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Dietary Reliance on Benthic Primary Production as a Predictor of Mercury Accumulation in Freshwater Fish and Turtles

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Abstract The feeding ecology of a species can affect the transfer and accumulation of contaminants such as mercury (Hg). Modeling the accumulation of Hg through food webs can help identify which animals are likely to be burdened by elevated Hg concentrations. In lakes, most of the Hg is sequestered in the sediments. Therefore, species ultimately relying on benthic primary production may experience a greater trophic transfer of Hg relative to species that rely on pelagic primary production. This hypothesis was tested in a simple food web using muscle tissue collected from three species of fish (*Lepomis gibbosus*, *Notropis heterodon*, and *Labidesthes sicculus*) and blood from two species of turtles (*Sternotherus odoratus* and *Chrysemys picta*) that differ in reliance on benthic primary production. Averaged multiple linear regression models were used to predict Hg concentrations in the five consumers with respect to reliance on benthic primary production, while controlling for other factors known to influence Hg accumulation (sex, size, lake, species identity, and trophic level). A positive and significant relationship was found between Hg burden and dietary reliance on benthic primary production, animal length, trophic level, and species identity in fish. In turtles, the relationship between Hg burden and dietary reliance on benthic primary production was not significant, but trophic level, animal length, and species identity significantly influenced Hg burden. Overall, reliance on benthic primary production was an important predictor of Hg burden for fish, but not for turtles. Future attempts to model Hg accumulation in similar study systems and/or fish species should include dietary reliance on benthic primary production as a predictor variable.

Keywords Stable isotopes · Mixing model · Food web · Ontario · Biomagnification

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1 Introduction

The ubiquitous presence of mercury (Hg) in the environment is a concern for wildlife (Eisler 1987). It can cause adverse health effects in freshwater vertebrate species, including reduced hatching success in freshwater turtles (Hopkins et al. 2013), and the suppression of egg production, decreased spawning success, and

suppression of the production of sex hormones in fish (Scheuhammer et al. 2007).

Hg accumulates in freshwater food webs predominantly in the form of monomethylmercury (MMHg) (Atwell et al. 1998; Boudou and Ribeyre 1997; Van der Velden et al. 2013), and species feeding at higher trophic levels tend to have higher MMHg tissue concentrations. For example, Bergeron et al. (2007) found a significant positive relationship between total mercury (THg) concentration [THg] and trophic level for four species of freshwater turtles. Among these four species, the snapping turtle (*Chelydra serpentina*) was the most carnivorous and had the highest [THg] in its blood (Bergeron et al. 2007). Similarly in freshwater fish, Depew et al. (2013) found a positive correlation between muscle [THg] and trophic level in 28 species.

Despite the correlation between trophic level and [THg] for freshwater fish and turtles, considerable unexplained variation in [THg] exists within and among lake populations. It has been a challenge to identify which food web processes and lake physico-chemistry variables are responsible for this variation and how these variables interact with one another (Lavoie et al. 2013). For instance, variation in THg biomagnification rates between species has 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), to the presence or absence of invasive species at the study sites (Hogan et al. 2007), and to water physicochemical variables such as pH or dissolved organic matter concentrations (Kelly et al. 1995; Wang and Wang 2010).

Stable isotope analyses have greatly improved our ability to model food web structure and dietary preferences in lake ecosystems. Stable nitrogen (N) isotope ratios ($\delta^{15}N$) are useful indicators of trophic level in food webs (Peterson and Fry 1987), and stable carbon isotope ratios ($\delta^{13}C$) provide information on dietary carbon (C) source (Hecky and Hesslein 1995). Previous studies have combined data from stable isotope analyses and data on contaminants to determine their inter-relationships. For example, Anderson et al. (2009) found that 91 % of the variation in [THg] in the blood of South Atlantic seabirds could be explained by variation in blood $\delta^{13}C$ and $\delta^{15}N$. Power et al. (2002) also attempted to explain variation in [THg] in a fish community from a subarctic lake using fish length, mass, age, $\delta^{15}N$, and $\delta^{13}C$; it was determined that $\delta^{15}N$ and $\delta^{13}C$ were the second and third most important variables explaining variation in [THg], respectively, and that in some fish species, including $\delta^{13}C$ as an explanatory variable in a multiple regression model could explain up to 93 % of the variation in [THg] (Power et al. 2002). Without the stable isotope data, the Power et al. (2002) models never explained more than 48 % of the variation in [THg]. These studies highlight the importance of considering both trophic level ($\delta^{15}N$) and reliance on benthic and pelagic primary production ($\delta^{13}C$) when modeling [THg] in aquatic species. However, the focus is often on well-studied fish, bird, and mammal species, and seldom on reptile species (Sparling and Gorsuch 2010).

The main objective of this study was to determine whether dietary reliance on benthic primary production affected THg accumulation in fish and turtles. This was done by building a food web model for three Ontario (Canada) lakes for which water and sediment [THg] were known, and in which a number of fish and turtle species that varied in dietary reliance on benthic primary production could be captured in sufficient numbers. The food web of the Ontario lakes considered here is simple, and the aquatic vertebrate biomass is composed mainly of species such as musk turtles (*Sternotherus odoratus*), painted turtles (*Chrysemys picta)*, pumpkinseeds (*Lepomis gibbosus*), brook silversides (*Labidesthes sicculus*), and blackchin shiners (*Notropis heterodon*).

Species that feed near or in lake sediments may be exposed to higher [THg] than those feeding in the water column since lake sediments can be a major repository for the different forms of Hg (Sorensen et al. 1990) and since methylation rates can be highest in lake surface sediments (Furutani and Rudd 1980). Based on this, we hypothesized that THg accumulation in fish and turtles could be predicted from their dietary reliance on benthic primary production. This hypothesis was tested while controlling for factors known to affect MMHg accumulation, including animal length, sex, provenance (lake), and species identity. Dietary reliance on benthic primary production was expected to be positively correlated with [THg] because at our study site, [THg] was higher in the sediments than in the water column (Parks Canada, unpublished data). Moreover, benthic MMHg production, rather than pelagic MMHg production, has been shown to be more closely related to the uptake of MMHg by invertebrates in some wetlands (Pinnegar and Polunin 1999).

2 Materials and Methods

2.1 Site Description

Sampling took place between May and August 2012 in three interconnected lakes—Indian Lake, Newboro Lake, and Upper Rideau Lake—in central Rideau Canal, a UN World Heritage Site in Ontario, Canada (Fig. 1). The three lakes are productive and shallow, and they are overlying dolomite, limestone, sandstone, and coarse conglomerate. These lakes are representative of the lakes in the Frontenac Arch of the Canadian Shield (Stuyt et al. 2015) and were chosen because THg concentrations had been documented in the surface sediments and in the water column. Newboro Lake has the highest mean surface sediment [THg] with 0.16± 0.01 μg/g THg (n=4), compared to Indian and Upper Rideau Lakes that had mean concentrations of 0.12± 0.07 μg/g THg (n=8) and 0.12±0.06 (n=17), respectively (Stuyt et al. 2015). Surface water [THg] was previously measured for all three lakes and was often found to be below detection limit (0.01 μg/l) and never over 0.02 μg/l in all three lakes (Parks Canada, unpublished data).

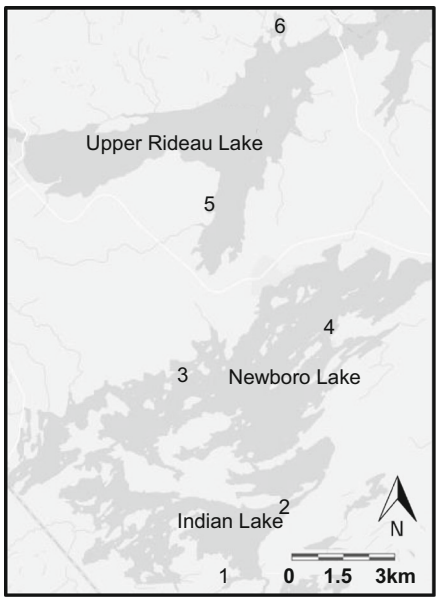

Fig. 1 Location of six sampling sites across three Rideau Lakes in Ontario, Canada: Indian Lake, Newboro Lake, and Upper Rideau Lake. Universal Transverse Mercator (UTM) coordinates are as follows: UTM zone 18, 394730E, 4937468N (site 1), UTM zone 18, 396097E, 4939690N (site 2), UTM zone 18, 394062E, 4942661N (site 3), UTM zone 18, 397549E, 4944225N (site 4), UTM zone 18, 394082E, 4946567N (site 5), UTM zone 18, 395773E, 4950965N (site 6)

2.2 Animal Collection and Sample Preparation

Musk turtles (n=39), painted turtles (n=60), and pumpkinseeds (n=20) were captured using fyke nets that were emptied every 24 h. Brook silversides (n=49) and blackchin shiners (n=50) were captured using seine nets that were dragged along the shore at each sampling site. Musk turtles are omnivorous and feed primarily on insects and snails. They are also known to eat earthworms, fish eggs, minnows, and some parts of higher plants. They forage along the bottom of soft-bottomed lakes where the water is shallow (Ernst and Barbour 1972). The painted turtle is also omnivorous: it feeds on aquatic vegetation, algae, insects, crustaceans, and fish (Ernst and Barbour 1972). Blackchin shiners feed on insects, algae, and crustaceans such as water fleas, while brook silversides eat mostly water fleas and small insects. Both species are well adapted to living in the surface layers of lakes (Scott and Crossman 1974). On the other hand, the larger pumpkinseed eats primarily insect larvae, nymphs, amphipods, and molluscs and forages for these prey items atop the sediments in the littoral zone (Scott and Crossman 1974).

For turtles, 0.7 ml of blood was taken by subcarapacial vein puncture (Dyer and Cervasio 2008) using 1-ml un-heparinized syringes fitted with 25-gauge, 38-mm needles. Blood samples were split in half in the field and immediately frozen at −20 °C pending analyses. In the laboratory, half of the blood volume per individual was then frozen at −60 °C in preparation for lyophilization and stable isotope analyses. Given the Species at Risk status of turtles in these lakes, collecting blood rather than muscle tissue was chosen as the acceptable minimally invasive tissue collection technique. For each turtle, carapace length was measured using a caliper and sex of each individual was determined using secondary sexual characteristics (Moll 1973). All fish were euthanized using sharp blows to the head followed by spinal cuts. Fish were frozen at −20 °C for transport back to the laboratory. Once in the laboratory, total length of thawed fish was measured and a muscle sample was collected using a filleting knife that was thoroughly cleaned between each use. Muscle samples were frozen and lyophilized.

Banded mystery snails (*Viviparus georgianus*) are grazers (Buckley 1986) and were selected in this study

to obtain $\delta^{13}C$ measures from a primary consumer relying on benthic primary production. Zebra mussels (*Dreissena polymorpha*), in contrast, are filter feeders (Horgan and Mills 1997) and were selected to obtain $\delta^{13}C$ measures from a primary consumer relying on pelagic primary production. Three composite samples of banded mystery snails and zebra mussels were collected from sampling sites 1 through 6 (Fig. 1). Each sample was composed of ten individuals collected at the same location. Both species of mollusks were collected by removing them from the surface of rocks or logs submerged in the water using a dip net or by hand. They were then transported to the laboratory in jars containing lake water. The live animals were depurated for 24 h in lake water. After depuration, the muscle from each mollusk was extracted and lyophilized.

All manipulations involving animals were pre-approved by the University of Ottawa Animal Care Committee, and an Animal Care Protocol was issued for this research project.

2.3 Stable Isotope Ratios

Carbon stable isotope ratio ($\delta^{13}C$) and nitrogen stable isotope ratio ($\delta^{15}N$) analyses were performed at the G.G. Hatch Laboratory at the University of Ottawa, Ontario, Canada. Internal standards used were as follows ($\delta^{15}N$, $\delta^{13}C$ in ‰): C-51 nicotiamide (0.07, −22.95), C-52 ammonium sulfate + sucrose (16.58, −11.94), C-54 caffeine (−16.61, −34.46), and blind standard C-55: glutamic acid (−3.98, −28.53). These standards cover the natural range. Blanks consisted of empty tin capsules. The analytical precision is based on the internal standard that is not used for calibration and averaged 0.05‰. Duplicate samples were analyzed for each ten-sample interval, and average relative difference between duplicates was 0.49‰. Details of the procedures can be found in Châteauvert (2013, MSc thesis, University of Ottawa, Ottawa, Ontario, Canada). The trophic level for each fish and each turtle was calculated using the two-source equation provided in Post (2002). Predators are typically enriched in the heavy isotope (higher $\delta^{13}C$ and $\delta^{15}N$) relative to their prey (Post 2002). To account for this enrichment in turtles, trophic enrichment factors (TEFs) of turtle blood based on laboratory experiments reported in the literature were used to correct $\delta^{13}C$ and $\delta^{15}N$: +0.23‰ for the $\delta^{13}C$ (Seminoff et al. 2007) and +2.2‰ for the $\delta^{15}N$ (Aresco, 2005, PhD thesis, Florida State University, Tallahassee, Florida, USA). For fish, TEFs of $\delta^{13}C$ and $\delta^{15}N$ were calculated with the equation provided by Caut et al. (2009) in their Fig. 4 for fish muscle. These TEFs were calculated for each individual fish and then averaged for all fish. The calculated TEF values used for the fish were +2.35 and +3.24‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively.

Although standardizing the stable isotope ratio of consumers to the base of the food web is often done for $\delta^{15}N$ (Kidd et al. 2012), very few studies have done so to reduce the ambiguities associated with comparing $\delta^{13}C$ across multiple food webs to derive a model for fish tissue MMHg concentration [MMHg], and to the best of our knowledge, no studies have done so for turtle tissue (Aubail et al. 2011; Riva-Murray et al. 2013). Using $\delta^{13}C$ to conduct between lake comparisons of the reliance of consumers on benthic or pelagic primary production is problematic because the $\delta^{13}C$ of primary producers can vary greatly between and within lakes (Syväranta et al. 2006). Stable isotope analyses ($\delta^{15}N$, $\delta^{13}C$) and Bayesian stable isotope mixing models (SIMMs) were used to examine the patterns of MMHg biomagnification in fish and turtle communities of the lakes. SIMMs were solved within a Bayesian framework because available applications based on the Bayesian framework currently allow users to include various sources of uncertainty more efficiently than the framework of frequentist SIMMs (Hopkins and Ferguson 2012), including basal variation in $\delta^{13}C$. The Bayesian mixing-model package Stable Isotope Analysis in R (SIAR) in R 2.15.3 (The R Foundation for Statistical Computing 2013) was used for the calculation of dietary reliance on benthic primary production (Parnell et al. 2008). Standard deviations (SD) for TEF values are required as input in the mixing-model package SIAR. We assigned larger-than-expected SD values to account for the fact that the TEFs were not obtained empirically. The lipid content of tissues used in stable isotope analyses can bias mixing models by causing an overestimation of the fraction of protein-rich foods in a consumer's diet (Phillips and Koch 2002). When lipids constitute a large fraction of the tissue analyzed (C/N> 3.5), lipid extraction or mathematical normalization of the $\delta^{13}C$ data is important (Post et al. 2007). Lipids did not constitute a large fraction of the tissue analyzed in neither fish nor turtles in our sample (C/N±SD ranged from 3.07±0.03 to 3.47±0.12), so we did not extract lipids or normalize $\delta^{13}C$ values prior to data analysis.

Finally, a difference of 10‰ in $\delta^{13}C$ is typically observed at the base of the pelagic and benthic food

chains (France 1995). In our study lakes, the difference in $\delta^{13}C$ between snails and zebra mussels was consistently smaller and averaged 4.49±1.71‰ (n=18). This did not, however, affect the performance of the mixing models (Châteauvert, 2013, MSc thesis, University of Ottawa, Ottawa, Ontario, Canada).

2.4 Mercury Analysis

[THg] was used to represent [MMHg] in animal tissue. MMHg biomagnifies more effectively in the food web relative to inorganic mercury compounds. Thus, the contribution of MMHg to the total Hg pool increases with an organism's trophic position, with mercury content of top predators being usually almost exclusively MMHg. Twenty-five percent of the samples were analyzed for both [MMHg] and [THg]. On average, the part of THg that was MMHg was 93.9±5.2 % in musk turtles (n=11), 86.9±19.5 % in painted turtles (n=16), 96.9±3.2 % in brook silversides (n=15), and 89.5±22.0 % in blackchin shiners (n=15). The percentage of MMHg to THg in pumpkinseeds was not examined, but it can be assumed to be above 90 % as well (Grieb et al. 1990). [THg] was thus used as a reasonable approximation of [MMHg].

Turtle blood and fish muscle were analyzed for [THg] by combustion, amalgamation, and cold vapor atomic absorption spectrophotometric detection following the Environmental Protection Agency (EPA) method 7473 and using a MA-3000 Mercury Analyzer (Nippon Instruments Corporation, Tokyo, Japan). For quality assurance, each group of ten samples included a standard reference material (SRM) obtained from the National Research Council of Canada (NRC). These were DORM-3, 382±60 ng/g THg, or DORM-4, 410 ±55 ng/g THg. Recovery on the SRMs averaged 93.2 % for DORM-3 (355.97±49.23 ng/g) and 87.3 % for DORM-4 (358±2.91). Each group of 10 samples also included a replicate sample, and each set of 100 samples was initiated by purging the instrument twice. Mean relative difference between replicates was 6.64 ng/g. Turtle blood and fish muscle were analyzed for [MMHg] by capillary gas chromatography coupled with atomic fluorescence spectrometry (GC-AFS) as described by Buckley (1986).

2.5 Statistical Analyses

Separate models were used to predict [THg] in fish and in turtles using multiple regression and the following predictor variables: relative body length, sex (only for turtles), lake identity, species, proportion of snails in the diet as a measure of dietary reliance on benthic primary production from the mixing models (PBENTHOS), and trophic level. Relative body length, calculated as the % of maximum body length recorded within each species across all lakes, was used instead of absolute body length as a means of standardization so that lengths could be compared across species. For the analysis, individuals were pooled across sites within each lake. Multicollinearity between predictor variables was assessed using variance inflation factors (VIFs) values and correlation coefficients. Collinearity was weak among all predictor variables. VIFs ranged from 1.1 to 1.6 and correlation coefficients varied between 0.02 and 0.40 (Châteauvert, 2013, MSc thesis, University of Ottawa, Ottawa, Ontario, Canada). Based on this, all predictor variables were included in the models for fish and turtles.

Using log-transformed [THg] to satisfy the assumptions of normality and homoscedasticity, models were built using the full dataset. Residual plots were examined to confirm the linear relationships between the dependent and independent variables. Akaike's information criterion (AICc) and Akaike weights (w) were used for model selection (Burnham and Anderson 2002). Models with ΔAICc <4 were used in the calculation of the parameters of the final average model (Burnham and Anderson 2002). The averaged predictive model also took into account the relative Akaike weights of each candidate model. Standard errors (SEs) and 95 % confidence intervals (95 % CIs) 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. p values were calculated on the same basis as the confidence intervals as described by Burnham and Anderson (2002).

Statistical analyses were performed with JMP 10.0 (SAS Institute, Cary, NC) and R 2.15.3 (The R Foundation for Statistical Computing) and the package MuMIn (Barton 2015).

3 Results and Discussion

The main objective of this study was to determine whether dietary reliance on benthic primary production affected THg accumulation in fish and turtles. In addition, instead of using $\delta^{13}C$ values as a direct way of

tracing carbon origins within the different lake zones, the ratio was converted to a measure of dietary reliance on benthic primary production using a mixing model to account for basal variations in $\delta^{13}C$. PBENTHOS was a significant predictor of [THg] in the fish model, but not in the turtle model.

3.1 Predictor Variables

The variability of predictor variables is presented in Table 1. Within lakes, blackchin shiners and brook silversides had the highest $\delta^{13}C$ values, except in Indian Lake where blackchin shiners had the lowest $\delta^{13}C$ values (Fig. 2). Blackchin shiners and brook silversides $\delta^{13}C$ values were on average 1.11–1.51‰ higher than the pumpkinseed values in Upper Rideau Lake. Musk turtle $\delta^{13}C$ values were on average 1.04–2.92‰ higher than painted turtle $\delta^{13}C$ values. Fish and turtle $\delta^{13}C$ values were comparable to $\delta^{13}C$ values found in the literature for similar species in similar environments (Bergeron et al. 2007; Hecky and Hesslein 1995). Snail and zebra mussel $\delta^{13}C$ averaged values varied across lakes. Snail $\delta^{13}C$ values were between −6.24 and −20.17‰ and zebra mussel $\delta^{13}C$ values were between −29.63 and −26.36‰ (Fig. 2). Zebra mussel $\delta^{13}C$ values were lower than snail $\delta^{13}C$ values by an average of 3.39–6.19‰, and there was no overlap in the range of $\delta^{13}C$ values of the two groups of mollusks within sampling sites. The $\delta^{13}C$ values for these primary consumers were similar to those in nearby Opinicon Lake (Bulté and Blouin-Demers 2008). For the three lakes, average % benthic carbon in the diet (PBENTHOS) varied less in fish (0.41–0.51 %) than in turtles (11–70 %; Table 1). This result is consistent with the fact that turtles are opportunistic feeders. Within a lake, interestingly, species with smaller $\delta^{13}C$ also had lower PBENTHOS with only one exception: Indian Lake painted turtles had smaller $\delta^{13}C$ values when compared to musk turtles, but had higher PBENTHOS values. This means that with the exception of Indian Lake painted turtles, both adjusted (PBENTHOS) and unadjusted ($\delta^{13}C$) measures of dietary reliance on benthic primary production in fish and turtles followed a similar trend.

Tissue [THg] varied between species and across the three lakes, and turtle blood consistently had lower [THg] than fish muscle (Table 1). Painted turtles from Upper Rideau Lake had lower blood [THg] than painted turtles in the other two lakes. Turtle [THg] was similar to previously published data for painted and musk turtles from non-contaminated sites (Bergeron et al. 2007). For fish, pumpkinseeds had the highest muscle [THg] and blackchin shiners had higher muscle [THg] than brook silversides. [THg] in fish from the three study lakes were similar to [THg] in the literature for similar species in similar lakes (Yu et al. 2011).

Total length and trophic level were included as variables in the multiple regression analysis to account for their effect on THg accumulation (Table 1). We did not sample across a wide range of values for length and trophic level as their effect on THg accumulation was not specifically being investigated in this study. Musk turtles had higher trophic levels than painted turtles in Indian Lake and in Newboro Lake. Also, brook silversides had lower trophic levels than blackchin shiners in Indian Lake and in Newboro Lake, but not in Upper Rideau Lake. Finally, in Upper Rideau Lake, pumpkinseeds had the highest trophic level. One-way ANOVAs revealed that, in general, all species of fish and turtles considered in this study had similar to marginally different trophic levels (fish: $F_{(2,\ 116)}=1.255$, $p>0.05$; turtles: $F_{(1,\ 97)}=4.72$, $p=0.04$), and this was consistent with the fact that a good portion of their diet is composed of primary producers (Ernst and Barbour 1972; Scott and Crossman 1973).

3.2 Turtle Model

The turtle sample size was not equal across the three lakes (no musk turtles were found in Upper Rideau Lake), so a comparison of the parameter coefficient estimations in the models with and without "lake identity" as a predictor was performed. Qualitatively, the imbalance in the sample size did not affect parameter coefficient estimation since parameter coefficients only varied by ±0.01 between the two models, and their statistical significance did not change. Using the full dataset for both species of turtles in the multiple regression analysis, seven models with ΔAICc <4 were obtained, and these models had between two and five parameters. 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 models. All coefficients except trophic level and animal length (as a % of the maximum) had high SE and large 95 % CI (Table 2(A)), and this indicated that the averaged multiple regression model did not fit the [THg] data well. Most notably, dietary

Table 1 Variability in the predictor variables used in modeling mercury concentrations in turtles and in fish in Ontario, Canada

	Lake	Species (*n*)	THg (ng/g)	Total length range (mm)	Total length (% max.)	TL	PBENTHOS
A. Turtles	Indian						
		Musk turtle (20)	5.42±0.90	80–119	86.44±1.85	2.97±0.10	0.62±0.02
		Painted turtle (20)	20.51±8.15	103–159	85.79±1.35	2.71±0.18	0.68±0.03
	Newboro						
		Musk turtle (19)	4.81±0.58	102–122	91.65±0.98	2.55±0.06	0.49±0.04
		Painted turtle (20)	20.50±1.06	114–160	89.00±1.35	1.82±0.11	0.11±0.02
	Upper Rideau						
		Painted turtle (20)	11.28±1.69	112–160	87.99±1.23	2.91±0.14	0.70±0.04
B. Fish	Indian						
		Blackchin shiner(20)	334.84±40.67	31.12–56.60	79.19±1.23	3.13±0.04	0.42±0.01
		Brook silverside (19)	161.44±11.07	49.42–75.17	72.77±1.59	3.08±0.03	0.45±0.03
	Newboro						
		Blackchin shiner (10)	321.93±46.63	43.46–53.57	84.89±2.09	3.23±0.02	0.50±0.01
		Brook silverside (10)	235.83±6.30	56.82–65.52	79.74±0.74	3.06±0.04	0.51±0.02
	Upper Rideau						
		Blackchin shiner (20)	138.56±10.68	23.13–51.47	73.34±2.27	2.77±0.05	0.46±0.03
		Brook silverside (20)	110.45±5.87	48.08–68.52	77.87±1.21	2.84±0.02	0.42±0.01
		Pumpkinseed (20)	409.13±42.12	107.46–182.56	71.24±2.17	3.08±0.07	0.41±0.04

Size of the animals is indicated as the percent maximum total length for that species at that location, and PBENTHOS is a proportion. Trophic level (TL) was calculated using the formula described by Post et al. (2007). All measurements are given as mean±standard error

reliance on benthic primary production (PBENTHOS) was not a significant predictor variable in the multiple regression model. Consequently, the initial prediction that THg burden should increase with PBENTHOS was not supported by the data on turtles.

Variation in turtle age may contribute to the unexplained variation in THg accumulation. Older individuals are expected to have higher [THg] in their tissues when compared to younger individuals due to the bioaccumulative nature of THg. However, because no reliable measure of age exists for adult freshwater turtles (methods are evaluated in Avens and Snover (2013)), the effect of age-related bioaccumulation could not be considered in the models, and may have obscured the effect of the other variables.

Although no effect of PBENTHOS on THg accumulation was uncovered in the analysis of the turtle data, [THg] significantly increased with length (as a % of the maximum) and trophic level, and mean [THg] varied significantly between species. The effect of length and trophic level can be explained by MMHg's ability to bioaccumulate and biomagnify, respectively. The comparatively large coefficient associated with species identity suggests that there are also some inherent qualities specific to each species of turtle that can influence their THg accumulation. In this case, painted turtles had higher [THg] in their blood than musk turtles. It is doubtful that diet alone could explain this difference in accumulation of THg since, on average, painted turtles had lower trophic levels. Instead, other factors which were not included in the models could be responsible for this interspecific difference in mercury accumulation between painted and musk turtles. For example, physiological turnover rates can vary across species and have been shown to affect metal accumulation in organisms such as polychaetes, mussels, and copepods (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). The results presented here could indicate a similar trend in turtles: the larger painted turtle could accumulate higher [THg] in its blood as a result of slower tissue turnover. In addition, the percent

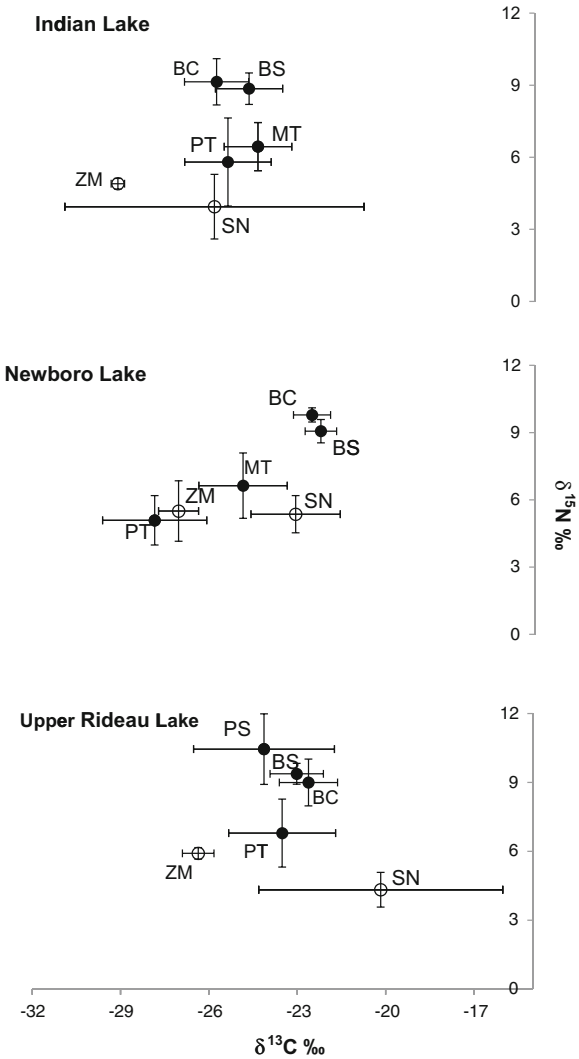

Fig. 2 Stable isotope diagram of the Rideau Canal Lakes food web. Means across lakes and standard deviations are presented. *SN* snail, *ZM* zebra mussels, *PT* painted turtle, *MT* musk turtle, *BC* blackchin shiner, *BS* brook silverside, *PS* pumpkinseed

hemoglobin in turtle blood varies between 5.9 and 11.2 in freshwater species (Dessauer 1970). Since MMHg has an affinity for proteins containing cysteine such as hemoglobin (Weed et al. 1962), those with higher blood hemoglobin concentrations may accumulate more MMHg and thus more THg in their blood. Consequently, although the models could only poorly predict THg accumulation in turtles, the modeling results support the inclusion of the covariates species identity, size, and trophic level in future attempts to model THg accumulation in these animals, but it does not support the inclusion of the variable of interest in this study, PBENTHOS.

3.3 Fish Model

Pumpkinseeds were only collected in one lake, but within species sample sizes were similar. A comparison of the parameter coefficient estimations in the models with and without lake identity as a predictor variable revealed that this imbalance was affecting the results in a biologically significant way. Thus, we excluded lake identity as a predictor variable in the model. The best predictive model based on the AICc values was the model that included all the other predictors (Akaike weight of 0.96). All continuous predictor variables had a significant effect on [THg], with length (as % of the maximum length), PBENTHOS, and trophic level having a positive relationship with [THg] (Table 2(B)). Thus, the results from this model support the prediction that [THg] in fish tissues should increase with PBENTHOS. In addition, the estimated coefficient for PBENTHOS was the highest of all estimated coefficients in this model, meaning that the positive estimated rate of change of the conditional mean of [THg] with respect to PBENTHOS, when all other predictor variable were fixed, was the most important. Eagles-Smith et al. (2008) found a similar trend when correlating percent diet derived from the benthos to [THg] for a variety of fish species similar to those considered here, including bluegills (*Lepomis macrochirus*) and inland silversides (*Menidia beryllina*). Lavoie et al. (2010) found that at the base of the food web, benthic species accumulated higher [THg] than pelagic species, whereas the opposite trend was true at the top levels of the food web. The positive trend observed between [THg] and PBENTHOS in our base-of-the-food-web dataset supports the conclusions of these studies.

Mean [THg] varied significantly between species of fish. Because most short-lived fish species such as the ones considered here grow continuously during their lives, the relationship between age and length is typically strong (Mommsen 2001). The positive relationship between [THg] and length is therefore consistent with the expected positive relationship between age and [THg] caused by the ability of mercury to bioaccumulate. [THg] also varied between species. Much like for turtles, the difference in accumulation of [THg] between species could be explained by differences in protein content in the muscle (Kinsella et al.

Table 2 Averaged parameter coefficients for two stepwise multiple regression models predicting THg concentrations in (A) turtles (n=99) and (B) fish (n=119) in Ontario, Canada

	Variable	Coefficient	SE	95 % CI	p Value
A. Turtles					
	Intercept	−1.03	0.55	−2.12, 0.06	>0.05
	LENGTH	*0.01*	*0.00*	*0.00, 0.02*	*0.04*
	SPECIES	*0.27*	*0.07*	*0.13, 0.42*	*0.0003*
	TL	*0.30*	*0.05*	*0.19, 0.42*	*<0.0001*
	PBENTHOS	0.20	0.15	−0.09, 0.49	>0.05
	SEX	0.03	0.08	−0.12, 0.19	>0.05
	LAKE	0.00	0.10	−0.20, 0.20	>0.05
B. Fish					
	Intercept	0.44	0.23	−0.02, 0.89	>0.05
	PBENTHOS	*0.76*	*0.15*	*0.46, 1.05*	*<0.0001*
	LENGTH	*0.01*	*0.00*	*0.01, 0.02*	*<0.0001*
	SPECIES	*0.25*	*0.04*	*0.12, 0.39*	*<0.0001*
	TL	*0.22*	*0.08*	*0.07, 0.38*	*0.004*

Standard error (SE), 95 % confidence intervals (95 % CI), and p values are given for each parameter. Significant parameter coefficients are italicized

1977) or to varying rates of tissue turnover between the different species of fish (MacAvoy et al. 2006). Finally, an increase in fish [THg] with trophic level was uncovered and is consistent with the fact that MMHg biomagnifies in the food web. The estimated coefficient for PBENTHOS was higher than that of trophic level, indicating that dietary reliance on benthic primary production had more influence on THg accumulation than trophic level. However, this result could be an artifact of the study design, which was restricted to a subset of the food web and included fish with a narrow range of trophic levels. Thus, including a broader range of prey items as well as fish from upper trophic levels would confirm whether this trend extends to a broader food web and whether the strength of influence of dietary reliance on benthic primary production is truly more important than that of trophic level for these animals.

3.4 Comparison Between Fish and Turtles

SE and 95 % CI of the coefficients were smaller for the fish average predictive model than for the turtle predictive model (Table 2), indicating that there was a better fit between the averaged multiple regression model and the [THg] data for fish than for turtles.

To make any further comparisons between the fish and turtle models, we must first consider the tissue types that were analyzed. Blood was used in the THg analyses for turtles, and muscle was used in the THg analyses for fish. [THg] was used as a good approximation of [MMHg] in both tissue types. Blood integrates MMHg in a similar manner as muscle: MMHg binds the sulfur-containing amino acids in proteins (Weed et al. 1962). Previous studies have shown that in turtles, blood [THg] is a good predictor of muscle [THg] for a variety of turtle species (Day et al. 2005; Golet and Haines 2001). Despite the significant correlation between blood [THg] and muscle [THg] in turtles, considerable variation exists in how the two concentrations covary (Golet and Haines 2001). This is because blood turnover rates are quite fast compared to turnover rates of other tissues such as muscle. If the turnover rate of the blood is so fast that the [THg] of an individual's blood fluctuates greatly in response to recently ingested meals, it would be difficult to disentangle the noise from the signal, and it would be difficult to relate blood [THg] to a crude measure of diet. Thus, the high turnover rate of blood

could explain why the turtle model could not predict [THg] in turtle blood. For this reason, a quantitative comparison between averaged model parameter coefficients in fish and in turtle models is not advisable. The model performance was better for fish because the tissue used in the analyses had a lower turnover rate for [Hg], and because in the three fish species considered length was a good predictor of age. Thus, [THg] was not influenced by recent dietary intakes and the variable length controlled for the effects of bioaccumulation in these species. For turtles, growth rate diminishes or stops in adulthood. Since all turtle samples were taken from adults, and since no accurate measure of age exists in turtles, the variance that bioaccumulation introduced into the data could not be quantified. Accordingly, the effect of animal length on the accumulation of [THg] in turtles was only marginally significant.

4 Conclusions

In summary, this study indicated that reliance on benthic primary production was an important predictor of THg burden in the fish species under study, but not in the turtle species. The lack of a relationship between PBENTHOS and [THg] in turtle blood may have been caused by the interference of variables not considered in this study, including animal age (bioaccumulation) and metabolic rate, or because of the high turnover rate of THg in blood. In addition, the models built with this set of variables cannot be used to predict [THg] in turtles accurately because the SE and 95 % CI of parameter coefficients estimations were large. For fish, however, the models were able to predict THg burdens well using trophic level, PBENTHOS, and size as predictor variables.

Most freshwater turtles in Ontario receive protection due to their Species at Risk status. This limits the types of tissue samples that can be collected. Obtaining muscle tissue samples from live individuals could potentially result in severe stress and potentially cause deaths. This highlights the importance of studies such as this one where non-lethal and low-stress methods of tissue sampling are investigated for their usefulness in predicting contaminant burdens. It is possible that the levels of mercury in Species at Risk turtles pose a risk to the health of turtles. However, very few studies have investigated mercury-associated health effects in freshwater turtles (Meyer et al. 2014; Hopkins et al. 2013). In our study system, lymphocyte counts in painted turtles were negatively correlated with blood mercury concentrations, but only marginally significantly (Slevan-Tremblay, 2013, BSc Thesis, University of Ottawa, Ottawa, Ontario, Canada).

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