Environmental Science
Processes & Impacts

Accepted Manuscript

ROYAL SOCIETY OF CHEMISTRY

rsc.li/process-impacts

Environmental Impact Statement

Mercury exposure through fish consumption may lead to adverse health effects, particularly impacting pregnant women and children. The Grand Lake watershed includes one of the largest reservoirs in Oklahoma and is a popular fishing destination for local recreational and subsistence fishermen, among whom consumption of local fish may account for the majority of dietary mercury intake. Moreover, reservoirs often have higher fish mercury concentrations than lakes due to their unique hydrodynamics. This study aims at exploring the key factors associated with fish mercury concentrations both within and among reservoirs through an extensive survey of over 30 fish species and 1300 samples in Grand Lake watershed and an inter-system analysis of 32 biogeochemical and ecological factors across 61 reservoirs.

Key Contributors to Variations in Fish Mercury Within and Among Freshwater Reservoirs in Oklahoma, USA

Zhao Dong[a*], Robert A. Lynch[b], Laurel A. Schaider[a1]

[a] *Harvard T.H. Chan School of Public Health, Boston, MA 02215, USA*

[b] *University of Oklahoma Health Sciences Center, Oklahoma City, OK 73126, USA*

** Corresponding author contact information: tel: +1-617-432-5554; fax:+1-617-432-3349; email: zdong@hsph.harvard.edu*

[1] *Present address: Silent Spring Institute, Newton, MA 02458, USA*

Abstract

Elevated fish mercury (Hg) concentrations in freshwater ecosystems worldwide are a significant human and ecological health concern. Mercury bioaccumulation and biomagnification in lakes and reservoirs are controlled by numerous biogeochemical and ecological factors, contributing to variability in fish Hg concentrations both within and among systems. We measured total mercury concentrations ([THg]) and stable isotopes ($\delta^{15}N$, $\delta^{13}C$) in over 30 fish species in two connected subtropical freshwater reservoirs (Grand Lake and Lake Hudson, Oklahoma, USA), their tributaries, and local farm ponds, all of which are potentially impacted by nearby atmospheric Hg sources. We also conducted an inter-system analysis among 61 reservoirs in Oklahoma to explore biological, chemical and physical factors associated with fish [THg] across systems. We found that [THg] for most species in Grand Lake and Lake Hudson were relatively low compared to other reservoirs in Oklahoma. There were significant spatial variations in many species within and between Grand Lake and Lake Hudson, even after accounting for length and/or trophic position (based on $\delta^{15}N$). Fish in local farm ponds, commonly used in the agricultural regions for raising game fish, had 2-17 times higher [THg] than fish of similar length in nearby reservoirs. The inter-system analysis revealed that pH, water color, rainfall, and nutrients are the best predictors of fish [THg] across systems. Our results provide insight into the key factors associated with fish [THg] variations both within and across systems, and may be useful for exposure assessment and for identifying sites and water bodies prone to high fish [THg] as monitoring priorities.

Keywords: fish; impoundment; mercury biomagnification; methylmercury; spatial variation

Introduction

Elevated concentrations of mercury (Hg) have been detected in freshwater fish globally[1-4]. Mercury bioaccumulation in commonly consumed fish has become a global health concern, primarily due to potential human health effects of exposure to methylmercury (MeHg), especially for fetuses and children[5], even at low levels associated with typical rates of fish consumption[6]. In the U.S., fish and shellfish consumption is the primary non-occupational source of MeHg exposure in the general population[7], and thousands of fish consumption advisories have been issued for freshwater bodies[8]. Elevated Hg concentrations in fish have also been associated with neurological and/or reproductive effects in fish[9], piscivorous birds and mammalian wildlife[10], particularly in systems close to major point sources and in regions that receive high levels of atmospheric deposition[11].

Mercury is released to the environment through both natural processes and human activities. Coal combustion is a major anthropogenic source, accounting for 24% of global emissions[12]. While Hg is primarily emitted to the atmosphere in inorganic species, a portion of Hg that enters aquatic systems is transformed into MeHg by sulfate- and iron-reducing bacteria in anoxic water, sediments, and wetlands[13, 14]. Biomagnification of MeHg results in orders of magnitude higher concentrations in top predators, many of which are consumed by humans, piscivorous birds and wildlife.

Variations in fish Hg concentrations are determined to some degree by the amount of Hg entering a system from atmospheric deposition[15, 16] and discharges from mines and other local sources[17, 18]. However, even among lakes and reservoirs located in the same region that receive similar levels of atmospheric Hg deposition, fish Hg concentrations can vary considerably[19] due

to the complex interactions among processes that determine net Hg methylation, bioavailability, uptake and trophic transfer.

Mercury bioaccumulation and biomagnification in freshwater ecosystems are influenced by numerous ecological, biogeochemical, and physical factors[20], which can lead to variation in MeHg in predator fish on multiple scales. First, among individuals of the same species, MeHg concentrations tend to be positively correlated with size and age[21], while low-Hg prey[22] and faster growth rates[23] have been associated with lower fish MeHg concentrations. Second, variations in MeHg concentrations among species are related to food chain structure and trophic position[24] and to species-specific efficiency of trophic transfer of MeHg[25]. Third, within a water body, rates of net methylation and supply of MeHg to primary producers are influenced by water quality parameters such as dissolved organic matter[26], pH[27, 28], temperature[29], and sulfate (SO_4^{2-}) concentration[30], as well as rates of primary productivity[31]. Fourth, characteristics of the water body and its watershed, such as surface area[32], age of reservoirs[33] or ponds[34], water level[35], wetland coverage[36], and precipitation[37], may also contribute to variations in MeHg production and fate.

Many studies have explored factors associated with spatial variations in fish Hg concentrations in lakes and reservoirs[24, 38-40]. However, very few studies have simultaneously examined variations both within and among systems, while the factors most closely associated with variations in fish Hg concentrations may be scale-dependent. For instance, across many lakes covering a range of geological settings, pH may vary widely and be a key variable in explaining variations in Hg bioavailability and uptake, whereas within a lake, the range may be too small to observe variations in Hg concentrations as a function of pH. In addition, most studies on Hg biomagnification in lakes and reservoirs in North America were conducted in

temperate zones[19, 24, 29, 41], and relatively few[42-44] have focused on subtropical regions despite higher Hg emissions from power plants in these areas[45].

To evaluate key factors that explain differences in fish Hg concentrations within and among reservoirs, we examined variations in Hg concentrations in fish occupying a range of habitats and trophic positions from two connected freshwater reservoirs in south central U.S., which are located within 100 km of six coal-fired power plants (CFPPs). In addition, we sampled fish from local farm ponds, commonly used to raise game fish in the U.S. (50,000 constructed annually[46]) and other parts of the world, and "may be one of the largest unstudied Hg pollution problems in the U.S" [47]. To interpret our findings in a broader geographical context, we assessed variations in fish Hg concentrations among 61 reservoirs in this region by conducting correlation and regression analyses using 32 biogeochemical and watershed parameters. Our results provide insights into the key factors that affect the distribution of fish Hg concentrations both within and among subtropical freshwater reservoirs, and may be helpful for identifying priorities for monitoring Hg in biota and for guiding health-oriented environmental regulations and management practices in general.

Method

Site characteristics

We conducted in-depth sampling in two connected reservoirs in northeastern Oklahoma, Grand Lake O' The Cherokees (Grand Lake) and Lake Hudson, located within the humid subtropical climate zone in south central U.S. Grand Lake, impounded in 1941, is the third largest reservoir in Oklahoma and a popular fishing destination, with a surface area of about 200 km^2 and mean and maximum depths of 11.1 and 40.5 m, respectively[48]. Its primary tributaries

are the Neosho River (69% of inflow), Spring River (16% of inflow), and Elk River (15% of inflow) (Figure 1). Grand Lake has been classified as eutrophic based on phosphorus concentrations (0.03-0.19 mg/L)[49]. There is limited wetland coverage, distributed primarily along the shorelines in the upper reaches, covering about 8.5 km^2. Lake Hudson is located downstream of Grand Lake (Figure 1), which is the main source of its flow, and was impounded in 1964. It covers approximately 45 km^2 and is also classified as eutrophic ([P]: 0.01–0.14 mg/L)[49]. It also has very limited wetland area, covering only 0.6 km^2 [48] mainly due to its steep shoreline and rocky substrates that limit plant growth.

Sample collection

Between April 2010 and February 2013, about 1300 fish representing more than 30 species were collected throughout Grand Lake and its tributaries, Lake Hudson, and nearby farm ponds. Most of the species are commonly consumed by local residents[50] or are sport fish. Around 68% of these fish were collected by the Oklahoma Department of Wildlife Conservation (ODWC) as part of routine fish population surveys, primarily using gill nets. The rest of the samples were donated by local anglers, caught by hook and line or noodling (hand fishing). To evaluate spatial variability, we divided Grand Lake and Lake Hudson into three sections based on hydrodynamics: upper (riverine), mid (transition zone), and lower (lacustrine). Hydrological differences were more pronounced in Grand Lake than Lake Hudson. Samples were also collected from five major tributaries of Grand Lake (Spring River, Neosho River, Elk River, Horse Creek and Honey Creek) and from the area below the Pensacola Dam (between the reservoirs). Monthly water chemistry data was provided by the Grand River Dam Authority (GRDA, unpublished data) for most sections of Grand Lake and its tributaries.

Samples of five species (N = 1-12) from seven farm ponds were donated by local pond owners. All of the ponds were located within the Grand Lake watershed. It is estimated that over 200,000 farm ponds have been built in Oklahoma, and they are commonly used as water sources for livestock and irrigation, and to grow game fish[51].

Fish were weighed and measured for total length upon collection, and a small piece of skinless fillet (about 30 g) was harvested from each fish along the spine and just behind the head. In addition, stomach contents were collected from several predator species. To obtain stomach contents, body cavity contents were removed through an incision in the abdomen of each fish and the stomach was then separated from the remainder of the contents. The stomach was sliced open and any relatively intact and identifiable prey fish were removed, weighed and measured. All samples were then placed in acid-washed, pre-weighed 50 mL polypropylene centrifuge tubes, frozen and shipped overnight on ice to the Trace Metals Laboratory at Harvard T.H. Chan School of Public Health (Boston, MA).

Our sampling protocols have been reviewed and approved by the HMA Standing Committee on Animals at the Harvard Medical School.

Hg analyses

All fish samples were freeze-dried in a benchtop FreeZone Freeze Dry System (Labconco, Kansas City, MO) for 72 hours. Wet and dry weights of each sample were obtained immediately before and after freeze-drying. The average water content in fish samples was 78 ± 7.3%. All Hg results hereafter were reported on a wet weight basis. Freeze-dried samples were homogenized manually within centrifuge tubes using acid-cleaned Teflon stir rods.

Total Hg concentrations ([THg]) were determined for 1179 fillet and stomach content samples on a DMA-80 Direct Mercury Analyzer (Milestone Inc., Shelton, CT), following a method of thermal decomposition, amalgamation, and atomic absorption spectrophotometry[52]. The DMA was calibrated with newly made $HgCl_2$ solutions, and the calibration curve was checked daily with varying masses of a certified reference material (CRM), usually fish protein (DORM-3). At least one method blank and one lobster hepatopancreas CRM (TORT-2) were analyzed with every 10 samples. The average recovery was 102 ± 8.5% for DORM-3 (n=106) and 107 ± 9.2% for TORT-2 (n=128). Duplicates were analyzed for 10% of the samples, with an average relative percent difference (RPD) of 14%.

Forty-five fillet samples from Grand Lake and Lake Hudson from 12 species were analyzed for MeHg at the Dartmouth College Trace Element Analysis Laboratory, by automated purge and trap gas chromatography interfaced with inductively coupled plasma mass spectrometry (GC-ICP-MS)[53]. A blank spike and a CRM, either mussel tissue (NIST 2976) or DORM-3, were analyzed with every 10 samples, with average recoveries of 94 ± 1.1% (n=3) and 92 ± 0.8% (n=3), respectively. Duplicates were analyzed for 10% of the samples, with RPDs ranging from 2 to 8%.

Stable isotope analyses

Nitrogen and carbon stable isotopes are commonly used ecological metrics of food chain dynamics. Stable nitrogen isotope ratio ($\delta^{15}N$) is a continuous and integrative index of trophic position[54] and fractionates approximately 3.4‰ at each trophic transfer[55]. Stable carbon isotope ratio ($\delta^{13}C$) reflects the dietary carbon source at the base of the food chain, and fractionates at a less consistent rate of <1‰ per trophic level[55].

A total of 460 fillet samples from Grand Lake were tested for $\delta^{13}C$ and $\delta^{15}N$ at the Boston University Stable Isotope Laboratory using automated continuous-flow isotope ratio mass spectrometry[56]. Duplicates were analyzed every three samples and had an average RPD of around 1% for both $\delta^{13}C$ and $\delta^{15}N$. One laboratory standard, either peptone (n=33) or glycine (n=25), was analyzed with every 10-15 samples. The average recoveries of these two standards were 100 ± 2 % for both $\delta^{13}C$ and $\delta^{15}N$.

Calculating trophic position

We used zooplankton for baseline correction for $\delta^{15}N$, similar to Kidd et al.[57] Zooplankton samples were collected monthly at two locations (upper Grand and mid Grand) between April and September, 2012 using a horizontal plankton tow near the shoreline with a net mesh size of 153-363 μm. Since zooplankton are short-lived and integrate over shorter periods of time than snails or mussels[44], we used average values over all six sampling events at each location. A trophic position (TP) was calculated for each fillet sample, according to the following equation[41, 55]:

$$TP = (\delta^{15}N_{sample} - \delta^{15}N_{zooplankton})/3.4 + 2$$

using an average enrichment factor of 3.4‰ per trophic level and assigning zooplankton TP=2. For fish samples collected in upper or mid Grand Lake or nearby tributaries, $\delta^{15}N_{zooplankton}$ values from these two locations (11.86 and 10.99‰) were used, respectively. For samples collected from lower Grand Lake or nearby tributaries, mid Grand $\delta^{15}N_{zooplankton}$ was used. Stable isotopes were not measured in samples from farm ponds due to lack of baseline data.

The C:N ratio (from %C and %N measured during the analyses for $\delta^{13}C$ and $\delta^{15}N$) in our samples varied from 3.1 ± 0.44 (crappie, *Promoxis spp*.) to 5.9 ± 3.5 (spoonbill, *Polyodon*

spathula), indicative of large variations in lipid content among species[58]. Therefore we calculated lipid normalized $\delta^{13}C$ ($\delta^{13}C_{adj}$), based on the following equation[58]:

$$\delta^{13}C_{adj} = \delta^{13}C - 3.32 + 0.99*C:N$$

Statistical analyses

We evaluated spatial differences in fillet [THg] within and between Grand Lake and Lake Hudson by performing a multivariate regression of [THg] with length, trophic position (for two species), and sampling location as independent variables for 10 species (or genera, when the actual species was unidentified or when multiple species were pooled; referred to as species hereafter) with N≥10 from multiple locations: blue catfish (*Ictalurus furcatus*), buffalo (*Ictiobus spp.*), channel catfish (*Ictalurus punctatus*), crappie (*Pomoxis spp.*), drum (*Aplodinotus grunniens*), flathead catfish (*Pylodictis olivaris*), largemouth bass (*Micropterus salmoides*), shad (*Dorosoma sp.*), sunfish (*Lepomis spp.*), white bass (*Morone chrysops*). The distribution of fish [THg] was positively skewed, so these data were natural log-transformed prior to statistical analyses (ln[THg] hereafter). The transformed [THg] values met the normality assumption for most species based on Shapiro-Wilk test at $p>0.05$, except for blue catfish, crappie and shad. Length and TP were not transformed since the studentized residuals of all regression models met assumptions of normality ($p>0.05$ for Shapiro-Wilk test and Kolmogorov-Smirnov test). Horse Creek was used as the reference location since all 10 species were sampled there.

In order to evaluate our results in a broader geographical context and to further examine the key factors that explain differences in Hg bioaccumulation among reservoirs in this region, we analyzed correlations between fish [THg] across reservoirs in Oklahoma and 32 aquatic biogeochemical and watershed parameters. Using a pooled dataset containing fish [THg]

measurement data from both national sampling campaigns[59] and ODEQ sampling events between 2007 and 2010 (ODEQ, unpublished data) in Oklahoma reservoirs, fish [THg] was regressed against length for largemouth bass, and sampling location (i.e., reservoir) was included in the model as a categorical variable. The resulting slope and intercepts were then used to predict [THg] in a 14″ (36 cm) largemouth bass in each reservoir. The regression and prediction were performed by ODEQ based on a model developed by U.S. Geological Survey[60]. Prior to our analysis, we performed natural log-transformations on these [THg] data to ensure a normal distribution ($p=0.35$, Shapiro-Wilk test).

In our inter-system analysis, we included parameters related to water chemistry (pH, alkalinity, true color, apparent color, Secchi depth, salinity, turbidity, temperature), biology (pheophytin, chlorophyll-a) and nutrients (nitrate nitrogen [NO_3-N], nitrite nitrogen [NO_2-N], ammonia nitrogen [NH_4-N], total phosphorus [P], N:P ratio, oxidation-reduction potential, trophic index, sulfate [SO_4], summer hypoxia frequency, etc.) in the bottom water. These data were provided by the Oklahoma Water Resources Board (OWRB) for over 120 lakes throughout Oklahoma between 2005 and 2012[49], nearly all of which were reservoirs. We also included total surface area, average annual rainfall, and watershed wetland coverage, which were all calculated from a spatial analysis, as well as reservoir age. For each lake, we calculated an average value for each variable and the final dataset included 61 reservoirs for which we had overlapping data on fish [THg] and explanatory variables (Supplemental Table S1). The reservoirs in this final dataset are all established reservoirs at least 17 years (average 54 years) since the impoundment, with surface areas ranging from 0.4 to 403 km^2. We calculated the Spearman's correlation coefficient (ρ) between fish [THg] and each variable, and also performed a multivariate linear regression, using the same parameters as predictors of fish ln[THg]. A bidirectional stepwise

regression based on AIC score (Akaike Information Criterion, a commonly used indicator for model goodness-of-fit) was performed to eliminate covariates that were not significant predictors of fish [THg]. The variance inflation factors (VIF) for all covariates in the final model were below 3.5, suggesting little multicollinearity. Results for all correlation tests and regressions were reported at a significance level of 0.05.

RStudio version 0.98.978 (with R version 3.1.1) was used to perform all statistical analyses. The spatial analysis was conducted in ArcGIS 10.1.

Results and Discussion

Fish Hg concentrations in Grand Lake and Lake Hudson

Most of the species examined in this study had relatively low [THg] (Table 1). With the exception of gar (*Lepisosteidae*), mean [THg] for all species was below the U.S. EPA's fish tissue residue criterion (TRC; 300 ng/g, based on an assumed fish consumption rate of 17.5 g /day for the general adult population[61]) and the EPA wildlife criterion (WC) value for trophic level 4 fish such as largemouth bass and flathead catfish (346 ng/g, for the protection of piscivorous mammalian wildlife[62]). Among fillet samples collected from Grand Lake, the average [THg] ranged from 15 ± 12 ng/g (shad) to 530 ng/g (gar, n=2). Average [THg] for fish species lower on the food chain, such as shad, carp (*Cyprinus carpio*), buffalo, and perch/sunfish, were all below the EPA WC for trophic level 3 fish (77 ng/g). No samples from Lake Hudson exceeded the EPA fish TRC or level 4 WC, with the average [THg] ranging from 9.5 ± 2.4 ng/g (shad) to 91 ng/g (gar, n=2). Stomach contents generally had [THg] below 40 ng/g, and the average concentrations were 3 (white bass) to 6 (flathead catfish) times lower than those in the corresponding fillet samples.

Fillet samples of all species contained 95% MeHg or above, except for shad (82%), which occupy a lower trophic level (Supplemental Table S2). Therefore, [THg] is a good surrogate for MeHg concentrations in our fillet samples.

Across both Grand Lake and Lake Hudson, average total length ranged from 15 ± 2.3 cm (sunfish) to 97 ± 15 cm (spoonbill; Table 2). In general, [THg] in fillets increased with length, with the exception of three lower trophic level species (sunfish, spoonbill, and shad). Among 11 species with a total sample size N≥10, [THg] was significantly ($p<0.01$) correlated with length in 8 species (blue catfish, buffalo, channel catfish, crappie, drum, flathead catfish, largemouth bass, and white bass), with a Spearman's ρ ranging from 0.43 (flathead catfish) to 0.88 (drum). The lack of correlation between length and [THg] for sunfish and shad may be partly due to the inclusion of multiple species. Overall, our results could be useful in setting guidelines for the types and sizes of fish that consumers should consider in assessing dietary Hg exposures. For example, flathead catfish, which has the highest average [THg] among all species, tends to have twice as much [THg] ($p<0.0001$) if the length is over 40 inches, with an average [THg] of 348 ng/g, above the EPA fish TRC of 300 ng/g.

Food chain dynamics in Grand Lake

The calculated average TP ranged from 2.8 ± 0.53 (shad) to 4.1 ± 0.31 (crappie) among species with N≥10 (Table 2). About 86% of samples had a TP between 3 and 4, consistent with the typical feeding behavior of these species[63, 64]. Within each species, the difference between maximum and minimum TP was above 1.0, which may be caused by a high degree of omnivory and/or a wide range of ages. For blue catfish and drum, TP and length were significantly correlated (Spearman's $\rho = 0.43$ and 0.51, respectively), suggesting that larger, older individuals

of these two species occupied a higher TP. The lack of correlation between TP and length for other species may be related to variations in $\delta^{15}N$ at the base of the food web that were not fully accounted for by our baseline correction or differences in food web dynamics among locations. Food web structure and chain length can vary by location and by season[65], so the same species of a certain length may occupy a range of TP among locations or across seasons.

Among fish from Grand Lake, TP had a significant positive correlation with [THg] for largemouth bass, drum and shad (Table 2). For all species combined, [THg] was significantly ($p=0.02$) associated with TP (Figure 2a), indicating that Hg biomagnification occurred along the food chain in Grand Lake, with a trophic magnification factor (TMF) of 1.6 (95% CI = 1.1 – 2.1; calculated as the exponential of slope from the regression of log10-transformed [THg] with TP). This TMF value is much lower than the average TMF (3.4) found in freshwater ecosystems around the world[66], but comparable to the low TMF (1.1-2.3) observed in fish from subtropical reservoirs in China[67, 68]. The lack of spatially and temporally resolved $\delta^{15}N$ values for primary consumers in baseline correction may have increased the uncertainty in our TP calculations. Nevertheless, the significant associations between fish [THg] and TP suggest that variations in $\delta^{15}N$ for zooplankton were smaller than variations among trophic levels.

Average $\delta^{13}C_{adj}$ values among species with $N \geq 10$ were generally similar, ranging from -28 ± 2.1‰ (spoonbill, pelagic) to -26 ± 1.5‰ (channel catfish, benthic). These values were within the ranges of $\delta^{13}C$ values for typical pelagic (-35 to -20‰) and littoral (-28 to -14‰) systems[55]. Overall, there was no significant correlation between [THg] and $\delta^{13}C_{adj}$ (Figure 2b), while for crappie, flathead catfish, blue catfish and drum, $\delta^{13}C_{adj}$ was positively correlated with [THg] (Table 2). There were no clear differences in [THg] among littoral, benthic, or pelagic species. The similarity in $\delta^{13}C_{adj}$ across most species indicates either that there was a substantial

overlap in the feeding habitats of these fish species in Grand Lake, or that the baseline values were similar across multiple habitats. The $\delta^{13}C$ in zooplankton did not vary much among mid Hudson, mid Grand and upper Grand, with average values in these three locations ranging from -27.5 to -26.4‰.

Across all tested samples from Grand Lake, TP was negatively correlated with $\delta^{13}C_{adj}$ (Figure 2c) with a highly significant linear trend ($p<0.0001$). Similar negative relationships between $\delta^{15}N$ and $\delta^{13}C$ have been observed in freshwater food chains in other parts of the world[69]. Each 1‰ increase in $\delta^{13}C$ was associated with a 1/3 lower trophic level. This indicates that the feeding behavior and habitats of these species were tightly linked to their trophic positions, with species on higher trophic levels showing a more pelagic diet.

Among the limited number of stomach content samples (total N=20 from 7 species), most had lower TP (~1 trophic level) and [THg] than corresponding fillet samples, while the $\delta^{13}C_{adj}$ values were close to or more negative than the $\delta^{13}C_{adj}$ in fillet (Supplemental Figure S1), suggesting again that these predator fish were primarily feeding in open water habitats. The only stomach sample (identified as a catfish) from drum and one sample from flathead catfish had a TP higher than the corresponding fillets (Figure 2a; Figure S1b), and they also showed higher TP and [THg] relative to other stomach samples, consistent with the highest average [THg] found in these two species. The only sample from channel catfish had the lowest TP and a much more positive $\delta^{13}C_{adj}$ (Figure 2a, b; Figure S1b, c) than the other samples, indicating this fish was on a mostly littoral/benthic diet and feeding lower on the food chain.

Spatial variations within and between Grand Lake and Lake Hudson

Within Grand Lake, fish [THg] varied substantially among locations for most species, mirrored by similar trends in length and TP (Figure 3), suggesting that much of the spatial variability in [THg] was caused by differences in fish size and trophic structure in different portions of the reservoir. However, even after accounting for length (and TP for flathead catfish and shad) in multivariate linear regressions, there were significant differences ($p<0.05$) among locations for all 10 species, and the ratio of the highest to lowest concentrations (calculated as the natural exponential of the maximum difference in model estimates among locations) ranged from 1.6 (buffalo) to 7.2 (flathead catfish; Table 3).

The spatial variations in fish [THg] may be related to different hydrodynamic conditions among sections of the Grand Lake. Overall, the lowest [THg] was often observed at Horse Creek, a shallow and rocky tributary with less sediment and anoxic bottom water for methylation. The highest [THg] was usually found at lower and mid Grand, which are the more lacustrine parts of the reservoir, and in the Neosho River. Extended periods of summer bottom water oxygen depletion during stratification in the deeper sections of Grand Lake may have promoted anoxic conditions in sediments that in turn enhanced Hg methylation. The relatively high [THg] for some species in the Neosho River may reflect conditions in the river sediment, such as higher organic matter and porewater dissolved organic carbon, that can promote Hg methylation[42]. While measurements of Hg speciation in water samples were beyond the scope of this study, another study on Hg fate and transport in Grand Lake found elevated bottom water MeHg in the summer near the transition zone of the lake (beginning of mid Grand section), where the combination of deeper water stratification and particle settling from river inputs may have provided optimal conditions for MeHg enrichment[70].

The main flow of Lake Hudson is discharge from the dam at lower Grand Lake. Within Lake Hudson, the upper section usually had higher [THg] than the mid and lower sections, although the difference was not statistically significant for any species.

Spatial differences were also found between Grand Lake and Lake Hudson. In general, samples collected from Lake Hudson were lower in [THg] than those from Grand Lake (Figure 3), especially at mid and/or lower Hudson, where [THg] was significantly lower than one or more Grand Lake sites for crappie, largemouth bass, shad and white bass (Table 3). This suggests that Grand Lake is not a net exporter of MeHg to Lake Hudson, and is consistent with the finding from Wildman (2015) that Grand Lake is a net sink for THg and MeHg by sequestering both THg and MeHg during the time of fall overturn. By contrast, some reservoirs can be a net source of MeHg to the downstream ecosystems, especially in newer reservoirs[71, 72].

Fish from farm ponds

About 32% of all fish samples collected from farm ponds had [THg] above the EPA TRC of 300 ng/g (Table 1). Compared to fish from sites in Grand Lake and Lake Hudson, [THg] in five species (blue catfish, channel catfish, crappie, largemouth bass and sunfish) were up to 2to 17times higher in farm ponds. These differences were significant even after accounting for length (Figure 3, Table 3). This may be explained by the accumulation of Hg over time in these ponds, which typically have no outlet. For example, a previous study on freshwater fish ponds[34] showed increasing MeHg in surface sediment over the age of the pond. Moreover, relatively high levels of organic material and greater interaction between the sediments and water column compared with much larger reservoirs and their tributaries may have promoted the methylation of inorganic Hg, which occurs primarily in the top layer of the sediment that exchanges into the

water column, and is incorporated into the base of the food web[34]. In addition to these potential differences in biogeochemistry between farm ponds and large reservoirs, the high [THg] in farm pond fish could also come from the feed or body burden accumulated prior to introduction to the pond. Although we were unable to obtain information for every pond in our study, most are not regularly stocked or frequently fed. In any case, our findings suggest that [THg] in fish raised in the farm ponds may be a health concern that needs further evaluation in future research. Our study region lies within an area highly concentrated (>3 per km^2) with small man-made ponds (<1 ha)[47], and according to our results, these ponds may be an largely overlooked source of dietary Hg for the local residents.

Inter-system analysis

Based on modeled [THg] in 14″ largemouth bass among 61 reservoirs throughout Oklahoma, Grand Lake and Lake Hudson fish had among the lowest [THg] despite their close proximity to CFPPs (Figure 4). Across all reservoirs evaluated, modeled fish [THg] ranged from 60 to 1100 ng/g, with a geometric mean of 320 ng/g (95% CI: 260 – 380 ng/g). Although reservoirs in southeast Oklahoma were expected to have the highest [THg] due to the presence of large CFPPs upwind in Texas (southerly winds are predominant in this region), modeled fish [THg] did not show a similar trend ($p = 0.3$ for an ANOVA test among the four quadrants of Oklahoma, defined by Interstates 35 and 40 and roughly representing regional differences in elevation and precipitation; delineation of the quadrants are shown in Figure 1), suggesting that rates of atmospheric deposition and proximity to CFPPs may not explain much of the variations in fish [THg]. Therefore, we explored the extent to which biogeochemical and physical factors explained variations in fish [THg] across reservoirs in the region.

Among the 32 water quality and physical parameters in our analysis, true color, apparent color, and redox potential all had a significant ($p<0.05$) positive correlation with fish [THg], while conductivity, salinity, pH, alkalinity, and chlorophyll-a had a significant negative correlation (Table 4). In addition, annual rainfall had a marginally significant positive correlation ($p<0.1$), while chloride concentration and surface area had a marginally significant negative correlation with fish [THg]. Correlation tests among parameters showed highly significant positive correlations among pH, alkalinity, salinity and conductivity ($\rho > 0.8$).

From the stepwise multivariate regression, pH, total P, annual rainfall, NO_3-N, apparent color, NH_4-N, and Secchi depth remained in the final model, which had an adjusted R^2 of 0.60 (Table 5). In this model, pH, total P, rainfall, and Secchi depth were negatively associated with fish [THg], while apparent color, NO_3-N and NH_4-N had a positive association. Among the significant variables, pH, apparent color and rainfall together explained 53% of the variability in fish [THg]. No significant interactions were found among these variables, suggesting that these factors contribute independently to explaining the variability in fish [THg].

Our results showed that among various biogeochemical parameters, water pH was the most significant predictor of species- and length-normalized fish [THg] across reservoirs. Consistent with previous research in both temperate and subtropical lakes in North America[3, 73, 74], we found that reservoirs with lower pH tend to have higher fish [THg]. Water pH alone explained 37% of the variability in fish [THg] among the reservoirs we examined, and according to the slope from a simple linear regression of ln[THg] and pH, a pH of 7.56 or lower corresponds to fish [THg] above the EPA fish TRC of 300 ng/g, and 85% of the reservoirs with a pH of 7.56 or lower in our study had average fish [THg] above 300 ng/g (Supplemental Figure S2). Water pH may also explain some of the variation in fish [THg] within Grand Lake and its

tributaries. Horse Creek, which had among the lowest [THg], had the highest average water pH (8.49 ± 0.45), while Neosho River, which had among the highest [THg], had the lowest pH (7.73 ± 0.39). This trend may be attributed to increased uptake by methylating microorganisms due to higher bioavailability of Hg in acidic water[75], as well as enhanced methylation[76] of the inorganic Hg (II) species by sulfate- and iron-reducing bacteria, since methylation is generally more efficient at lower pH with more sulfide species available and with more soluble Hg compounds as the dominant species[75]. In addition, fish in higher pH water may gain more weight at a certain length, resulting in less [THg] per unit body mass through growth dilution[19]. However, the interaction between pH and Hg bioaccumulation is complex. For instance, enhanced demethylation and volatilization of Hg^0 have also been observed for lower water pH, leading to decreased production of MeHg[77]. Thus, while lower pH reservoirs tended to have higher fish [THg], our analysis does not indicate which mechanisms or pathways were most sensitive to differences in pH.

Water color was also found to be an important predictor of fish [THg] among reservoirs; both apparent (representing both suspended and dissolved substances, especially organic matter) and true (representing dissolved substances only) color were positively correlated with fish [THg]. Previous studies have found both positive and negative correlations between lake water DOC and fish [THg]. DOC can reduce the bioavailability of Hg for methylation by binding to Hg(II) species[78] and by enhancing photo-reduction of Hg(II) to Hg^0 [79]. Conversely, increased DOC can enhance Hg methylation by increasing bioavailability of Hg attached to humic matter in the hypolimnion of deep lakes[80], and by providing a carbon substrate for sulfate-reducing bacteria in the sediment[81]. Although true color had a stronger correlation with fish [THg] in our data, apparent color was a better predictor of fish [THg] after accounting for pH, suggesting that

both dissolved and suspended organic matter may have played a role in facilitating Hg methylation. The apparent and true color measured at Grand Lake and Lake Hudson were lower than most of the other reservoirs in Oklahoma, partially explaining the low fish [THg] in these two reservoirs (Figure 4).

We found that higher total P levels and trophic index were associated with lower fish [THg], which was observed by previous research[82, 83] and might be due to biodilution[31] from elevated inputs of growth-limiting nutrients that lead to dilution of Hg in primary producers. The negative correlation between chlorophyll-a and fish [THg] in our data provides evidence of algal biodilution. Low fish [THg] in eutrophic reservoirs can also result from growth dilution of the fish[67, 82], since food is more readily available in nutrient-rich waters, allowing fish to accumulate relatively more biomass. Thus, because most freshwater systems are P-limited, the negative correlation between total P and fish [THg] suggests that growth dilution and biodilution may have limited Hg biomagnification, which is supported by the low TMF observed in Grand Lake. In some systems, eutrophication caused by higher total P also can lead to more reducing bottom water conditions that enhance methylation. However, in our results, more reducing conditions (lower redox potential) were correlated with lower [THg] in fish, suggesting that biological factors outweighed redox effects on methylation. Grand Lake and Lake Hudson were both eutrophic and had total P concentrations in the top quartile of Oklahoma reservoirs, consistent with their relatively low fish [THg] (Figure 4). Overall, differences in nutrients levels and trophic status explained a small portion of variability in fish [THg] among reservoirs.

Among lake and watershed characteristics, average annual rainfall in the watershed was positively correlated with fish [THg], while this relationship became negative after accounting for pH. This suggests that the effect of rainfall on Hg dynamics among these reservoirs was

primarily through increasing water acidity[37], rather than through acting as a source of Hg wet deposition. Moreover, smaller surface area was associated with higher fish [THg], which may be another explanation for the low [THg] observed in Grand Lake and Lake Hudson, which are among the largest reservoirs in Oklahoma, and the high concentrations we observed in local farm ponds. Wetland coverage of the watershed was not found to be predictive of fish [THg] as in many other studies[81, 84, 85], probably because the wetlands were generally small and scattered in Oklahoma and thus relatively unimportant for MeHg production.

Overall, different variables described above seem to have contributed concurrently to changes in fish [THg] across reservoirs examined in this study, while each variable alone may not be adequate in explaining the variability in fish [THg] (Supplemental Figure S2), especially the low fish [THg] observed in Grand Lake and Lake Hudson. In addition, around 40% of the total variability in the data was not explained by variables included in our model, suggesting that other unexamined factors, such as local Hg emission and deposition, point sources, and hydrodynamics, may have also played a role.

Unique hydrodynamics and Hg cycling in reservoirs

As water bodies created, operated and managed by humans, reservoirs often have elevated fish Hg concentrations compared to natural water bodies[86]. This difference can be partially attributed to water-level fluctuations resulting from dam operations and the subsequent redox cycles that increase the availability of Hg sulfides for MeHg production[35]. It may also be caused by the reservoir effect, that is, the initial impoundment of water leads to decomposition of plant materials, creating a favorable environment for methylation with abundant organic matter

and low dissolved oxygen[87]. However, enhanced methylation in new reservoirs usually starts to decline 2-3 years after impoundment and Hg in fish can drop to background levels after 20-30 years[88], likely caused by increased demethylation[71] or depletion of labile organic carbon over time. This may explain why we did not observe a significant correlation between reservoir age and fish [THg] in our inter-system analysis, since these are all established reservoirs, mostly between 40 and 80 years old. The water levels in Grand Lake and Lake Hudson also do not fluctuate as much as some of the higher Hg reservoirs in this region, which could be another explanation for their low fish [THg]. For instance, the maximum difference in monthly average water elevation in 2012 was 0.83 m and 0.97 m in Grand Lake and Lake Hudson, respectively, compared to 2.0 m in Lake Eufaula and 3.6 m in Lake Hugo[89], two of the reservoirs with the highest fish [THg] in Oklahoma.

Strengths and limitations

This study benefited from its large sample size, with many different species collected from a variety of locations. By including fish samples donated by local anglers, our study represented a sustainable design and promoted greater community involvement. However, while our non-systematic sampling regime may have increased the relevance of our results to a related study of Hg exposure in local residents[50], it limited our ability to investigate seasonal changes in fish [THg] that may explain some of the variability observed in our data, since the majority (71%) of our samples were collected in fall 2010, fall 2011 and spring 2011, and after accounting for length, TP and location, sampling year was not a significant predictor of fish [THg] for any species. In addition, it may have decreased the representativeness of our results in characterizing

these systems as a whole. In general, donated fish (32% of total samples) tended to be longer than fish collected as part of routine population assessments by ODWC ($p<0.05$ for blue catfish, channel catfish, largemouth bass, crappie and shad; Wilcoxon rank-sum test), since anglers were more likely to keep or donate their largest catch. For four species (i.e., blue catfish, largemouth bass, sunfish and shad), donated samples had significantly higher [THg] than those collected by ODWC ($p<0.05$) even after controlling for length. This may be caused by differences in Hg bioaccumulation between the ODWC sampling sites and where the anglers usually go fishing. The donated blue catfish and crappie had significantly higher $\delta^{13}C_{adj}$ than ODWC samples, while donated shad had significantly lower $\delta^{13}C_{adj}$ ($p<0.05$, Wilcoxon rank-sum test). This difference also suggest that samples collected during routine fish population assessments may underestimate [THg] in locally caught and consumed fish.

Conclusions

In this study, we surveyed [THg] in a range of fish species and examined spatial variability both within and between two connected freshwater reservoirs, which are among the largest in Oklahoma and potentially impacted by CFPPs. While [THg] for most species were relatively low in these two reservoirs, we found significant spatial variations in many species even after accounting for length and/or trophic position. Our results suggest that within reservoirs, where water chemistry factors such as pH and nutrients varied in smaller ranges, ecological factors such as fish size and trophic position seemed to explain much of the variation in fish [THg]. In addition, fish from nearby farm ponds, which are small, isolated water bodies commonly used to raise fish in the U.S., generally had higher [THg] than those of the same species and length in larger reservoirs nearby and may need further attention in future research.

To further explore the biogeochemical mechanisms that may have led to the low fish [THg] in these two reservoirs despite their proximity to atmospheric sources, we examined the key factors contributing to spatial variability in fish [THg] on a broader geographic scale, and found that differences in fish [THg] among reservoirs could be best explained by abiotic factors such as pH, nutrients, rainfall and water color. Our study demonstrates that inter- and intra-system spatial variations in fish [THg] among freshwater ecosystems may be influenced by different biological, chemical and physical factors. Considering spatial variability on different scales simultaneously would deepen our understanding of the complex linkage between sources of Hg inputs into water bodies and biomagnification in fish. Furthermore, focusing on key parameters that affect fish Hg concentrations, especially those on the water body or watershed level, could not only help us identify monitoring priorities, but also help tailor exposure

assessment and fish consumption guidelines to specific water bodies and locations for better protection of human and ecological health.

Acknowledgements

This study was funded by National Institute of Environmental Health Sciences (NIEHS) grant number 1R21ES017941 and NIEHS Center Grant 2 P30-ES00002. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIEHS. We thank Brent Gordon, Ashley Foster, Oklahoma Department of Wildlife Conservation, and numerous local anglers for fish collection; Rebecca Jim, Gina Manders, Kindel Maymi, Earl Hatley, and Mary Daugherty, L.E.A.D. Agency, for coordinating fish sample donations; Jay Wright and Randy Parham, Oklahoma Department of Environmental Quality, for providing state-wide fish Hg data; Monty Porter and Julie Chambers, Oklahoma Water Resources Board, for providing water chemical and depth profile data on Oklahoma lakes; Darrell Townsend and Sam Ziara, Grand River Dam Authority, for providing logistical support and water quality depth profile data; James Shine, Harvard T.H. Chan School of Public Health, for input on study design, sample analysis and data interpretation; Joshua Bridges, University of Oklahoma, for assistance on collecting plankton tows; Brian Jackson, Dartmouth College Trace Metals Laboratory, for MeHg analyses; and Robert Michener, Boston University Stable Isotope Laboratory, for stable C and N isotope analyses.

References

1. P. A. Amundsen, F. J. Staldvik, A. A. Lukin, N. A. Kashulin, O. A. Popova and Y. S. Reshetnikov, *Sci. Total Environ.*, 1997, **201**, 211-224.
2. L. M. Campbell, D. G. Dixon and R. E. Hecky, *J. Toxicol. Environ. Health, Pt. B Crit. Rev.*, 2003, **6**, 325-356.
3. C. T. Driscoll, Y.-J. Han, C. Y. Chen, D. C. Evers, K. F. Lambert, T. M. Holsen, N. C. Kamman and R. K. Munson, *Bioscience*, 2007, **57**, 17-28.
4. M. S. Evans, W. L. Lockhart, L. Doetzel, G. Low, D. Muir, K. Kidd, G. Stephens and J. Delaronde, *Sci. Total Environ.*, 2005, **351**, 479-500.
5. P. Grandjean, E. Budtz-Jorgensen, R. F. White, P. J. Jorgensen, P. Weihe, F. Debes and N. Keiding, *Am. J. Epidemiol.*, 1999, **150**, 301-305.
6. M. R. Karagas, A. L. Choi, E. Oken, M. Horvat, R. Schoeny, E. Kamai, W. Cowell, P. Grandjean and S. Korrick, *Environ. Health Perspect.*, 2012, **120**, 799-806.
7. K. R. Mahaffey, R. P. Clickner and C. C. Bodurow, *Environ. Health Perspect.*, 2004, **112**, 562-570.
8. U.S. EPA, *Fish Consumption Advisories: What You Need to Know About Mercury in Fish and Shellfish*, U.S. Environmental Protection Agency, Washington D.C., 2004.
9. K. L. Crump and V. L. Trudeau, *Environ. Toxicol. Chem.*, 2009, **28**, 895-907.
10. M. F. Wolfe, S. Schwarzbach and R. A. Sulaiman, *Environ. Toxicol. Chem.*, 1998, **17**, 146-160.
11. A. M. Scheuhammer, M. W. Meyer, M. B. Sandheinrich and M. W. Murray, *Ambio*, 2007, **36**, 12-18.
12. UNEP, *Global Mercury Assessment 2013: Sources, Emissions, Releases and Environmental Transport*, United Nations Environment Programme Chemicals Branch, Geneva, Switzerland, 2013.
13. F. M. M. Morel, A. M. L. Kraepiel and M. Amyot, *Annu. Rev. Ecol. Syst.*, 1998, **29**, 543-566.
14. E. J. Kerin, C. C. Gilmour, E. Roden, M. T. Suzuki, J. D. Coates and R. P. Mason, *Appl. Environ. Microbiol.*, 2006, **72**, 7919-7921.
15. C. R. Hammerschmidt and W. F. Fitzgerald, *Environ. Sci. Technol.*, 2006, **40**, 7764-7770.
16. R. C. Harris, J. W. M. Rudd, M. Amyot, C. L. Babiarz, K. G. Beaty, P. J. Blanchfield, R. A. Bodaly, B. A. Branfireun, C. C. Gilmour, J. A. Graydon, A. Heyes, H. Hintelmann, J. P. Hurley, C. A. Kelly, D. P. Krabbenhoft, S. E. Lindberg, R. P. Mason, M. J. Paterson, C. L. Podemski, A. Robinson, K. A. Sandilands, G. R. Southworth, V. L. S. Louis and M. T. Tate, *Proc. Natl. Acad. Sci. USA*, 2007, **104**, 16586-16591.
17. T. H. Suchanek, C. A. Eagles-Smith, D. G. Slotton, E. J. Harner, A. E. Colwell, N. L. Anderson, L. H. Mullen, J. R. Flanders, D. P. Adam and K. J. McElroy, *Ecol. Appl.*, 2008, **18**, A177-A195.
18. B. K. Greenfield, D. G. Slotton and K. H. Harrold, *Environ. Toxicol. Chem.*, 2013, **32**, 2728-2737.
19. J. A. Dittman and C. T. Driscoll, *Biogeochemistry*, 2009, **93**, 179-196.
20. M. G. Clayden, K. A. Kidd, B. Wyn, J. L. Kirk, D. C. G. Muir and N. J. O'Driscoll, *Environ. Sci. Technol.*, 2013, **47**, 12047-12053.
21. A. Farkas, J. Salanki and A. Specziar, *Water Res.*, 2003, **37**, 959-964.
22. J. M. Lepak, K. D. Kinzli, E. R. Fetherman, W. M. Pate, A. G. Hansen, E. I. Gardunio, C. N. Cathcart, W. L. Stacy, Z. E. Underwood, M. M. Brandt, C. A. Myrick and B. M. Johnson, *Can. J. Fish. Aquat. Sci.*, 2012, **69**, 122-135.
23. M. Simoneau, M. Lucotte, S. Garceau and D. Laliberte, *Environ. Res.*, 2005, **98**, 73-82.
24. P. R. Gorski, L. B. Cleckner, J. P. Hurley, M. E. Sierszen and D. E. Armstrong, *Sci. Total Environ.*, 2003, **304**, 327-348.
25. C. A. Eagles-Smith, T. H. Suchanek, A. E. Colwell and N. L. Anderson, *Ecol. Appl.*, 2008, **18**, A196-A212.
26. M. Ravichandran, *Chemosphere*, 2004, **55**, 319-331.
27. T. A. Haines, V. Komov and C. H. Jagoe, *Environ. Pollut.*, 1992, **78**, 107-112.
28. D. J. Spry and J. G. Wiener, *Environ. Pollut.*, 1991, **71**, 243-304.
29. R. C. Harris and R. A. Bodaly, *Biogeochemistry*, 1998, **40**, 175-187.
30. C. C. Gilmour, E. A. Henry and R. Mitchell, *Environ. Sci. Technol.*, 1992, **26**, 2281-2287.
31. P. C. Pickhardt, C. L. Folt, C. Y. Chen, B. Klaue and J. D. Blum, *Proc. Natl. Acad. Sci. USA*, 2002, **99**, 4419-4423.
32. R. A. Bodaly, J. W. M. Rudd, R. J. P. Fudge and C. A. Kelly, *Can. J. Fish. Aquat. Sci.*, 1993, **50**, 980-987.
33. R. A. Bodaly, V. L. St. Louis, M. J. Paterson, R. J. P. Fudge, B. D. Hall, D. M. Rosenberg and J. W. M. Rudd, in *Metal Ions in Biological Systems, Vol 34: Mercury and Its Effects on Environment and Biology*, eds. A. Sigel and H. Sigel, CRC Press, 1997, vol. 34, pp. 259-287.
34. D. D. Shao, P. Liang, Y. Kang, H. S. Wang, Z. Cheng, S. C. Wu, J. B. Shi, S. C. L. Lo, W. X. Wang and M. H. Wong, *Chemosphere*, 2011, **83**, 443-448.

35. J. A. Sorensen, L. W. Kallemeyn and M. Sydor, *Environ. Sci. Technol.*, 2005, **39**, 9237-9243.
36. V. L. St. Louis, J. W. M. Rudd, C. A. Kelly, K. G. Beaty, N. S. Bloom and R. J. Flett, *Can. J. Fish. Aquat. Sci.*, 1994, **51**, 1065-1076.
37. S. G. Downs, C. L. Macleod and J. N. Lester, *Water Air Soil Pollut.*, 1998, **108**, 149-187.
38. X. Yu, C. T. Driscoll, M. Montesdeoca, D. Evers, M. Duron, K. Williams, N. Schoch and N. C. Kamman, *Ecotoxicology*, 2011, **20**, 1543-1554.
39. C. Mathieu, C. V. Furl, T. M. Roberts and M. Friese, *Arch. Environ. Contam. Toxicol.*, 2013, **65**, 122-131.
40. M. C. Gabriel, R. Kolka, T. Wickman, E. Nater and L. Woodruff, *Sci. Total Environ.*, 2009, **407**, 4117-4126.
41. R. A. Lavoie, C. E. Hebert, J.-F. Rail, B. M. Braune, E. Yumvihoze, L. G. Hill and D. R. S. Lean, *Sci. Total Environ.*, 2010, **408**, 5529-5539.
42. J. C. Becker, A. W. Groeger, W. H. Nowlin, M. M. Chumchal and D. Hahn, *Environ. Toxicol. Chem.*, 2011, **30**, 2300-2311.
43. M. M. Chumchal and K. D. Hambright, *Environ. Toxicol. Chem.*, 2009, **28**, 962-972.
44. M. M. Chumchal, T. R. Rainwater, S. C. Osborn, A. P. Roberts, M. T. Abel, G. P. Cobb, P. N. Smith and F. C. Bailey, *Environ. Toxicol. Chem.*, 2011, **30**, 1153-1162.
45. P. J. Miller and C. Van Atten, *North America Power Plant Air Emissions*, Commission for Environmental Cooperation of North America, Montreal, QC, Canada, 2004.
46. C. D. Regan, Farm Pond Management: Observation of Farm Pond Is the Key to Keeping Your Water Sources in Top Health., http://www.grit.com/departments/farm-pond-management.aspx, (accessed September 1, 2015).
47. M. M. Chumchal and R. W. Drenner, *Environ. Toxicol. Chem.*, 2015, **34**, 1197-1205.
48. OWRB, Lakes of Oklahoma, http://www.owrb.ok.gov/news/publications/lok/lok.php, (accessed October 20, 2014).
49. OWRB, *Bump Lakes Report 2012*, Oklahoma Water Resources Board, Oklahoma City, OK, 2012.
50. Z. Dong, R. C. Jim, E. L. Hatley, A. S. N. Backus, J. P. Shine, J. D. Spengler and L. A. Schaider, *Environ. Res.*, 2015, **136**, 155-162.
51. K. W. Williams, The Encyclopedia of Oklahoma History and Culture - Farm Ponds, http://www.okhistory.org/publications/enc/entry.php?entry=FA014, (accessed April 17, 2015).
52. U.S. EPA, *Method 7473: Mercury in Solids and Solutions by Thermal Decomposition, Amalgamation, and Atomic Absorption Spectrophotometry*, U.S. Environmental Protection Agency, Washington D.C., 2007.
53. B. Jackson, V. Taylor, R. A. Baker and E. Miller, *Environ. Sci. Technol.*, 2009, **43**, 2463-2469.
54. G. Cabana and J. B. Rasmussen, *Nature*, 1994, **372**, 255-257.
55. D. M. Post, *Ecology*, 2002, **83**, 703-718.
56. N. J. P. Owens and A. P. Rees, *Analyst*, 1989, **114**, 1655-1657.
57. K. A. Kidd, D. C. G. Muir, M. S. Evans, X. Wang, M. Whittle, H. K. Swanson, T. Johnston and S. Guildford, *Sci. Total Environ.*, 2012, **438**, 135-143.
58. D. M. Post, C. A. Layman, D. A. Arrington, G. Takimoto, J. Quattrochi and C. G. Montana, *Oecologia*, 2007, **152**, 179-189.
59. U.S. EPA, *Update: National Listing of Fish and Wildlife Advisories.*, U. S. Environmental Protection Agency, Office of Water, Washington D.C., 2003.
60. S. P. Wente, *A Statistical Model and National Data Set for Partitioning Fish-Tissue Mercury Concentration Variation between Spatiotemporal and Sample Characteristic Effects*, Report USGS 2004-5199, U.S. Geological Survey, Reston, VA, 2004.
61. U.S. EPA, *Water Quality Criterion for the Protection of Human Health: Methylmercury*, Report EPA-823-R-01-001, Office of Science and Technology and Office of Water, Washington D.C., 2001.
62. U.S. EPA, *Mercury Study Report to Congress. Volume Vi: An Ecological Assessment for Anthropogenic Mercury Emissions in the United States*, Report EPA-452/R-97-008, U.S. Environmental Protection Agency, Washington D.C., 1997.
63. M. F. Mettee, P. E. O'Neil and J. M. Pierson, *Fishes of Alabama and the Mobile Basin*, Oxmoor House, Birmingham, AL, 1st edn., 1996.
64. H. W. Robinson and T. M. Buchanan, *Fishes of Arkansas*, University of Arkansas Press, Fayetteville, AR, 1988.
65. P. H. Warren, *Oikos*, 1989, **55**, 299-311.
66. R. A. Lavoie, T. D. Jardine, M. M. Chumchal, K. A. Kidd and L. M. Campbell, *Environ. Sci. Technol.*, 2013, **47**, 13385-13394.

67. R. N. Razavi, M. Qu, D. Chen, Y. Zhong, W. Ren, Y. Wang and L. M. Campbell, *Limnol. Oceanogr.*, 2015, **60**, 386-401.
68. R. N. Razavi, M. Qu, B. Jin, W. Ren, Y. Wang and L. M. Campbell, *Ecotoxicology*, 2014, **23**, 133-146.
69. T. M. Tadiso, R. Borgstrom and B. O. Rosseland, *Ecotoxicol. Environ. Saf.*, 2011, **74**, 953-959.
70. R. A. Wildman, *Lake Reserv. Manage.*, 2015, **In Press**.
71. V. L. St. Louis, J. W. M. Rudd, C. A. Kelly, R. A. Bodaly, M. J. Paterson, K. G. Beaty, R. H. Hesslein, A. Heyes and A. R. Majewski, *Environ. Sci. Technol.*, 2004, **38**, 1348-1358.
72. C. Y. Chen, C. T. Driscoll and N. C. Kamman, in *Mercury in the Environment: Pattern and Process*, ed. M. S. Bank, University of California Press, Berkeley Los Angeles London, 1st edn., 2012, ch. 9, p. 155.
73. J. G. Wiener, B. C. Knights, M. B. Sandheinrich, J. D. Jeremiason, M. E. Brigham, D. R. Engstrom, L. G. Woodruff, W. F. Cannon and S. J. Balogh, *Environ. Sci. Technol.*, 2006, **40**, 6261-6268.
74. T. R. Lange, H. E. Royals and L. L. Connor, *Trans. Am. Fish. Soc.*, 1993, **122**, 74-84.
75. C. A. Kelly, J. W. M. Rudd and M. H. Holoka, *Environ. Sci. Technol.*, 2003, **37**, 2941-2946.
76. B. M. Miskimmin, J. W. M. Rudd and C. A. Kelly, *Can. J. Fish. Aquat. Sci.*, 1992, **49**, 17-22.
77. W. F. Fitzgerald, R. P. Mason and G. M. Vandal, *Water Air Soil Pollut.*, 1991, **56**, 745-767.
78. J. M. Benoit, R. P. Mason, C. C. Gilmour and G. R. Aiken, *Geochim. Cosmochim. Acta*, 2001, **65**, 4445-4451.
79. N. J. O'Driscoll, S. D. Siciliano, D. Peak, R. Carignan and D. R. S. Lean, *Sci. Total Environ.*, 2006, **366**, 880-893.
80. A. Nilsson and L. Hakanson, *Hydrobiologia*, 1992, **235**, 675-683.
81. B. D. Hall, G. R. Aiken, D. P. Krabbenhoft, M. Marvin-DiPasquale and C. M. Swarzenski, *Environ. Pollut.*, 2008, **154**, 124-134.
82. T. E. Essington and J. N. Houser, *Trans. Am. Fish. Soc.*, 2003, **132**, 57-68.
83. K. A. Kidd, M. J. Paterson, R. H. Hesslein, D. C. G. Muir and R. E. Hecky, *Can. J. Fish. Aquat. Sci.*, 1999, **56**, 2193-2202.
84. J. P. Hurley, J. M. Benoit, C. L. Babiarz, M. M. Shafer, A. W. Andren, J. R. Sullivan, R. Hammond and D. A. Webb, *Environ. Sci. Technol.*, 1995, **29**, 1867-1875.
85. P. Selvendiran, C. T. Driscoll, J. T. Bushey and M. R. Montesdeoca, *Environ. Pollut.*, 2008, **154**, 46-55.
86. N. C. Kamman, N. M. Burgess, C. T. Driscoll, H. A. Simonin, W. Goodale, J. Linehan, R. Estabrook, M. Hutcheson, A. Major, A. M. Scheuhammer and D. A. Scruton, *Ecotoxicology*, 2005, **14**, 163-180.
87. D. C. Evers, Y. J. Han, C. T. Driscoll, N. C. Kamman, M. W. Goodale, K. F. Lambert, T. M. Holsen, C. Y. Chen, T. A. Clair and T. Butler, *Bioscience*, 2007, **57**, 29-43.
88. M. R. Anderson, D. A. Scruton, U. P. Williams and J. F. Payne, *Water Air Soil Pollut.*, 1995, **80**, 927-930.
89. USACE, Grand Lake O' the Cherokees, Pensacola Dam - Monthly Charts of Reservoir Data, http://www.swt-wc.usace.army.mil/PENS.lakepage.html, (accessed April 17, 2015).
90. OCS, *The Climate of Ottawa County*, Oklahoma Climatological Survey, Norman, OK, 2004.

Figure Captions

Figure 1. A map of Grand Lake and Lake Hudson, with major sections and tributaries. The top inset shows delineation of the quadrants and locations of all reservoirs included in this study. The lower inset shows the coal-fired power plants located near the Grand Lake watershed. The wind rose depicts average predominant wind direction from 1994 to 2001 at Miami, OK.[90]

Figure 2. Relationship between (a) ln[THg] and trophic position, (b) ln[THg] and lipid normalized $\delta^{13}C$ ($\delta^{13}C_{adj}$), and (c) trophic position and $\delta^{13}C_{adj}$ in Grand Lake fish fillets and stomach contents (SC). Each symbol represents the mean value of each species, and error bars represent standard deviations. Species were sorted and color-coded by trophic position (a, c) or by typical habitat (b). Equations are based on linear regressions of the means for the fillet samples, and gray areas indicate 95% confidence regions.

Figure 3. Spatial variations across Grand Lake, Lake Hudson, and local farm ponds in ln[THg], length and trophic position among 10 fish species with N≥10. Each point represents at least 3 samples. Error bars represent standard deviations. Black triangles indicate tributaries. Length is shown in decimeters here so that variation in length is on a scale comparable to variations in both ln[THg] and trophic position.

Figure 4. Distribution of water pH, true color, annual rainfall, total phosphorus, and modeled [THg] in 14″ largemouth bass (LMB) among reservoirs analyzed in this study.

Table 1. A summary of fish samples collected for this study, including sample type (fillet unless otherwise specified), sample size, total mercury concentrations ([THg]) (shown as mean ± standard deviation, wet weight) in ng/g, and percentage of samples above EPA fish TRC of 300 ng/g. Species were color-coded by typical trophic position and sorted by [THg]. Standard deviations were only calculated when N>2.

Species	Grand Lake and Tributaries			Lake Hudson			Farm Ponds		
	N	Mean [THg] ± SD	% > TRC	N	Mean [THg] ± SD	% > TRC	N	Mean [THg] ± SD	% > TRC
Gar[a] *(Lepisosteidae)*	2	530	**100**	2	91	0			
Flathead Catfish *(Pylodictis olivaris)*									
Fillet	38	220 ± 150	**24**						
Stomach Contents	2	34	0						
Spotted Bass *(Micropterus punctulatus)*	2	79	0						
Largemouth Bass *(Micropterus salmoides)*									
Fillet	99	78 ± 60	**1.0**	4	41 ± 33	0	12	400 ± 250	**33**
Stomach Contents	9	15 ± 8.9	0						
Wiper *(M. chrysops x M. saxatilis)*	4	72 ± 43	0						
White Bass *(Morone chrysops)*									
Fillet	165	47 ± 34	0	59	52 ± 51	0			
Stomach Contents	2	16	0						
Striped Bass *(Morone saxatilis)*	2	42	0						
Smallmouth Bass *(Micropterus dolomieu)*	3	34 ± 22	0						
Crappie[b] *(Pomoxis spp.)*									
Fillet	130	30 ± 24	0	14	18 ± 25	0	3	86 ± 110	0
Stomach Contents	3	6.2 ± 0.3	0	4	7.1 ± 2.3	0			
Drum *(Aplodinotus grunniens)*									
Fillet	29	130 ± 190	**10**	10	31 ± 18	0			
Stomach Contents	1	41	0						
White Catfish *(Ameiurus catus)*	5	72 ± 53	0						
Blue Catfish *(Ictalurus furcatus)*									
Fillet	116	59 ± 55	**1.8**	69	40 ± 26	0	2	172	0

Stomach Contents	2	14	0	2	8.8	0			
Channel Catfish *(Ictalurus punctatus)*									
Fillet	102	48 ± 28	0	18	38 ± 17	0	1	560	**100**
Stomach Contents	1	15	0						
Sunfish[c] *(Lepomis spp.)*	48	31 ± 27	0				11	120 ± 110	**50**
Catfish *(Ictalurus sp.)*	1	27	0						
Common Carp *(Cyprinus carpio)*	4	67 ± 54	0	3	28 ± 17	0			
Buffalo[d] *(Ictiobus spp.)*	50	54 ± 39	0	18	52 ± 33	0			
River Carpsucker *(Carpiodes carpio)*	1	48	0						
Spoonbill *(Polyodon spathula)*	43	40 ± 24	0	1	7.1	0			
Shad[e] (*Dorosoma sp.)*	39	15 ± 12	0	36	9.5 ± 2.4	0			
Turtle (unidentified)									
Fillet	1	3.0	0						
Leg	2	84	0						
Crayfish (Whole)	4	23 ± 26	0						
Total N	910			240			29		

[a] includes shortnose gar and longnose gar;
[b] includes white crappie, black crappie, and unidentified species of crappie;
[c] includes bluegill sunfish, green sunfish, redear sunfish, warmouth, longear sunfish and unidentified species of sunfish;
[d] includes largemouth buffalo and smallmouth buffalo;
[e] includes gizzard shad and unidentified species of shad.

Table 2. Summary of length, trophic position (TP), lipid normalized $\delta^{13}C$ ($\delta^{13}C_{adj}$) for 11 species with N≥10, and Spearman's correlation coefficients of [THg] (ng/g) and length, TP, and $\delta^{13}C_{adj}$ for each species. Species are color-coded according to typical trophic position.

Species	N	Mean ± sd			Spearman's Correlation with [THg]		
		Length[a] (cm)	TP[b]	$\delta^{13}C_{adj}$ (‰)[b]	Length	TP	$\delta^{13}C_{adj}$
Crappie	148	27 ± 4.4	4.1 ± 0.31	-28 ± 0.86	0.55***	0.12	0.45***
White Bass	225	31 ± 7.8	4.0 ± 0.35	-28 ± 0.52	0.61***	0.19	0.035
Largemouth Bass	121	37 ± 6.0	3.9 ± 0.46	-27 ± 1.0	-0.46***	0.45***	0.10
Flathead Catfish	46	82 ± 25	3.7 ± 0.32	-27 ± 1.2	0.43**	0.31^	0.49**
Blue Catfish	192	47 ± 18	3.6 ± 0.42	-28 ± 1.0	0.76***	0.25	0.58***
Drum	39	30 ± 13	3.5 ± 0.44	-28 ± 1.9	0.88***	0.47*	0.68***
Sunfish	59	15 ± 2.3	3.4 ± 0.21	-27 ± 1.4	0.18	-0.32*	0.039
Channel Catfish	127	41 ± 13	3.2 ± 0.53	-26 ± 1.5	0.51***	0.21	-0.016
Spoonbill	56	97 ± 15	3.4 ± 0.24	-28 ± 2.1	0.21	-0.072	0.47^
Buffalo	73	42 ± 11	3.2 ± 0.27	-28 ± 1.6	0.81***	0.23	0.15
Shad	75	23 ± 4.6	2.8 ± 0.53	-27 ± 1.4	-0.13	0.41*	0.16

[a] Include samples from all locations;
[b] Only include samples from Grand Lake and its tributaries.
^: 0.05<p<0.1
*: 0.01<p<0.05
**: 0.001<p<0.01
***: p<0.001

Table 3. Multivariate regression of ln[THg] (ng/g) on length, trophic position (TP), and location for 10 fish species with N≥10. Estimated coefficients are shown, with 95% confidence intervals in parentheses.

Species	Blue Catfish	Buffalo	Channel Catfish	Crappie	Drum
Adjusted R^2	0.65	0.68	0.44	0.49	0.81
Length (cm)	0.027 (0.023, 0.032)***	0.067 (0.055, 0.079)***	0.019 (0.012, 0.026)***	0.076 (0.056, 0.095)***	0.094 (0.064, 0.12)***
TP	NA[a]	NA[a]	NA[a]	NA[a]	NA[a]
Location within Grand Lake Watershed (Referent = Horse Creek)					
Spring River	0.63 (-0.13, 1.4)	0.28 (-0.12, 0.68)	-0.40 (-0.81, 0.020)^	NA	1.6 (0.65, 2.6)**
Neosho River	0.42 (0.18, 0.66)***	NA	0.38 (0.094, 0.66)**	NA	1.6 (0.80, 2.3)***
Upper Grand	0.18 (-0.11, 0.47)	0.30 (-0.24, 0.83)	0.084 (-0.21, 0.38)	0.33 (0.031, 0.63)*	1.5 (0.49, 2.6)**
Elk River	0.14 (-0.072, 0.35)	0.45 (0.094, 0.80)*	0.18 (-0.042, 0.39)	0.42 (0.16, 0.68)**	1.6 (0.61, 2.6)**
Mid Grand	0.83 (0.36, 1.3)***	NA	0.77 (-0.097, 1.6)^	0.60 (0.076, 1.1)*	NA
Honey Creek	0.50 (-0.25, 1.3)	NA	0.32 (-0.54, 1.2)	0.31 (-0.40, 1.0)	1.1 (0.091, 2.0)*
Lower Grand	0.077 (-0.38, 0.53)	0.46 (0.0057, 0.86)*	0.28 (-0.13, 0.68)	0.65 (0.20, 1.1)**	1.7 (0.49, 2.9)**
Dam	0.051 (-0.23, 0.33)	NA`	0.32 (-0.20, 0.85)	0.36 (-0.069, 0.78)^	-0.59 (-1.8, 0.61)
Upper Hudson	0.18 (-0.036, 1.4)	0.26 (-0.18, 0.69)	0.066 (-0.45, 0.58)	-0.10 (-1.1, 0.85)	1.4 (0.31, 2.5)*
Mid Hudson	0.044 (-0.16, 0.25)	0.62 (0.14, 1.1)*	0.063 (-0.35, 0.47)	-0.46 (-0.86, -0.069)*	0.94 (-0.015, 1.9)^
Lower Hudson	0.086 (-0.12, 0.29)	0.51 (0.036, 0.98)*	-0.085 (-0.39, 0.22)	-0.24 (-0.83, 0.34)	1.4 (0.16, 2.6)
Farm Pond	1.1 (0.50, 1.6)***	NA	2.5 (1.7, 3.4)***	1.6 (1.0, 2.2)***	NA

Table 3. (Cont.)

Species	Flathead Catfish	Largemouth Bass	Shad	Sunfish	White Bass
Adjusted R^2	0.42	0.68	0.58	0.51[c]	0.41
Length (cm)	0.00022 (-0.0086, 0.013)	0.078 (0.061, 0.095)***	-0.0078 (-0.077, 0.061)	0.12 (0.066, 0.18)***	0.061 (0.051, 0.072)***
TP	1.1 (0.12, 2.2)*	NA[a]	0.56 (0.22, 0.91)**	NA[a]	NA[a]
Location within Grand Lake Watershed (Referent = Horse Creek)					
Spring River	NA	0.33 (-0.18, 0.84)	-0.18 (-1.1, 0.75)	NA	-0.27 (-1.4, 0.83)
Neosho River	1.3 (0.56, 2.0)**	0.54 (-0.078, 1.2)^	-0.072 (-1.2, 1.1)	NA	1.3 (0.18, 2.4)*
Upper Grand	1.1 (0.27, 1.9)*	0.29 (-0.12, 0.70)	-1.0 (-2.1, 0.036)^	0.22 (-0.25, 0.69)	0.17 (-0.091, 0.42)
Elk River	0.90 (-0.10, 1.9)^	0.26 (-0.059, 0.58)	NA	0.47 (-0.21, 1.1)	0.26 (0.0027, 0.52)*
Mid Grand	0.69 (-0.30, 1.7)	0.10 (-0.28, 0.48)	NA	NA	0.19 (-0.29, 0.67)
Honey Creek	NA	0.0014 (-0.46, 0.46)	NA	0.58 (-0.17, 1.3)	0.12 (-0.53, 0.78)
Lower Grand	2.0 (0.74, 3.2)**	0.60 (0.30, 0.91)***	0.16 (-0.18, 0.50)	0.61 (0.073, 1.1)*	0.58 (0.23, 0.93)**
Dam	NA	-0.16 (-0.78, 0.46)	-0.73 (-1.1, -0.34)***	NA	0.62 (-1.0, -0.22)**
Upper Hudson	NA	NA	-0.47 (-0.97, 0.029)^[b]	NA	-0.00047 (-0.33, 0.32)
Mid Hudson	NA	0.19 (-0.43, 0.81)	-0.51 (-0.84, -0.19)**[b]	NA	-0.28 (-0.60, 0.031)^
Lower Hudson	NA	-0.11 (-1.2, 0.96)	-0.71 (-1.0, -0.39)***[b]	NA	-0.15 (-0.45, 0.15)
Farm Pond	NA	2.3 (1.9, 2.7)***	NA	1.7 (1.2, 2.2)***	NA

[a] Model did not include TP as a covariate due to much smaller sample size, lower adjusted R^2 compared to model with length and location only, and lack of correlation with [THg].
[b] Estimates were based on model without TP, since TP data were not available at these locations.
[c] For sunfish, a model including length and TP without location yielded a better fit (adj. R^2=0.61).
^: 0.05<p<0.1
*: 0.01<p<0.05
**: 0.001<p<0.01
***: p<0.001

Table 4. Summary of bottom water chemistry and watershed parameters among 61 reservoirs in Oklahoma, and Spearman's correlation coefficients (ρ) between these parameters and modeled [THg] (μg/g) in a 14″ largemouth bass.

Parameters	N	Mean ± SD	ρ	P-value
True color (standard unit)	57	57 ± 55	0.58	<0.001
Conductivity (μS/m)	59	410 ± 470	-0.55	<0.001
pH	59	7.4 ± 0.56	-0.55	<0.001
Salinity (ppt)	59	0.23 ± 0.31	-0.54	<0.001
Alkalinity (mg/L)	58	87 ± 55	-0.54	<0.001
Redox Potential (mV)	59	340 ± 60	0.35	0.016
Apparent Color (standard unit)	54	150 ± 82	0.34	0.020
Chlorophyll-a (mg/m^3)	60	9.7 ± 5.7	-0.29	0.046
Chloride (mg/L)	59	44 ± 79	-0.28	0.055
Area (km^2)	55	28 ± 41	-0.26	0.073
Annual Rainfall (inch)	55	41 ± 7.0	0.26	0.082
Nitrogen, Total (mg/L)	57	0.81 ± 0.30	-0.23	0.13
Nitrogen, Kjeldahl (mg/L)	59	0.58 ± 0.20	-0.22	0.14
Resistivity (kΩ·cm)	59	950 ± 1300	0.22	0.14
Reservoir Age (year)	61	59 ± 20	-0.21	0.15
Trophic Index	59	53 ± 6.4	-0.21	0.17
Phosphorus, Total (mg/L)	60	0.082 ± 0.051	-0.19	0.20
Dissolved Oxygen (mg/L)	59	7.3 ± 0.99	-0.19	0.21
Perimeter (km)	55	110 ± 140	-0.19	0.21
Dissolved Oxygen Saturation (%)	59	70 ± 9.8	-0.18	0.23
Phosphorus, Ortho (mg/L)	57	0.044 ± 0.040	-0.17	0.26
Turbidity (NTU)	59	27 ± 27	0.16	0.27
Temperature (°C)	59	16 ± 2.4	-0.14	0.34
Nitrogen, Nitrite (mg/L)	57	0.054 ± 0.0080	0.11	0.44
Sulfate (mg/L)	59	45 ± 58	-0.11	0.45
Pheophytin (mg/m^3)	60	2.6 ± 1.9	-0.11	0.45
Secchi Depth (cm)	59	72 ± 48	-0.098	0.51
Nitrogen, Nitrate (mg/L)	57	0.17 ± 0.18	-0.031	0.83
N:P Ratio	59	24 ± 17	-0.018	0.90
Wetland Coverage (%)	55	0.97 ± 1.6	0.015	0.92
Summer Hypoxia (%)	59	41 ± 24	-0.014	0.93
Nitrogen, Ammonia (mg/L)	57	0.14 ± 0.12	0.0069	0.96

Table 5. Stepwise regression results on ln[THg] (μg/g) in a normalized 14″ largemouth bass among reservoirs in Oklahoma.

Variable	Estimate	95% CI	P value
pH	-1.1	(-1.5, -0.70)	<0.0001
Total Phosphorus (mg/L)	-8.4	(-14, -2.9)	0.0034
Annual Rainfall (inch)	-0.047	(-0.078, -0.016)	0.0042
Nitrogen, Nitrate (mg/L)	1.7	(0.45, 3.0)	0.0095
Apparent Color (Standard Unit)	0.0029	(0.00072, 0.0051)	0.011
Nitrogen, Ammonia (mg/L)	1.2	(-0.087, 2.5)	0.067
Secchi Depth (cm)	-0.0032	(-0.0067, 0.00034)	0.076

Figure 1

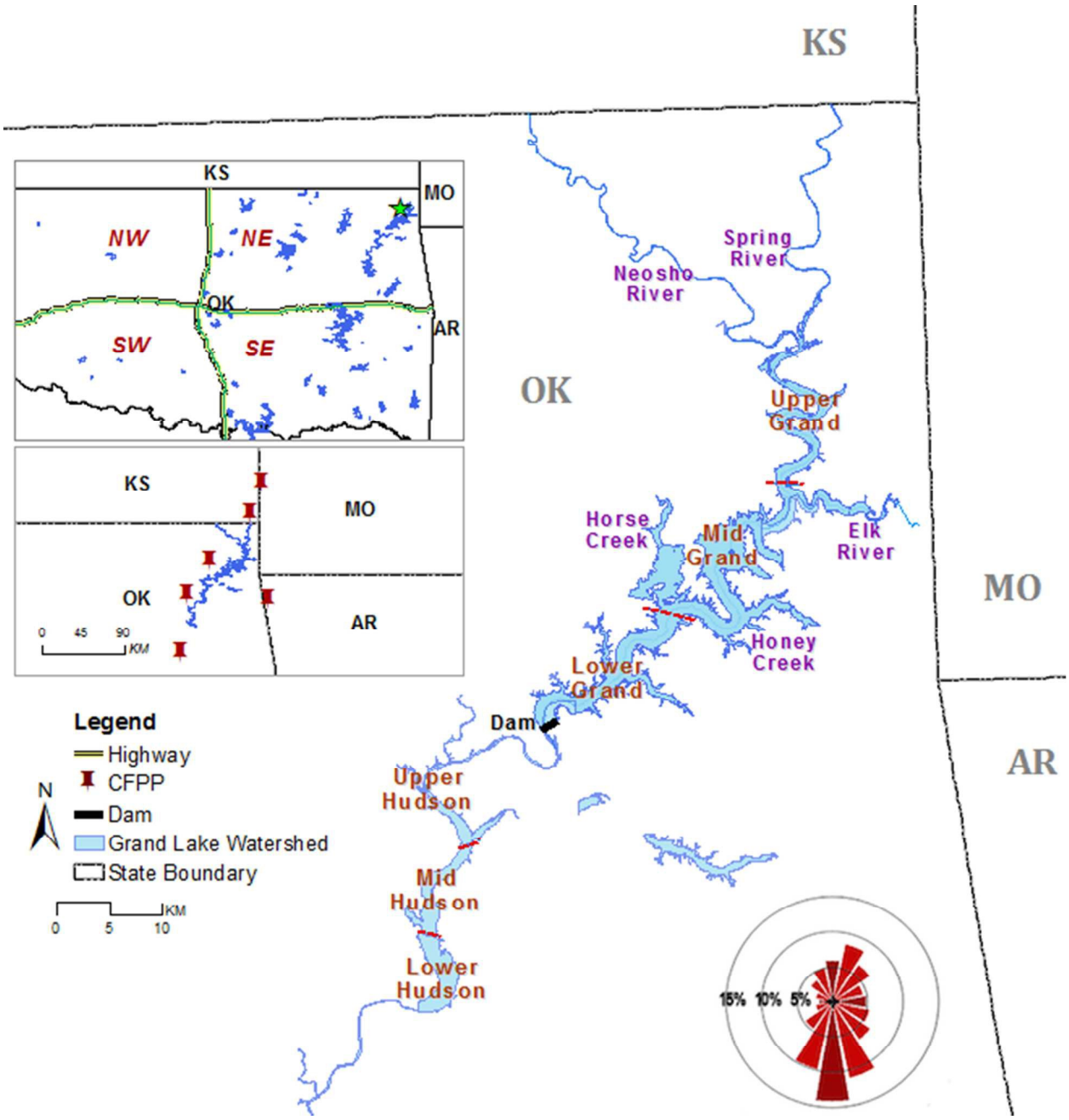

Figure 2

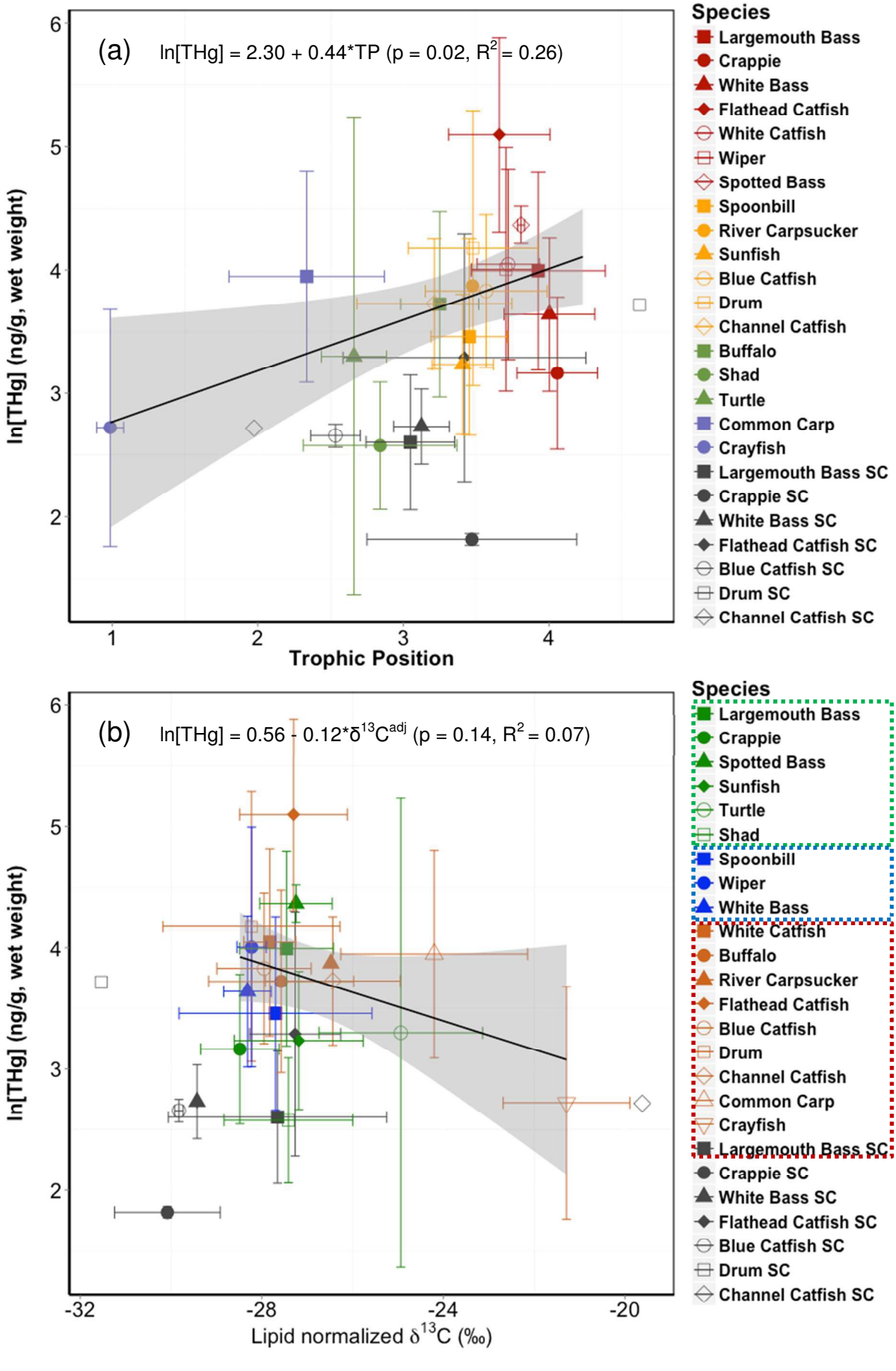

Figure 2 (Cont.)

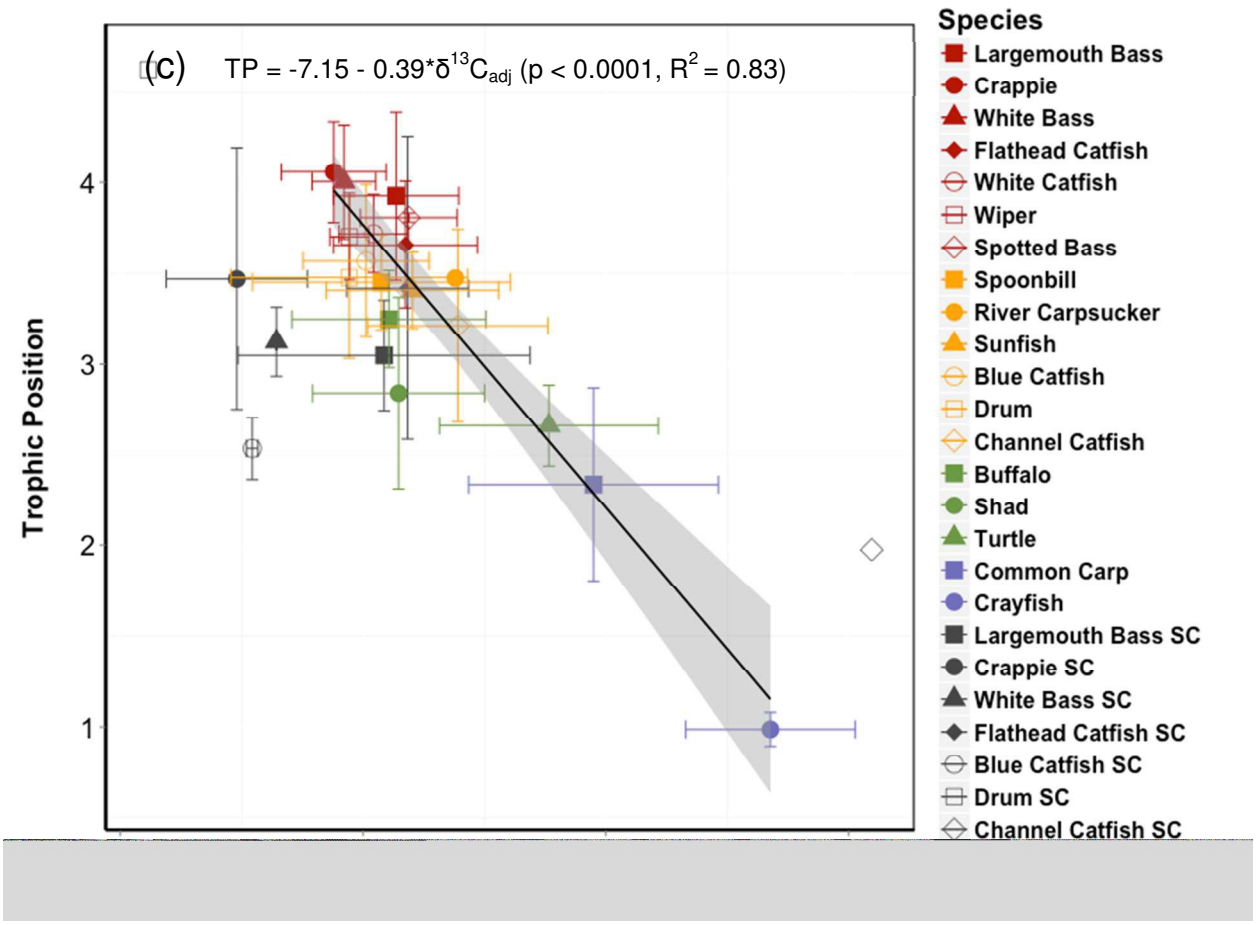

Figure 3

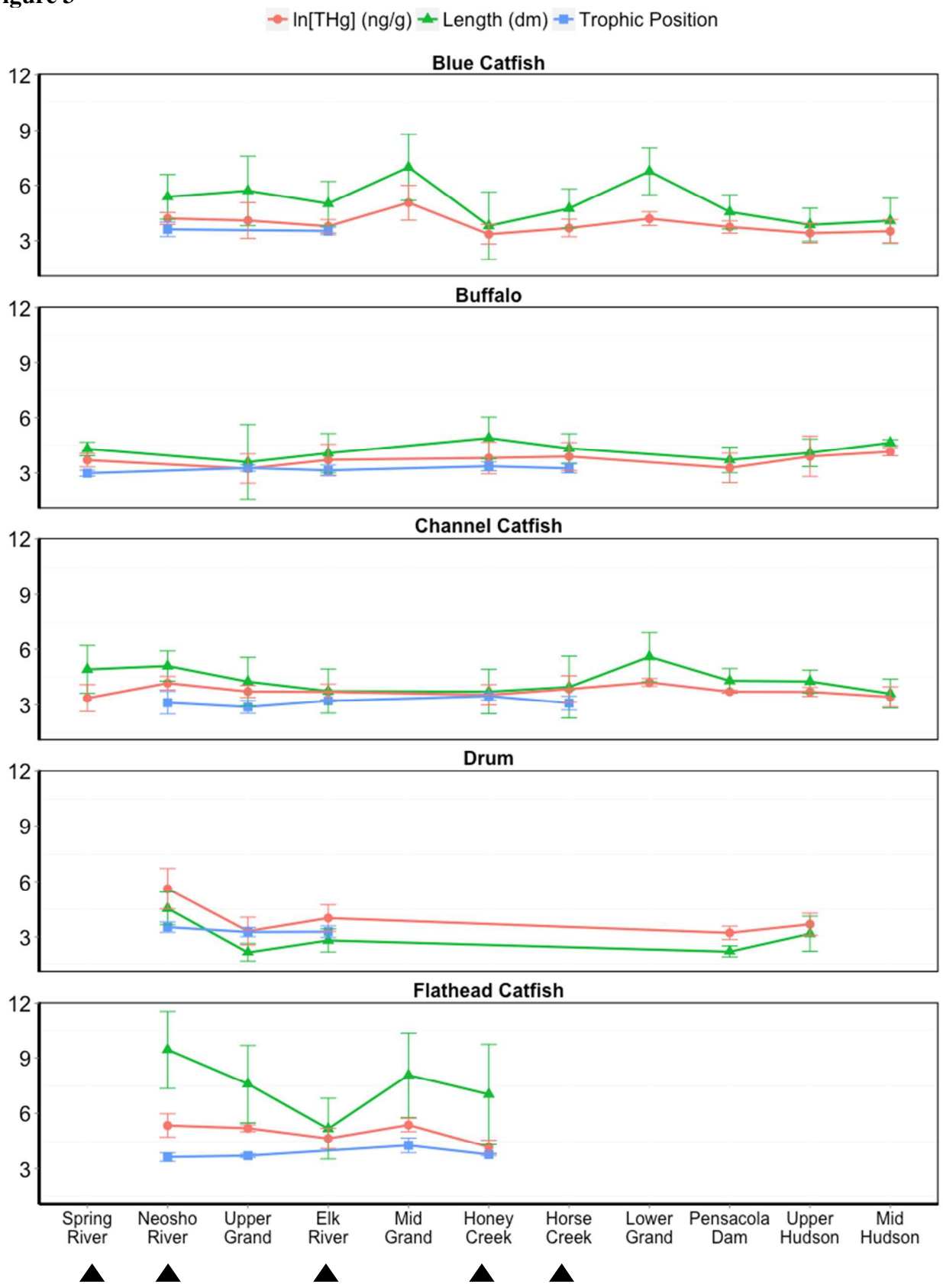

Figure 3 (Cont.)

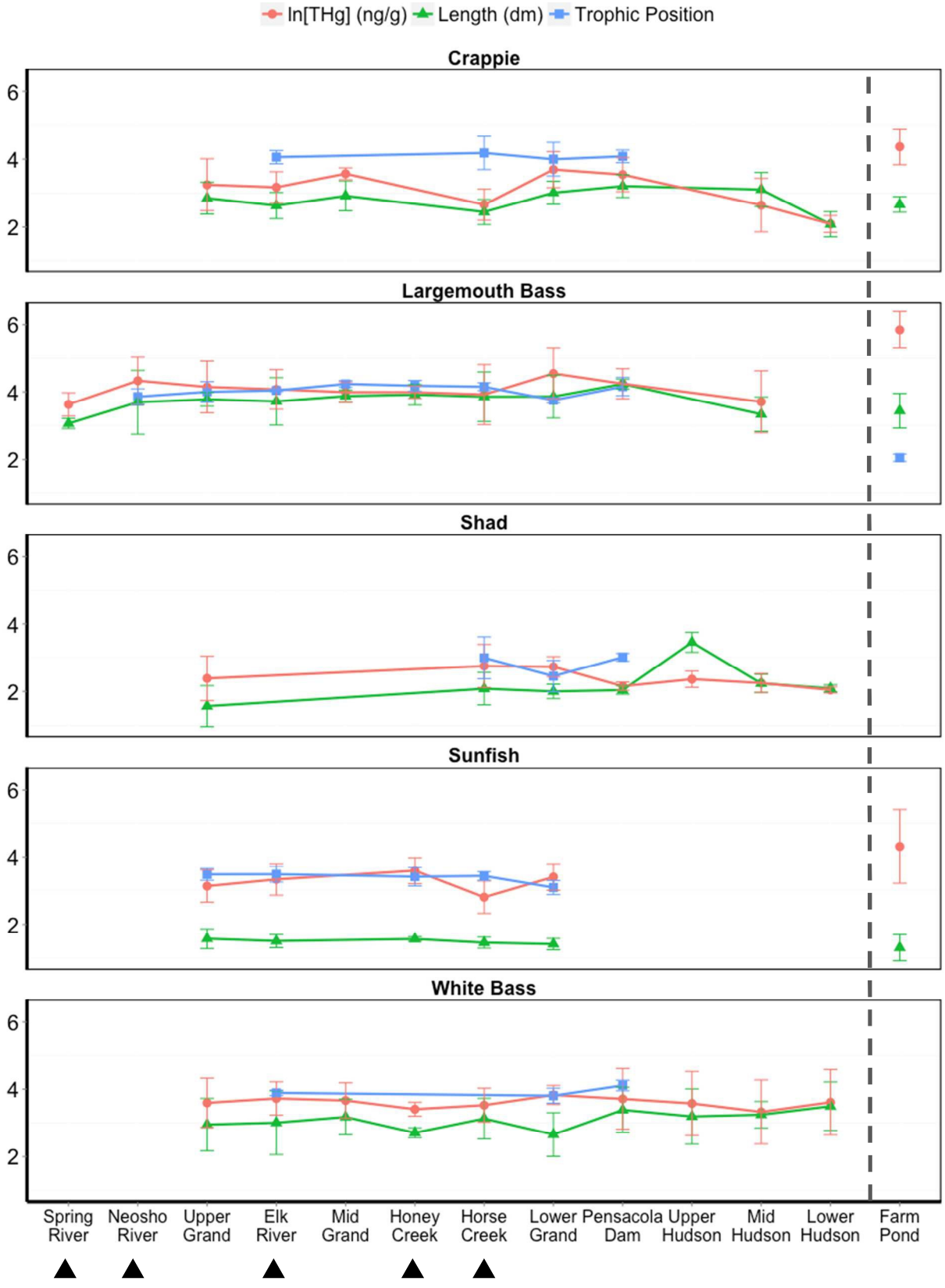

Figure 4