Influence of feeding ecology on mercury accumulation in turtles and fish of the Rideau Canal, Ontario, Canada.

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ABSTRACT

Pollution is a major cause of biodiversity declines worldwide. Therefore, understanding exposure and uptake mechanisms for contaminants such as mercury (Hg) is a crucial step in our efforts to understand the causes of species decline. I investigated the influence of dietary reliance on the benthic food chain, and the influence of the proportion of zebra mussels in the diet, on the accumulation of Hg in freshwater fish and turtle species. I collected turtle blood samples and fish muscle samples in 2012 and analyzed these tissue samples for carbon and nitrogen isotope ratios ($\delta^{13}C$ and $\delta^{15}N$), and for Hg concentrations. Isotopic ratios were used to calculate trophic level, dietary reliance on the benthic food chain, and the proportion of zebra mussels in the diet. Reliance on the benthic food chain was a good predictor of Hg concentration in fish muscle, but not in turtle blood. The proportion of zebra mussels in the diet was not a good predictor of Hg in turtles or in fish. My results indicate that dietary reliance on the benthos should be considered in future tissue Hg modelling studies for fish, and that this predictor variable could be used to identify other fish species likely to be burdened by high concentrations of Hg.

RÉSUMÉ

La pollution est une des causes principales du déclin de la biodiversité dans le monde. Par conséquent, une bonne compréhension des mécanismes d'exposition et d'absorption des contaminants tels que le mercure (Hg) est une étape importante dans nos efforts pour comprendre la hausse du taux d'extinction associée à la pollution. J'ai étudié l'influence de la dépendance de la diète à la chaîne alimentaire benthique, et l'influence de la proportion de moules zébrées dans la diète, sur l'accumulation de Hg chez les poissons et les tortues d'eau douce. J'ai recueilli des échantillons de sang de tortue et de muscle de poisson en 2012. J'ai ensuite analysé ces échantillons de tissus pour obtenir les ratios d'isotopes d'azote et de carbone ($\delta^{13}C$ et $\delta^{15}N$) et la concentration de Hg. J'ai utilisé les ratios isotopiques pour calculer le niveau trophique, la dépendance de la diète à la chaîne alimentaire benthique et la proportion des moules zébrées dans la diète. Mes résultats démontrent que la dépendance de la diète à la chaîne alimentaire benthique est un bon prédicteur de la concentration de Hg dans le muscle de poisson, mais pas dans le sang de tortue. La proportion des moules zébrées dans la diète n'était pas un bon prédicteur de la concentration en Hg chez les tortues ou les poissons. Mes résultats indiquent que la dépendance alimentaire sur le benthos est une variable qui devrait être considérée dans les études futures de modélisation chez les poissons. De plus, cette variable pourrait être utilisée pour identifier les espèces de poissons susceptibles d'être affectés par des concentrations élevées de Hg.

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GENERAL INTRODUCTION

Biodiversity loss is a current global concern. Numerous factors are thought to contribute to biodiversity loss, but the most important threats are habitat destruction, climate change, pollution, invasion by non-native species, and increases in atmospheric carbon dioxide (CO_2) (Dudgeon et al., 2006; Sala et al., 2000; Strayer and Dudgeon, 2010; Wilcove et al., 1998). Pollution ranks as the third most important threat to terrestrial species and as the second most important threat to aquatic species (Richter et al., 1997; Wilcove et al., 1998). Globally, over 50% of threatened freshwater vertebrate species are affected by pollution (Collen et al., 2013). Because biodiversity losses are observed in terrestrial, freshwater, and marine biomes (Burkhead, 2012; Gray, 1997; Payne et al., 2013), pollution can undoubtedly be considered a conservation and ecosystem health issue of capital importance.

Heavy metals are a large class of environmental pollutants and, with other common pollutants such as dioxins and Polychlorinated biphenols (PCBs), they constitute a chemical mixture that seeps into water bodies and sediments and that causes adverse health effects (Järup, 2003). The effects to such chemical mixtures in the wild are often difficult to quantify because toxicants can have combined effects (Pietrock and Marcogliese, 2003). Even though heavy metals occur naturally in the Earth's crust, they qualify as pollutants because they can be anthropogenically introduced in the environment in concentrations that exceed background levels, and because they can cause negative health effects. Whereas some pollutants are biodegradable, heavy metals

are transported in the atmosphere, displaced by water currents, and deposited in soils and sediments where they can remain and accumulate indefinitely. Within heavy metals, lead (Pb), cadmium (Cd), and mercury (Hg) are some that have been studied extensively because they pose a threat to human health. For centuries, these three heavy metals have been used for various purposes and released into the environment. For instance, Pb piping was once used to transport water. Although Pb emissions are generally decreasing worldwide (Pacyna and Pacyna, 2001), remnants of Pb piping can still be found in older constructions and infrastructures (Hodge, 1981). On the contrary, Cd emissions have greatly increased in the 20th century, mainly because Cd-containing items such as re-chargeable nickel (Ni) – Cd batteries are rarely re-cycled (Hellström et al., 2007). Cigarettes are the main source of Cd exposure for tobacco smokers (Satarug and Moore, 2004). Similarly, various human enterprises have accelerated the release of Hg into the environment. Global anthropogenic emissions of Hg come primarily from the combustion of fossil fuels in industrial and residential boilers and gold mining (Pacyna et al., 2006), whereas Hg-releasing natural processes include volcanic eruptions and forest fires (Nriagu, 1989). Historically in Canada, electricity generation, waste incineration, and non-ferrous mining and smelting constituted the main sources of atmospheric Hg. Recently, however, a Risk Management Strategy for Mercury was implemented after Hg was declared a toxic substance under the Canadian Environmental Protection Act of 1999; Canadian emissions have been significantly reduced since then. In the early 1990's, for example, non-ferrous mining and smelting released over 26 000 kg of Hg into the atmosphere annually whereas presently only about 210 kg are released annually from this industrial activity (Environment Canada, 2011). Although

Canada is working to reduce Hg emissions, other regions of the world are not. In 1995, for example, total North American emissions were estimated at 153 000 kg, whereas emissions from Asia were estimated at 1 281 000 kg (UNEP 2013). Since Hg can be disseminated from its emission point source in the atmosphere through a process called global distillation, high concentrations of Hg can be found in remote regions of the world. Consequently, decreasing Hg emissions in Canada may only offset the increase in emissions in other parts of the world (UNEP 2008). In fact, Hg is ubiquitous in the atmosphere and the rate at which is it deposited in the soils and sediments is thought to be a function of elevation, land cover, and proximity to urban areas (Miller et al., 2005). Although Hg liberated from the weathering of rock formations contributes to sediment and soil Hg concentrations, most of the Hg found in rural and remote sites comes from atmospheric transport and deposition of global anthropogenic emissions (Pacyna et al., 2010; Thomas, 1972).

In aquatic ecosystems, Hg is found in the sediments, in the water column, and in the biota. Fugacity-based mass-balance models suggest that sediments are most often its major repository (Chon et al., 2012). Three forms of Hg are typically found in these aquatic environments: metallic Hg (Hg^0), divalent Hg (Hg^{2+}), and methylmercury (MeHg). Together, these three forms are referred to as total Hg (THg). Recent studies focus on the presence and accumulation of MeHg in aquatic ecosystems because this form accumulates in animal tissues (Barrocas et al., 2010), and because it can cause adverse health effects (Eisler 1987, Scheuhammer et al., 2007). Figure 1 reviews Hg transformations and depicts the occurrence of the three forms within a lake ecosystem.

The rates at which Hg cycles between its different forms depend on several chemical and physical environmental factors. As a result, the concentration of bioavailable MeHg can vary within water bodies, even over very short distances. Water turbidity, for example, can increase physical transport and abundance of Hg in the water column by re-suspending the surface layer of deposited sediment particles (Gray et al., 2002). In addition, low pH can cause an increase in the rate at which Hg in the sediments is rendered bioavailable in a specific location (Kelly et al., 2003). Another example of a factor that plays a role in determining the rate of methylation of Hg is the amount of dissolved organic carbon (DOC) in the water column (Driscoll et al., 1995). DOC is created when water makes contact with organic soils causing organic compounds to leach into the water body. DOC plays a dual role in this context as it can either increase or decrease the amount of Hg that is rendered bioavailable through methylation. For instance, there is a positive correlation between Hg and DOC concentrations in water because Hg in the soil and sediments binds to organic materials that are carried away with the water in turbid areas. In addition, DOC reduces the amount of ultraviolet light penetrating the water column (Morris et al., 1995). Reducing ultraviolet light penetration inhibits the reduction of Hg^{2+} to Hg^{0}, thereby increasing the amount of Hg^{2+} available for methylation. Organic matter also serves as carbon source for bacteria that can add a methyl group to the metal so these can remain and thrive in areas where DOC is present (Bisinoti et al., 2007). On the other hand, DOC can bind MeHg, thereby limiting its bioavailability in the water column and in the surface sediments (Driscoll et al., 1995). Still, the processes by which these factors interact with one another and with other lake parameters remain poorly understood. Nevertheless, these environmental

factors have often been used to explain within-lake and between-lake differences in THg concentrations (Bloom et al., 1991; Burger et al., 2004; Kamman et al., 2005; Riget et al., 2007).

Regardless of its concentration, bioavailable MeHg will eventually enter the food web. In lake ecosystems, MeHg enters the food web through micro-organisms who assimilate it directly from the water. For example, primary producers bioaccumulate MeHg by sequestering it in their cells and, as a result, their internal MeHg concentration comes to exceed that of the surrounding environment. MeHg has restricted lipophilic properties and a strong affinity for some proteins such as sulfur-containing amino acids (Carty and Malone, 1979). Thus, in consumers, MeHg tends to be found in muscles and fat tissues. MeHg biomagnifies up food chains because of the tendency of primary producers and consumers to accumulate MeHg. Therefore, animals at the top of the food web tend to have higher concentrations of MeHg in their tissues when compared to those feeding at lower trophic levels (Boudou and Ribeyre, 1997).

Once incorporated in animal tissues, MeHg causes biochemical, physiological, and neurological effects. The presence and intensity of these biological effects depend on the MeHg dose-response relationship and on the particular organism. In laboratory tests, freshwater fish vital functions such as reproduction, osmoregulation, foraging activities, anti-predator behaviours, and communication may be disrupted as a result of the accumulation of high concentrations of MeHg (Zillioux et al., 1993). These negative effects included impaired spermatogenesis and reduced egg deposition in fish exposed to concentrations ranging from 0.1 to 1.0 µg THg/L, and impaired spermatogenesis and

decreased intestinal nutrient transport in fish exposed to concentrations ranging from 1.0 to 2.0 µg THg/L. In amphibians, exposure to 1.0 µg THg/L was related to decreased rates of successful metamorphosis and high embryo mortality (Eisler, 1987). More recent evidence reports similar findings, along with additional biochemical, genetic, and histological effects possible at concentrations of 0.1 µg Hg/g (Figure 2 and references therein). Still, most documented health effects of MeHg are the consequence of exposure to levels of MeHg that exceed environmental concentrations (Fimreite, 1974; Kamman et al., 2005; Monteiro and Furness, 2001), which typically range from 0.0001 to 0.5 µg Hg/L in north-American freshwater bodies (Mousavi et al., 2011).

Instead of examining for the health effects of MeHg in natural populations, the majority of studies focus on Hg distribution by trophic level. Initially, information from gut content analysis and inferred feeding habits were used to estimate trophic level (e.g. Nriagu, 1989; Richter et al., 1997; Wilson, 1992). These methods had limitations since they provided no effective way of characterizing complex food webs, and because they only offered information on recent dietary intakes. Because these methods were not very precise and only reflected recent dietary intake, opposing conclusions were reached by different researchers: some were able to detect a correlation between trophic level and tissue Hg concentration (e.g. Mason et al., 1996) while others found no such relationship (e.g. Williams and Weiss, 1973). A more recent technique than gut content analysis involving stable isotope ratios offers an alternative way to quantify trophic position. With this technique, a more precise characterization of contaminant transfer in food webs is possible. The stable nitrogen (N) isotopes are useful because ^{15}N predictably increases in

abundance relative to ^{14}N with each trophic transfer (Cabana and Rasmussen, 1996). Therefore, depending on the turnover rate of the tissue used for isotope analysis, the trophic level can be estimated over several seasons. Following the adoption of this technique for quantifying trophic position, the positive correlation between trophic level and tissue MeHg concentration became apparent in the literature, and comparing biomagnification rates became possible (e.g. Bergeron et al., 2007; Campbell et al., 2008; Campbell et al., 2005; Chen et al., 2008). One way to compare biomagnification rates across food chains is to plot the linear relationships between the logarithm of MeHg concentration (log μg MeHg/g in the tissue, wet weight) and $\delta^{15}N$ values, and then to use the regression slope as a measure of the rate of biomagnification (Kidd et al., 1995). In this graphical representation, the y-axis intercept can be thought of as a baseline input of the pollutant at the level of the primary producers. Using this method, a range of biomagnification rates for MeHg have been calculated for various food webs and these usually fall between 0.1 - 0.3 (Chen et al., 2008; Dennis et al., 2005). The mechanisms underlying the variation in tissue burdens and in biomagnification rates for MeHg are unknown, but they may be related to differences in the number of trophic levels used in the calculation of the rate (Atwell et al., 1998), to the seasonally variable food web structure (Zhang et al., 2012), or to the presence or absence of invasive species at the study sites (Hogan et al., 2007).

Overall, only a small proportion of studies focus on factors, other than trophic level, that may affect MeHg distribution in the food web. In fact, the United Nations Environment Programme (UNEP) (2013) stated in their most recent report that there is a major gap in our ability to predict MeHg uptake by living organisms, and that this gap warrants further

investigation. Therefore, there is a need to be able to forecast which species are likely to be burdened with high concentrations of MeHg with some level of confidence, especially for species at risk. The goal of my thesis is to identify key components of animal feeding ecology that are likely to influence MeHg accumulation, and to determine how these components influence MeHg accumulation. Furthermore, I want to create models capable of predicting MeHg concentrations in the tissues of freshwater species of fish and turtles.

In chapter one, I attempt to determine whether the MeHg load of freshwater species is a function of the horizontal food web structure, i.e., the dietary reliance on the benthic food web versus the pelagic food web. Since the highest concentration of all forms of Hg (THg) in lakes is usually found in the sediments, and since species vary in their accumulation of MeHg, I test the hypothesis that the variation in the amount of MeHg accumulated is a function of the proximity to the benthos in the food web. I use N and C isotope ratios to quantify the proportion of the diet that is tightly linked to the benthic food chain and compare it to animal tissue MeHg burdens using a Bayesian mixing-model.

In chapter two, I investigate how the zebra mussel (*Dreissena polymorpha*), an invasive bivalve, influences the trophic transfer of MeHg to lake predators. Zebra mussels filter large volumes of water while feeding compared to other freshwater filter-feeding mollusks (Strayer et al., 1999). Zebra mussels therefore accumulate more MeHg than other freshwater bivalves (Figure 2-1). Since their accidental introduction in North American water bodies, some native predatory species have changed their diet to include this new prey item (Bulté and Blouin-Demers, 2008; Molloy et al., 1997). Because freshwater vertebrate predators vary in their consumption of zebra mussels, I test the hypothesis that

the amount of MeHg accumulated in freshwater vertebrates that feed on zebra mussels is a function of the proportion of zebra mussels in their diet. Predators that feed heavily on zebra mussels should have a heavier MeHg burden.

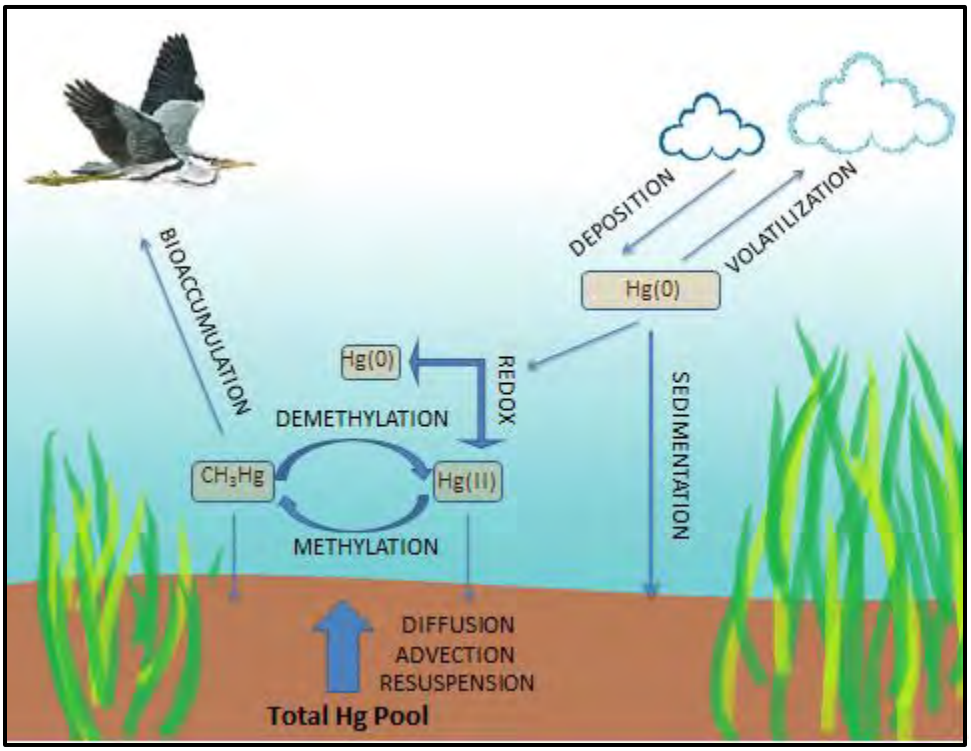

Figure 1. The generalized Hg cycle in freshwater ecosystems: Hg from the atmosphere comes primarily in the metallic form (Hg^0). In an aquatic system, Hg^0 is either deposited, or it is oxidized to the divalent form (Hg^{2+}). In turn, Hg^{2+} can be deposited into the sediments or rendered bioavailable by bacteria who add one or two methyl groups to the ion to form methylmercury (MeHg). These transformations are also reversible so that Hg^{2+} can be reduced to Hg^0, and MeHg can be demethylated. MeHg can be incorporated in the sediments or it can enter the food web. Once in the food web, MeHg bioaccumulates and biomagnifies so top predators are likely to have high concentrations of MeHg in their tissues.

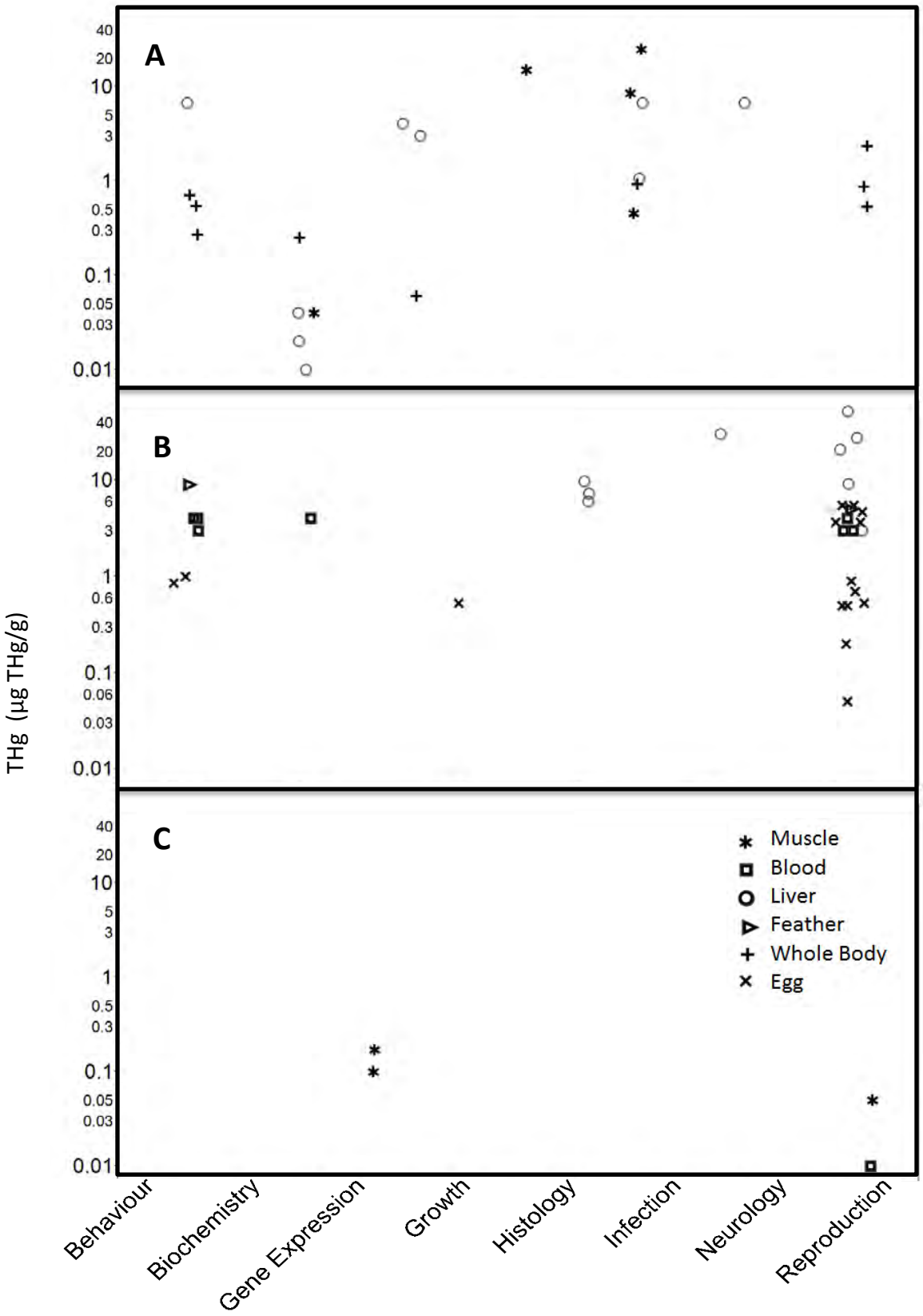

Figure 2. Concentration of mercury (µg THg/g) in various types of animal tissues and associated effects on biochemistry, gene expression, behaviour, reproduction, histology, growth, and infection in fish (A), birds (B), and turtles (C). Values are either mean effects concentrations or minimum concentrations at which effects were observed. THg concentration values are as cited in Scheuhammer et al. (2007), Burger et al. (1997), and Wolfe et al. (1998) for birds, in Sandheinrich and Wiener (2011) for fish, and from Hopkins et al. (2013) and Meyer-Schöne (1993) for turtles. The x-axis is log-transformed to facilitate visualization.

CHAPTER 1: DIETARY RELIANCE ON THE BENTHIC FOOD CHAIN INFLUENCES MERCURY ACCUMULATION IN FRESHWATER TURTLES AND FISH

ABSTRACT

Differences in feeding ecology are known to affect the accumulation of contaminants such as mercury (Hg) in aquatic animals. Modelling the accumulation of Hg in animals can help identify which animals are likely to accumulate high concentrations of Hg. Since most of the Hg in lakes is found in the sediments, I predicted that Hg burden should increase with dietary reliance on the benthic food chain. I created averaged multiple linear regression models to predict Hg burdens in fish and turtles species from their dietary reliance on the benthic food chain, while controlling for other factors known to influence Hg accumulation (sex, size, location, and trophic level) using training and testing sets. There was a positive relationship between Hg burdens and dietary reliance on the benthic food chain for both fish and turtles. For turtles, however, the averaged models explained only a small portion of the observed variation in tissue Hg concentration, with R^2 values ranging between -0.05 and 0.74. For fish, a larger proportion of the variation was explained by the models, with R^2 values ranging between 0.50 and 0.77. The results indicate that the reliance on the benthic food chain is an important Hg predicting variable for fish, but not as much for turtles. In addition, model generalization to independent data sets is a possibility for the fish models. Future attempts to model Hg accumulation in fish should include dietary reliance on the benthic food chain as a predictor variable.

INTRODUCTION

Pollution from mercury (Hg) is a global concern. Various industrial activities release Hg into the environment where it disperses around the globe by atmospheric transport. The most important source of Hg in the environment is the burning of fossil fuels and the industrial mining and smelting of metals (UNEP, 2013). In Canada, Hg was declared a toxic substance under the Canadian Environmental Protection Act of 1999 and emissions decreased significantly after a Risk Management Strategy for Mercury was implemented (Environment Canada, 2011). In the early 1990's, for example, non-ferrous mining and smelting released over 26 000 kg of Hg into the atmosphere annually whereas presently only about 210 kg are released annually from this industrial activity in Canada (Environment Canada, 2011). Although Hg emissions rates in Canada have decreased in the past decades, rates in other parts of the world have increased (UNEP, 2013). Since Hg travels in the atmosphere to remote regions of the globe, and since it gets deposited in soils, water bodies, sediments, and biota, decreasing emission rates in Canada may only serve to offset emission increases in other parts of the world.

Once deposited from the atmosphere, Hg can be chemically transformed through biotic and abiotic processes. Recent studies focus on the presence and accumulation of methylmercury (MeHg) in aquatic ecosystems since this form accumulates in animal tissues (Barrocas et al. 2010), and since it can cause adverse health effects (Eisler 1987, Scheuhammer et al., 2007). The threshold level at which MeHg can cause adverse health effects (TEL), however, is not well defined in the literature. Sandheinrich and Wiener (2011)

conclude from reviewing recent findings that adverse health effects in fish are possible at muscle concentrations of 0.3–0.7 µg MeHg/g. In addition, others have recently reported detectable effects in fish with muscle tissue concentrations that were below 0.1 µg MeHg/g (Drevnick et al., 2008; Larose et al., 2008; Moran et al., 2007; Webb et al., 2006). Direct mortality has only been observed in systems subjected to extreme contamination where animal tissues had 6 to 20 µg Hg/g (Wiener and Spry, 1996). Even though MeHg is conventionally defined as a neurotoxin, these studies suggest that it could also cause alterations in gene transcription, histological changes, and increases in macrophage aggregates (Figure 2).

MeHg biomagnifies along food chains (Atwell et al., 1998; Boudou and Ribeyre, 1997; van der Velden et al., 2013). Therefore, top predator tissue concentrations often exceed the threshold effect level (Kamman et al., 2005), making health effects possible in wild animal populations. How MeHg is incorporated at the base of the pelagic and benthic food chains, and how it is distributed in the food web remains poorly understood. This makes it difficult to explain why MeHg concentration in animal tissues varies within species and between species (e.g. Bates and Hall, 2012; Burger et al., 2010; Coelho et al., 2006; van der Velden et al., 2013). For instance, variations in MeHg tissue concentrations between animals have been related to differences in the number of trophic levels present in an ecosystem (Atwell et al. 1998), to the seasonally variable food web structure (Zhang et al., 2012), and to the presence or absence of invasive species at the study sites (Hogan et al., 2007). Lake sediments are considered a major repository for all forms of Hg (Chon et al.,

2012). Overall, only a small proportion of studies focus on factors that determine which animals are likely to accumulate large concentrations of MeHg, other than trophic level.

Recently, stable isotope analyses have improved our understanding of food web structures. The stable nitrogen (N) isotopes are useful because ^{15}N predictably increases in abundance relative to ^{14}N with each trophic transfer (Cabana and Rasmussen, 1996). Therefore, the N stable isotope ratio ($\delta^{15}N$) offers a way to estimate trophic level averaged over long time intervals. Depending on the turnover rate of the tissue used for isotope analysis, the trophic level can also be estimated over several seasons. Similarly, the carbon isotope ratio ($\delta^{13}C$) provides information on which carbon (C) source is preferentially used. In aquatic ecosystems, pelagic primary producers have lower ratios than benthic primary producers. Because the $\delta^{13}C$ is defined at the level of the primary producer and maintained along food chains, a consumer's reliance on the benthic or pelagic food chains can be estimated. Together, $\delta^{13}C$ and $\delta^{15}N$ can be used to conceptualize a two-dimensional food web in which trophic level and reliance on the benthic food chain are the dimensions. Previous studies have combined data from stable isotope analyses and data on contaminants, but these typically focus on fish and birds (Anderson et al., 2009; Riget et al., 2007). Moreover, these studies typically use the raw $\delta^{15}N$ as a representation of the trophic level, and the raw $\delta^{13}C$ as a measure of carbon sources in the food web. However, $\delta^{13}C$ and $\delta^{15}N$ comparisons across systems are complicated by the intrinsic differences in isotopic ratios that exist at the base of the food chains within and between lakes (Cabana and Rasmussen, 1996). Therefore, existing studies cannot be used to make predictions in other locations and cannot be compared to one another.

In this chapter, I use $\delta^{15}N$, $\delta^{13}C$, and Bayesian mixing model analyses to examine the patterns of MeHg biomagnification in the fish and turtle community of three Rideau Canal lakes. I test the hypothesis that MeHg accumulation in these animals can be predicted from their position in the vertical and horizontal food web structure. Since most MeHg in a lake is usually found in or near the sediments (Chon et al., 2012), I predict that MeHg burden should increase with dietary reliance on the benthic food chain. To my knowledge, no previous study has employed a mixing model to eliminate the ambiguities associated with using raw isotope data to derive a predictive model for turtle and fish tissue MeHg concentrations.

METHODOLOGY

Study area — Sampling took place in eastern Ontario (Canada) on the Rideau Canal, a series of lakes, rivers, and human-made canals linking Ottawa to Kingston. I captured turtles, fish, and prey samples in Indian, Newboro, and Upper Rideau Lakes. Hg concentrations in the sediments of the three lakes ranged from 0.01 to 0.22 µg/g dry weight (LeBlond, 2009). In each lake, I trapped animals at two sites. The sites were located at 44° 34' 58.276" N, 6° 19' 33.837" W and 44° 36' 10.986" N, 76° 18' 33.479" W for Indian Lake, 44° 37' 46.178" N, 76° 20' 7.976" W and 44° 38' 38.674" N, 76° 17' 30.885" W for Newboro Lake, and 44° 39' 52.744" N, 76° 20' 9.974" W, and 44° 42' 16.131" N, 76° 18' 56.427" W for Upper Rideau Lake. Every site was a shallow bay where turtles and fish were likely to be found.

Turtle capture — I captured painted and musk turtles in May and June 2012 using two sets of paired fyke nets. Each net was 3.5 m long and composed of seven 0.9 m diameter steel

rings. Two throats were fitted at the second and fourth rings in each net to prevent the escape of captured animals. Two 4.6 m wings and one 10.7 m long lead were also fastened to each net. Nets, throats, wings and leads were built with 5.08 cm knotted nylon mesh. I emptied the nets every 24 hours to avoid deaths by asphyxia. The turtles I captured were transported in plastic bins to my field laboratory (Queen's University Biological Station) where I collected blood samples, and where I recorded morphological measurements. I marked each turtle with a notch on the carapace using a file to avoid resampling the same individual. I kept turtles overnight and released them at the site of capture on the next fair-weather day.

Fish capture — Pumpkinseeds (*Lepomis gibbosus*) were captured at the same time as turtles using the same fyke nets. Blackchin shiners (*Notropis heterodon*) and brook silversides (*Labidesthes sicculus*) were captured using a seine net, which was dragged along the shores at each site. I euthanized all fish (50 Blackchin shiners, 20 pumpkinseeds, and 49 brook silversides) using sharp blows to the head followed by spinal cuts. I then sealed fish individually in polyethylene bags and put them on ice for transport back to our field laboratory (Queen's University Biological Station). There, I kept them frozen at -20°C.

Blood sampling — I collected 0.5 ml of blood from 39 musk turtles and 60 painted turtles by subcarapacial vein puncture (Dyer and Cervasio, 2008) using 1 ml un-heparinized syringes fitted with 25 gauge, 38 mm needles. The total blood volume for each individual was split in two and frozen in two separate 1.5 ml microcentrifuge tubes. These were immediately frozen at -20^{o}C pending analyses. Half of the blood volume per individual was frozen at

-60°C and then lyophilized for isotopic analyses. For each turtle, I also measured the carapace length using a 50 cm tree caliper, and recorded the mass using a 300g Pesola spring scale. I determined the sex of each individual using secondary sexual characteristics (Moll, 1973).

Fish muscle sampling — Frozen whole fish were thawed in warm waters. I weighed pumpkinseeds using a Scout scale with 600 x 0.1 g capacity, and I weighed blackchin shiners and brook silversides with a 600 x 0.1 mg capacity microscale. Fish were also measured (length, height, and width) using an electronic Powerfist calliper with a 300 mm capacity. The length of the fish was measured from the tip of the snout to the base of the tail. To measure the maximal height and width, the calliper was held parallel (lengthwise) to the fish. Using a filleting knife, I made a transverse cut behind the gills, downward with a slight angle towards the head. Then, holding the fish by the tail, I made a longitudinal cut from the base of the tail to the first cut behind the gills. For pumpkinseeds, only one side of the fish was filleted. For blackchin shiners and brook silversides, I filleted both sides of each fish. I store muscle samples in 15 ml flat cap centrifuge tubes. I then weighed the muscle samples, froze them at -60° C, and lyophilized them.

Prey species sampling —In lakes, carbon isotopic differences exist between primary producers that are pelagic and those that are benthic: pelagic primary producers are depleted in ^{13}C when compared to benthic primary producers because the periphyton that covers submerged surfaces reduces access of the benthic primary producers to the underlying inorganic carbon (Hecky and Hesslein, 1995). Because the trophic fractionation of the carbon isotopes between trophic levels is low (France, 1995), the carbon isotope ratio ($\delta^{13}C$)

defined at the level of the primary producer is maintained in the food web. Therefore, the $\delta^{13}C$ of prey items can be used to calculate the proportion of energy that a consumer derived from the benthic and pelagic zones (Post, 2002). To determine fish and turtle reliance on both prey items, I obtained three composite samples of banded mystery snails *(Viviparus georgianus)* and of zebra mussels *(Dreissena polymorpha)* from each sampling site. Because snails are grazers (Buckley, 1986), they represent the benthic food chain prey items. Because zebra mussels are filter feeders (Horgan and Mills, 1997), they represent the pelagic food chain prey items. I collected both species of mollusks by removing them from the surface of rocks or logs submerged in the water using a dip net or by hand. The mussel and snail samples were transported on ice to our field laboratory, and depurated for 24 hours in lake water to allow for their gut contents to be excreted. Each composite prey sample consisted of 10 individual snails or zebra mussels. I extracted the muscle from each mollusk, placed them in a 45 ml screw-cap centrifuge tube, froze them at -60°C, and then lyophilized them.

Mercury analyses

Total mercury (THg) and methylmercury (MeHg) analyses were performed on 25% of the samples. The results from this analysis indicated that, on average, the part of THg that was MeHg was 95% in musk turtles, 83% in painted turtles, 100% for brook silversides, and 91% in blackchin shiners (Figure A1 – 1). Therefore, I used THg concentrations as a good approximation of MeHg concentrations in my analyses (see THg Concentration as a

Measure of MeHg Concentration in APPENDIX I, and Figure A1-1). In all subsequent sections, I use THg concentrations to represent MeHg concentrations.

Total Mercury — Whole blood samples from each turtle, lyophilized muscle samples from each fish, and lyophilized composite prey samples were analyzed for total mercury (THg) content by combustion-amalgamation-cold-vapor atomic absorption spectrophotometry following the Environmental Protection Agency (EPA) method 7473 and using the MA-3000 Mercury Analyzer Latest Direct Combustion Technology from Nippon Instruments (detection limit was 0.002 ng THg). For quality assurance, each group of 10 samples included a standard reference material (DORM-3 or DORM-4), and each set of 100 samples was initiated by purging the instrument twice.

Methylmercury — Organomercury (MeHg) concentrations were determined in 25% of the fish and turtle tissue samples and in one snail and one zebra mussel composite prey sample at each site by capillary gas chromatography coupled with atomic fluorescence spectrometry (GC-AFS) as described by (Cai et al., 1997). Initial extracts of fish tissue and turtle blood samples were subjected to sodium thiosulfate clean-up and the organomercury species were isolated as their bromide derivatives by acidic KBr and $CuSO_4$ and subsequent extraction into a small volume of dichloromethane. Hg analysis was then performed using the P S Analytical Hg speciation system model PSA 10.723. This is an integrated gas chromatography - Hg atomic fluorescence instrument which is comprised of an Ai Cambridge (UK) model GC 94 gas chromatograph equipped with a CTC A200S autosampler, an optic injector module, and coupled to the PSA Merlin Detector via a pyrolysis oven held at 800°C. A fused silica analytical column with dimensions of 15 m x 0.53 mm i.d.

(Megabore), coated with a 1.5 µm film of DB-1 (J&W Scientific), was used. The column temperature was held at 40°C for 30 seconds, programmed at 30°C/min to 85°C, which was held for 1 min, then programmed at 20°C/min to a final temperature of 200°C, and then held for 1 min. A split/splitless injector was used in the splitless mode and maintained at 150°C. The carry gas and make-up gas flows were 4.0 ml/min of helium and 60 ml/min of argon, respectively. For the PSA Merlin detection system, the sheath gas flow was 150 ml/min of argon. Other parameter settings were the same as those reported previously. Data were acquired by a real-time chromatographic control and data acquisition system (EzChromTM, Scientific Software Inc., CA).

Carbon and Nitrogen Isotope Ratios – Samples and standards were weighed into tin capsules and loaded into an elemental analyser (Isotope Cube manufactured by Elementar, Germany) interfaced to an isotope ratio mass spectrometer (Delta Advantage manufactured by Thermo, Germany) (IRMS). Sample/Std was flash combusted at about 1800C (Dumas combustion) and the resulted gas products was carried by helium through columns of oxidizing/reducing chemicals optimised for CO_2 and N_2. The gases were separated by a "purge and trap" adsorption column and sent to IRMS interface (Conflo III manufactured by Thermo, Germany) then to IRMS. Internal standards used were (d15N,d13C in ‰): C-51 Nicotiamide (0.07,-22.95), C-52 mix of ammonium sulphate + sucrose (16.58,-11.94), C-54 caffeine (-16.61,-34.46), blind std C-55: glutamic acid (-3.98, -28.53). These cover the natural range. These analyses were performed at the G.G. Hatch Lab at the University of Ottawa, Ontario, Canada. The analytical precision is based on the internal std (C-55) which is not used for calibration and is usually better than 0.2 ‰.

Trophic Level and Reliance on the Benthos — I calculated trophic level using the equation suggested by Post (2002): $\lambda + (\delta^{15}N_{\text{secondary consumer}} - [\delta^{15}N_{\text{base1}} * \alpha + \delta^{15}N_{\text{base2}} * (1 - \alpha)]/\Delta_n$, where λ is the trophic position of primary consumers, α is the proportion of N in the consumer derived from the base of food chain one (benthic food web), $\delta^{15}N_{\text{base1}}$ is the N isotope ratio for the primary consumer at the base of food chain one, $\delta^{15}N_{\text{base2}}$ is the N isotope ratio for the primary consumer at the base of food chain two, and Δ_n is the trophic fractionation of N. For the calculation of the reliance on the benthos in terms of diet, I used the Bayesian mixing-model package SIAR in R 2.15.3 (©The R foundation for Statistical Computing). The N and C isotopic ratios of snails and zebra mussels were used to represent the benthic and pelagic food chains, respectively. Predators are slightly enriched in $\delta^{13}C$ and significantly enriched in $\delta^{15}N$ relative to their prey (Post, 2002). To correct for this enrichment, I added 0.23‰ and 2.2 ‰ to the $\delta^{13}C$ and $\delta^{15}N$ of turtles respectively (Seminoff et al., 2007). For fish, the values added to the $\delta^{13}C$ and $\delta^{15}N$ values were calculated following the equation provided by Caut et al. (2009).

Modeling — I built separate models to predict the THg concentration in fish and in turtles using multiple regression. I used the following predictor variables where applicable: SIZE (as the % maximum within each species), SEX (only for turtles), sampling lake (LAKE), SPECIES, proportion of snails in the diet (PSNAIL), and trophic level (TL). SIZE and TL were included because previous studies showed that they can influence the level of THg accumulation in animals (Canli and Atli, 2003; Kidd et al., 1995).

I looked for evidence of multicollinearity among the possible THg predicting variables (See Collinearity, APPENDIX I) (Smith et al., 2009). Then, for each group of animals,

I first used multiple regressions to build models including all the predictors on the full data set. I used the log transformed THg data to satisfy the assumptions of normality and homoscedasticity. Using the MuMIn package in R, I calculated the second order Akaike's Information Criterion (AICc) for each candidate model, and made a final model selection based on ΔAICc and Akaike weights (Burnham and Anderson, 2002). Models with ΔAICc < 4 were used in the calculation of the parameters of the final average model. The averaged predictive model also took into account the relative Akaike weight of each candidate model. I also calculated the relative importance of the predictive variables for each model using the MuMIn package. All averaged models included the full list of parameters originally considered. Standard Errors (SE) and 95% confidence intervals (95% CI) of each average model coefficient were used to validate the model since these measures provide information on the uncertainty related to a coefficient's predictive ability.

To further validate the ability of these models in predicting THg concentrations in animals, I also used multiple regressions to build models including all the predictors on several training sets. These were then validated using their complementary testing sets. There were 8 testing sets in total for each group of animals; 4 of the testing sets were composed of site-specific data, and 4 others were composed of random sampling points taken out of the original data set. Using the MuMIn package in R, I calculated the second order Akaike's Information Criterion (AICc) for each candidate training model, and final model selection was based on ΔAICc and Akaike weights (Burnham and Anderson, 2002). Model with ΔAICc < 4 were used in the calculation of the parameters in the average final model. The averaged predictive model also took into account the relative Akaike weight of

each candidate model. All averaged models included the full list of parameters originally considered, and the relative importance of the predictive variables for each model was also calculated using the MuMIn package. I then used the resulting predictive models to calculate predicted THg concentrations in the testing sets. I plotted observed (OBS) THg values against predicted (PRE) THg values in the testing set and compared the slope and intercept of this regression line to the best possible predictive model line (1:1). In the best predictive model, plotting OBS vs. PRE values should give a regression line (mx + b) in which m = 1 and b = 0 (Piñeiro et al., 2008). I performed statistical analyses with JMP 10.0 (SAS Institute, Inc., Cary, NC; http://www.jmp.com) and R 2.15.3 (©The R foundation for Statistical Computing). Power analyses were performed to calculate the minimum required sample size for the multiple regression analyses using G*Power 3.1.7 © Franz Faul, Universität Kiel, Germany 1992 – 2013.

RESULTS

Turtles

Model Variables – I measured the concentration of THg in the blood of 39 musk turtles (30 males and 9 females) and 60 painted turtles (40 males and 20 females). The average THg concentration was 5.04 ± 1.89 ng/g and 10.53 ± 1.53 ng/g for musk and painted turtles, respectively. The concentration of THg was significantly different between the two species of turtles ($t_{(97)} = 2.26$, $p = 0.03$) (Figure 1 – 1A).

I examined multicollinearity between each continuous predictor variable and found that VIFs values and correlation coefficients were low, indicating that the predictors used in

the models were not strongly correlated with one another (see Collinearity in APPENDIX I, and Figure A1 – 2A). As a result, all predictor variables were considered. SIZE showed little variability, but in contrast, PSNAIL and TL were measured over a large range of possible values for both species of turtles (Table 1 – 1A).

Modeling and Validation – Using the full dataset in the multiple regression analysis, I obtained seven models with ΔAICc < 4 and these models had between two and five parameters (Table A1-1A). Akaike weights for the models ranged from 0.05 to 0.31. Since Akaike weights were generally low (< 0.90), the final averaged model took into account all seven candidate predictive models. The most important variables in the averaged final model were TL and SPECIES. The least important variable was LAKE. All three continuous variables had a positive relationship with THg, but only the effect of TL was significant ($z = 5.31$, $p < 0.0001$). The only categorical variable to significantly affect THg was SPECIES ($z = 3.66$, $p = 0.0003$). To validate the model, I looked at the SE and 95%CI for each averaged coefficient. All parameters except TL had high SE and 95% CI (Table 1 – 2A).

Cross-Validation – Using multiple regression analysis, I obtained between 3 and 11 candidate models with ΔAICc < 4 for both site-specific and random training sets (Table A1 – 2). Candidate models for all the training sets had between two and five parameters. Akaike weights ranged from 0.03 to 0.62 and were generally low. Therefore, instead of picking the best model, I averaged all candidate models for each training set. The most important variable in all eight training models were SPECIES and TL. The least important variable varied between PSNAIL, SEX, and LAKE. PSNAIL, TL, and SIZE all had a positive relationship with THg, with the exception of PSNAIL who had a negative relationship with THg in one of

the training sets. Across all predictive models, only TL and SPECIES consistently and significantly affected THg concentration in blood.

Six out of eight averaged models significantly predicted THg in the testing sets. The model obtained for each training set was used to calculate predicted THg concentrations in testing sets. Then, OBS and PRE THg concentrations in the testing set were examined for correlation. R^2 values from the six averaged models that significantly predicted THg were generally low, and between 26 to 82% of the variation remained unexplained by the models (Table 1 – 3A). Moreover, the slope of these relationships deviated from the 1:1 slope expected from a good predictive model by 2 to 122% (Figure 1 – 2), and these deviations averaged 51.88 ± 15.88 %. THg in turtles from the third site-specific training set (S3) seemed to be best predicted by the corresponding average model (R^2 = 0.74, m = 0.98, b = 0.12). All other models had lower predictive power.

Fish

Model variables – I measured the muscle THg concentration in 50 blackchin shiners, 20 pumpkinseeds, and 49 brook silversides. The concentrations of THg were significantly different between the three species of fish ($F_{(2, 116)}$ = 24.85 , $p < 0.0001$) (Figure 1 – 1B). Blackchin shiners had 234.55 ± 20.43 ng THg/g, brook silversides had 158.27 ± 18.09 ng THg/g, and pumpkinseeds had 409.13 ± 29.72 ng THg/g.

I examined multicollinearity between each continuous predictor variable and found that VIFs values and correlation coefficients were low. This indicates that the predictors used in the models were not strongly correlated with one another (see Collinearity in

APPENDIX I and Figure A1 – 2B). As a result, all predictor variables were considered. Fish trophic level showed little variability between and within species, but PSNAIL and SIZE had high variability within species (Table 1 – 1B).

Modeling and Validation – From the multiple regression analysis that considered the full dataset, I obtained two models with ΔAICc < 4 (Table A1 – 1). These models had four and five parameters. Akaike weights for the models were 0.85 and 0.15. Since Akaike weights were low (< 0.90), the final averaged model took into account the two candidate predictive models. All predictive variables were as important in the model, with the exception of trophic level which was less important. All continuous predictor variables had a significant effect on THg, with SIZE ($z = 9.07$, $p < 0.0001$) and PSNAIL ($z = 2.61$, $p = 0.01$) having a positive linear relationship, and TL having a negative linear relationship with THg ($z = 2.34$, $p = 0.02$). The only categorical variable that had a significant effect on THg was SPECIES ($z = 7.78$, $p < 0.0001$). To validate the model, I looked at SE and 95% CI, and these were smaller for the fish average predictive model than for the turtle predictive model (Table 1 – 2B).

Cross-Validation – I obtained between 2 and 4 models with ΔAICc < 4 for both site-specific and random training sets (Table A1 – 3). All candidate models had between three and five parameters. Akaike weights ranged between 0.11 and 0.88, so models were averaged in every case instead of relying on the model with the highest weight. The most important variable in all training sets were LAKE, SPECIES, and SIZE and these variables were all ranked equally in terms of importance. The least important variable alternated between PSNAIL, TL, or a combination of both, depending on the training set. Even though these

variables ranked as less important, they were only slightly less important than LAKE, SPECIES and SIZE. In every training set, PSNAIL and SIZE had a positive relationship with THg, whereas TL has a negative relationship with THg, and this negative relationship was significant in half the training sets (coefficient = -0.00 – -0.32, z = 0.03 – 2.75, p = 0.01 – 0.98).

All training sets significantly predicted THg in the testing sets. Plotting OBS vs. PRE THg concentrations in the testing sets showed that between 50 and 77 % of the variation in THg is explained by the models (Table 1 – 3B). Moreover, the slope of the relationship between OBS and PRE THg deviated from the expected 1:1 slope indicative of a good predictive model by 2 – 19 % and these deviations averaged 11.75 ± 1.91 % (Figure 1 – 3). THg in fish from site 2 (S2) and from the third random training set (R3) seemed to be best predicted by their corresponding training set (R^2 = 0.77, m = 1.02, b = 0.14 and R^2 = 0.77, m = 1.11, b = -0.25), and the model predictions at S2 were the least biased due to the predictive model's minimal deviation from the 1:1 expected regression line.

DISCUSSION

A number of factors are known to influence the accumulation of THg in animal tissues. These include age and size of the animal (Farkas et al., 2003), trophic level (Atwell et al., 1998; Coelho et al., 2006; Kidd et al., 2011; Power et al., 2002), and surrounding wetland morphology (Snodgrass et al., 2000). Only a few studies attempt to create predictive models for animal tissue THg concentration using physiological and ecological factors (e.g. Greenfield et al., 2001; Qian et al., 2001). The influence of other factors, such

as the dietary reliance on various carbon sources, as measured by $\delta^{13}C$ signatures, is still debated. Some show that dietary reliance on various carbon sources can influence the accumulation of MeHg (Bergeron et al., 2007; Power et al., 2002), while others do not consider it a useful predictor of tissue concentrations (Chumchal and Hambright, 2009). The lack of consensus may be due to the fact that basal $\delta^{13}C$ varies inexplicably and significantly between and within lakes (Doucett et al., 1996). When included as a variable in models, it introduces a lot of unexplained variance, making it difficult to discern its main effect. Nevertheless, creating a simple model capable of predicting which species is likely to be burdened by high levels of THg from known feeding ecology parameters is important in a conservation context.

In this study, I attempted to create a predictive model capable of forecasting THg burdens in freshwater animal tissues based on feeding ecology and relative body size. I use trophic position and reliance on the benthic food chain as feeding ecology parameters. Instead of using $\delta^{13}C$ as a direct way of tracing carbon origins within the different lake zones, however, I converted the ratio to a measure of dietary reliance on the benthic food chain using a mixing model. I then created predictive models using data obtained from two species of turtles and three species of fish, and these models were validated using SE and 95% CIs. In addition, I created a series of training sets and corresponding testing sets. Models created from the training sets were validated on their matching testing sets as a way to assess whether the results of this analysis could be generalized to independent data sets. My prediction was that there should be an increase in tissue THg concentration with an increase in dietary reliance on the benthic food chain. The ability of all models to predict

THg from feeding ecology parameters and from relative size was greater in fish than in turtles so my prediction was supported by the fish data.

Turtles

In general, the predictive models of THg accumulation performed poorly for this group. The large SE and 95% CI obtained on the parameter coefficients from the full averaged model indicate that this model did not fit the THg concentration data well, and that predictions could only be made with low precision. Similarly, in the cross-validation exercise, an average of over 75% of the variation in THg concentration remained unexplained by the models, suggesting that they made poor predictions. In addition, the large deviations from the 1:1 line obtained when plotting OBS vs. PRE THg concentrations indicates that predictions made from these models were biased. It is therefore not advisable to generalize these models to independent data sets.

Of all the variables included in the training models, trophic level (TL) seemed to have the overall strongest relationship with turtle blood THg concentration. In fact, TL was the most important variable in all the models, and the relationship between THg and TL was constantly positive (Table 1 – 4A). This supports the prediction that THg (as a measure of MeHg concentration) biomagnifies in the food web. The variable SPECIES was equally important in all the models. The magnitude of its effect on THg concentration, as indicated by its comparatively large coefficients, suggests that there are some inherent qualities specific to each species of turtles that can influence their THg accumulation. In this case, painted turtles had more THg in their blood than musk turtles (Figure 1 – 1). Although I

cannot determine which species-specific quality is responsible for the differences in accumulation of THg from the results of this study, potential explanations can be found in the literature. For example, physiological turnover rates can vary across species and can affect metal accumulation (Wang and Fisher, 1999). In mammals, metabolic rate can be used to estimate blood turnover rate: larger animals have slower metabolic rates per unit mass, and have slower blood turnover rates (MacAvoy et al., 2006). My results could indicate a similar trend in turtles: the larger painted turtle could accumulate more THg in its blood as a result of slower tissue turnover. In addition, the percent haemoglobin in turtle blood varies between 5.9 and 11.2 in freshwater species (Dessauer, 1970). Since the methylated form of Hg, MeHg, preferentially binds proteins, those with higher blood haemoglobin concentrations may accumulate more THg in their blood. Thus, although the models could only poorly predict THg accumulation in turtles, my results support the inclusion of the variables SPECIES and TL in future attempts to model THg accumulation in these animals.

PSNAIL, or reliance on the benthic food chain, consistently ranked as one of the least important variables in the training models. Moreover, the relationship between THg and PSNAIL was not consistently positive in the cross-validation exercise (Table 1 – 4A), and no statistically significant linear dependence of the mean THg concentration on PSNAIL was detected. This makes it impossible to interpret the effect of PSNAIL on THg concentration's conditional mean. Similarly, the PSNAIL coefficient in the model based on the full dataset had high SE and 95% CI (Table 1 – 2A), indicating that it could not be quantified with certainty in the stepwise regression analysis. Consequently, my initial prediction that THg

burden should increase with dietary reliance on the benthic food chain is not supported by my data on turtles. Moreover, I did not reach my goal of creating a predictive model for THg burden in turtles since the cross-validation exercise showed that the application of the model could not be extended to other sites of study.

Fish

Models made far more accurate predictions of THg accumulation for fish than for turtles. The SE and 95% CI on the averaged model coefficients created using the full fish dataset were smaller than for turtles, meaning that the model parameters were estimated with more accuracy for fish (Table 1 – 2). Similarly, in the cross-validation exercise, I found that the training models explained on average 64% of the variation in THg concentration in the testing sets, suggesting that generalization to independent data set could be possible. Moreover, whereas I could only make biased estimates of THg concentration in turtle blood, predictive models provided THg concentration estimates in fish muscle that were less biased since the OBS vs. PRE regression line deviated less from the 1:1 linear fit expected from the best possible predictive model.

Interestingly, the variables that were the most important in the models were LAKE, SIZE, and SPECIES, and not the variable related to fish feeding ecology. Because most fish species grow continuously during their lives, the relationship between age and size is strong (Mommsen, 2001). The positive relationship between THg concentration and size for this variable is therefore consistent with the expected positive relationship between age and THg concentration caused by the ability of the methylated form of Hg to bioaccumulate.

LAKE and SPECIES were as important as SIZE in the models. Much like it was the case for turtles, the effect of SPECIES could be explained by differences in protein content in the muscle (Kinsella et al., 1977) or it could be due to varying rates of tissue turnover between the different species of fish (MacAvoy et al., 2006). In this case, the pumpkinseeds had the most THg in their muscles, and the brook silverside had the lowest concentration of THg in the muscle (Figure 1 – 1B). Yet, I cannot determine the exact reason why THg concentration varied between species from my data set. Likewise, the significant effect of LAKE cannot be explained from my data set. However, I can speculate that ecological parameters such as the variation in methylation rates of Hg across the three lakes due the presences and size of surrounding wetlands (Ullrich et al., 2001), or the differences in amounts of THg found across and within the three lakes (LeBlond, 2009) could explain the effect of LAKE. In fact, fish from Newboro Lake had relatively more THg in their tissues than fish from Upper Rideau and Indian Lakes. Likewise, Newboro Lake has the highest average surface sediment concentration of THg with 0.16 ± 0.03 μg/g compared to Indian and Upper Rideau Lakes which had an average of 0.11 ± 0.02 μg/g THg in the surface sediments (LeBlond, 2009). Since the models predicted THg concentration in fish with some degree of accuracy, my results support the inclusion of these predictive variables in future similar modeling exercises.

Trophic level (TL) consistently ranked as the least important variable, and PSNAIL alternated between being as important as SIZE, LAKE and SPECIES, and being the second least important variable. However, TL and PSNAIL were always only slightly less important than the other variables. Thus, all the predictor variables that I considered in the models

were valuable. My initial prediction was that there would be an increase in THg concentration in animal tissues with an increase in dietary reliance on the benthic food chain as measured by the proportion of the diet that was derived from the benthos (PSNAIL). As predicted, PSNAIL had a consistent positive relationship with THg across all training models and in the full model (Table 1 – 4B). In addition, the estimated rate of change of the conditional mean of THg concentration with respect to PSNAIL, when all other predictor variables were fixed, was the highest of all the predictor variables. This means that PSNAIL had the most conditional influence on THg concentration in fish muscle. On the contrary, the relationship between TL and THg concentration in fish muscle was consistently negative, and the z-scores for the TL coefficients were only significant in some of the models, making any interpretation regarding its meaning unadvisable. In summary, my study indicates that the reliance of the benthic food web is an important predictor of THg burden in fish.

Applications and future modeling exercises

The models I built with my set of variables cannot be used to predict THg burden in turtles accurately for a number of reasons: the training models poorly predicted THg in testing sets, the THg estimations were biased, and SE and 95% CI of the full dataset model parameter coefficients were large. For fish however, the models built with the same variables were able to predict THg burdens with some accuracy. Therefore, the fish models could be used to predict THg concentrations in external data sets, as long as the data come from a site with similar concentrations of THg in the sediments. This restriction is necessary

because when THg is present in concentrations typical of industrially polluted areas, the ecosystem's Hg methylation capacity gets overloaded, leaving higher concentrations of THg available in the ecosystem. Because such systems end up with more THg than MeHg, THg tends to accumulate at a faster rate in organisms (Bergeron et al., 2007). In areas of high concentration then, it cannot be assumed that THg concentration equals MeHg concentration in animal tissues. The rates of accumulation could therefore be influenced by other unknown factors.

Based on the results of this study, I recommend that conservation strategies focus on trophic level to identify turtle species that are likely to be burdened by THg. For fish, I recommend that reliance on the benthic food web be considered. Future modelling studies should use transformed isotopic ratios to represent trophic level and reliance on the different food chains. This is especially important when sampling occurs in several locations because a lot of unexplained variation exists in isotopic signatures within and between lakes. Furthermore, the C and N isotopes give a coarse estimate of diet composition (i.e. benthic vs. pelagic). Within each source, there exist a lot of prey items that may vary in their ability to accumulate and transfer Hg. I therefore also recommend including a more refined estimate of diet composition in future models.

A major issue that I encountered was that the snail species I chose to represent the benthic food chain, the banded mystery snail (*Viviparus georgianus*), is an occasional filter-feeder. I chose this species of snail because it is the most abundant in the study lakes, and because it is the only one that can be found throughout the sampling season. This means that, at least in some instances, this primary consumer integrates both the pelagic and

benthic food chains. This is a potential problem because when I calculated the reliance on the benthos using Bayesian mixing models, I assumed that my representatives of the benthic and pelagic food chains were sufficiently distinct from one another in terms of isotopic signatures. Since the snail has the option of filter-feeding, its carbon isotopic signature can resemble that of the pelagic representative. To determine whether this was an issue in my data set, I plotted the isotopic signatures of the benthic and pelagic representatives and looked for overlap in carbon isotope ratios. In some locations, the $\delta^{13}C$ of the pelagic primary consumers resembled that of the benthic primary consumer, but there was never any overlap in the range of $\delta^{13}C$ values. For that reason, the assumption that the representatives must be distinct is met within my data set. In addition, I ran the mixing-model analysis a few times using snail and zebra mussel $\delta^{13}C$ values typical of a situation in which the zebra mussel filter-feeds and the snail grazes, and concluded from the results of this hypothetical scenario that the fact that snails can filter-feed should not affect the predictive ability of the models (see Snails and Filter-Feeding in APPENDIX II and Figure A2 – 2). Still, reliance on the benthic food web may have been more accurately measured if the benthic representative had been an obligate grazer.

Another issue I encountered is related to the measure of age in turtles. The methylated form of Hg (MeHg) bioaccumulates in turtles, so older individuals are expected to have more THg in their tissues when compared to younger individuals. However, there is currently no accurate way to measure turtle age for wild individuals. Whereas size can be used as a surrogate of age in fish because they grow indeterminately, turtle growth rates diminish significantly or stops completely in adults (Galbraith et al., 1989), making size a

poor predictor of age. It is possible then that having a way to estimate turtle age, and including turtle age as a variable in the models, could improve its ability to predict THg concentration in turtle blood.

Table 1 – 1. Variability in measurements of the predictors used in modeling THg concentrations in turtles (n = 99) and in fish (n = 119). Size of the animals is indicated in centimeters (cm) and PSNAIL is a proportion. Trophic level (TL) was calculated using the formula described in Post et al. (2002). All measurements are given as average (M) ± standard error (SE) (minimum – maximum).

A. Turtles				
	Musk	10.82 ± 0.13 (8.00 - 12.20)	0.56 ± 0.03 (0.11 - 0.85)	2.76 ± 0.07 (1.86 - 3.52)
	Painted	13.97 ± 0.12 (10.30 - 16.00)	0.49 ± 0.04 (0.02 - 0.95)	2.47 ± 0.10 (0.71 -4.48)
B. Fish				
	Blackchin Shiner	4.35 ± 0.10 (2.31 - 5.66)	0.45 ± 0.01 (0.33 - 0.78)	3.00 ± 0.04 (2.38 - 3.44)
	Brook Silverside	5.76 ± 0.07 (4.81 - 7.52)	0.45 ± 0.01 (0.36 - 0.99)	2.98 ± 0.02 (2.68 - 3.37)
	Pumpkinseed	13.23 ± 0.27 (9.59 - 19.00)	0.41 ± 0.04 (0.22 - 0.79)	3.08 ± 0.07 (2.47 - 3.68)

Table 1 – 2. Averaged parameter coefficients for two logistic regression models predicting THg concentrations in A. turtles (n = 99) and B. fish (n = 119). Standard error (SE) and 95% confidence intervals (95% CI) are given for each parameter.

	Variable	Coefficient	SE	95% CI
A. Turtles				
	Intercept	-1.03	0.55	-2.12, 0.06
	SIZE	0.01	0.00	0.00, 0.02
	SPECIES	0.27	0.07	0.13, 0.42
	TL	0.30	0.05	0.19, 0.42
	PSNAIL	0.20	0.15	-0.09, 0.49
	SEX	0.03	0.08	-0.12, 0.19
	LAKE	0.00	0.10	-0.20, 0.20
B. Fish				
	Intercept	1.73	0.35	1.03, 2.43
	LAKE	-0.13	0.05	-0.21, -0.05
	PSNAIL	0.41	0.15	0.10, 0.71
	SIZE	0.01	0.00	0.01, 0.02
	SPECIES	0.17	0.04	0.09, 0.25
	TL	-0.22	0.10	-0.41, -0.04

Table 1 – 3. Predictive ability assessment for 16 averaged models predicting turtle and fish tissue THg concentration on internal data (n = 19 – 30). Coefficient of determination (R^2), p-value (p), and slope (m) and intercept (b) of the linear fit between observed (OBS) and predicted (PRE) THg concentrations (ng/g) are presented. The name of the training set refers to the data that was taken out of the full data set to create the testing set.

	Training Set	R^2	p	m	b
A. Turtles	Site 1	0.26	0.0121	1.13	-0.24
	Site 2	-0.05	0.8977	0.06	0.60
	Site 3	0.74	p < 0.0001	2.46	0.84
	Site 4	-0.05	0.8307	0.08	0.54
	Random 1	0.47	0.0005	2.22	-0.84
	Random 2	0.53	0.0002	1.44	-0.32
	Random 3	0.30	0.0071	1.14	-0.09
	Random 4	0.29	0.0080	1.34	-0.13
B. Fish	Site 1	0.50	0.0004	1.19	-0.64
	Site2	0.77	< 0.0001	1.02	0.14
	Site 5	0.70	< 0.0001	0.90	0.19
	Site 6	0.65	< 0.0001	0.91	0.30
	Random 1	0.66	< 0.0001	0.89	0.26
	Random 2	0.57	< 0.0001	0.82	0.36
	Random 3	0.77	< 0.0001	1.11	-0.25
	Random 4	0.52	< 0.0001	0.86	0.31

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Table 1 – 4. Parameter coefficients for eight averaged multiple linear regression models predicting THg for A. turtles (n = 19 – 20) and B. fish (n = 19 – 30). The number of the study site S1 – S4, and the number of the random sub-samples R1 – R4 refer to the data excluded from each training set to create the testing set. The z-score of the coefficients highlighted in grey were not significant.

A. Turtles	***S1***	***S2***	***S3***	***S4***	***R1***	***R2***	***R3***	***R4***
Intercept	-0.59	-1.69	-1.32	-0.47	-0.27	-0.76	-0.65	-0.62
Lake	0.00	0.00	0.02	0.00	0.06	0.05	0.00	0.00
PSNAIL	0.30	0.00	0.16	0.18	0.27	0.17	0.16	0.08
Size	0.01	0.01	0.01	0.00	0.01	0.01	0.01	0.01
Sex	-0.07	0.01	-0.04	0.04	0.02	0.05	0.13	0.05
Species	0.30	0.29	0.32	0.25	0.20	0.25	0.21	0.24
TL	0.24	0.35	0.30	0.63	0.24	0.28	0.30	0.32
B. Fish								
Intercept	1.84	1.98	2.11	1.34	1.80	1.02	1.66	0.95
Lake	-0.20	-0.04	-0.13	-0.12	-0.12	-0.13	-0.12	-0.07
PSNAIL	0.69	0.35	0.34	0.21	0.40	0.86	0.48	0.92
Size	0.01	0.01	0.02	0.02	0.01	0.01	0.01	0.01
Species	-0.52	0.18	0.17	0.17	0.15	0.16	0.15	0.16
TL	-0.07	-0.27	-0.32	-0.14	-0.25	-0.03	-0.23	0.00

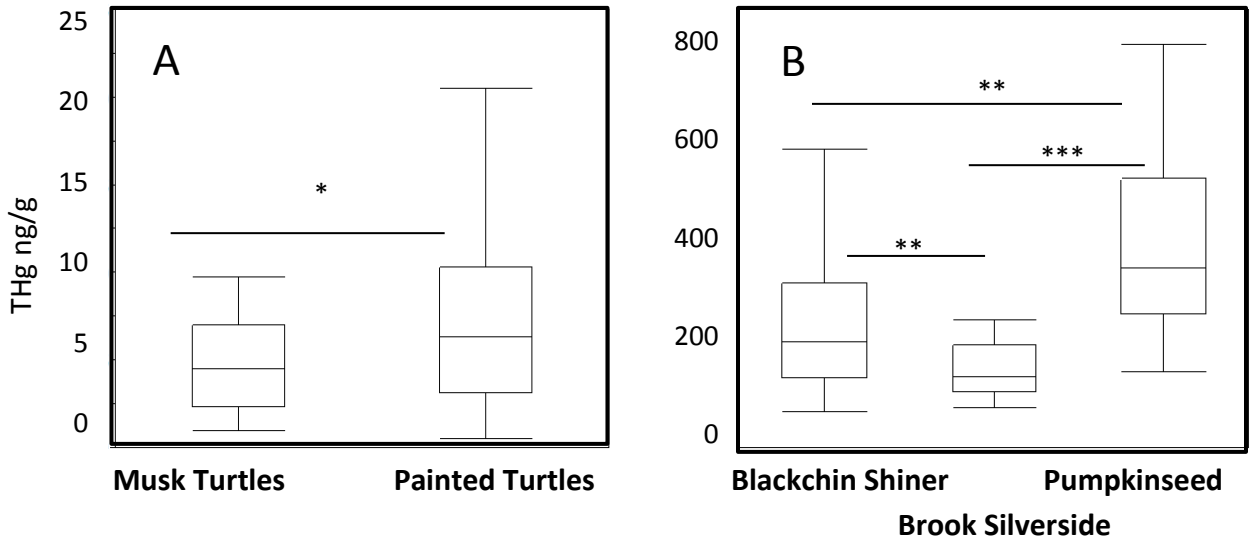


Figure 1-1. Total mercury (THg) concentration (ng/g) in A. musk (n = 39) and painted (n = 60) turtle blood and B. blackchin shiner (n = 50), brook silverside (n = 49), and pumpkinseed muscle (n = 20) across lakes Indian, Newboro, and Upper Rideau. * $p < 0.05$, ** $p < 0.001$ *** $p < 0.0001$

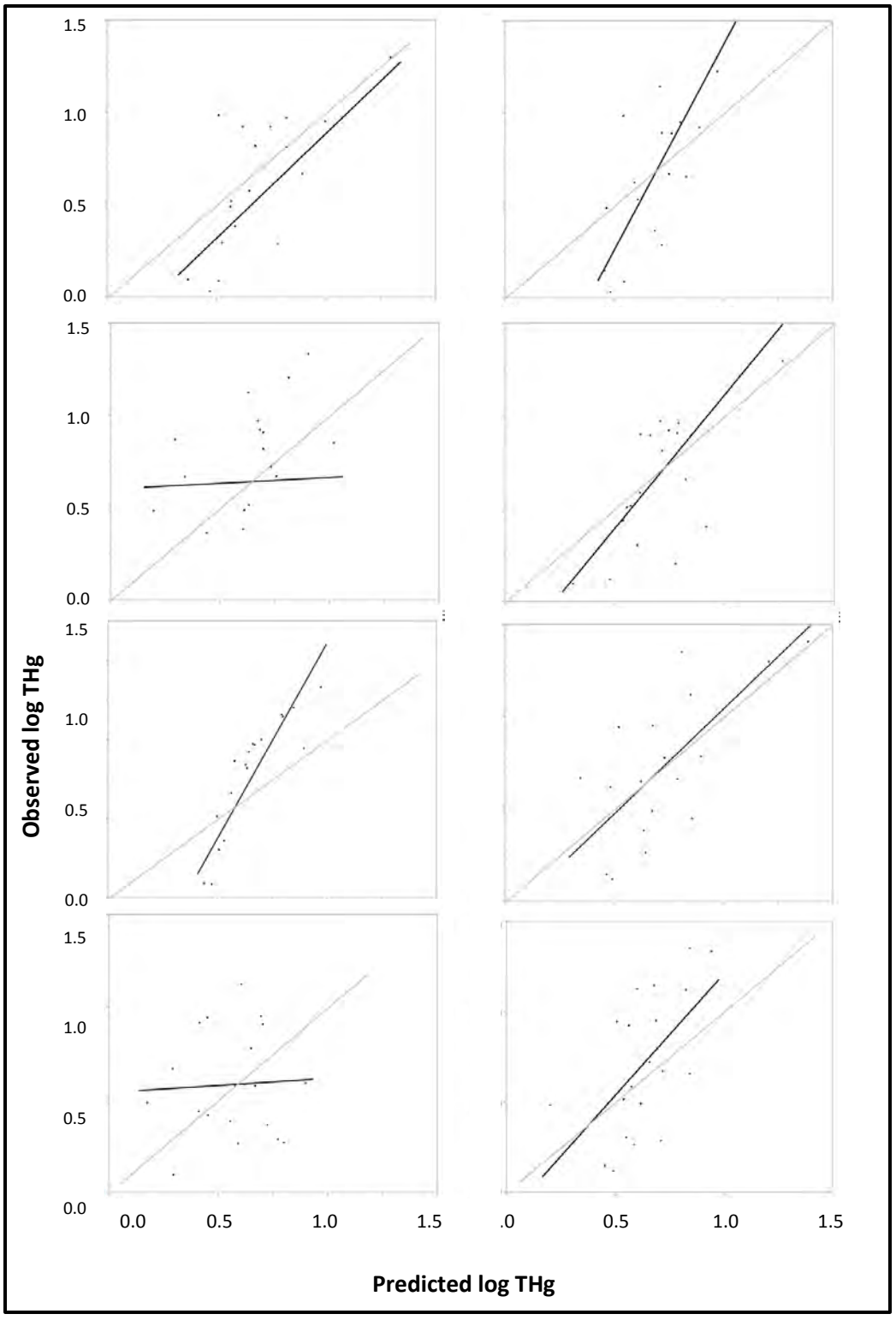


Figure 1 – 2. Correlations between predicted and observed THg concentrations in turtles in log-transformed ng/g (n = 19 – 20). The dark line represents the model linear fit and the pale line is the 1:1 linear fit within the boundaries of the axes.

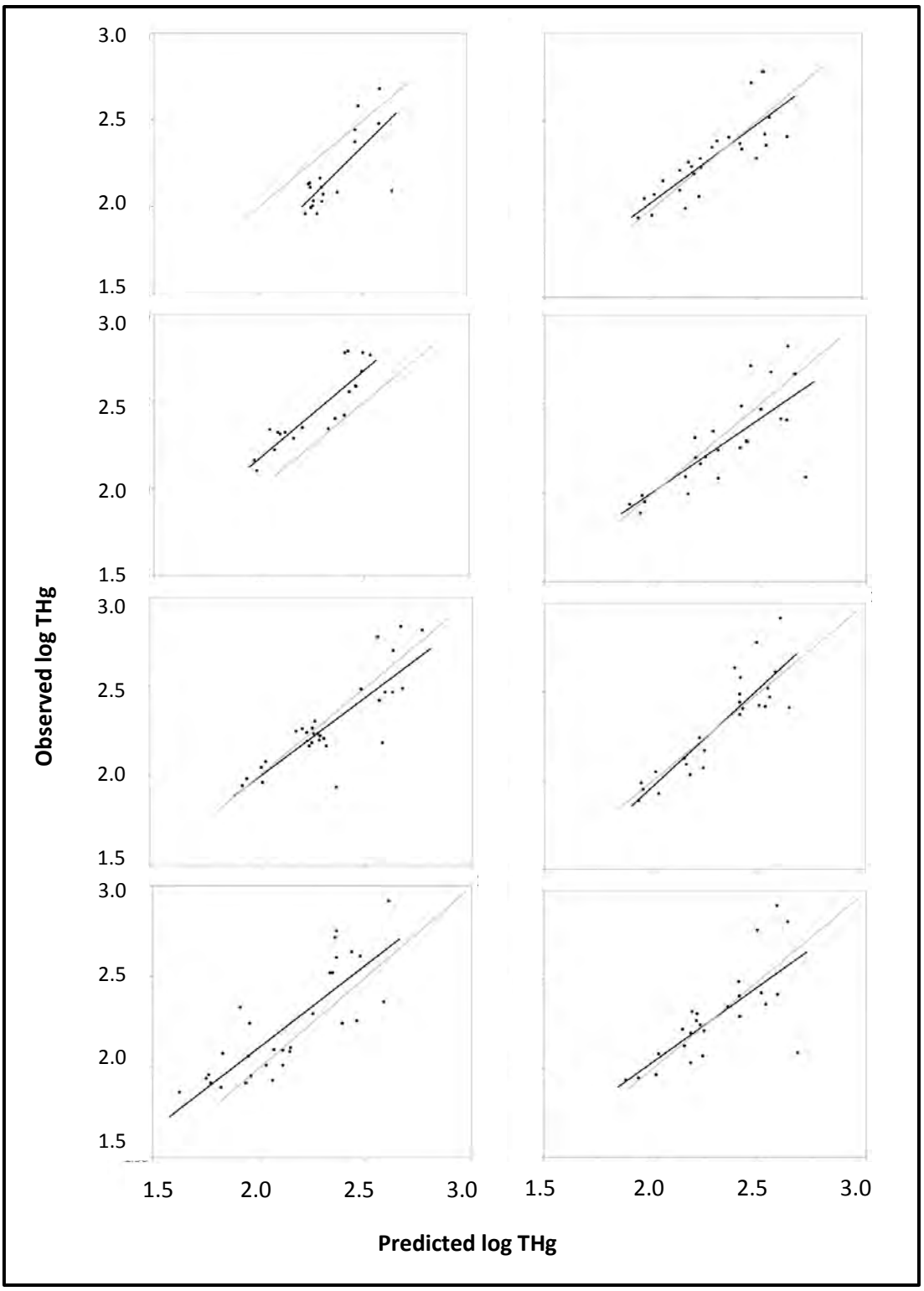


Figure 1 – 3. Correlations between predicted and observed THg concentrations in fish in log-transformed ng/g (n = 19 – 30). The dark line represents the model linear fit and the pale line is the 1:1 linear fit within the boundaries of the axes.

CHAPTER 2: THE PROPORTION OF ZEBRA MUSSELS (DREISSENA POLYMORPHA) IN THE DIET OF MUSK TURTLES (STERNOTHERUS ODORATUS) AND PUMPKINSEEDS (LEPOMIS GIBBOSUS) DOES NOT INFLUENCE THE ACCUMULATION OF MERCURY

ABSTRACT

Differences in feeding ecology are known to affect the accumulation of contaminants such as mercury (Hg) in aquatic animals. Modelling the accumulation of Hg in animals can help identify which animals are likely to accumulate high concentrations of Hg. Since zebra mussels can accumulate more Hg than native mussels in their tissues due to their relatively high filtering rates, I predict that Hg burden should increase with an increase in dietary reliance on zebra mussels as a prey item. I created averaged multiple linear regression models to predict Hg burdens in pumpkinseeds and musk turtle, two species that consume zebra mussels, from the proportion of zebra mussels in their diets. I also controlled for other factors known to influence Hg accumulation (sex, size, location, and trophic level). The proportion of zebra mussels in the diet was the most influential variable in both fish and turtle models. However, none of the regression coefficients could be estimated significantly, making any interpretation of my results not advisable. Errors in predictions were mostly due to unexplained variance, and R^2 values between observed and predicted Hg values were 0.11 for musk turtles, and 0.22 for pumpkinseeds. The results indicate that the proportion of zebra mussels in the diet of musk turtles and pumpkinseeds is not a good predictor of Hg burdens.

INTRODUCTION

Biotic exchanges, defined as the successful accidental or intentional introduction of plants or animals to an ecosystem, is ranked in the top five threats to biodiversity (Sala et al., 2000), and as the second most important threat after habitat destruction (Lowe et al., 2000). Generally, biotic exchanges are a relatively more important threat in freshwater ecosystems because of activities such as fish stocking, long distance navigation by ship, and because the spread of non-native species may be facilitated by water currents and by the lack of physical barriers within water bodies (Lodge et al., 1998). Non-native species can disrupt native communities and even cause extinctions. For instance, the Nile perch was introduced into Lake Victoria in the 1950s and caused the disappearance of 200 of the 300 species of endemic cichlids that were present before the invasion (Witte et al., 1992). Invasive species can also alter levels of primary productivity. In plants, for example, biomass, primary productivity, nitrogen (N) availability, and N fixation rates all increase significantly in communities with invasive species (Ehrenfeld, 2003). Furthermore, invasive species can shift the balance of energy transfers between pelagic and benthic food chains. The introduction of largemouth bass (*Micropterus salmoides*) and rock bass (*Ambloplites rupestris*) in Canadian lakes, for instance, caused a decrease in trophic level and a shift towards the pelagic food chain in *Salvelinus namaycush*, the native lake trout (Vander Zanden et al., 1999). Effects can also cascade down the food web. For example, after the introduction of the opossum shrimp (*Mysis relicta*), zooplankton populations decreased due to predation by the introduced shrimp. As a result, planktivorous kokanee salmon

(*Oncorhynchus nerka)* population collapsed, which lead to the displacement of a number of salmon predators, including grizzlies and bald eagles (Ellis et al., 2011; Spencer et al., 1991). As globalization causes biogeographic barriers to disappear, invasive species are becoming more common and prevalent (Mooney and Hobbs, 2000; Vince, 2011). However, the impact that invasive species have on ecosystems often remains only partially characterized.

Zebra mussels (*Dreissena polymorpha*) are bivalves endemic to most of Europe, but invasive in North America. This mollusk is believed to have reached North American waters via ballast water discharge of Atlantic-crossing vessels (Hebert et al., 1989). The invasion was discovered in 1988 in Lake St-Clair, Ontario, Canada. Since 1988, zebra mussels have colonized the majority of the Great Lakes basin (Griffiths et al., 1991), they are now part of the primary threat to the viability of native species in near-shore zones in some of the Great Lakes (Lake Ontario Biodiversity Strategy Working Group 2009), and they are considered one of world's worst 100 invaders (Lowe et al., 2000). Zebra mussels are filter-feeders with a high filtration rates (10 to 100 ml/individual/day: Mackie, 1991), and they can remove from the water column particles that are 150 µm to 1.22 mm in size (Horgan and Mills, 1997). Zebra mussels can also re-suspend bottom sediments and filter them. Since sediments are a repository for contaminants such as polychlorinated biphenyls (PCBs) and heavy metals (Chon et al., 2012; Martinez et al., 2010), zebra mussels may be exposed to more contaminants while re-suspending sediments and, as a result, accumulate more contaminants than other bivalves or gastropods. In fact, sediment contaminant concentrations often correlate well with zebra mussel contaminant burdens (e.g. Kwan et al., 2003; Regoli et al., 2001).

One contaminant of concern is mercury (Hg). Hg is a heavy metal that has anthropogenic and natural origins. Its natural origins include volcanic eruptions and the weathering of rocks (Nriagu, 1989). Its anthropogenic sources include industrial smelting and gold mining (UNEP, 2013). Once Hg is in the atmosphere, it is transported to remote regions of the globe and deposited in soils, water bodies, and sediments. Hg is therefore ubiquitous: it can be found in industrial regions of the globe as well as in remote regions.

In aquatic environments, three forms of Hg are typically found: metallic Hg (Hg^0), divalent Hg (Hg^{2+}), and methylmercury (MeHg). Once in the water column or in the sediments, Hg^0 is either deposited, or it is oxidized to Hg^{2+}. In turn, Hg^{2+} can be deposited into the sediments or rendered bioavailable by bacteria who add one or two methyl groups to the ion. These transformations are also reversible so that Hg^{2+} can be reduced to Hg^0, and MeHg can be demethylated. Studies on Hg focus on MeHg because it is bioavailable (Barrocas et al., 2010), because it is the form that dominates in vertebrate tissues (Bloom, 1992), and because it is has known toxic effects (Mark and James, 2011; Scheuhammer et al., 2007).

Since the introduction of the zebra mussel, several species have changed their diets to include them as a food item. This is the case for several species of waterfowl in the Great Lakes including the lesser scaups (Aythya affinis), the greater scaups (Aythya marila), the buffleheads (Bucephala albeola), the canvasback (Aythya valisineria), and Bucephala clangula, the common golden eye (Custer and Custer, 1996). Reptiles and fish, such as map turtles (*Graptemys geographica*), musk turtles (*Sternotherus odoratus*), pumpkinseed (*Lepomis gibbosus*), and carps (*Cyprinidae* family) have also altered their dietary habits to

include zebra mussels (Bulté and Blouin-Demers, 2008; French, 1993; Patterson and Lindeman, 2009). The zebra mussel is also an important prey item for the round goby (*Neogobius melanostomus)*, an invasive and extremely abundant species in Lake Erie (Marsden and Jude, 1995; Ray and Corkum, 1997). All have become predators of the invasive mussel, but to varying degrees. In the Great Lakes waterfowl community, for instance, gastrointestinal content analyses revealed that lesser scaup stomachs contained 98.6% zebra mussels whereas canvasback stomachs contained 9% zebra mussels (Custer and Custer, 1996). Since zebra mussels can contain more MeHg than native food items, predators of zebra mussels may accumulate more MeHg post zebra mussel invasion. Because MeHg causes several deleterious health effects in animals (Wolfe et al., 1998), an increase in MeHg burden via the consumption of zebra mussels is cause for concern.

MeHg causes several deleterious health effects in animals: it influences development, behaviour, and reproduction. Because MeHg negatively affects reproduction, it not only affects the health of individuals, but also impacts populations (Barr, 1986). Most studies look at total Hg tissue concentrations (THg). Since MeHg is the predominant form in animal tissues, measuring THg concentrations often provides a good estimate of THg concentrations. Deleterious effects associated with THg are often caused by exposure to doses that exceed environmentally relevant concentrations (Fimreite, 1974; Kamman et al., 2005; Monteiro and Furness, 2001), but more subtle cellular, histological, and genetic effects are possible at THg concentrations typical of North-American freshwater bodies (0.0001 to 0.5 µg/L: Zillioux et al., 1993). For example, white sturgeons with muscle THg concentrations of 0.04 to 0.52 µg/g showed reduced plasma testosterone and estradiols,

were smaller, and were in poorer overall condition when compared to sturgeons with lower muscle THg concentrations (Webb et al., 2006). In addition, gene transcription in cutthroat trout (*Oncorhynchus clarkii*) was altered at whole body concentration of 0.06 µg THg/g ww (Moran et al., 2007), and histological changes were observed in brook trout (*Salvelinus fontinalis*) with whole body concentrations ranging from 0.05 to 0.29 µg THg/g ww (Schwindt et al., 2008). Effects such as altered gene transcription, decreased feeding, increased time to first spawn, and decreased antioxidant enzyme activity have also been observed in other freshwater fish (reviewed in Sandheinrich and Wiener, 2011). Similarly, in birds effects like the production of fewer fledged young, elevated levels of corticosteroid hormone in blood, and decreased foraging and brooding have been reported in individuals contaminated with THg (reviewed in Scheuhammer et al., 2007).

Knowing which environmental factors are likely to influence the accumulation of the methylated form of Hg in wild animal populations is crucial. In fact, the United Nations Environment Program (UNEP) (2013) stated in their most recent report that there is a major gap in our ability to predict MeHg uptake by living organisms. There is therefore a need to be able to forecast, with some level of confidence, which species are likely to be burdened with high concentrations of MeHg.

I tested the hypothesis that the proportion of zebra mussels in the diet of consumers influences their accumulation of MeHg. To test my hypothesis, I measured the proportion of the diet composed of zebra mussels and the concentration of MeHg in two species known to consume zebra mussels: pumpkinseeds and musk turtles. I predicted a

positive relationship between the proportion of zebra mussels in the diet of those two consumers and the concentration of MeHg in their tissue.

METHODOLOGY

Study area — Sampling took place in eastern Ontario (Canada) on the Rideau Canal, a series of lakes, rivers, and human-made canals linking Ottawa to Kingston. I captured turtles, fish, and prey samples in Indian, Newboro, and Upper Rideau Lakes. Hg concentrations in the sediments of the three lakes ranged from 0.01 to 0.22 µg THg/g dry weight (LeBlond, 2009). In each lake, I trapped animals from two distinct sites. The sites were located at 44° 34' 58.276" N, 76° 19' 33.837" W and 44° 36' 10.986" N, 76° 18' 33.479" W for Indian Lake, 44° 37' 46.178" N, 76° 20' 7.976" W and 44° 38' 38.674" N, 76° 17' 30.885" W for Newboro Lake, and 44° 39' 52.744" N, 76° 20' 9.974" W, and 44° 42' 16.131" N, 76° 18' 56.427" W for Upper Rideau Lake. Every site was a shallow bay where turtles and fish are likely to be found.

Turtle and fish capture — I captured musk turtles and pumpkinseeds in May and June of 2012 using two sets of paired fyke nets. Each net was 3.5 m long and composed of seven 0.9 m diameter steel rings. Two throats were fitted at the second and fourth rings in each net to prevent the escape of captured animals. Two 4.6 m wings and one 10.7 m long lead were also fastened to each net. Nets, throats, wings and leads were built with 5.08 cm knotted nylon mesh. I emptied the nets every 24 hours to avoid deaths by asphyxia. The turtles I captured were transported in plastic bins to my field laboratory (Queen's University Biological Station) where I collected blood samples from turtles, and where I recorded

morphological measurements. I marked each turtle with a notch on the carapace using a file to avoid recapture. I kept turtles overnight at my field laboratory and released them at the site of capture on the next fair-weathered day. Pumpkinseeds were euthanized at the site of capture using sharp blows to the head followed by spinal cuts. I then sealed fish individually in polyethylene bags and put them on ice for transport back to my field laboratory. There, I kept them frozen at -20° C.

Blood sampling — I collected 0.5 ml of blood from 39 musk turtles by subcarapacial vein puncture (Dyer and Cervasio, 2008) using 1 ml un-heparinized syringes fitted with 25 gauge, 38 mm needles. The total blood volume for each individual was split in two and frozen in two separate 1.5 ml microcentrifuge tubes. These were immediately frozen at -20^{o}C pending analyses. Half of the blood volume per individual was frozen at -60°C and then lyophilized for isotopic analyses. For each turtle, I also recorded the carapace length using a 50 cm tree caliper, and the mass using a 300g Pesola spring scale. I determined the sex of each individual using secondary sexual characteristics (Moll, 1973).

Fish muscle sampling — Frozen whole fish were thawed in warm water. I weighed pumpkinseeds using a Scout scale with 600 x 0.1g capacity. Pumpkinseeds were also measured (length, height and width) using an electronic Powerfist calliper with a 300 mm capacity. The length of the fish was measured from the tip of the snout to the base of the tail. To measure the maximal height and width, the calliper was held parallel (lengthwise) to the fish. Using a filleting knife, I made a transverse cut behind the gills, downward with a slight angle towards the head. Then, holding the fish by the tail, I made a longitudinal cut

from the base of the tail to the first cut behind the gills. Only one side of the fish was filleted. I stored muscle samples in 15 ml flat cap centrifuge tubes. I then weighed the muscle samples, froze them at -60° C, and lyophilized them.

Prey species sampling — In lakes, carbon isotopic differences exist between primary producers that are pelagic and those that are benthic: pelagic primary producers are depleted in ^{13}C when compared to benthic primary producers (Hecky and Hesslein, 1995). Because the trophic fractionation of the carbon isotopes between trophic levels is low (France, 1995), the carbon isotope ratio ($\delta^{13}C$) defined at the level of the primary producer is maintained in the food web. Therefore, the $\delta^{13}C$ of prey items can be used to calculate the proportion of energy that a consumer derived from the benthic and pelagic zones (Post, 2002). To determine fish and turtle reliance on both prey items, I obtained three composite samples of banded mystery snails *(Viviparus georgianus)* and of zebra mussels *(Dreissena polymorpha)* from each sampling site. Because snails are grazers (Buckley, 1986), they represent the benthic food chain prey items. Because zebra mussels are filter feeders (Horgan and Mills, 1997), they represent the pelagic food chain prey items. I collected both species of mollusks by removing them from the surface of rocks or logs submerged in the water using a dip net or by hand. The mussel and snail samples were transported on ice to our field laboratory, and depurated for 24 hours in lake water to allow for their gut contents to be excreted. Each composite prey sample consisted of 10 individual snails or zebra mussels. I extracted the muscle from each mollusk, placed them in a 45 ml screw-cap centrifuge tube, froze them at -60°C, and then lyophilized them.

Mercury analyses

Total Mercury — Whole blood samples from each turtle, lyophilized muscle samples from each fish, and lyophilized composite prey samples were analyzed for total mercury (THg) content by combustion-amalgamation-cold-vapor atomic absorption spectrophotometry following the Environmental Protection Agency (EPA) method 7473 and using the MA-3000 Mercury Analyzer Latest Direct Combustion Technology from Nippon Instruments (detection limit of 0.002 ng THg). For quality assurance, each group of 10 samples included a standard reference material (DORM-3 or DORM-4), and each set of 100 samples was initiated by purging the instrument twice.

Methylmercury — Organomercury (MeHg) concentrations were determined in 25% of the fish and turtle tissue samples and in one snail and one zebra mussel composite prey sample at each site by capillary gas chromatography coupled with atomic fluorescence spectrometry (GC-AFS) as described by (Cai et al., 1997). Initial extracts of fish tissue and turtle blood samples were subjected to sodium thiosulfate clean-up and the organomercury species were isolated as their bromide derivatives by acidic KBr and $CuSO_4$ and subsequent extraction into a small volume of dichloromethane. Hg analysis was then performed using the P S Analytical Hg speciation system model PSA 10.723. This is an integrated gas chromatography - Hg atomic fluorescence instrument which is comprised of an Ai Cambridge (UK) model GC 94 gas chromatograph equipped with a CTC A200S autosampler, an optic injector module, and coupled to the PSA Merlin Detector via a pyrolysis oven held at 800°C. A fused silica analytical column with dimensions of 15 m x 0.53-mm i.d.

(Megabore), coated with a 1.5-µm film thickness of DB-1 (J&W Scientific) was used. The column temperature was held at 40°C for 30 seconds, programmed at 30°C/min to 85°C, which was held for 1 min, then programmed at 20°C/min to a final temperature of 200°C, and then held for 1 min. A split/splitless injector was used in the splitless mode and maintained at 150°C. The carry gas and make-up gas flows were 4.0 ml/min of helium and 60 ml/min of argon, respectively. For the PSA Merlin detection system, the sheath gas flow was 150 ml/min of argon. Other parameter settings were the same as those reported previously. Data was acquired by a real-time chromatographic control and data acquisition system (EzChromTM, Scientific Software Inc., CA).

The analyses of both forms of Hg on 25% of the samples in chapter 1 indicated that THg concentrations are a good approximation of MeHg concentrations in my system (see THg Concentration as a Measure of MeHg Concentration in APPENDIX I, and Figure A1-1). For this reason, in the results and discussion sections, THg is used interchangeably with MeHg.

Carbon and Nitrogen Isotope Ratios – Samples and standards were weighed into tin capsules and loaded into an elemental analyser (Isotope Cube manufactured by Elementar, Germany) interfaced to an isotope ratio mass spectrometer (Delta Advantage manufactured by Thermo, Germany) (IRMS). Sample/Std were flash combusted at about 1800C (Dumas combustion) and the resulted gas products carried by helium through columns of oxidizing/reducing chemicals optimised for CO2 and N2. The gases were separated by a "purge and trap" adsorption column and sent to IRMS interface (Conflo III manufactured by Thermo, Germany) then to IRMS. Internal standards (std) used were

(d15N, d13C in ‰): C-51 Nicotiamide (0.07,-22.95), C-52 mix of ammonium sulphate + sucrose (16.58,-11.94), C-54 caffeine (-16.61,-34.46), blind std C-55: glutamic acid (-3.98, -28.53). These cover the natural range. These analyses were performed at the G.G. Hatch Lab at the University of Ottawa, Ontario, Canada. The analytical precision is based on the internal std (C-55) which is not used for calibration and is usually better than 0.2 ‰.

Trophic Level — I calculated trophic level using the equation suggested by Post (2002): $\lambda + (\delta^{15}N_{secondary\ consumer} - [\delta^{15}N_{base1} * \alpha + \delta^{15}N_{base2} * (1 - \alpha)]/\Delta_n$, where λ is the trophic position of primary consumers, α is the proportion of N in the consumer derived from the base of food web one (benthic food web), $\delta^{15}N_{base1}$ is the N istope ratio for the primary consumer at the base of food web one, $\delta^{15}N_{base2}$ is the N istope ratio for the primary consumer at the base of food web two, and Δ_n is the trophic fractionation of N.

Proportion of zebra mussels in the diet — Musk turtles feed almost exclusively on caddisfly larvae (order *Trichoptera*), snails, and zebra mussels (Patterson and Lindeman, 2009). Pumpkinseeds feed on a variety of gastropods and arthropods, but recent evidence from my study site reveals that their diet is mainly composed of zebra mussels and snails (Locke et al., 2013). To obtain the proportion of the diet that is composed of zebra mussels, I converted fish and turtle $\delta^{13}C$ into proportions of pelagic (zebra mussels) and benthic (snails) prey with a two end-member mixing model using the Bayesian mixing-model package SIAR in R 2.15.3 (©The R foundation for Statistical Computing). Predators are slightly enriched in $\delta^{13}C$ and significantly enriched in $\delta^{15}N$ relative to their prey (Post, 2002). To correct for this enrichment in the model, I added 0.23‰ and 2.2 ‰ to the $\delta^{13}C$ and $\delta^{15}N$ of turtles respectively (Seminoff et al., 2007). For fish, the values added to the $\delta^{13}C$ and

$\delta^{15}N$ values were calculated following the equation provided in Caut et al. (2009). Because the diet of both the musk turtle and the pumpkinseed are not exclusively composed of zebra mussels and snails, the ratios obtained by the two end-member mixing model may be biased. However, since more than 85% of the food items consumed by both predators are zebra mussels and snails, I believe it is unlikely that the presence of other prey items would bias the mixing model results significantly.

Modeling — I built separate models to predict the THg concentration in pumpkinseeds and musk turtles using multiple regressions. I used the following predictor variables where applicable: SIZE (as the % maximum within each species), SEX (only for turtles), sampling LAKE, proportion of zebra mussels in the diet (PZEBRA), and trophic level (TL). SIZE and TL were included because previous studies showed that they can influence the level of Hg accumulation in animals (Canli and Atli, 2003; Kidd et al., 1995).

I looked for evidence of multicollinearity among the possible Hg-predicting variables using the variance inflation factors (VIFs) method and the commonly used cut off value of 5. The VIFs were calculated using the "car" package in R. The results from the VIF method were corroborated with the calculation of correlation coefficient for each pair of continuous variables by restricted maximum likelihood (REML) method. For this, I used a threshold value of 0.70 (See Collinearity, APPENDIX II) (Smith et al., 2009).

I used multiple regressions to build models including all the predictors on the full data set for the musk turtle and the pumpkinseed separately. Using the MuMIn package in R, I calculated the second order Akaike's Information Criterion (AICc) for each candidate

model, and made a final model selection based on ΔAICc and Akaike weights (Burnham and Anderson, 2002). Model with ΔAICc < 4 were used in the calculation of the parameters in the average final model. The averaged predictive model also took into account the relative Akaike weight of each candidate model. I also calculated the relative importance of the predictive variables for each model using the MuMIn package. All averaged models included the full list of variables originally considered. Standard Errors (SE) and 95% confidence intervals (95% CI) of each average model coefficient were used to validate the model since they provide information on the uncertainty related to a coefficient's predictive ability. I further validated the models using Theil's coefficients and the root mean squared deviation (RMSD) (Paruelo et al., 1998; Piñeiro et al., 2008). I performed statistical analyses with JMP 10.0 (SAS Institute, Inc., Cary, NC; http://www.jmp.com) and R 2.15.3 (©The R foundation for Statistical Computing). Power analyses were performed to calculate the minimum required sample size for the multiple regression analyses using G*Power 3.1.7 © Franz Faul, Universität Kiel, Germany 1992 – 2013.

RESULTS

Variables – Zebra mussels had a significantly higher proportion of MeHg (t_5 = -7.54, p = 0.0007, n = 6) and a higher concentration of MeHg (t_5 = 3.30, p = 0.02, n = 6) in their tissues than snails (Figure 2 – 1). The average MeHg concentration was 23.49 ± 4.58 ng/g and 33.09 ± 3.03 ng/g in snails and zebra mussels, respectively.

I measured the concentration of THg in the blood of 39 musk turtles (30 males and 9 females) and 20 pumpkinseeds. Musk turtle blood had 5.04 ± 1.89 ng THg/g and pumpkinseed muscle had 409.13 ± 29.72 ng THg/g.

I examined multicollinearity between each continuous predictor variable (SIZE, TL, PZEBRA) and found that, for turtles, VIF values and correlation coefficients were low, whereas they were high for fish. (see Collinearity in APPENDIX II, and Figure A2 – 2). All predictor variables had high variability for both musk turtles and pumpkinseeds (Table 2 – 1).

Modeling and Validation

Musk Turtles – In the multiple regression analysis, I obtained 17 models with ΔAICc < 4 and these models had between one and four parameters (Table A2 – 1A). Akaike weights for the models ranged from 0.02 to 0.17. Since Akaike weights were low (< 0.90), the final averaged model took into account all 17 candidate predictive models. The most important variable in the averaged model were PZEBRA and the least important variable was SEX (Table 2 – 2A). All three continuous predictor variables had a positive relationship with THg concentration, but none of these were significant (Table 2 – 3A). None of the categorical variables had a significant effect on THg. To validate the model, I looked at the SE and 95% CI for each averaged coefficient. All parameters had high SE and 95% CI.

The linear regression of observed (OBS) values versus predicted (PRE) values should have a slope of 1 and an intercept of 0 if the model predicted THg concentrations with perfect accuracy. Therefore, the OBS vs. PRE regression analysis can provides a series of

parameters that are indicative of model performance (Paruelo et al., 1998; Piñeiro et al., 2008). In my model, the proportion of the linear variation in OBS values that is explained by the variation in PRE values (R^2) was 0.11. I also calculated Theil's partial inequality coefficients (U_{bias}, U_{slope}, and U_{error}) to separate the error of the predictions to further characterize the model performance. These coefficients divide the variance in the observed values that is not explained by the predicted values. U_{bias} and U_{slope} were close to 0, whereas U_{error} was close to 1, indicating that most the total error in prediction is due to unexplained variance. The value of RMSD, which is the mean deviation of the predicted values from the observed ones, was high (Table 2 – 4). The slope of the regression line between OBS and PRE values did not differ significantly from the value of 1 since the 95% CI on the slope encompassed the value 1. Similarly, the intercept did not differ from zero as zero was within the 95% CI.

Pumpkinseeds – In the multiple regression analysis, I obtained four models with $\Delta AICc < 4$ and these models had zero, one or two parameters (Table A2 – 1B). Akaike weights for the models ranged from 0.14 to 0.35, so all candidate models were used to calculate average parameters. The most important predictor in the averaged model was PZEBRA, which was followed closely by SIZE (Table 2 – 2B). PZEBRA had a negative relationship with THg concentration and SIZE had a positive relationship with THg concentration, but none of these were significant (Table 2 – 3B). To validate the model, I looked at the SE and 95% CI for the averaged coefficients. All coefficient estimates had high SE and 95% CIs. The R^2 value was larger for the pumpkinseed model than for the musk turtle model (Table 2 – 4), and as is expected with an increase in the R^2 value, the RMSD was smaller. As was the case for the

turtle model, most of the error on the predictions can be attributed to unexplained variance since U_{error} was relatively high. The slope of the regression line between OBS and PRE values did not differ significantly from the value of 1 since the 95% CI on the slope encompassed the value 1. Similarly, the intercept did not differ from zero as zero was within the 95% CI.

DISCUSSION

The factors governing the accumulation of THg in animals and the distribution of THg along food chains remain poorly understood. Several factors play a role, including trophic level (Atwell et al., 1998; Kidd et al., 2011; Power et al., 2002), age and size (Farkas et al., 2003), and the availability of THg in the environment (Snodgrass et al., 2000). The introduction of non-native species has also been shown to affect contaminant transfer in the food web by creating new pathways by which contaminants can enter the food web (Hogan et al., 2007). Similarly, the invasive zebra mussels in my study system accumulated more THg in their tissues than snails, and a higher proportion of the THg in their tissues was MeHg (Figure 2 – 1). I therefore tested the hypothesis that the proportion of zebra mussels in the diet of consumers influences their accumulation of THg. To test my hypothesis, I measured the proportion of the diet composed of zebra mussels and the concentration of THg in two species known to consume zebra mussels: pumpkinseeds and musk turtles. I predicted a positive relationship between the proportion of zebra mussels in the diet of those two consumers and the concentration of THg in their tissue.

In this study, I also attempted to create a predictive model capable of forecasting THg burdens in musk turtles and pumpkinseeds, two freshwater species that have altered their diet to include the invasive zebra mussel as a prey item. I first calculated the proportion of zebra mussels in the diet of both species, and then I created predictive models and validated these models using SE and 95% CI for each parameter coefficient, and by analysing the regression between OBS and PRE THg values.

In general, the models performed poorly. The high SE and large 95% CI for each parameter coefficient from the average models indicate that the model did not fit the THg concentration well and that it could only make low precision predictions. The predictions made by the models were not biased according to the U_{bias} values and according to the fact that the slopes of the OBS vs. PRE regression did not significantly differ from 1, but the larger values for U_{error} indicates that the error on the predictions were mostly due to unexplained variance in THg. This means that factors that I did not include in the models are responsible for much of the variation in THg concentrations in both musk turtles and pumpkinseeds. As shown in my first chapter, the reliance on the benthic food web can influence the accumulation of THg in aquatic organisms, and within-species variability in the reliance on the benthic food chain exists. It is possible that adding this variable in my models could have increased their performance. However, it was not possible for me to add a variable representing the link to the benthic food web in this study because of the strong correlation between the variable PZEBRA and the reliance on the benthic food web.

The fact that none of the coefficients could be estimated at a significant level is probably related to my small sample size, at least for the pumpkinseed model (n = 20). In

addition, because the correlation between SIZE and TL, and PZEBRA and TL, was strong for fish, TL had to be removed from the model. In the turtle model, the coefficients were not significant either, but the reasons for this might differ. MeHg bioaccumulates in animals (Kidd et al., 2011) and musk turtles can live many decades so age is likely to be an important predictor of THg tissue concentration in this species. However, there is no way to accurately age a turtle in the field, so this variable could not be included in my model. Still, for both pumpkinseed and musk turtle models, the small sample size made it impossible to estimate generalization error to other data sets since cross-validation by training and testing sets was not possible. A power analysis revealed that to obtain a probability equal to 0.8 of detecting the true effect of the predictor variable PZEBRA, a minimum sample size of 55 individuals was required for both musk turtles and pumpkinseeds. Since my sample size was smaller than 55 for both musk turtles and pumpkinseeds, it would not have been useful to split the data set into training sets and testing sets since that would only further decrease my ability to detect an effect of the predictor variables. However, judging by the large SE and 95% CI on the regression coefficients, and from the RMSD and Theil's coefficients calculated from the regression between OBS and PRE THg values, I can conclude that the models would not fit external data well.

Overall, the most important predictor variable in both the pumpkinseed and musk turtle model was PZEBRA (Table 2 – 2), indicating that amongst the variables chosen to predict THg, the proportion of zebra mussels in the diet was the most influential when all other variables were held constant. However, the relationship between PZEBRA and THg was positive for the musk turtles and negative for the pumpkinseeds. In addition, the z-

scores for the parameter coefficients were not statistically significant. Thus, it is difficult to interpret the significance of the coefficients.

A major issue that I encountered was that the snail species I chose to represent benthic prey items, the banded mystery snail, is an occasional filter-feeder. I chose this species of snail because it is the most abundant in my study lakes, and because it is the only one that can be found throughout the sampling season. This means that, at least in some instances, the snail integrates both the pelagic and benthic food chains. This is a potential problem because when I calculated the proportion of zebra mussels in the diet using Bayesian mixing models, I assumed that zebra mussels and snails were sufficiently distinct from one another in terms of isotopic signatures. Since the snail has the option of filter-feeding, its carbon isotopic signature can resemble that of the zebra mussel, thereby influencing the resulting mixing-model proportions. To determine whether this was an issue in my data set, I plotted the isotopic signatures of the benthic and pelagic representative isotopic signatures and looked for overlap in carbon isotope ratios. In some locations, the $\delta^{13}C$ of the pelagic primary consumers resembled that of the benthic primary consumer, but there was never any overlap in the range of $\delta^{13}C$ values. For that reason, the assumption that the representatives must be distinct is met within my data set. In addition, I ran the mixing-model analysis a few times using snail and zebra mussel $\delta^{13}C$ values typical of a situation in which the zebra mussel filter-feeds and the snail grazes, and concluded from the results of this hypothetical scenario that the fact that snails can filter-feed should not affect the predictive ability of the models (see Snails and Filter-Feeding in APPENDIX II

and Figure A2 – 2). Still, the proportion of the diet that is zebra mussels may have been more accurately measured if the benthic representative had been an obligate grazer.

Based on the results of this study, I conclude that the proportion of zebra mussels in the diet does not significantly influence the accumulation of MeHg in musk turtle blood and pumpkinseed muscle, even though zebra mussels have a higher MeHg/THg proportion, and a higher concentration of MeHg in their tissues. Although the difference in MeHg concentration was significantly different between zebra mussels and snails, the difference might not be large enough be transferred to higher trophic levels.

Table 2 – 1. Variability in measurements of the predictors used in modeling THg concentrations in musk turtles (n = 39) and in pumpkinseeds (n = 20). Size of the animals is indicated in centimeters (cm) and PZEBRA is a proportion. Trophic level (TL) was calculated using the formula described in Post et al. (2002). All measurements are given as an average ± standard error (SE) (min – max).

	SIZE	PZEBRA	TL
Musk	10.82 ± 0.13 (8.00 - 12.20)	0.44 ± 0.03 (0.15 - 0.39)	2.76 ± 0.07 (1.86 - 3.52)
Pumpkinseed	13.23 ± 0.27 (9.59 - 19.00)	0.44 ± 0.04 (0.21 - 0.78)	3.08 ± 0.07 (2.47 - 3.68)

Table 2 – 2. Relative importance of each predictor variable in the averaged model for A. musk turtles and B. pumpkinseeds.

		Relative Variable Importance
A.	PZEBRA	0.69
	TL	0.57
	SIZE	0.42
	LAKE	0.21
	SEX	0.16
B.	PZEBRA	0.52
	SIZE	0.49

Table 2 – 3. Averaged parameter coefficients for two logistic regression models predicting THg concentrations in A. musk turtles (n = 39) and B. pumpkinseeds (n = 20). Standard error (SE), z-scores, p-values, and 95% confidence intervals (95% CI) are given for each parameter.

		Model averaged coefficients	SE	z-score	p-value	95% CI
A.	**Intercept**	-0.12	0.73	0.16	0.88	-1.58, 1.34
	PZEBRA	0.44	0.32	1.36	0.17	-0.20, 1.09
	SIZE	0.01	0.01	1.30	0.19	0.00, 0.02
	TL	0.15	0.13	1.11	0.27	-0.12, 0.42
	SEX	0.02	0.13	0.15	0.88	-0.24, 0.28
	LAKE	0.05	0.13	0.40	0.69	-0.21, 0.31
B.	**Intercept**	2.42	0.33	6.94	$p < 0.0001$	1.74, 3.10
	Pzebra	-0.61	0.38	1.53	0.13	-1.40, 0.17
	Size	0.01	0.01	1.47	0.14	0.00, 0.02

Table 2 – 4. Slope (m) and y-intercept (b) for the regression between observed and predicted THg concentrations, along with the 95% confidence intervals (95% CI) are given for the musk turtle and pumpkinseed models. R^2, RMSD, U_{bias}, U_{slope}, and U_{error} are also given.

	Musk Turtle	**Pumpkinseed**
m	2.27	1.57
95% CI	0.37, 4.17	0.25, 2.89
b	-0.76	-1.45
95% CI	-2.78, 0.39	-4.82, 1.92
R^2	0.11	0.22
RMSD	0.29	0.19
U_{bias}	0.00	0.00
U_{slope}	0.05	0.04
U_{error}	0.95	0.14

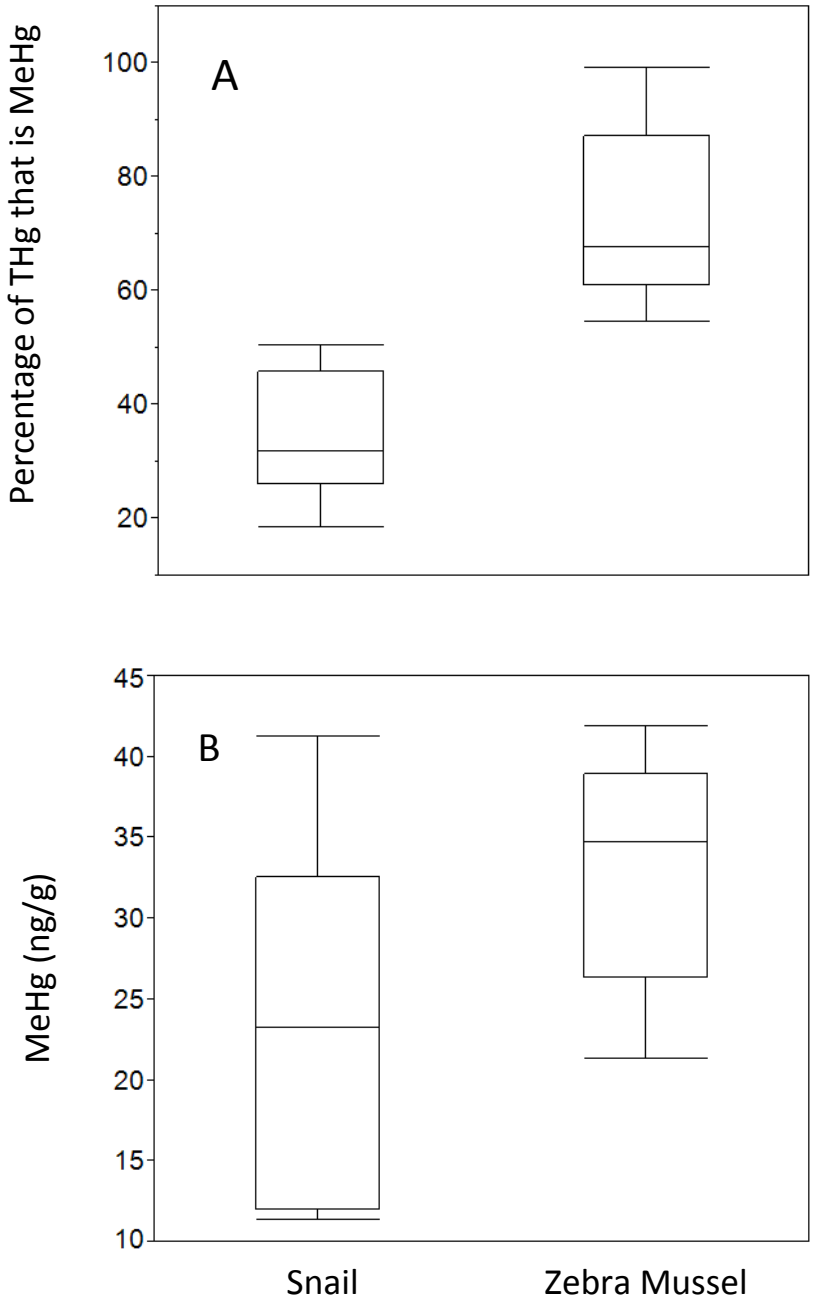


Figure 2 – 1. Percent (%) THg that is MeHg in snails and zebra mussels (A), and MeHg concentration in snails and zebra mussels given in ng/g.

GENERAL CONCLUSION

The main goals of the first chapter were to create multiple regression models capable of predicting total mercury (THg) concentrations in turtle and fish species from the Rideau Canal Lakes, and to determine whether dietary reliance on the benthic food chain influences the accumulation of THg in turtles and in fish. Because lake sediments are often a major repository for THg (Chon et al., 2012), I hypothesized that the variation in THg accumulation between species is a function of the dietary reliance on the benthic food chain. My prediction was that there would be an increase in THg concentration with an increase in dietary reliance on the benthic food chain. I built multiple linear regression models independently for fish and turtles on the full data sets. The analysis of the parameter coefficients obtained in the multiple regression models indicated that there was a significant positive effect of dietary reliance on the benthic food chain on the accumulation of THg in fish, but not in turtles. In addition, cross-validation by data-splitting showed that fish models could be used to predict THg concentrations in independent data sets, but not turtle models. It is possible that models made poor predictions for THg concentrations for turtles because these animals are omnivorous. Because omnivores consume a wide variety of prey items, the intraspecific variation in their isotopic signatures can be more marked than that of animals feeding on a narrow range of prey items. There is thus inherently more variation in the turtle isotope data than in the fish isotope data. Consequently, the error in the estimation of model coefficients is larger. Alternatively, because THg bioaccumulates, having a way by which to estimate turtle age could have

improved the model predictions by increasing the amount of variance that is explained by the model.

My second chapter was conceptualized *post hoc* when I noticed that zebra mussels consistently accumulated more THg (and proportionally more MeHg) than the banded mystery snail (Figure 2-1). I therefore hypothesized that the variation in accumulation of THg could be a function of the proportion of zebra mussels in the diet of turtles and fish. I predicted that there would be an increase in THg concentration with an increase in consumption of zebra mussels in musk turtles and in pumpkinseeds. I built a multiple regression model using the proportion of zebra mussels in the diet as one of the predictor variables. Because the parameter coefficients for this variable could not be estimated with accuracy, and because a power analysis revealed that my sample size was small, I could not determine whether the consumption of zebra mussels influenced the accumulation of THg, but the data suggested that it did not. Future research should attempt to determine why zebra mussels accumulate more THg and MeHg, and why this increased contaminant burden is not transferred to higher trophic levels.

APPENDIX I

Complementary results for THg concentration modelling as a function of reliance on the benthic food chain in turtles and fish

THG CONCENTRATION AS A MEASURE OF MEHG CONCENTRATION

I used THg concentration as a measure for MeHg concentration in both turtle blood and fish muscle. Since MeHg bioaccumulates, most of the THg found in animals that feed in the higher trophic levels is MeHg (Lasorsa and Allen-Gil, 1995). For turtles, preliminary analyses showed that MeHg constituted 95.07 ± 2.46 % of the THg in the blood in musk turtles, and 83.20 ± 7.20 % of the THg in the blood of painted turtles. These ratios did not vary significantly from each other ($t_{(18.24)}$ = -1.56, p = 0.14), and were similar to other previously published ratios (Bergeron et al., 2007; Turnquist et al., 2011). For fish, MeHg made up 91.37 ± 6.05 % of the THg in the muscle of blackchin shiners, and 101.14 ± 2.73 % if the THg in the muscle of brook silversides. These percent ratios did not differ from one another ($t_{(19.46)}$ = 1.47, p = 0.16), and were similar to those previously published in the literature (Bloom, 1992). I therefore considered THg concentration a good approximation of MeHg concentration in turtle blood and fish muscle.

COLLINEARITY

When two predictor variables co-vary, it can be difficult to disentangle each predictor's individual effect. Correlations between predictors can cause problems in the analysis and interpretation of model averaging results (Freckleton, 2011). For this reason, I tested the whole data set for multicollinearity amongst the predictor variables. I used the variance inflation factors (VIFs) method and the commonly used cut off value of 5. The VIFs were calculated using the "car" package in R. The results from the VIF method were corroborated with the calculation of correlation coefficient for each pair of continuous

variables by restricted maximum likelihood (REML) method. For this, I used a threshold value of 0.70 (Smith et al., 2009).

I found that VIFs values and correlation coefficients were low, indicating that the predictors used in the models were not strongly correlated with one another. All VIFs were between 1.07 and 1.57, and correlation coefficients were between 0.02 and 0.4 (Figure A1 – 2A). As a result, all predictor variables were considered in the model averaging exercise.

CANDIDATE MODELS

The predictor variables I used to model MeHg concentrations in animals tissues were sex (SEX), size (SIZE), species (SPECIES), lake (LAKE), trophic level (TL), and reliance on the benthic food chain (PSNAIL). THg concentration in fish and turtles were log-transformed to satisfy assumption of normality and homoscedasticity. For fish and turtles separately, I built a model using the full data set, and eight training models using a subset of the data. Four of the training sets excluded site-specific data, and four other training sets excluded random data points. The models considered in the averaging exercise were those with $\Delta AICc < 4$ (Burnham and Anderson, 2002). The MuMIn package in R was used to create the candidate models (Tables A1 – 1 to A1 – 3).

Table A1-1. Candidate multiple linear regression models predicting THg concentrations in A. turtles (n = 99) and B. fish (n = 119) for the full data set. Models are ranked by increasing order of second order Akaike Information Criterion (AICc and ΔAICc < 4). The number of parameters (k), as well as the Akaike weights (w), are listed.

	Model	k	AICc	ΔAICc	w
A. Turtle					
	SIZE, SPECIES, TL	3	75.60	0.00	0.31
	PSNAIL, SIZE, SPECIES, TL	4	75.96	0.36	0.26
	SEX, SIZE, SPECIES, TL	4	77.68	2.08	0.11
	SPECIES, TL	2	78.00	2.40	0.09
	PSNAIL, SEX, SIZE, SPECIES, TL	5	78.12	2.52	0.09
	PSNAIL, SPECIES, TL	3	78.18	2.58	0.09
	LAKE, SIZE, SPECIES, TL	4	79.10	2.58	0.05
B. Fish					
	SPECIES,LAKE,SIZE,PSNAIL,TL	5	-135.91	0.00	0.85
	SPECIES,LAKE,SIZE,PSNAIL	4	-132.41	3.49	0.15

Table A1-2. Candidate multiple linear regression models predicting THg concentrations in painted (n = 60) and musk turtles (n = 39) in the cross-validation exercise. Models are ranked by increasing order of second order Akaike Information Criterion (AICc and ΔAICc < 4). The number of parameters (k) as well as the Akaike weights (w), are listed. Training set refers to the data excluded from the models to create the testing sets.

Training Set	Model	K	AICc	ΔAICc	w
S1					
	PSNAIL, SIZE, SPECIES, TL	4	59.48	0.00	0.25
	PSNAIL, SPECIES, TL	3	60.04	0.57	0.19
	SIZE, SPECIES, TL	3	60.47	1.00	0.15
	SPECIES, TL	2	61.19	1.72	0.11
	PSNAIL, SEX, SIZE, SPECIES, TL	5	61.22	1.75	0.10
	PSNAIL, SEX, SPECIES, TL	4	61.44	1.96	0.09
	SEX, SIZE, SPECIES, TL	4	62.40	2.92	0.06
	SEX, SPECIES, TL	3	62.82	3.35	0.05
S2					
	SIZE, SPECIES, TL	3	43.63	0.00	0.62
	SEX, SIZE, SPECIES, TL	4	45.96	2.33	0.19
	PSNAIL, SIZE, SPECIES, TL	4	45.97	2.34	0.19
S3					
	SIZE, SPECIES, TL	3	56.57	0.00	0.40
	PSNAIL, SIZE, SPECIES, TL	4	57.65	1.08	0.23
	SEX, SIZE, SPECIES, TL	4	58.66	2.09	0.14
	LAKE, SIZE, SPECIES, TL	4	59.81	3.23	0.08
	PSNAIL, SEX, SIZE, SPECIES, TL	5	59.81	3.24	0.08
	SPECIES, TL	2	60.11	3.54	0.07
S4					
	SPECIES, TL	2	66.85	0.00	0.39
	PSNAIL, SPECIES, TL	3	68.21	1.36	0.20
	SIZE, SPECIES, TL	3	68.83	1.98	0.14
	SEX, SPECIES, TL	3	68.95	2.10	0.14
	PSNAIL, SIZE, SPECIES, TL	4	70.31	3.46	0.07
	PSNAIL, SEX, SPECIES, TL	4	70.32	3.47	0.07

Tables A1-2. Continued.

Training Set	Model	k	AICc	ΔAICc	w
R1					
	PSNAIL, SPECIES, TL	3	53.99	0.00	0.26
	SPECIES, TL	2	55.08	1.09	0.15
	PSNAIL, SIZE, SPECIES, TL	4	55.18	1.19	0.14
	SIZE, SPECIES, TL	4	55.95	1.96	0.10
	PSNAIL, SEX, SPECIES, TL	4	56.32	2.33	0.08
	LAKE, TL	2	57.16	3.17	0.05
	SEX, SPECIES, TL	3	57.26	3.27	0.05
	LAKE, SPECIES, TL	3	57.39	3.40	0.05
	PSNAIL, SEX, SIZE, SPECIES, TL	5	57.46	3.47	0.05
	LAKE, PSNAIL, SPECIES, TL	4	57.74	3.74	0.04
	SEX, SIZE, SPECIES, TL	4	57.96	3.97	0.04
R2					
	SIZE, SPECIES, TL	3	68.91	0.00	0.27
	SPECIES, TL	2	69.87	0.96	0.17
	PSNAIL, SIZE, SPECIES, TL	4	70.23	1.31	0.14
	SEX, SIZE, SPECIES, TL	4	70.78	1.87	0.11
	PSNAIL, SPECIES, TL	3	71.11	2.20	0.09
	LAKE, SIZE, SPECIES, TL	4	71.73	2.82	0.07
	PSNAIL, SEX, SIZE, SPECIES, TL	5	72.08	3.17	0.06
	SEX, SPECIES, TL	3	72.09	3.18	0.06
	LAKE, SPECIES, TL	3	72.55	3.64	0.04
R3					
	SEX, SIZE, SPECIES, TL	4	59.12	0.00	0.20
	SPECIES, TL	2	59.44	0.33	0.17
	SIZE, SPECIES, TL	3	59.83	0.71	0.14
	SEX, SPECIES, TL	3	59.87	0.76	0.14
	PSNAIL, SPECIES, TL	3	60.59	1.47	0.10
	PSNAIL, SEX, SIZE, SPECIES, TL	5	60.70	1.59	0.09
	PSNAIL, SIZE, SPECIES, TL	4	61.06	1.94	0.08
	PSNAIL, SEX, SPECIES, TL	4	61.30	2.18	0.07
	TL	1	62.90	3.73	0.03
R4					
	SPECIES, TL	2	65.34	0.00	0.31
	SIZE, SPECIES, TL	3	65.57	0.23	0.27
	SEX, SPECIES, TL	3	67.41	2.07	0.11
	PSNAIL, SPECIES, TL	3	67.41	2.07	0.11
	SEX, SIZE, SPECIES, TL	4	67.47	2.13	0.11
	PSNAIL, SIZE, SPECIES, TL	4	67.75	2.41	0.09

Table A1-3. Candidate multiple linear regression models predicting THg concentrations in brook silversides (n = 49), blackchin shiners (n = 50), and pumpkinseeds (n = 20). Models are ranked by increasing order of second order Akaike Information Criterion (AICc and ΔAICc < 4). The number of parameters (k), as well as the Akaike weights (w), are listed. Training set refers to the data excluded from the models to create the testing sets.

Training Set	Model	k	AICc	ΔAICc	w
S1					
	LAKE, PSNAIL, SIZE, SPECIES	4	-131.02	0.00	0.73
	LAKE, PSNAIL, SIZE, SPECIES, TL	5	-129.01	2.01	0.27
S2					
	LAKE, PSNAIL, SIZE, SPECIES, TL	5	-120.26	0.00	0.88
	LAKE, SIZE, SPECIES, TL	4	-116.26	4.00	0.12
S3					
	LAKE, PSNAIL, SIZE, SPECIES, TL	5	-99.80	0.00	0.76
	LAKE, SIZE, SPECIES, TL	4	-97.53	2.27	0.24
S4					
	LAKE, SIZE, SPECIES	3	-104.60	0.00	0.37
	LAKE, PSNAIL, SIZE, SPECIES	4	-103.99	0.61	0.27
	LAKE, SIZE, SPECIES, TL	4	-103.61	0.99	0.22
	LAKE, PSNAIL, SIZE, SPECIES, TL	5	-102.69	1.92	0.14
R1					
	LAKE, PSNAIL, SIZE, SPECIES, TL	5	-100.28	0.00	0.70
	LAKE, PSNAIL, SIZE, SPECIES	4	-97.60	2.67	0.18
	LAKE, SIZE, SPECIES, TL	4	-96.61	3.66	0.11
R2					
	LAKE, PSNAIL, SIZE, SPECIES	4	-111.25	0.00	0.77
	LAKE, PSNAIL, SIZE, SPECIES, TL	5	-108.87	2.38	0.23
R3					
	LAKE, PSNAIL, SIZE, SPECIES, TL	5	-106.89	0.00	0.80
	LAKE, PSNAIL, SIZE, SPECIES	4	-104.07	2.82	0.20
R4					
	LAKE, PSNAIL, SIZE, SPECIES	4	-113.29	0.00	0.77
	LAKE, PSNAIL, SIZE, SPECIES, TL	5	-110.85	2.45	0.23

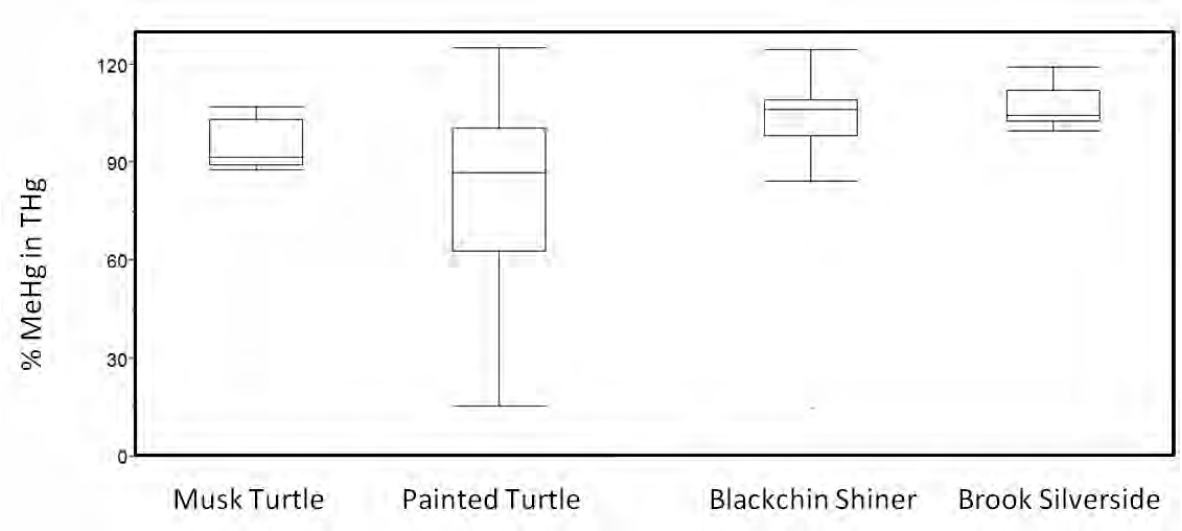


Figure A1 – 1. Percentage of THg that is MeHg in musk turtles (n = 39), painted turtles (n = 60), blackchin shiners (50), and brook silversides (49).

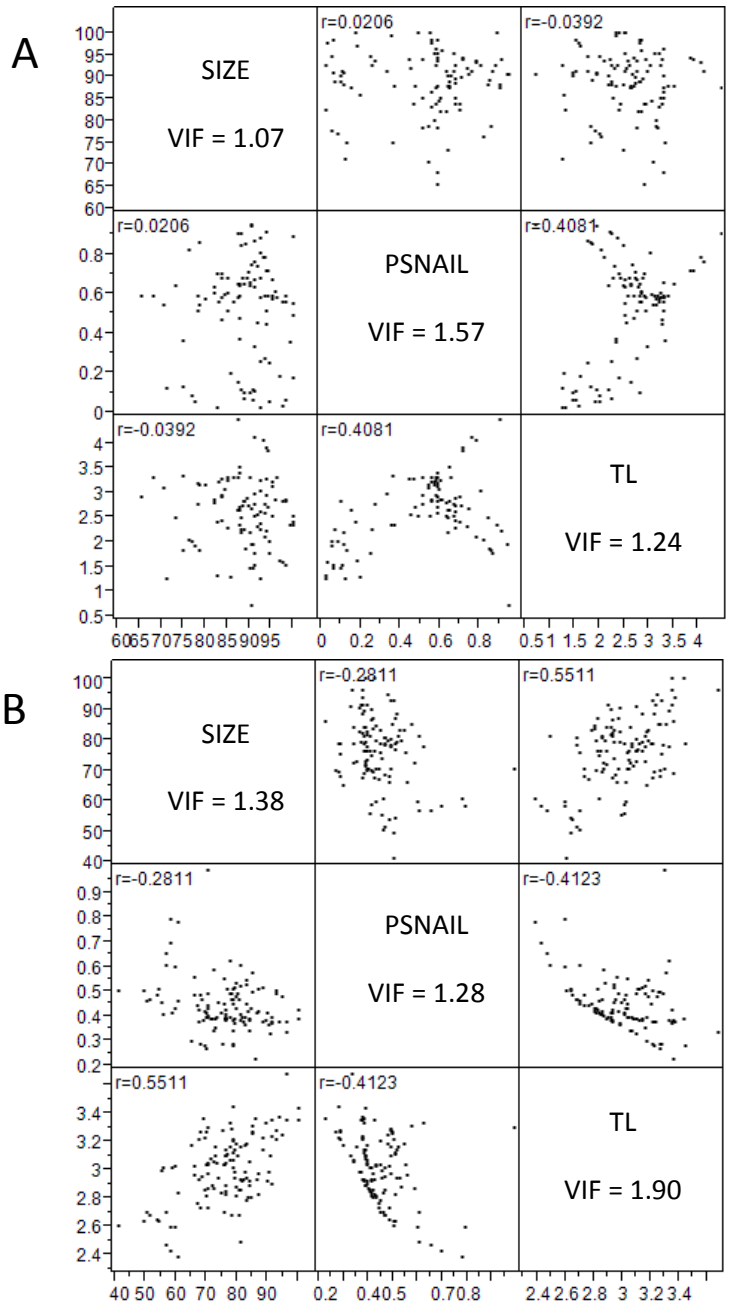


Figure A1 – 2. Correlation matrix of continuous predictor variables in A. turtles (n = 99) and B. fish (n = 119): Trophic level (TL), reliance on the benthic food web (PSNAIL) and size (SIZE). Correlation coefficients by REML method (r) and variance inflation factors (VIF) are given.

APPENDIX II

Complementary results for THg concentration modelling in turtles and fish as a function of the proportion of zebra mussels in the diet

THG CONCENTRATION AS A MEASURE OF MEHG CONCENTRATION

I used THg concentration as a measure for MeHg concentration in both turtle blood and fish muscle. Since MeHg bioaccumulates, most of the THg found in animals that feed in the higher trophic levels is MeHg (Lasorsa and Allen-Gil, 1995). For turtles, preliminary analyses showed that MeHg constituted 95.07 ± 2.46 % of the THg in the blood in musk turtles. This ratio was similar to other previously published ratios for freshwater turtles (Bergeron et al., 2007; Turnquist et al., 2011). For fish, MeHg made up 91.37 ± 6.05 % of the THg in the muscle of blackchin shiners, and 101.14 ± 2.73 % if the THg in the muscle of brook silversides. These percent ratios did not differ from one another ($t_{(19.46)}$ = 1.47, p = 0.16), and were similar to those previously published in the literature (Bloom, 1992). I therefore considered THg concentration a good approximation of MeHg concentration in turtle blood and fish muscle.

COLLINEARITY

When two predictor variables co-vary, it can be difficult to disentangle each predictor's individual effect. Correlations between predictors can cause problems in the analysis and interpretation of model averaging results (Freckleton, 2011). For this reason, I tested the whole data set for multicollinearity amongst the predictor variables. I used the variance inflation factors (VIFs) method and the commonly used cut off value of 5. The VIFs were calculated using the "car" package in R. The results from the VIF method were corroborated with the calculation of correlation coefficient for each pair of continuous

variables by restricted maximum likelihood (REML) method. For this, I used a threshold value of 0.70 (Smith et al., 2009).

I found that, for turtles, VIF values and correlation coefficients were low, whereas they were high for fish. This indicates that predictor variables were not strongly correlated with one another for turtles, but that collinearity may occur for fish model predictor variables. Turtle VIFs ranged between 1.17 and 1.82, and fish VIFs ranged between 2.19 and 5.11 (Figure A2 – 2). As a result, all predictor variables were considered in the model averaging exercise for turtles, but TL (VIF = 5.11) was left out of the model averaging exercise for fish.

CANDIDATE MODELS

The predictor variables I used to model MeHg concentrations in animals tissues were sex (SEX), size (SIZE), species (SPECIES), lake (LAKE), trophic level (TL), and the proportion of zebra mussels in the diet (PZEBRA). THg concentration in fish and turtles were log-transformed to satisfy assumption of normality and homoscedasticity. For fish and turtles separately, I built a model using the full data set. The models considered in the averaging exercise were those with $\Delta AICc < 4$ (Burnham and Anderson, 2002). The MuMIn package in R was used to create the candidate models (Tables A2 – 1).

SNAILS AND FILTER-FEEDING

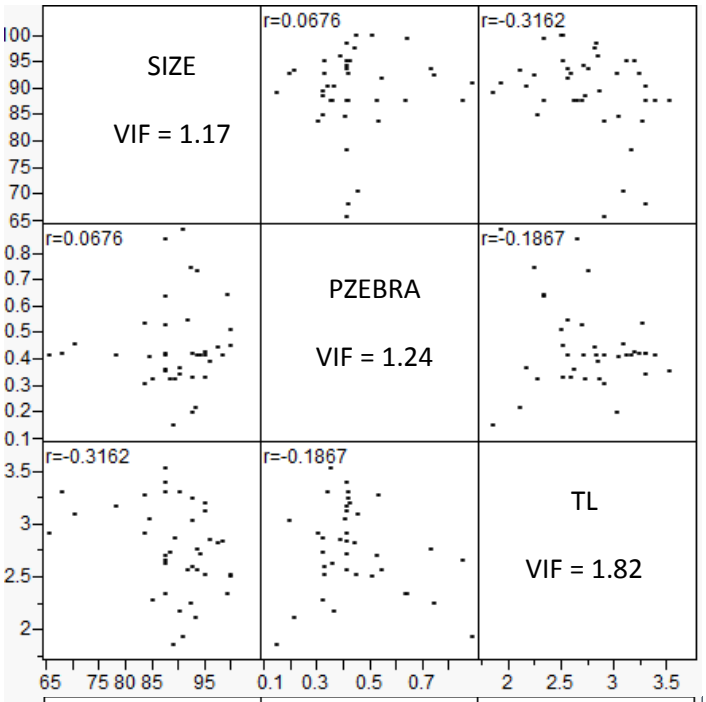
Because the banded mystery snail is an occasional filter-feeder, its ability to accurately represent the benthic food chain can be questioned. This is because filter-

feeding causes the snail's $\delta^{13}C$ to resemble that of the zebra mussels. A difference of 10‰ in $\delta^{13}C$ is typically observed at the base of the pelagic and benthic food chains (France, 1995). In my data set however, the difference in $\delta^{13}C$ between snails and zebra mussels, the chosen representatives of the benthic and pelagic food chains, was consistently smaller and averaged 4.49 ± 1.71 ‰. To determine whether this could affect the predictive ability of my models, I ran the mixing model analysis a few times using hypothetical $\delta^{13}C$ values for snails and zebra mussels. I then compared the results of two scenarios: I compared the output of the model in which the $\delta^{13}C$ for snails and zebra mussels were similar and the output for the model in which the $\delta^{13}C$ values were 10‰ apart. The results show that the fact that snails were occasional filter-feeders in my system shifts the distribution to the right, i.e. the mixing model overestimates the proportion of snails in the diet. It does not affect the shape of the distribution however (Figure A2 – 2). Therefore, I conclude that using this species of filter-feeding snail as a representative of the benthic food chain should not affect the predictive ability of my models.

Table A2 – 1. Candidate multiple linear regression models predicting THg concentrations in A. musk turtles (n = 39) and B. pumpkinseeds (n = 20) for the full data set. Models are ranked by increasing order of second order Akaike Information Criterion (AICc and ΔAICc < 4). The number of parameters (k), as well as the Akaike weights (w), are listed.

	Model	k	AICc	ΔAICc	w
A.	PZEBRA	1	22.25	0	0.17
	PZEBRA, SIZE	2	23.41	1.17	0.1
	PZEBRA, SIZE, TL	3	23.49	1.24	0.09
	PZEBRA, TL	2	23.49	1.25	0.09
	SIZE, TL	2	23.55	1.3	0.09
	TL	1	23.58	1.33	0.09
	PZEBRA, SEX	2	24.5	2.25	0.06
	LAKE, PZEBRA	2	24.74	2.49	0.05
	LAKE, TL	2	24.78	2.54	0.05
	LAKE, SIZE, TL	3	25.39	3.14	0.04
	PZEBRA, PZEBRA, TL	3	25.76	3.51	0.03
	PZEBRA, SEX, SIZE	3	25.82	3.57	0.03
	LAKE, PZEBRA, SIZE	3	25.9	3.65	0.03
	SEX, SIZE, TL	3	26.04	3.79	0.03
	SEX, TL	2	26.05	3.81	0.03
	PZEBRA, SEX, TL	3	26.13	3.88	0.02
	LAKE, PZEBRA, SIZE, TL	4	26.18	3.94	0.02
B.	PZEBRA, SIZE	2	-2.37	0	0.35
	Null	0	-2.34	0.03	0.35
	PZEBRA	1	-0.85	1.52	0.16
	SIZE	1	-0.46	1.91	0.14

A



B

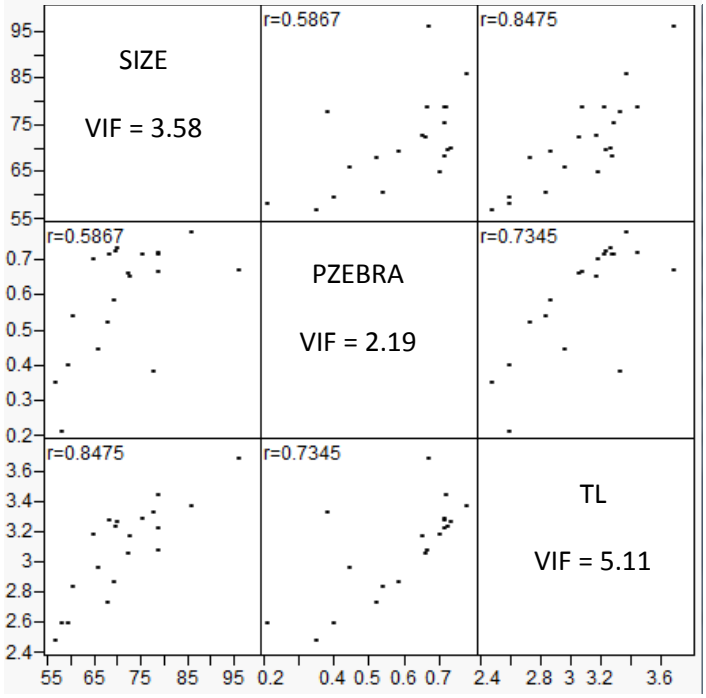


Figure A2 – 1. Correlation matrix of THg continuous predictor variables in A. musk turtles (n = 39) and B. pumpkinseeds (n = 20): Trophic level (TL), proportion of zebra mussels in the diet (PZEBRA) and size (SIZE). Correlation coefficients by REML method (r) and variance inflation factors (VIF) are given.

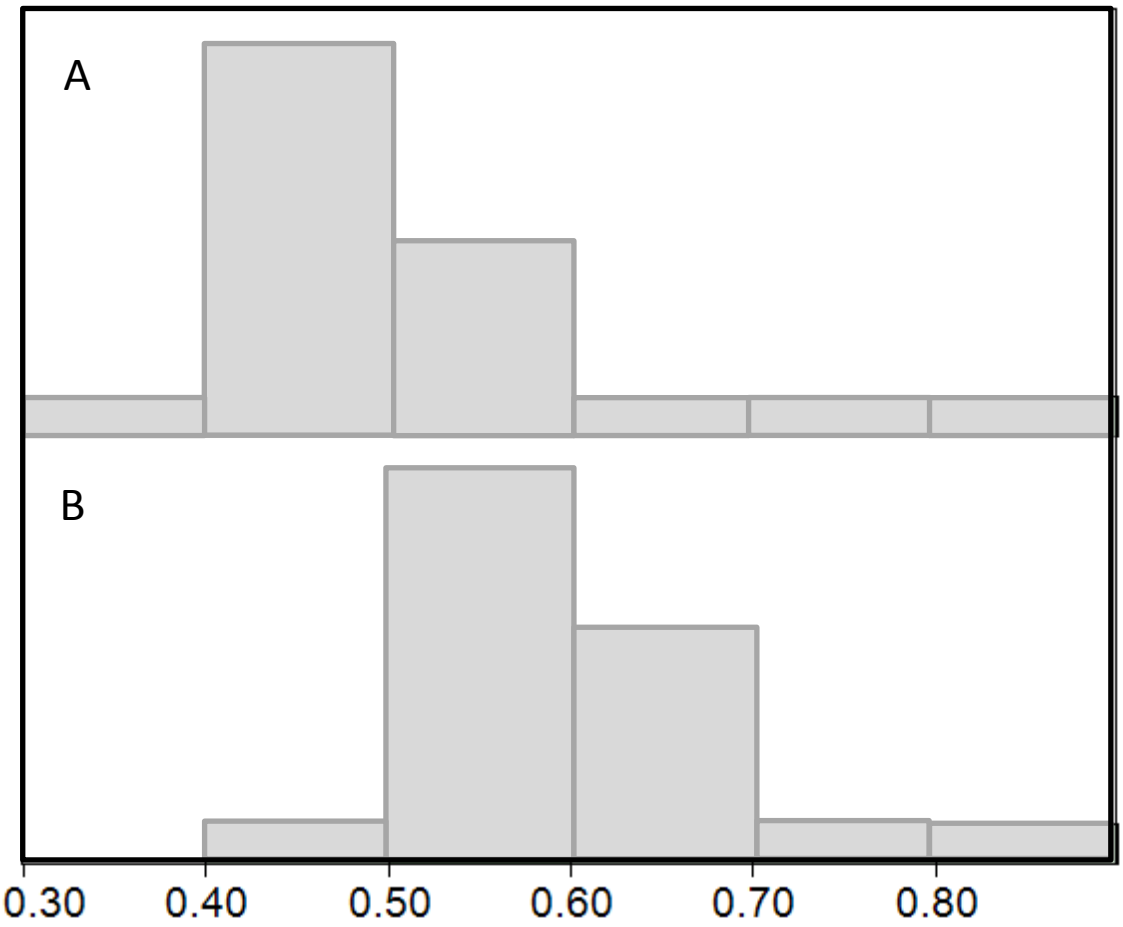


Figure A2 – 2. Frequency distribution of the proportion of snails in the diet of musk turtles when obtained by two end-member mixing model analysis when A. hypothetical $\delta^{13}C$ values are used to create a 10‰ difference in ratio between snails and zebra mussels, and B. when the measured $\delta^{13}C$ from the data set are used.

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