra mussels.

To avoid the confounding effects of lipid content on the δ13C signature some researchers normalize their data for lipid content either by chemically extracting the lipid fraction (DeNiro and Epstein, 1977), or by using a mathematical correction (Power et al., 2002; McIntyre et al., 2006). As the chemical extraction method (e.g. using a methanol–chloroform solution) can affect the δ13N signature (Sweeting et al., 2006), mathematical normalization is often preferred. However, these lipid correction models can also be quite variable in their predictive ability (Boecklen et al., 2011). Most mathematical corrections work on the premise that due to the high C content of lipid, the C/N ratio of the sample is linearly correlated to the lipid content and therefore the C/N ratio can be used as a proxy for the lipid content. We used the linear lipid correction model proposed by Post et al. (2007) as this approach is considered most effective when lipid contents are relatively low (i.e. 2 < C/N < 10) (Boecklen et al., 2011). If seasonal variations in the δ13C values were solely the result of variations in lipid content, we would expect the seasonal trends in the δ13C values to become insignificant once the data have been lipid-corrected. However, this was not the case as we found that the seasonal variation of δ13C values was similar between the corrected and non-corrected values. This suggests that some of the variation in the δ13C could be attributed to variation in other environmental factors such as diet. The seasonal variation in δ13C values suggests that zebra mussels may be relying on more pelagic carbon sources in the summer months (i.e. more depleted δ13C signature) and more littoral carbon sources in the fall and winter months (i.e. more enriched δ13C signature) (France, 1995).

Based on these results, it is difficult to say whether variation in (1) diet, (2) seasonal changes in body condition and energy stores, or (3) a combination of these two factors is causing the seasonal variation in Hg concentrations in zebra mussels in our study. For example, the reproductive cycle has previously been reported to influence Hg concentrations in two species of bivalves (Mytilus galloprovincialis and Ostrea edulis), likely due to changes in mussel condition (Najdek and Sapunar, 1987). Maximum weight-per-shell-length was observed in zebra mussels sampled from the St. Clair River in the spring followed by decreases in the summer (Nalepa et al., 1993). Garton and Haag, 1993 found two periods of weight loss in zebra mussels sampled from western Lake Erie: late spring (corresponding to low phytoplankton abundance) and summer (corresponding to spawning). Spawning typically occurs in late spring and summer when water temperatures reach approximately 15 °C (Mackie, 1991). Swanson et al. (2011) found a negative relationship between Hg concentrations in arctic char (Salvelinus alpinus) and the C/N ratio. These results are consistent with research that indicates that Hg binds to sulfur groups of proteins and not lipids (Mason et al., 1995). The C/N ratio provides an indication of energy reserves as tissue nitrogen content is related to tissue protein and therefore a decline in the C/N ratio would indicate a loss of nonproteinaceous energy reserves (i.e. lipids and carbohydrates). We therefore speculate that this loss of reserves is causing Hg concentrations to increase in the spring and summer months.

In addition to variations in lipid content, previous studies have also shown a relationship between carbon source and Hg concentrations. For example, an inverse relationship between δ13C signatures and Hg concentrations in fish was reported by Power et al. (2002) leading these authors to conclude that species connected to the benthic algal food chain had lower Hg concentrations than species connected to the pelagic algal food chain. Stewart et al. (2008) also found slightly higher MeHg concentrations in pelagic foodwebs than in benthic foodwebs. Watanabe et al. (2008) reported that autochthonous carbon sources had higher metal concentrations than allochthonous carbon sources, leading these authors to hypothesize that macroinvertebrates highly reliant on autochthonous carbon would have higher metal concentrations. However, their study concluded that the relative contribution of autochthonous and allochthonous carbon sources to aquatic macroinvertebrates was not predictive of their metal concentration.

Although we have looked for relationships between Hg accumulation in zebra mussels and their stable isotope signature, the turnover rates of Hg and stable isotopes may be very different in zebra mussels. We have looked at total Hg in zebra mussels, and approximately 60% of the total Hg is methyl mercury (L. Kraemer, unpublished results). Methyl mercury has a high assimilation efficiency and a low elimination rate (Trudel and Rasmussen, 1997). Therefore it is likely that the turnover rate of total Hg in zebra mussels is much slower than the turnover rate of stable isotopes of carbon and nitrogen. However, there is little known about the turnover rates of stable isotopes in mussels living in natural environments (Dubois et al., 2007).

5. Conclusions

Our two hypotheses were: (1) that DOC concentrations would be related to Hg concentrations in zebra mussels and (2) that seasonal dietary changes would be related to Hg concentrations in zebra mussels sampled monthly from the same site. We found that DOC concentrations were negatively related to Hg concentrations in zebra mussels sampled from a series of lakes, likely the result of less Hg taken up in phytoplankton in lakes with higher DOC concentrations. We did not find any conclusive evidence to support our second hypothesis that only seasonal dietary changes affected Hg concentrations, but rather in addition to possible seasonal dietary changes, seasonal variation in energy stores also influences Hg concentrations. Collectively, results from this study suggest that DOC concentrations, seasonal variation in diet and seasonal variation in energy stores are all important variables to consider when understanding Hg accumulation in zebra mussels.

References


