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Metal accumulation in Lake Michigan prey fish: Influence of ontogeny, trophic position, and habitat ☆

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ABSTRACT

Developing an understanding of factors that influence the accumulation and magnification of heavy metals in fish of the Laurentian Great Lakes is central to managing ecosystem and human health. We measured muscle tissue concentrations of heavy metals in Lake Michigan prey fish that vary in habitat use, diet, and trophic position, including alewife, bloater, deepwater sculpin, round goby, rainbow smelt, and slimy sculpin. For each individual, we measured tissue concentrations of four metals (chromium [Cr], copper [Cu], manganese [Mn], and total mercury [THg]), stable isotope ratios for trophic position ($\delta^{15}N$ and $\delta^{13}C$), and individual fish attributes (length, mass). Total mercury concentration was positively related to total length and $\delta^{15}N$. Of all species, round goby had among the greatest increases in mercury per unit growth and was most isotopically distinct from other species. Profundal species (bloater, deepwater sculpin, slimy sculpin) had similar high THg tissue concentrations, possibly due to slower growth due to cold temperatures, whereas other species (alewife, round goby, rainbow smelt) showed more variation in THg. In contrast, other metals (Cr, Cu, Mn) had either a negative or no relationship to total length and $\delta^{15}N$, suggesting no bioaccumulation or biomagnification. Potential incorporation of mercury by sportfish may thus be related to species, age, diet, trophic position, and habitat of prey fish. Our findings serve as a foundation for understanding how heavy metals accumulate in Lake Michigan food webs and highlight the continued need for management of metal input and cycling in Lake Michigan.

Introduction

Metals in aquatic ecosystems are introduced through both natural and anthropogenic sources. While some metals, such as magnesium, are essential trace elements required for a healthy immune system and bone maintenance in animals, other metals are toxic even at low concentrations (e.g., mercury, lead). In addition, some essential trace metals, such as zinc and copper, can sometimes accumulate to concentrations that can harm aquatic life (Chen et al., 2000; Mebane et al., 2020). Further, certain metals, like mercury, can bioaccumulate in organisms and biomagnify with each trophic transfer as a result of dietary food web linkages and habitat use (Arcagni et al., 2018; Clayden et al., 2013). For example, mercury can reach sufficiently high concentrations in large piscivorous fish to warrant concern for fish health and lead to human consumption risks (Gandhi et al., 2017).

The Laurentian Great Lakes have a long legacy of environmental contamination from industrial, urban, and agricultural activities (Venier et al., 2014). For example, the shores of these lakes have petroleum refineries, landfills, foundries, and smelting factories that have released metals including cadmium, copper, iron, lead, manganese, and mercury (Dakiky et al., 2002; Jarup and Akesson, 2009). Impacts resulting from industrial effluents are highlighted most notably by the 31 Areas of Concern (AOCs) designated by the United States Environmental Protection Agency that dot the Great Lakes region, with 10 of these AOCs occurring in the Lake Michigan watershed (USEPA, 2020). While point source inputs of many of these metals have been largely halted by regulatory action, contaminants continue to be introduced through accidental spills, tributary loading, and atmospheric deposition (Lepak et al., 2015b; Sherman et al., 2015). Once introduced, metals cycle in

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the lakes due to persistence in sediments long after the source has been eliminated.

Mercury is methylated in aquatic environments by sulfate-reducing and iron-reducing bacteria found in anoxic conditions (Benoit et al., 2003; Kerin et al., 2006). The transformation of mercury from an inorganic form to an organic form (methylmercury) makes mercury highly bioavailable, which can lead to bioaccumulation in aquatic organisms and biomagnification in the food web. In Lake Michigan, sulfate-reducing bacteria are abundant in anoxic regions created by dense assemblages of algae (largely *Cladophera*) and quagga mussels (Lepak et al., 2015a). Other metals, like cadmium and lead, can also be methylated in aquatic environments by sulfate-reducing bacteria but are either unstable or insoluble in water, thereby making their presence short-lived (Craig and Rapsomanikis, 1985; Feldmann, 2003; Wong et al., 1975). Metals that behave similarly to cadmium and lead have almost no susceptibility to biomagnification across trophic levels.

Another group of metals, the essential trace metals, serve important roles and functions for life. For example, the metals chromium, copper, and manganese aid in multiple metabolic functions including glucose metabolism, synthesis of proteins used for bone and blood formation, and essential enzyme activation in vertebrates (Ebdon et al., 2001). These and other trace metals typically partition or accumulate directly from water, and therefore do not biomagnify or bioaccumulate (Ali and Khan. 2019). While the body normally regulates the concentration of essential trace metals, examples exist of high metal-contaminated sites leading to organisms bioaccumulating these metals with deleterious effects (e.g., Chen et al., 2000; Mebane et al., 2020).

Food web studies are an effective means of assessing the health of an ecosystem and the impact of environmental stressors, such as the introduction and proliferation of invasive species or the fate of environmental contaminants (Lepak et al., 2015b; Turschak et al., 2014). The Lake Michigan food web has been transformed by invasive dreissenid mussels (*Dreissena bugensis* and *D. polymorpha*), which are efficient benthic filter-feeders that sequester nutrients otherwise available to the pelagic food web (Nalepa et al., 2009). This food web alteration has reduced the amount of energy available to the pelagic food web and led to declines in water column phosphorus, chlorophyll, and prey fish biomass (Bunnell et al., 2014; Fahnenstiel et al., 2010; Hecky et al., 2004). The net result has shifted energy pathways from pelagic to benthic habitats (e.g., Kao et al., 2018; Madenjian et al., 2015) and from offshore to nearshore habitats (e.g., Turschak et al., 2014) which, in turn, has altered pathways of contaminant transfer (Lepak et al., 2015a).

Despite the dynamic nature of food webs and contaminant pathways, no previous study has examined how heavy metal concentrations vary among the prey fish community in Lake Michigan. Although often similar in size and trophic position, prey fish have diverse life history strategies and exploit different habitats in the lake (e.g., nearshore or offshore, pelagic or benthic zones, or a combination). For example, native bloater (*Coregonus hoyi*) complete most of their life cycle in the profundal zone (i.e., benthic offshore), whereas non-indigenous round goby (*Neogobius melanostomus*) spend the summer and fall in the benthic nearshore zone, but migrate to the profundal zone during winter and early spring (Mychek-Londer et al., 2013; Walsh et al., 2007). Estimating variation in heavy metal concentrations for different prey fish species can improve understanding of the pathways through which these contaminants accumulate in piscivorous sportfish, such as Chinook salmon (*Oncorhynchus tshawytscha*) and lake trout (*Salvelinus namayacush*) (Gerig et al., 2019). Likewise, stable isotope ratios are useful for analyzing trophic relationships and positions, habitat use, and general food web structure (Post, 2002; Vander Zanden et al., 1997, 1999). Specifically, $\delta^{13}C$ ratios ($^{13}C/^{12}C$) aid in identifying what a species consumes while $\delta^{15}N$ ratios ($^{15}N/^{14}N$) can infer consumer trophic position (Vander Zanden et al., 1999). When combined, $\delta^{13}C$ and $\delta^{15}N$ ratios can be used to calculate isotopic niche widths and dispersion to compare different species in an ecosystem (Jackson et al., 2011).

In this study, we coupled an analysis of heavy metal concentration with an assessment of trophic position and habitat use to identify factors influencing the concentration of heavy metals in Lake Michigan prey fish. We had three main objectives in this study. Our first objective was to determine if bioaccumulation or biomagnification occurs for six heavy metals – cadmium (Cd), chromium (Cr), copper (Cu), manganese (Mn), lead (Pb), and mercury (Hg) – in six common Lake Michigan prey fish species. We predicted that only Hg concentrations would increase with increasing body size and $\delta^{15}N$ values. Our second objective was to relate metal concentrations to fish biometric and habitat variables to discern species-specific differences. We predicted that species identity would be the most important variable for predicting tissue contamination levels but that habitat fidelity could also influence metal concentrations. Our third objective was to compare isotopic niches among prey fish using stable isotope ratios to determine if dietary overlaps can explain variations in metal concentrations. We predicted that invasive round gobies would have the most distinctive isotopic niche, and therefore metal signature, based on their unique habitat, resource use, and seasonal migrations.

Methods

Fish collection

Six species of prey fish — alewife (*Alosa pseudoharengus*), bloater (*Coregonus hoyi*), deepwater sculpin (*Myoxocephalus thompsoni*), round goby (*N. melanostomus*), rainbow smelt (*Osmerus mordax*), and slimy sculpin (*Cottus cognatus*) — were collected by the U.S. Geological Survey (USGS) research vessel *Arcticus* at ten locations across Lake Michigan from May to October 2015 using bottom trawl nets and by a University of Notre Dame (Notre Dame, Indiana) crew at one location in August 2016 using minnow traps (Fig. 1). Samples across all seasons were pooled. Overall, 295 individual prey fish were caught — 70 alewife, 40 bloater, 59 deepwater sculpin, 14 rainbow smelt, 55 round goby, and 57 slimy sculpin. However, sample sizes for the different analyses differ due to tissue availability (Table 1). Upon collection, fish were identified to species, annotated with depth captured and closest port (Electronic Supplementary Material (ESM) Table S1), and then immediately frozen.

Fish tissue preparation

Fish samples were transferred frozen to the University of Notre Dame where species identities, lengths, and mass were recorded. For each fish, dorsal muscle tissue was extracted, freeze-dried, and then homogenized to a fine powder. Wet fish tissue was weighed immediately prior to freeze-drying and immediately after to calculate percent water so that later measurements could be converted from dry weight to wet weight. Prepared fish tissue was used to measure total mercury (THg), the five other heavy metals (Cd, Cr, Cu, Mn, Pb), and stable carbon and nitrogen isotope ratios.

Total mercury analysis

Total mercury (THg) concentrations were measured using a Direct Mercury Analyzer (DMA-80, Milestone Srl, Sorisole, Italy) located in the University of Notre Dame Center for Environmental

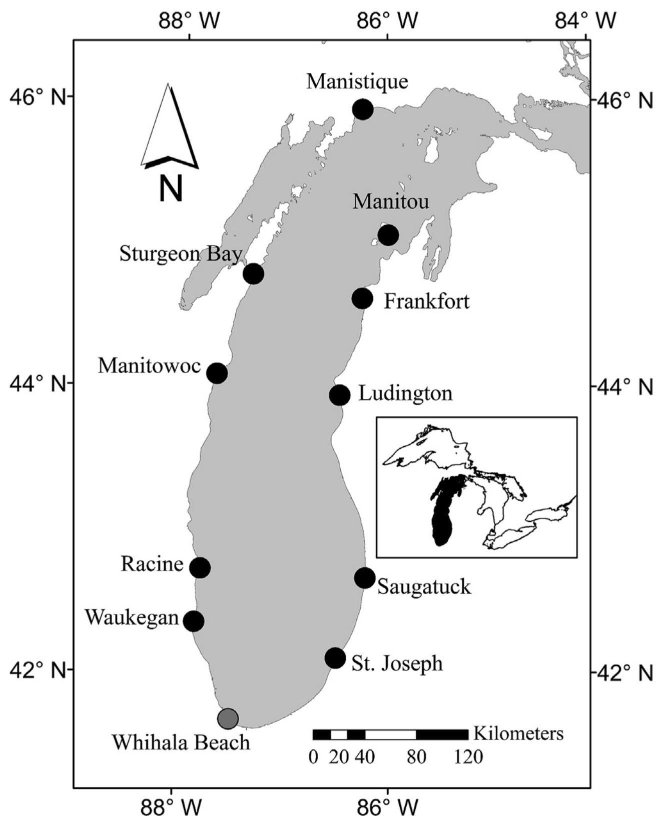

Fig. 1. Lake Michigan prey fish sampling ports from which transects into the lake were sampled by USGS (black circles), and point samples were taken by Notre Dame (grey circle).

Science and Technology (CEST). The DMA-80 measures THg, which includes MeHg, Hg^{2+}, and Hg. However, MeHg and Hg^{2+} are the predominant forms of mercury incorporated into fish tissue. Previous studies have found that 95% of Hg in fish is MeHg and advise that measuring either MeHg or THg are acceptable for fish samples (Bloom, 1992; Watras and Bloom, 1992). Approximately 20 mg of homogenized fish tissue was weighed into ashed nickel boats and analyzed for THg (cf. Gerig et al., 2018). Each run included standard reference material (DORM-4, National Research Council Canada), blanks, and sample duplicates for quality control. Sample duplicates yielded an average relative standard deviation (RSD) of 1.4%. DORM-4 had a percent recovery rate of 100.6% ± 6.8% (mean ± standard deviation, n = 45) and the detection limit was 0.21 ng/g. Measurements are reported in ng/g wet weight.

Cadmium, chromium, copper, lead, and manganese analyses

Freeze-dried fish tissue was digested using methods described in Dabeka et al. (2002) and Smith et al. (2016). Approximately 0. 3 ± 0.1 g (mean ± standard error) of homogenized freeze-dried fish tissue was placed in metal-free centrifuge tubes, 5 mL of nitric acid was added, and samples were incubated at room temperature for 6 h on an orbital shaker. Subsequently, 3.5 mL of hydrochloric acid was added, samples were covered with digestion reflux caps, and the resulting liquid was heated at 60 °C for 6 h. Then, 2.5 mL of 30% hydrogen peroxide was added, and samples were incubated at 60 °C for 2 h. Digestion reflux caps were removed, and samples were heated at 60 °C until liquid was fully evaporated. For uniform acidity of metal matrices, samples were resuspended in 5% nitric acid and filtered through 0.45 µm cellulose acetate filters to remove any remaining solid organic matter.

Metal concentrations (other than THg) were measured using a Perkin Elmer Inductively Coupled Plasma Optimal Emission Spectrometer (ICP-OES; Perkin Elmer, Inc., Waltham, Massachusetts) located at CEST. Each run included standard reference material (DORM-4) and blanks for quality control. DORM-4 values were within the published acceptable range for each metal. The instrument internal standard of yttrium had a recovery rate of 98.1% ± 0.98%. Each sample was measured in triplicate and the average RSD was 4.2%. Detection limits were 0.11 µg/g for Cd and Cu, 0.15 µg/g for Cr and Mn, and 1.5 µg/g for Pb. Measurements are reported in µg/g wet weight.

Stable isotope analyses

Stable nitrogen and carbon isotope ratios and C:N ratios were measured with an Elemental Analyzer (Costech, Valencia, California, USA) paired to a Delta Plus Isotope Ratio Mass Spectrometer (Thermo Scientific, Waltham, Massachusetts, USA) located at CEST. Stable nitrogen and carbon isotope ratios were expressed as $\delta^{13}C$ or $\delta^{15}N = [(R_{sample}/R_{standard}) - 1] \times 1000$ where R = $^{13}C/^{12}C$ or $^{15}N/^{14}N$. Isotope ratios were corrected using a three-point standard curve after each run using known isotopic standards (EA Consumables, Pennsauken, New Jersey, USA). The standard deviations of these standards were: protein ($\delta^{15}N$ = 0.15, $\delta^{13}C$ = 0.05), sorghum ($\delta^{15}N$ = 0.20, $\delta^{13}C$ = 0.08), and wheat flour ($\delta^{15}N$ = 0.14, $\delta^{13}C$ = 0.08). Acetanilide was used as the standard for calculating tissue C:N (cf., Gerig et al., 2017). The standard deviation for acetanilide nitrogen was 0.35‰ and for carbon was 0.13‰. If the C:N ratio was greater than 4, $\delta^{13}C$ values were lipid-corrected using an arithmetic mass balance (McConnaughey and McRoy, 1979). Specifically, we used a $C:N_{lipid\ free}$ of 3.5 and lipid discrimination of −6.5‰ as reported by Hoffman et al. (2015).

Table 1
Mean ± standard deviation for total length (mm), tissue metal concentrations (ng/g for THg and µg/g for other metals, all wet weight), sample sizes (n), $\delta^{15}N$ and lipid-corrected $\delta^{13}C$ isotope values (‰), C/N ratio, and standard ellipse area (SEA_C, ‰^{2}) for each prey fish species. – = no data.

	Alewife	Bloater	Deepwater Sculpin	Rainbow Smelt	Round Goby	Slimy Sculpin
Total Length	129.2 ± 33.4	172.4 ± 44.0	81.5 ± 25.2	62.0 ± 30.0	72.3 ± 13.3	77.9 ± 18.3
THg	50.7 ± 37.8	97.2 ± 47.9	60.3 ± 39.5	10.7 ± 11.8	27.5 ± 14.1	71.6 ± 34.8
THg n	70	40	59	14	55	57
Cr	0.4 ± 0.2	0.4 ± 0.2	0.6 ± 0.3	–	0.4 ± 0.2	0.5 ± 0.3
Cu	1.6 ± 0.3	1.1 ± 0.3	1.0 ± 0.4	–	1.2 ± 0.5	1.3 ± 0.5
Mn	1.1 ± 0.4	0.7 ± 0.3	1.7 ± 1.0	–	1.3 ± 1.0	1.7 ± 0.6
Cr, Cu, Mn n	51	22	18	0	31	23
Isotope n	67	22	34	12	48	32
$\delta^{15}N$	8.7 ± 1.4	11.4 ± 0.6	10.6 ± 0.9	8.7 ± 1.4	9.1 ± 0.6	11.2 ± 1.1
$\delta^{13}C$	−25.0 ± 0.6	−25.5 ± 0.6	−24.5 ± 0.6	−23.5 ± 0.8	−20.2 ± 1.2	−23.6 ± 1.2
C/N Ratio	3.8 ± 0.8	3.5 ± 0.2	3.5 ± 0.3	3.6 ± 0.3	3.3 ± 0.1	3.6 ± 0.4
SEA_C	2.7	1.2	1.6	1.6	2.3	3.0

THg = total mercury, Cr = chromium, Cu = copper, Mn = manganese.

Statistical analyses

Metal tissue concentration measurements that fell below the lower limit of detection (LLD) were replaced with 50% LLD. Cu and THg had no samples below the LLD. Cr had three measurements and Mn had one measurement below the LLD. Cd and Pb measurements were either below LLD (0.11 µg/g and 1.5 µg/g) or triplicate measurements were too inconsistent near the LLD to confidently report. For this reason, Cd and Pb measurements are excluded from analysis. Additionally, because of small sample size, rainbow smelt were not analyzed for Cd, Cr, or Mn because no tissue remained following THg and stable isotope ratio measurements.

We used analysis of variance (ANOVA) to analyze differences in tissue metal concentration among all species. To parse out species-specific differences in accumulation, we used analysis of covariance (ANCOVA) to determine if the relationship between metal concentration and total length or δ^{15}N was influenced by species identity (cf., Cabana and Rasmussen, 1994; Clements et al., 2012). Post-hoc Tukey multiple comparisons tests were used for both ANOVA and ANCOVA approaches to determine differences among species (α = 0.05). Assumptions of ANOVA and ANCOVA were assessed by visually inspecting plots of residuals. Due to non-normality of data, metal concentrations were ln-transformed prior to analysis. To better understand the relationship between tissue metal concentration and total length, δ^{15}N, and δ^{13}C, we used simple linear regressions to evaluate if a linear relationship existed and then compared slopes among species using ANCOVA to determine if differences were significant. R statistical software was used for all analysis (https://www.r-project.org/, 4.0.3). The stats, car, TukeyHSD, and multcomp packages were used for ANOVA, ANCOVA, and post-hoc Tukey tests.

To examine stable isotopic niche space and overlaps of Lake Michigan prey fish, we used the Stable Isotope Bayesian Ellipses (SIBER, Jackson et al., 2011) and the NicheRover (Swanson et al., 2015) packages in R. SIBER uses isotope data (δ^{15}N and δ^{13}C) with species identity and returns the Bayesian posterior draws after 10,000 iterations for the Standard Ellipse Area core (SEAc) for each species corrected by sample size. SIBER accomplishes this by approximating the total extent of trophic diversity by first using a convex hull (cf., Layman et al., 2007). The resulting SEAc area represent 40% credible interval of isotopic niche area for each individual fish species (Jackson et al., 2011). In doing so, outlier individuals are excluded leaving the resulting ellipses as strong examples of each species' δ^{15}N and δ^{13}C values. Using NicheRover, we calculated the amount of SEAc ellipse overlap among species (unit = ‰^{2}, Swanson et al., 2015). The resulting percent niche overlap among species is two-way and allowed us to examine similarities and differences in prey fish stable isotope niche spaces.

Results

Tissue metal concentrations

Tissue metal concentration for all measurable metals (Cr, Cu, Mn, THg) was influenced by species identity (ANOVA, $p < 0.05$). THg concentration differed among species (ANOVA, $F_{5,\ 289}$ = 31.0, $p < 0.001$), ranging from 10.7 ± 11.8 ng/g (mean ± standard deviation) in rainbow smelt to 97.2 ± 47.9 ng/g in bloater (Table 1). All species differed from all other species in THg except for 4 out of 15 total pairwise species comparisons (Fig. 2). THg concentration also differed among habitats (ANOVA, $F_{5,\ 292}$ = 39.4, $p < 0.05$). Profundal species (bloater, deepwater sculpin, slimy sculpin) had higher THg concentrations than pelagic species (alewife, rainbow smelt) and benthic, nearshore-offshore seasonally migrating species (round goby) (Tukey HSD, $p < 0.05$).

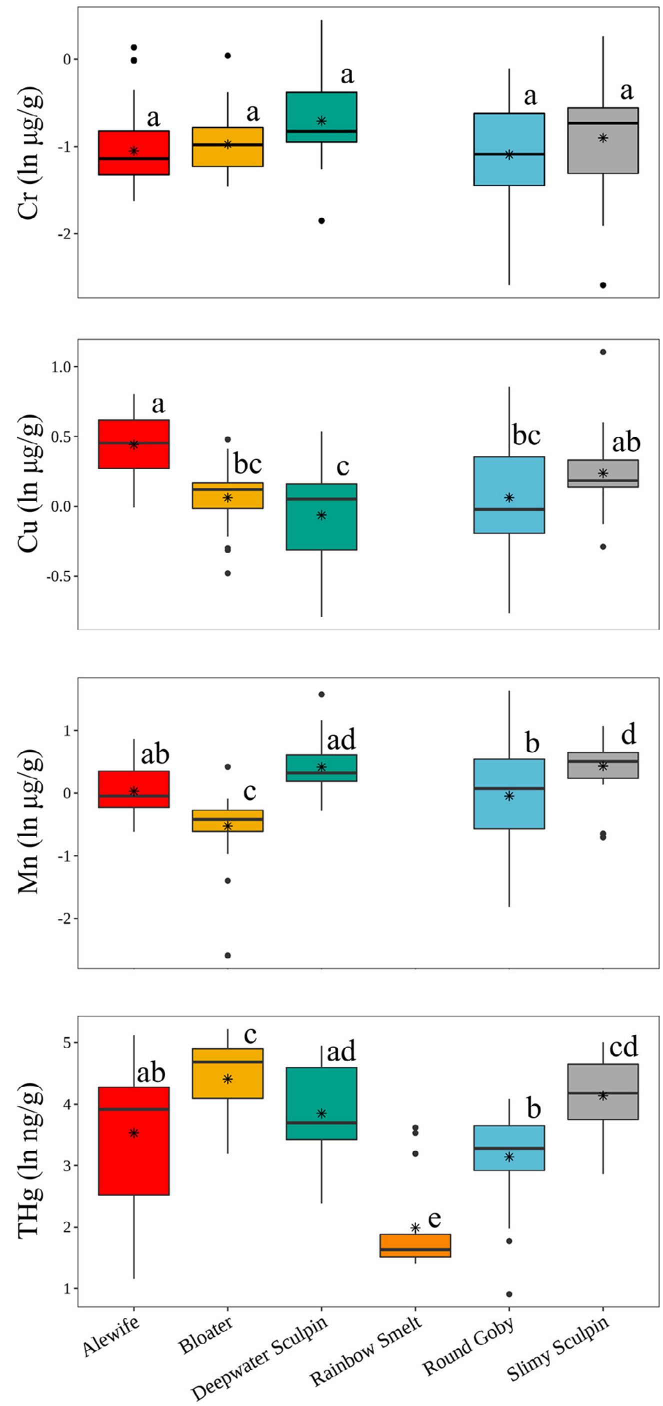

Fig. 2. Tissue metal concentration (ng/g for THg and µg/g for other metals, all wet weight) across species. Boxplot shows median (dark horizontal line), mean (asterisk), upper and lower quartiles (box), smallest and largest values within 1.5 times the interquartile range (vertical line), and outliers greater or smaller than 1.5 times the interquartile range (black points). Species are represented by different colors. Rainbow smelt was not analyzed for Cr, Cu, and Mn due to small sample sizes. Letters above each barplot represent ANOVA Tukey groupings across metals, with different letters indicating different statistical groups ($p < 0.05$).

For other metals, Cu concentration also differed among species (ANOVA, $F_{4,\ 140}$ = 14.2, $p < 0.05$), ranging from 1.0 ± 0.4 µg/g in deepwater sculpin to 1.6 ± 0.3 µg/g in alewife (Table 1). Cu concentration differed for 4 out of 15 total pairwise comparisons (Fig. 2). Cr concentration did not differ among species (ANOVA, $F_{4,140}$ = 2.0, $p > 0.05$), ranging from 0.4 ± 0.2 µg/g in alewife to 0.6 ± 0.3 µg/g in deepwater sculpin (Table 1). Mn concentration differed among

Table 2
Least-squares regression slopes for total length and $\delta^{15}N$ relationships with metal concentration along with sample size (n) for THg and all other metals for each prey fish species. – = no relationship or low n; * = p < 0.05, ** = p < 0.01, *** = p < 0.001.

Total Length	n	THg	n	Cr	Cu	Mn
Alewife	70	0.028***	51	–	–	–
Bloater	40	0.014**	22	–	−0.004**	−0.005*
Deepwater Sculpin	59	0.028**	18	–	−0.010*	–
Rainbow Smelt	14	0.023**	0	–	–	–
Round Goby	55	0.034**	31	–	–	−0.04**
Slimy Sculpin	57	0.022**	23	–	−0.013*	–
$\delta^{15}N$	n	THg	n	Cr	Cu	Mn
Alewife	67	0.61***	51	–	−0.049*	0.12**
Bloater	22	–	22	–	−0.21*	–
Deepwater Sculpin	34	0.64***	18	–	–	–
Rainbow Smelt	12	0.54***	0	–	–	–
Round Goby	48	0.45**	31	–	–	–
Slimy Sculpin	32	0.18*	23	–	–	–

species (ANOVA, $F_{4,\,140}$ = 11.5, p < 0.05), ranging from 0.7 ± 0.3 µg/g in bloater to 1.7 ± 1.0 µg/g and 1.7 ± 0.6 µg/g in deepwater sculpin and slimy sculpin, respectively (Table 1). Mn concentrations differed among all species except for 3 out of 15 total pairwise species comparisons (Fig. 2).

The relationship between tissue metal concentration and fish total length varied among metals (Table 2). For all species combined, only THg tissue concentration had a positive ln-linear relationship with total length (slope b = 0.01) and $\delta^{15}N$ (b = 0.43) (Fig. 3). Mn showed a weak negative ln-linear relationship between tissue concentration and total length (b = -0.006), while Cr and Cu showed no ln-linear relationship between concentration and total length (p > 0.05, Table 2). For $\delta^{15}N$, Cu tissue concentration showed a weak negative ln-linear relationship (b = -0.08) while Cr and Mn showed no ln-linear relationship to $\delta^{15}N$ (p > 0.05) (Table 2). THg and Cu showed a weak negative relationship with $\delta^{13}C$ (b = -0.1 and −0.04, respectively) (ESM Fig. S1).

Species-specific differences in THg

The relationship between THg tissue concentration and total length varied among species (ANCOVA, $F_{5,\,281}$ = 13.9, p < 0.001) but all slopes were positive (Fig. 4). The strongest slopes for the ln-linear relationship between THg and total length was observed in round goby (b = 0.034) followed by alewife and deepwater sculpin (b = 0.028 for both), rainbow smelt (b = 0.023), slimy sculpin (b = 0.022), and bloater (b = 0.014). The relationship between THg tissue concentration and total length differed for 4 out of 15 total pairwise comparisons (Bonferroni-corrected α = 0.003; Fig. 4). Additionally, alewife and bloater length data had bimodal distributions suggesting the presence of at least two age classes. Trends for metal concentrations were consistent between these age classes and all lengths combined.

We also found a positive relationship between THg tissue concentration and $\delta^{15}N$ that varied among species (ANCOVA, $F_{5,\,203}$ = 4.2, p < 0.01) (ESM Fig. S2). The strongest ln-linear relationship was observed in deepwater sculpin (b = 0.64) followed by alewife (b = 0.61), rainbow smelt (b = 0.54), round goby (b = 0.45), and slimy sculpin (b = 0.18). Bloater showed no ln-linear relationship between THg and $\delta^{15}N$ (p > 0.05). The relationship between THg tissue concentration and $\delta^{15}N$ differed among two species pairs–slimy sculpin:alewife and slimy sculpin:deepwater sculpin (pairwise Bonferroni correction, α = 0.003; ESM Fig. S2).

The relationship between THg tissue concentration and $\delta^{13}C$ varied among species (ANCOVA, $F_{5,\,203}$ = 8.0, p < 0.001). Alewife, bloater, and rainbow smelt had positive relationships with $\delta^{13}C$ (respectively, b = 0.67; b = 0.56; b = 0.93). Deepwater sculpin and round goby showed no ln-linear relationship with $\delta^{13}C$ (p > 0.05) while slimy sculpin had a negative relationship with $\delta^{13}C$ (b = -0.18) (ESM Fig. S3). The relationship between THg tissue concentration and $\delta^{13}C$ differed significantly for 6 of 15 total pairwise species comparisons (pairwise Bonferroni correction, α = 0.003; ESM Fig. S3).

Species-specific differences in stable isotope ratios

We found evidence for species-specific differences in isotopic niche size; slimy sculpin had the largest niche (3.0 ‰^{2}) whereas bloater had the smallest niche (1.2 ‰^{2}) (Table 1). Deepwater sculpin and rainbow smelt also had relatively narrow niches at 1.6 ‰^{2}. Different fish species also displayed varying ranges in their stable isotope axes. For example, alewife had variable $\delta^{15}N$ values but similar $\delta^{13}C$ values. Other species, such as round goby, had the reverse relationship of similar $\delta^{15}N$ values but variable $\delta^{13}C$ values (Fig. 5). Many variables inform $\delta^{15}N$ and $\delta^{13}C$ values, such as habitat, age, and also isotopic baseline, which we were not able to account for here and often differs by habitat. Fish $\delta^{13}C$ values can help infer foraging habitat (France, 1995; Sierszen et al., 2014). For example, rainbow smelt are typically a pelagic, offshore species but in this study some rainbow smelt $\delta^{13}C$ values were similar to round goby (Fig. 5). Stable isotope ratios for all fish species centered around a $\delta^{15}N$ value of 9.7 ± 1.6 (mean ± SD) and $\delta^{13}C$ value of −23.6 ± 2.1. In addition, $\delta^{15}N$ increased with increasing water depth (b = 0.03) (ESM Fig. S4) whereas $\delta^{13}C$ decreased with increasing depth (b = −0.02) (ESM Fig. S5).

The largest isotopic niche overlap was between deepwater sculpin and alewife (85.5%). However, isotope niche overlap is independently bi-directional, and alewife shared only 38.5% of their isotopic niche with deepwater sculpin (ESM Table S2). Round goby was the most isotopically differentiated from other species (Fig. 5) and had <5% isotopic niche overlap with all other species with the exception of a 24.2% overlap with slimy sculpin (ESM Table S2). Prey fish taxa with the greatest niche overlap also had the most similar Hg concentrations. Bloater, slimy sculpin, and deepwater sculpin were positioned closely in isotopic space (Fig. 5), had the highest mean THg concentrations of the six prey species (ESM Table S2), and had the highest $\delta^{15}N$ values. Alewife, round goby, and rainbow smelt were more isolated in isotopic space (Fig. 5) and had different THg concentrations (Fig. 2).

Discussion

Bioaccumulation differs among metals, prey fish species, and habitat use

For all six Lake Michigan prey fish species, we found measurable tissue metal concentrations of Cr, Cu, Mn, and THg. Of these, only THg displayed clear patterns of increasing tissue concentration

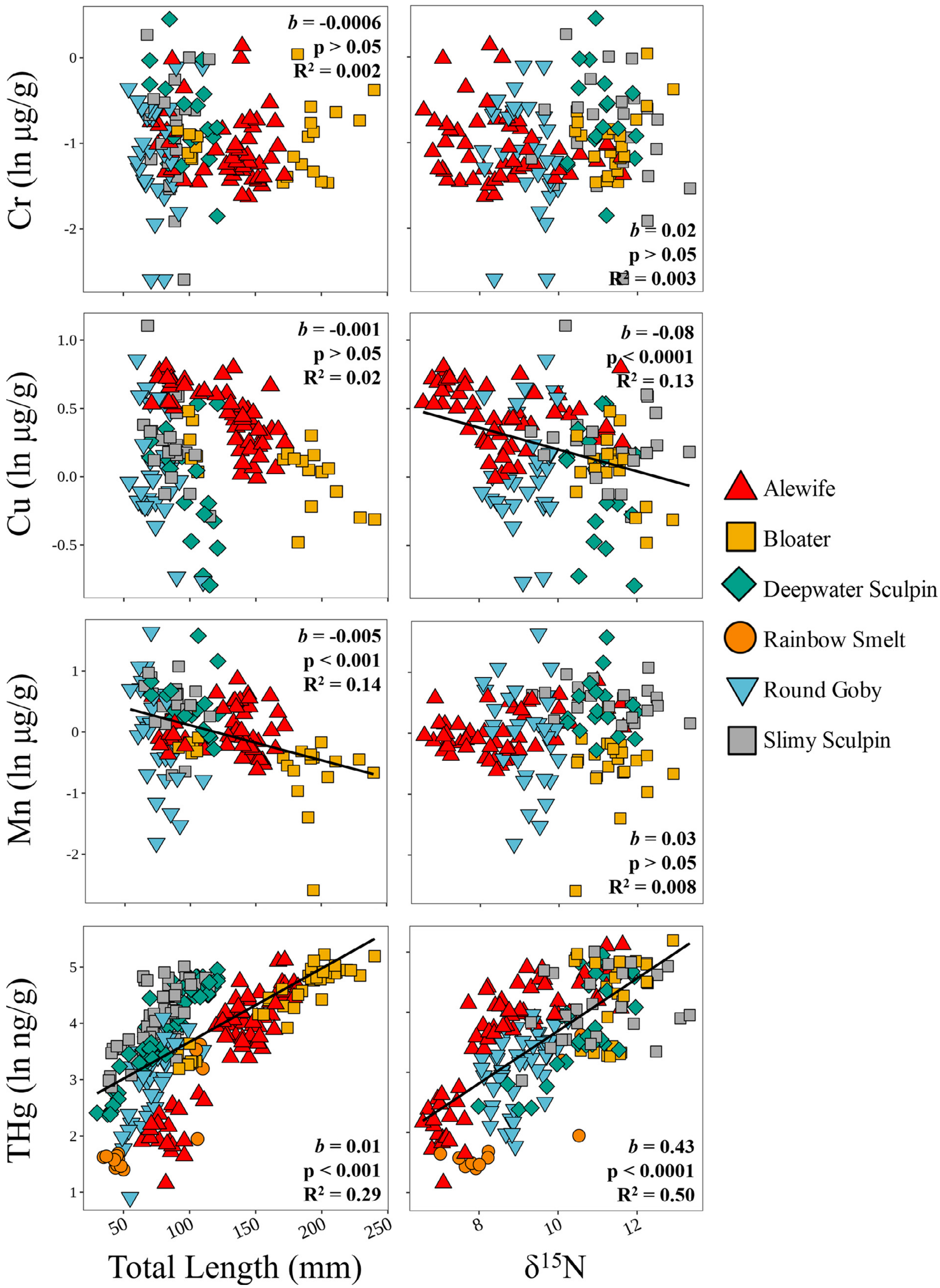

Fig. 3. Total length (mm) and $\delta^{15}N$ relationship with ln-transformed metal concentration (ng/g for THg and µg/g for all other metals, all wet weight) for all six prey fish species. Black line represents least-squares regression if significant. Species are represented by different colors and shapes.

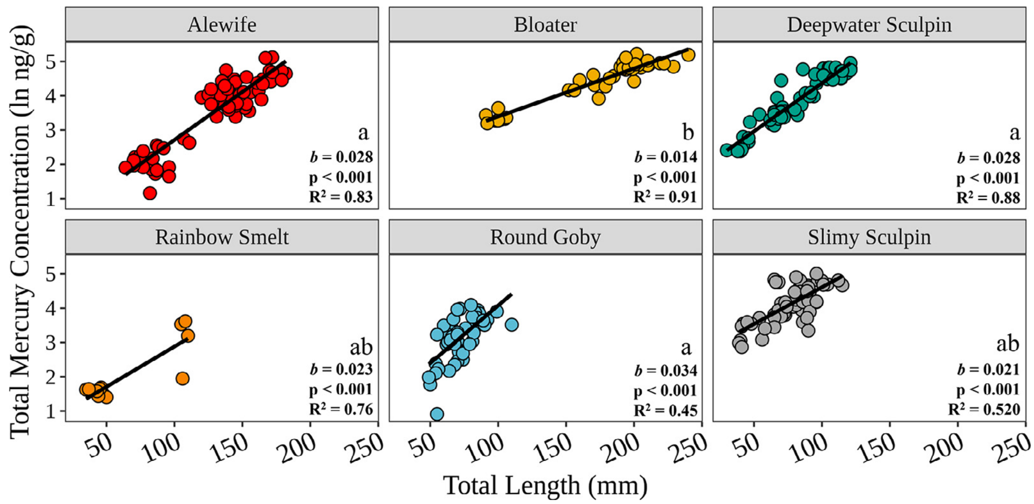

Fig. 4. Relationship between total length (mm) and total mercury (THg) concentration (ln ng/g wet weight) for each fish species. Black line represents least-squares regression if significant. Letters represent pairwise Bonferroni-corrected differences across species slopes, with different letters indicating different statistical groups ($\alpha = 0.003$). Overall ANCOVA, $p < 0.001$, $R^2 = 0.84$.

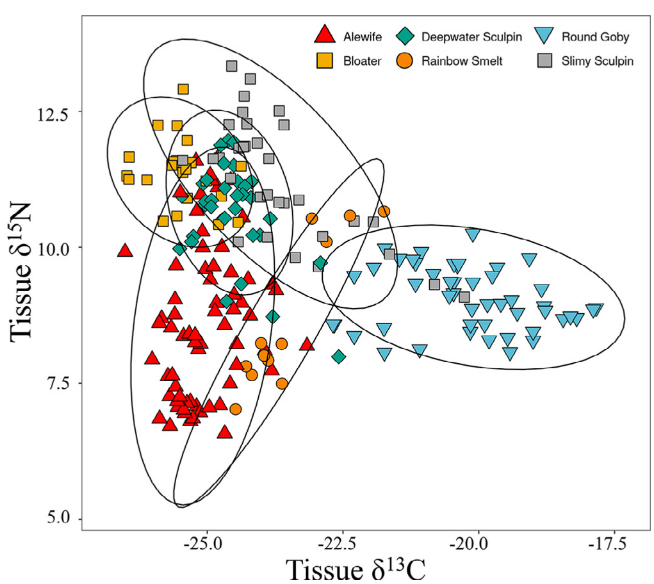

Fig. 5. Cross-plot of δ^{15}N and lipid-corrected δ^{13}C isotope values with ellipses representing 95% credible interval. Species are represented by different colors and shapes.

with increasing total length and δ^{15}N, suggesting biomagnification of this metal. However, δ^{15}N is influenced by many factors besides trophic position, such as isotopic baseline differences across different habitats (Sierszen et al., 2014), which we could not account for in our study. The metals Cr, Cu, and Mn exhibited either no or weak negative relationships with total length or δ^{15}N, suggesting that these elements do not magnify but rather accumulate directly in Lake Michigan prey fish. Cr, Cu, and Mn are all considered essential trace elements in vertebrates that aid in multiple metabolic functions including glucose metabolism, synthesis of proteins used for bone and blood formation, and essential enzyme activation (Ebdon et al., 2001). These metals are taken up from the water directly, and therefore, do not biomagnify or bioaccumulate (Ali and Khan, 2019).

Total mercury concentrations increased with increasing size of all prey fish species, but the rate of THg accumulation rates (i.e., slope) differed among species. Mercury concentration is known to correlate with fish size (i.e., total length; Gewurtz et al., 2010; Sonesten, 2003), which is a strong indicator of trophic position (i.e., δ^{15}N; Chouvelon et al., 2014; Nakazawa et al., 2010). Therefore, the slope of the relationship between length and Hg may reflect ontogenetic variation in growth. round goby, alewife, and deepwater sculpin had the highest THg accumulation rates (per mm of growth), slimy sculpin and rainbow smelt were intermediate, and bloater had the lowest accumulation rate. Furthermore, although not directly addressed in our study, species-specific absorption, distribution, metabolism, and excretion (ADME) processes can influence metal concentrations and therefore bioaccumulation (Campbell et al., 2003).

In terms of habitat occupancy, profundal species (bloater, slimy sculpin, deepwater sculpin) had the highest THg tissue concentrations. One possible explanation for higher concentrations but lower accumulation rates in these species is that profundal species experience colder temperatures, on average, than alewife and round goby that spend more time in nearshore or pelagic environments where water temperatures are warmer. Both sculpin species spend their entire day in the profundal environment, whereas bloater can migrate from the bottom during the daytime to up near the metalimnion during the night (TeWinkel and Fleischer, 1999). Round goby generally occur in nearshore waters during the growing season, and some may migrate into offshore, relatively warmer waters during winter (Kornis et al., 2012). Alewife, like bloater, also are found in the profundal area during the daytime, but relative to bloater they migrate farther up into the warmer epilimnion at night (Janssen and Brandt, 1980). Furthermore, adult alewife move into the nearshore to spawn in early summer, where their offspring often spend their first year of life (Madenjian et al., 2008; Tin and Jude, 1983; Wells, 1968). As poikilotherms, water temperature is one of the many variables that can affect fish growth rates and, in turn, THg accumulation rates. An alternative explanation for profundal species having the highest THg concentrations is that THg concentrations of invertebrate prey could vary across habitats. For example, the primary invertebrate prey for these profundal species is *Mysis diluvania* (Bunnell et al., 2015), a relatively large, high-lipid animal that is not found in nearshore waters but whose methylmercury concentrations were up to four times higher than zooplankton in the late 1990s (Mason and Sullivan, 1997).

Our findings of species and habitat-specific accumulation of Hg have important implications for the transfer of mercury to popular sportfishes that are commonly caught and consumed by anglers.

Alewife and round goby are the most important and commonly consumed prey fish in Lake Michigan. In fact, alewife and round goby make up the majority of diet for Chinook salmon (Jacobs et al., 2013; Leonhardt et al., 2020), lake trout (Luo et al., 2019; McKenna, 2014), smallmouth bass (*Micropterus dolomieu*; Crane and Einhouse, 2016; Happel et al., 2017), steelhead (*Oncorhynchus mykiss*; Turschak and Bootsma, 2015), walleye (*Sander vitreus*; Pothoven et al., 2017), and lake whitefish (*Coregonus clupeaformis*; Pothoven and Madenjian, 2013). Benthic, nearshore sportfish (e.g., lake whitefish, walleye) rely heavily on round gobies while pelagic, offshore sportfish (e.g., Chinook salmon, steelhead) readily consume alewives. As invasive species, alewives and round gobies can reach higher densities than other native fish, thereby making them more accessible to predators and potentially a lower energetic investment to consume (Bunnell et al., 2019; Johnson et al., 2005). The importance of alewives and round gobies in the Lake Michigan food web and biomagnification of THg in these prey fish supports continued periodic monitoring of prey fish, which may provide an early warning sign of higher metal concentrations in Great Lakes sportfish that humans consume.

Influence of food web alterations on heavy metal accumulation and transfer

Altered food web dynamics resulting from invasive species have implications for the trophic transfer of contaminants in Lake Michigan. The widespread establishment of dreissenid mussels has redirected energy flow from the pelagic zone to the nearshore benthic zone (Cuhel and Aguilar, 2013). High rates of P-excretion by dreissenids and enhanced light conditions resulting from filtration have facilitated the proliferation of filamentous *Cladophora* algal mats that create biogeochemical hotspots for methylation of Hg (Lepak et al., 2015a). In addition, the invasion of round gobies has been associated with higher bioaccumulation levels of Hg (Hogan et al., 2007) and polychlorinated biphenyls (PCBs) (Kwon et al., 2006) in the food web due to this novel prey conduit. Before round gobies invaded Lake Michigan, sediment contaminants were largely isolated to dreissenid mussels and benthic invertebrates, but few native benthic fish consumed dreissenid mussels. Round gobies, however, co-evolved with dreissenid mussels in the Ponto-Caspian region (Kornis et al., 2012). When gobies invaded Lake Michigan they quickly exploited the abundant dreissenid prey, thereby providing a new pathway for contaminants to move higher in the food web (Hogan et al., 2007; Johnson et al., 2005). Indeed, at the basin scale, the decades-long decline in Great Lakes fish Hg levels began to reverse in the mid-1990s (Blukacz-Richards et al., 2017; Lepak et al., 2019), coincident with the invasion of round gobies throughout the Great Lakes basin. In the face of climate change and food web shifts due to aquatic invaders, prey fish could be used as an early warning signal of food web shifts leading to enhanced or reduced metal mobility. For example, metal concentrations should be measured more frequently when food web changes have occurred and efforts could focus on piscivores that rely on round gobies and alewives as their primary food source.

Stable isotope ratios can reveal resource use and overlap contributing to contamination

Fish contaminant magnification reflects past dietary sources (Trudel and Rasmussen, 2006) and trophic position (Pereira et al., 2010), while C and N stable isotope ratios provide proxies for diet, habitat, and approximate trophic position (Parnell et al., 2013). Stable isotope ratios can therefore help to illuminate reasons for observed differences in THg tissue concentration among species and habitats. For example, stable isotopic niche area reflects differences in isotope ratios of diet items, which should be positively related to the diversity of prey items (Layman et al., 2007; Parnell et al., 2010). Slimy sculpin had the largest niche area (SEAc = 3.0) closely followed by alewife (SEAc = 2.7), suggesting more diverse, generalist diets compared to other prey fish. Indeed, recent diet studies are consistent with this prediction, as slimy sculpin diets include *Mysis*, *Diporeia*, and chironomids (Bunnell et al., 2015; Hondorp et al., 2011a). Likewise, alewife diets are seasonally dynamic and variable, with primary prey including calanoid copepods, *Mysis*, and *Bythotrephes* (Bunnell et al., 2015). Round goby are seasonal migrators between nearshore and offshore habitats (Blair et al., 2019, Pennuto et al., 2010), and their diets are dominated by dreissenid mussels (Bunnell et al., 2015; Kornis et al., 2012). Based on this seasonal habitat shift, we would expect their isotopic niche area to expand to represent more diverse habitats. However, round goby isotopic niche area may be constrained by their specialized diet. Round goby niche area (SEAc = 2.3) was comparable to other species, suggesting that both a specialized diet and habitat shifts influence their isotopic niche area. In contrast, bloater had the smallest niche area (SEAc = 1.2) followed by deepwater sculpin and rainbow smelt (SEAc = 1.6 for both), suggesting more specialized diets for these species such as mostly *Mysis* for bloater and deepwater sculpin (Bunnell et al., 2015; Hondorp et al., 2011b).

The largest niche overlap was found among profundal species (bloater, deepwater sculpin, and slimy sculpin), which all had the highest $\delta^{15}N$ values. Furthermore, these profundal species displayed the lowest $\delta^{13}C$ values consistent with their deepwater habitat. These three prey fish also had the highest mean concentrations of THg, consistent with benthic habitats where mercury methylation is optimized in anaerobic microzones. The offshore pelagic species, alewife and rainbow smelt, had modest isotopic niche overlap with one another, which was consistent with their different mean concentrations of THg. Alewife had both low $\delta^{13}C$ and $\delta^{15}N$ suggesting use of multiple habitats and the presence of multiple size classes. Finally, round gobies inhabit both benthic nearshore and offshore environments for portions of the year and had a distinct isotopic niche from other species, consistent with their unique seasonal migration pattern and dietary reliance on dreissenid mussels. The stable isotope ratios and THg concentration of round goby were also distinct from other prey fish species and may reflect a specialized diet of dressenid mussels contaminated with Hg (Lepak et al., 2019).

Conclusions

In our study, we detected several metals in six Lake Michigan prey fish species, but only mercury showed strong evidence of increasing concentration with total length and $\delta^{15}N$ values. Mercury in aquatic food webs is transferred primarily through diet (Clayden et al., 2013; Wu et al., 2018), and therefore prey fish provide a potential conduit for Hg to accumulate in sportfish caught and consumed by anglers. Offshore pelagic and nearshore species had variable THg tissue concentrations whereas profundal species had similar high THg tissue concentrations, possibly due to higher Hg methylation rates in profundal habitats. We have further shown that several key prey fish species that are important to sportfish diet have the potential to mediate contaminant movement in an aquatic food web. Based on our findings, Great Lakes fishery managers and public health would likely benefit from continuing to monitor prey fish Hg concentrations and even intensifying monitoring efforts when food web structure shifts due to trophic interactions.

CRediT authorship contribution statement

Whitney M. Conard: Conceptualization, Investigation, Formal analysis, Data curation, Writing - original draft. **Brandon S. Gerig:**

Conceptualization, Investigation, Formal analysis, Writing - review & editing. **Lea M. Lovin:** Conceptualization, Investigation, Formal analysis, Writing - review & editing. **David B. Bunnell:** Resources, Writing - review & editing. **Gary A. Lamberti:** Formal analysis, Writing - review & editing, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Any use of trade, product, or firm names is for descriptive purposes only and does not imply endorsement by the U.S. Government. All sampling and handling of fish were carried out in accordance with guidelines for the care and use of fishes by the American Fisheries Society (http://fisheries.org/docs/wp/Guidelines-for-Use-of-Fishes.pdf).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jglr.2021.08.019.

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