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FACTORS AFFECTING MERCURY CONTAMINATION IN FISH FROM CADDO LAKE, TEXAS

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FACTORS AFFECTING MERCURY CONTAMINATION IN FISH FROM CADDO LAKE, TEXAS

A DISSERTATION APPROVED FOR THE
DEPARTMENT OF ZOOLOGY

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ABSTRACT

The concentration of mercury in the biosphere has increased, primarily due to anthropogenic activities like the burning of coal, and now constitutes one of the most important environmental problems facing the planet. Mercury is toxic and has negative effects on the neurological, cardiovascular, and reproductive systems of both humans and wildlife, particularly during development. Humans and wildlife are exposed to mercury primarily by consuming mercury-contaminated fish. Although mercury is ubiquitous in fish tissue, there is a tremendous amount of variation in the concentration of mercury between fish, with some individuals having concentrations high enough that they could suffer negative health effects or pose a risk to organisms that consume them. Determining which factors regulate the level of mercury contamination in fish is critical to understanding which organisms and ecosystems are at risk.

Previous research on mercury contamination in fish focused on factors responsible for between-lake variation, and identified pH, dissolved organic carbon, and connections to wetlands as important predictors of mercury in fish. Within-lakes, fish also exhibit variability in mercury concentration but within-lake differences have received limited attention. Further, mercury contamination in fish from the southeastern United States has received less attention than in other regions, despite high levels of mercury emissions from coal-burning power plants and high rates of mercury deposition. In this dissertation I used Caddo Lake, a shallow reservoir on the border of northern Texas and Louisiana as a model system to study factors related to within-lake variation in the mercury concentrations of fish.

Chapter one is a survey of mercury contamination in largemouth bass (*Micropterus salmoides*) from Caddo Lake. We found that largemouth bass collected from forested wetland habitats had higher concentrations of mercury relative to largemouth bass collected from open water habitats. Habitat-specific differences in largemouth bass size, age, growth rate, trophic position (based on $\delta^{15}N$), and horizontal food web position (based on $\delta^{13}C$), characteristics known to influence mercury accumulation, did not explain the observed differences in mercury contamination. Rather, differences in mercury concentrations in a primary consumer, grass shrimp (*Palaemonetes kadiakensis*) across the two habitat types indicated that food webs in forested wetland habitats may be more contaminated with mercury. Spatial variation in mercury contamination within lakes and elevated mercury levels in forested wetlands should be of special concern, not only to researchers, but to public and environmental health officials dealing with mercury contamination in aquatic environments and human health risks associated with consumption of mercury-laden fish.

In Chapter two, we present the results of a survey of mercury contamination in ten species of fish from Caddo Lake. We also examined how size, age, and food web position (estimated using $\delta^{13}C$ and $\delta^{15}N$) were related to mercury concentrations in fish. Concentrations of mercury in the Caddo Lake fish assemblage were high enough to pose a threat to human and wildlife health. Similar to Chapter 1, we found that mercury concentrations in fish were elevated in forested wetland habitats relative to open water habitats most likely because there is more mercury entering the base of the food web in wetland habitats relative to open water habitats. Trophic position was the best predictor of mercury concentrations between species and the biomagnification coefficient (the

slope of the relationship between trophic position and mercury) was similar to that found in other studies. Age and size were the best predictors of mercury concentration within species. In contrast to previous studies, we did not find a relationship between horizontal food web position and mercury in Caddo Lake fish. This study indicates that there are similarities and differences in the processes governing mercury accumulation in fish that inhabit shallow lakes and reservoirs (common in subtropical regions) relative to large lakes (often found in cold temperate and arctic regions) where most previous studies were conducted.

Chapter three describes a survey of mercury contamination in three species of planktivorous fish, brook silverside (*Labidesthes sicculus*), threadfin shad (*Dorosoma petenense*) and gizzard shad (*Dorosoma cepedianum*), from Caddo Lake, Texas in which we identified species-specific differences in mercury contamination. We also examined trophic position (determined using $\delta^{15}N$), growth rate, and horizontal food web position (determined using $\delta^{13}C$) of planktivorous fish as factors that could have led to species-specific differences in mercury contamination. Mean trophic position differed by less than one trophic level between planktivorous fish species but this difference was likely responsible for species-specific differences in mercury concentration. We observed biomagnification between the species and the biomagnification coefficient (the slope of the relationship between trophic position and mercury) was similar to a previous study that examined the biomagnification in the Caddo Lake fish assemblage. Species-specific differences in growth rate and horizontal food web position could not explain differences in mercury concentrations between planktivorous fish. This implies that trophic position is one of the most important predictors of interspecific mercury concentration in fish,

even among species within a trophic guild that only exhibit fine scale differences in trophic position.

Chapters 1-3 demonstrated the utility of stable isotope analyses (SIA) for interpreting patterns of mercury contamination in fish. However, fish tissues have historically been prepared for mercury analysis and SIA using different techniques. United States Environmental Protection Agency protocols for analyzing total mercury concentration in fish tissue recommend using wet tissues, but SIA requires that tissues be dried. In Chapter four we compared total mercury concentration from wet and dry tissues, using 30 individual fish representing 11 freshwater and estuarine species. After correcting for water content, estimates of mercury concentrations from dry tissue were not significantly different from estimates of mercury concentrations from wet tissue. Variation in estimates of mercury concentration from wet and dry tissues was random and was not related to the level of contamination of the tissue. Our data indicate that dried fish tissues are suitable for estimating mercury concentration, and give results equivalent to those of wet tissues.

Chapter 1: Habitat-specific differences in mercury concentrations in a top predator from a shallow lake

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Abstract

We conducted a survey of mercury contamination in largemouth bass (*Micropterus salmoides*) from Caddo Lake, Texas, and found that largemouth bass collected from forested wetland habitats had higher concentrations of mercury relative to largemouth bass collected from open water habitats. Habitat-specific differences in largemouth bass size, age, growth rate, trophic position (based on $\delta^{15}N$), and horizontal food web position (based on $\delta^{13}C$), characteristics known to influence mercury accumulation, did not explain the observed differences in mercury contamination. Rather, differences in mercury concentrations in a primary consumer, grass shrimp (*Palaemonetes kadiakensis*) across the two habitat types indicated that food webs in forested wetland habitats may be more contaminated with mercury. Spatial variation in mercury contamination within lakes and elevated mercury levels in forested wetlands should be of special concern, not only to researchers, but to public and environmental health officials dealing with mercury contamination in aquatic environments and human health risks associated with consumption of mercury-laden fish.

Introduction

Since the industrial revolution (ca. 1850), mercury deposition rates have increased by a factor of three to four (Swain et al. 1992), with some regions experiencing 11 fold increases in mercury deposition (Schuster et al. 2002). The largest anthropogenic source of environmental mercury is emissions from coal-burning power plants (Pacyna and Pacyna 2002). Power plants release inorganic mercury into the atmosphere where it resides until being deposited onto the earth's surface (Morel et al. 1998; Pacyna and Pacyna 2002). In aquatic ecosystems, bacteria convert inorganic mercury to highly toxic methylmercury (Morel et al. 1998; Ullrich et al. 2001). Organisms at the base of the food web such as phytoplankton and periphyton absorb methylmercury directly from the water (Miles et al. 2001), while consumers, including fish, are primarily exposed to methylmercury through their diet (Hall et al. 1997; Tsui and Wang 2004). Methylmercury bioaccumulates in aquatic organisms (Wiener et al. 2003) and most of the mercury found in fish is in the form of methylmercury (Bloom 1992).

The primary source of methylmercury in humans is consumption of mercury-contaminated fish (NRC 2000) and methylmercury is detrimental to human health. Even low doses of methylmercury can damage the nervous and cardiovascular systems of humans (NRC 2000; Clarkson 2002). Fetuses are particularly sensitive to methylmercury consumed by pregnant women, and prenatal exposure to low levels of methylmercury can cause developmental and cognitive problems (NRC 2000; Clarkson 2002). To better understand these human health-related issues, we must first understand the ecological factors that regulate mercury accumulation in fish.

Fish that live in ecosystems with high methylmercury availability (i.e., high net methylmercury production or bioavailability) have elevated concentrations of mercury because more mercury is available for incorporation into the food web (Wiener et al. 2003). However, many biological characteristics of fish can also strongly influence mercury accumulation. For example, mercury concentrations in fish are positively correlated with fish size, age, and trophic position (i.e., the vertical trophic level at which fish feed) (Johnels et al. 1967; Cabana and Rasmussen 1994; McClain in press) and negatively correlated with fish growth rate (Rodgers 1996; Stafford and Haines 2001; Simoneau et al. 2005). Because pelagic food webs (i.e., those based on phytoplankton production) are more contaminated with mercury than littoral/benthic food webs (i.e., those based on periphyton production) (Lindqvist et al. 1991; Power et al. 2002; Gorski et al. 2003; Kidd et al. 2003) horizontal food web position (sensu Leibold et al. 1997) also influences mercury concentrations in fish.

Within lakes, mercury concentrations in fish can exhibit spatial variation (Munn and Short 1997; Cizdziel et al. 2002; Campbell et al. 2003a; Burger et al. 2004; Stafford et al. 2004; Simoneau et al. 2005), presumably due to heterogeneity in methylmercury availability (Munn and Short 1997; Cizdziel et al. 2002; Campbell et al. 2003a; Stafford et al. 2004). However most of these studies were not able to rule out habitat -specific differences in fish size, age, growth rate, trophic position, and horizontal food web position, factors that could confound conclusions of heterogeneity in methylmercury availability (but see Campbell et al. 2003a,b).

We conducted a survey of mercury contamination in largemouth bass (*Micropterus salmoides*) from Caddo Lake, a lake located on the border of Texas and

Louisiana. Largemouth bass collected from forested wetland habitats (sensu Cowardin et al. 1979) characterized by the presence of bald cypress (*Taxodium distichum*), shallow depths (approximately 1 m), and abundant aquatic vegetation had higher concentrations of mercury than largemouth bass collected from open water habitats. Here we present these results and our investigation of some of the mechanisms that may explain the observed patterns of habitat-specific mercury contamination in Caddo Lake largemouth bass**.** Habitat-specific differences in mercury contamination of largemouth bass would be expected if biological characteristics of largemouth bass that influence mercury accumulation or methylmercury availability differed between the two habitats. To distinguish between these alternatives we compared largemouth bass size, age, growth rate, trophic position (determined using $\delta^{15}N$), and horizontal food web position (determined using $\delta^{13}C$) between the two habitat types. We also examined mercury concentrations in grass shrimp (*Palaemonetes kadiakensis*) to determine if there were habitat-specific differences in the mercury concentrations of organisms near the base of the food web, indicative of differences in methylmercury availability (Lindqvist et al. 1991).

Methods

Study site

Caddo Lake, located on the border of northern Texas and Louisiana (Figure 1), is approximately 107 km^2 in surface area (Van Kley and Hine 1998), with average and maximum depths of 1.4 m and 8.2 m, respectively (Ensminger 1999). The western portion of the lake (approx. 40 km^2, and mostly in Texas) is composed primarily of a

forested wetland (hereafter wetland) dominated by bald cypress, water elm (*Planera aquatica*), and other aquatic vegetation including fanwort (*Cabomba caroliniana*), common waterweed (*Egeria densa*), and yellow pond-lily (*Nuphar luteum*) (Van Kley and Hine 1998). The eastern portion of Caddo Lake (mostly in Louisiana) is primarily open water habitat, though submerged vegetation can be extensive in summer months (M.M. Chumchal personal observation).

The primary anthropogenic sources of mercury in the region are coal-burning power plants (Crowe 1996; TDH 1999). Caddo Lake is located within 250 km of five of the 20 highest mercury-emitting power plants in North America (Miller and Van Atten 2004). A fish consumption advisory has been issued for largemouth bass in Caddo Lake by the Texas Department of State Health Services (DSHS) (DSHS 1995). The DSHS recommends that consumption of largemouth bass be limited to two meals per month (serving size = 227 and 113 g for adults and children, respectively). The Louisiana Department of Environmental Quality (DEQ) monitors largemouth bass on the Louisiana side of Caddo Lake but they have not issued an advisory (DEQ 2005).

Fish and invertebrate collection

We collected largemouth bass, with assistance from biologists from the Texas Parks and Wildlife Department (TPWD), during the early evening of 10 May 2004 and the morning of 12 May 2004 using a boat-mounted electrofishing unit. Largemouth bass were collected from five sites in the wetland habitat (n = 44) and from four sites in the open water habitat (n = 47) (Figure 1). After collection, fish were placed on ice and

transported to a lab where total length (TL) was measured and otoliths were dissected. Fish were then frozen for subsequent mercury and stable isotope analyses.

To provide insight into mercury concentrations near the base of the food web, we collected grass shrimp, a common macroinvertebrate in Caddo Lake. Differences in the mercury concentrations of short–lived consumers would imply that there may be differences in methylmercury availability between habitats (Lindqvist et al. 1991). Grass shrimp were collected with a dip net from three sites in the wetland habitat (n = 26) and three sites in the open water habitat (n = 29) on 10 May 2006. Grass shrimp were placed on ice and transported to a lab where they were identified to species and measured (TL) under a dissecting microscope. Grass shrimp were then frozen for subsequent mercury analyses.

Mercury analysis

Largemouth bass and grass shrimp were processed separately for mercury concentration. Fillets of largemouth bass epaxial muscle were dissected from each fish and a small subsample of skinless tissue was collected from the center of each fillet using a scalpel and forceps and weighed to the nearest 0.1 mg. Whole grass shrimp were dried at 60°C for 48 hours, homogenized with a ball-mill grinder, and weighed to the nearest 0.1 mg. All lab-ware was rinsed with 50% HNO_3 solution (largemouth bass) or 95% ethanol (grass shrimp) and deionized water between samples.

Total mercury concentrations in fish and shrimp tissue were analyzed with a direct mercury analyzer (DMA-80, Milestone Inc. Monroe, CT) that uses thermal decomposition, gold amalgamation, and atomic absorption spectrometry (USEPA 1998)

and are reported as ng total mercury/$g_{wet\ weight}$ of fish or ng total mercury/$g_{dry\ weight}$ of shrimp tissue. We used total mercury as a proxy for methylmercury, the predominant form of mercury in fish and grass shrimp (Bloom 1992; Cleckner et al. 1998).

For largemouth bass mercury analyses, a calibration curve was generated using three reference materials from the National Research Council of Canada Institute for National Measurement Standards: MESS-3 (marine sediment, certified value = 91 ± 9 ng/$g_{dry\ weight}$ total mercury (average ± 95% C.I.)), PACS-2 (marine sediment, certified value = 3040 ± 200 ng/$g_{dry\ weight}$ total mercury) and DORM-2 (dogfish muscle, certified value = 4,640 ± 260 ng/$g_{dry\ weight}$ total mercury). Quality assurance included reference and duplicate samples. During largemouth bass mercury analyses, reference samples of MESS-3 or DORM-2 were analyzed approximately every 10 samples and the mean percent recovery was 100 ± 1% (range = 92–107%; n = 41) and 100 ± 2% (range = 95–104%; n = 11), respectively. Duplicate samples were analyzed approximately every 20 samples and the mean relative percent difference was 3.6 ± 1.3% (range = 0.3–11.4%; n = 28). During grass shrimp mercury analyses, reference samples of MESS-3 were analyzed approximately every 10 samples and the mean percent recovery was 98.8 ± 0.6% (range = 98– 100%; n = 6). Duplicate samples were analyzed approximately every 20 samples and the mean relative percent difference was 0.2 ± 0.3% (range = 0.0 –0.4%; n = 3). In order to compare grass shrimp and largemouth bass mercury concentrations, we converted dry weight-based values for grass shrimp to wet weight equivalents assuming a dry weight:wet weight ratio of 0.2 (Vernberg and Piyatiratitivorakul 1998).

Largemouth bass age and growth rates

We examined otolith annuli to estimate the age of a subset of largemouth bass (33 from wetland and 32 from open water habitats). Otoliths were broken perpendicular to the longest axis through the nucleus and polished using 400 and 600-grit sandpaper (Buckmeier and Howells 2003). Annuli were counted at 8-40× magnification under a dissecting microscope with a fiber-optic light source. Two readers independently estimated the ages of fish without knowledge of fish length, and disagreements were resolved by reexamining otoliths and mutually agreeing on age. Growth rates were determined as TL divided by age.

Largemouth bass trophic position and diet

Stable nitrogen and carbon isotope ratios in largemouth bass and primary consumers (unionid clams and gastropods) were used to examine differences in largemouth bass trophic positions and horizontal food web position across the two habitat types. Stable nitrogen isotopes are used differentially in cellular processes (Fry 2006) resulting in a predictable increase in the heavy isotope, ^{15}N, relative to ^{14}N with each increase in vertical trophic level (Minagawa and Wada 1984). Horizontal food web position can be determined using stable carbon isotopes (^{13}C and ^{12}C) because benthic and pelagic primary producers have distinct carbon isotope signatures (Hecky and Hesslein 1995).

Largemouth bass fillet subsamples and foot muscle from gastropods and unionid clams were dried in a 60°C oven and homogenized using a ball mill grinder (Dentsply, Inc, York, PA). Sixty-one largemouth bass (31 from wetland and 30 from open water habitats) were analyzed at Louisiana State University (LSU) for isotopic composition

using a Thermoquest Finnigan Delta Plus isotope ratio mass spectrometer (IRMS). The remaining largemouth bass (13 from wetland and 17 from open water habitats) and primary consumers were analyzed at the University of California-Davis (UC-Davis) stable isotope facility using a Europa Hydra 20/20 continuous flow IRMS. Tank nitrogen and carbon dioxide gases calibrated with known standards were used as working reference materials in daily laboratory operation. Carbon and nitrogen isotope results are given as:

$$\delta^{13}C \text{ or } \delta^{15}N = (R_{sample}/R_{standard} - 1) \times 1000 \quad (1)$$

where R is $\delta^{13}C/\ \delta^{12}C$ for $\delta^{13}C$ and $^{15}N/\ ^{14}N$ for $\delta^{15}N$. Standards for $\delta^{13}C$ and $\delta^{15}N$ are Vienna Pee Dee Belemnite (VPDB) and air N_2, respectively. Analysis of replicate samples of dried bovine liver (National Institute of Standards and Technology) indicated good agreement between the results from each lab (mean $\delta^{15}N$ and $\delta^{13}C$ differed by 0.5‰ and 0.2‰, respectively).

To calculate trophic position, $\delta^{15}N$ values in largemouth bass were first corrected for habitat-specific differences in basal $\delta^{15}N$ using $\delta^{15}N$ and $\delta^{13}C$ of primary consumers according to the method of Vander Zanden and Rasmussen (1999). Primary consumers that utilize littoral sources of carbon are less enriched in ^{15}N than organisms that utilize pelagic sources of carbon (Vander Zanden and Rasmussen 1999) so we collected gastropods and unionid mussels as representatives of littoral and pelagic primary consumers, respectively (Post et al. 2000). We assumed that largemouth bass were resident in the habitat in which they were collected. Thus, ^{15}N values of largemouth bass

collected from wetland and open water habitats were corrected for differences in ^{15}N at the base of the food web using gastropods and unionid mussels collected from the corresponding habitat. Gastropod $\delta^{15}N$ and $\delta^{13}C$ was 4.32 ± 0.69‰ and -29.4 ± 1.16‰, respectively for the wetland habitat (*n*= 5) and 2.23‰ and -25.8‰, respectively for the open water habitat (*n* = 1). Unionid mussel $\delta^{15}N$ and $\delta^{13}C$ was 6.50 ± 0.66 ‰ and -33.5 ± 0.13‰, respectively for wetland habitat (*n* = 6) and 4.85 ± 0.22 ‰ and -32.4 ± 0.53‰, respectively for open water habitat (*n* = 5). We used the corrected $\delta^{15}N$ ($\delta^{15}N_{corrected}$) values of largemouth bass to calculate trophic position (TP_{bass}) as:

$$TP_{bass} = \delta^{15}N_{corrected}/3.4 + 2 \quad (2)$$

We corrected $\delta^{13}C$ of each largemouth bass ($\delta^{13}C_{bass}$) for trophic enrichment according to method of Fry (2006) as:

$$\delta^{13}C_{corrected} = \delta^{13}C_{bass} - 0.5 \text{ x } (TP_{bass} - 1) \quad (3)$$

Corrected $\delta^{13}C$ values of largemouth bass were compared to $\delta^{13}C$ of gastropods and unionid mussels to determine if largemouth bass were feeding predominately in either pelagic or littoral food webs.

Statistical analyses

Because age and TL are correlated with mercury concentrations in fish (Wiener et al. 2003), we tested for habitat-specific differences in largemouth bass mercury

concentrations after controlling for the effects of age and TL. Specifically, we tested for within-group effects (two levels of habitat) on largemouth bass mercury concentrations, after removing the effect of a covariate (age or TL). Some variables were transformed to maximize correlation coefficients and increase linearity. If the slopes of the relationships between the covariate and dependent variable were homogeneous between habitats (i.e., habitat x covariate = $P > 0.05$), we removed the interaction term from the model and tested for main effects of habitat and the covariate using analysis of covariance (ANCOVA) (SPSS Inc., version 11.5.0, Chicago, IL). If the slopes of the covariate were not homogenous between habitats we tested for an effect of habitat using the Wilcox procedure (Quinn and Keough 2002) that determines the range of the covariate for which the within-group means are significantly different (WILCOX, version 3.2, Constable 1989).

We also tested for habitat-specific differences in growth rate, trophic position, and $\delta^{13}C$ (i.e., horizontal food web position) as additional factors that can affect mercury concentrations in fish. Specifically we tested for within-group effects (two levels of habitat) on dependent variables (trophic position, $\delta^{13}C$, or TL) after removing the effect of a covariate (age or TL) as described above for mercury analysis.

We tested for habitat-specific differences in mercury concentrations of grass shrimp with a one-way analysis of variance (ANOVA). Statistical significance was determined at $P \leq 0.05$.

Results

Largemouth bass collected from wetland habitats had 2.4 times higher mercury concentrations than largemouth bass collected from open water habitats (Table 1). Largemouth bass collected from wetland habitats were of similar size but 1.5 times older than the largemouth bass collected from open water habitats (Table 1). After controlling for the effects of TL and age, largemouth bass collected from wetland habitats still had significantly higher concentrations of mercury than largemouth bass collected from open water habitats (Table 2; Figure 2).

Largemouth bass collected from wetland habitats had mean growth rates that were 1.4 times slower than largemouth bass from open water habitats (Table 1). Largemouth bass collected from wetland habitats were significantly smaller than similar aged largemouth bass from open water habitats (Table 2; Figure 3).

Largemouth bass collected from wetland habitats had a slightly lower mean trophic position than largemouth bass collected from open water habitats (Table 1). The observed habitat specific difference in trophic position was significant after controlling for the effect of TL (Table 2; Figure 4A).

Mean horizontal food web position ($\delta^{13}C$) in largemouth bass was similar between habitats (Table 1) but was dependent on largemouth bass TL (Table 1; Figure 4B). Small largemouth bass (< 251 mm) collected from wetland habitats had significantly higher $\delta^{13}C$ values than largemouth bass from open water habitats. Comparison of largemouth bass $\delta^{13}C$ values with $\delta^{13}C$ values of primary consumers indicated that small largemouth bass from wetlands were feeding in food webs based on littoral primary production (i.e., $\delta^{13}C$ values in largemouth bass were similar to those in gastropods), whereas small largemouth bass in open water habitats were feeding in food

webs based on pelagic primary production (i.e., $\delta^{13}C$ values in largemouth bass were similar to those in unionids). The $\delta^{13}C$ values of medium-sized largemouth bass (251-386 mm) were not significantly different between habitats and were intermediate between the $\delta^{13}C$ values of unionid mussels and gastropods. Large largemouth bass (> 386 mm) collected from wetland habitats had significantly lower $\delta^{13}C$ values than largemouth collected from open water habitats after controlling for the effects of TL. These data indicate that large largemouth bass (> 386 mm) collected from wetland habitats were more dependent on pelagic food webs than similar-sized largemouth bass collected from open water habitats, however $\delta^{13}C$ values of largemouth bass from both habitats were intermediate between the $\delta^{13}C$ values of unionid mussels and gastropods.

Grass shrimp from the wetland and open water habitats had a TL of 29.0 ± 0.9 and 31.2 ± 1.5 mm, respectively. Grass shrimp from wetland habitats had significantly higher concentrations of mercury than grass shrimp from open water habitats (P = 0.004). Grass shrimp collected from wetland habitats had a mean mercury concentration of 69.5 ± 6.2 $ng/g_{\text{dry weight corrected}}$ while grass shrimp collected from open water habitats had a mercury concentration of 57.4 ± 5.0 $ng/g_{\text{dry weight corrected}}$.

Discussion

In this study we made the novel observation that largemouth bass collected from wetland habitats were more than twice as contaminated with mercury than largemouth bass collected from open water habitats. Spatial and habitat variability in the mercury concentrations of fish have been observed in lakes and reservoirs located in temperate and tropical regions (Munn and Short 1997; Cizdziel et al. 2002; Campbell et al. 2003a;

Burger et al. 2004; Stafford et al. 2004; Simoneau et al. 2005). However, within lakes, fish of a given species, age, and size are generally assumed to have relatively homogeneous mercury concentrations. The diversity of systems in which this phenomenon has been reported and the large habitat-specific difference in mercury concentration observed in this study, implies that spatial variation in mercury contaminations is ubiquitous and may confound efforts to assess bioaccumulation in fish. Thus spatial variation in mercury concentrations of fish should be of concern to basic researchers and public and environmental health officials responsible for monitoring contaminant levels in fish.

In this study we were also interested in determining potential causes of habitat-specific differences in mercury contamination of largemouth bass. Although largemouth bass biological characteristics differed between the two habitats, our data suggest that the primary factor responsible for habitat-specific differences in largemouth bass mercury levels was a more contaminated food web in the wetland habitat.

Despite differences between habitats, largemouth bass growth rates do not appear to explain the pattern of mercury contamination observed in this study. Consistent with the hypothesis that fish with slow growth rates have elevated concentrations of mercury (Rodgers 1996; Stafford and Haines 2001; Simoneau et al. 2005), largemouth bass collected from wetland habitats had slower growth rates and higher concentrations of mercury than largemouth bass collected from open water habitats. However, scrutiny of our data reveals that growth rate is not a sufficient explanation for habitat-specific differences in mercury concentrations. Using a series of models, Rodgers (1996) examined the effects of changes in dietary mercury levels and growth rate on mercury

concentrations in fish tissue. Populations of fish with different levels of mercury in their diets exhibited size- and age-specific concentrations of mercury that were distinct from populations of fish with different growth rates. Fish with high levels of mercury in their diets had higher size- and age-specific concentrations of mercury in their tissues than fish with low levels of mercury in their diet. Fish with slow growth rates had higher size-specific concentrations of mercury in their tissues but *lower* age-specific concentrations of mercury in their tissues relative to fish with fast growth rates. In this study, fish from wetland habitats had higher length-specific and age-specific concentrations of mercury in their tissues relative to fish from open water habitats. Therefore, differences in growth rate can not explain the habitat-specific differences in mercury concentration we observed. Rather, TL- and age-specific patterns in mercury contamination of largemouth bass indicate that the mercury concentration of largemouth bass diets differed between habitats.

Relative to largemouth bass collected from open water habitats, largemouth bass collected from wetland habitats would have had diets that were elevated in mercury if they were feeding at a higher trophic position, relying more heavily on pelagic food webs, or if the entire wetland food web was more contaminated with mercury. We were able to distinguish between these alternative hypotheses by comparing the trophic position and horizontal food web position of largemouth bass, and mercury concentrations of grass shrimp collected from the two habitat types.

Variation in mercury concentration was not caused by habitat-specific differences in largemouth bass trophic position. Because mercury is a biomagnifying contaminant (Cabana and Rasmussen 1994), it would be predicted that largemouth bass

with higher trophic positions would also have higher mercury concentrations. However, even though trophic positions were lower, the mercury concentrations of fish from wetland habitats were significantly higher than fish from open water habitats. Thus, we conclude that some other factor overrode the effect of trophic position on mercury concentration in largemouth bass.

Variation in mercury concentration was not caused by habitat-specific differences in horizontal food web position. Fish feeding in food webs based predominantly on pelagic primary production have elevated concentrations of mercury (Lindqvist et al. 1991; Power et al. 2002; Gorski et al. 2003; Kidd et al. 2003). Despite being dependent on food webs based on pelagic primary production, small largemouth bass collected from open water habitats had lower concentrations of mercury relative to similar-sized individuals collected from wetland habitats. For small largemouth bass, the patterns exhibited by carbon isotopes were opposite of what would be expected if differences in reliance on pelagic primary production were driving habitat-specific differences in mercury concentration (Power et al. 2002). It is worth noting, that the hypothesized inverse relationship between $\delta^{13}C$ values and mercury in fish has only been reported in deep lakes with extensive pelagic zones (Power et al. 2002; Gorski et al. 2003; Kidd et al. 2003). However in shallow ecosystems, littoral zones are important sites of mercury methlyation (Cleckner et al. 1999). Therefore the relationship between $\delta^{13}C$ values and mercury in fish may be ecosystem specific. Regardless of the relationship between $\delta^{13}C$ values and mercury in fish, the pattern of $\delta^{13}C$ values exhibited by medium and large largemouth bass indicate that some other factor was responsible for habitat-specific differences in mercury concentration. Medium and large

largemouth bass exhibited both the greatest difference in mercury concentration and the most similar $\delta^{13}C$ values. Thus, differences in reliance on pelagic primary production between the two habitats were not likely responsible for the observed habitat-specific differences in mercury concentration in largemouth bass.

We suggest that the observed pattern in largemouth bass mercury concentrations was due to elevated mercury concentrations in the wetland food web. Largemouth bass stable isotopes analyses revealed that largemouth bass diets do not differ in ways that can explain the observed pattern in mercury accumulation. Thus the food web in wetland habitats may be inherently more contaminated with mercury than the food web in open water habitats. The higher concentrations of mercury in grass shrimp collected from wetland habitats provide additional evidence that mercury concentrations at the base of the food web differed between habitats and implies that there were differences in methylmercury availability between habitats (Lindqvist et al. 1991).

All wetland types, including forested wetlands, have features that may make them conducive to mercury methylation (Zilloux 1994; Ullrich et al. 2001; Wiener et al. 2003). Relative to open water habitats, wetland habitats in Caddo Lake have low pH (Hartung 1983; Darville et al. 1998), low dissolved oxygen concentrations (Hartung 1983; Darville et al. 1998), and high organic carbon concentrations in sediments (Wilson 2003). In contrast to open water habitats, wetland habitats in Caddo Lake have direct connections to seasonally flooded areas (Van Kley and Hine 1998) and sulfate concentrations that are optimal for mercury methylation (i.e., 0.2-0.5 mM SO_4^{2-}) (Hartung 1983).

In general, wetlands are sources of methylmercury to lakes and rivers (St. Louis et al. 1994; Hurley et al. 1995; Lee et al. 1998; Paller et al. 2004; Warner et al. 2005). However, the impact of methylmercury production in wetlands on mercury contamination in aquatic organisms has not been well studied (Wiener et al. 2003). Fish from lakes and reservoirs with wetlands in their watershed have elevated concentrations of mercury (Greenfield et al. 2001; Warner et al. 2005) and rivers that drain wetlands contain invertebrates with elevated concentrations of mercury (Paller et al. 2004). Based on our results we hypothesize that biota in lakes or reservoirs with connections to wetlands will exhibit spatial variation in mercury contamination, with organisms living in or near wetlands exhibiting elevated levels of mercury contamination.

Forested wetlands may be more at risk for containing organisms with elevated mercury concentrations than has been appreciated. There are more than 210,000 km^2 of forested wetlands in the conterminous United States and forested wetland habitats are becoming more common due to succession of other wetlands types and ecosystem restoration projects (Dahl 2006). Because mercury contaminated fish have negative health effects on humans and wildlife, forested wetlands should be of special concern to public and environmental health officials, especially in the southern United States where these habitats are extensive (Conner and Buford 1998) and atmospheric deposition of mercury is elevated (NADP 2005).

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Table 1. Mean characteristics (± CI) of largemouth bass collected from forested wetland and open water habitats in Caddo Lake.

	Forested wetland habitat	Open water habitat
Mercury ($ng/g_{wet\ weight}$)	465 ± 113	193 ± 54
Total length (mm)	287 ± 37	260 ± 33
Age (years)	3.9 ± 0.7	2.6 ± 0.5
Growth rate (mm/year)	101 ± 11	138 ± 13
Trophic position	3.9 ± 0.1	4.2 ± 0.1
$\delta^{13}C$ (‰)	-29.5 ± 0.4	-30.0 ± 0.3

Table 2. Significance values associated with analysis of covariance (ANCOVA) and Wilcox procedure.

Dependent variable[a]	Covariate[a]	Covariate x habitat *P*	ANCOVA		Wilcox significance range
			Habitat *P*	Covariate *P*	
Log mercury	TL[b]	0.1	<0.001	<0.001	-
Mercury	Age	0.1	<0.001	<0.001	-
Trophic position	TL[b]	0.6	<0.001	<0.001	-
$\delta^{13}C$	Ln TL[b]	<0.001	-	-	< 251 mm, > 386 mm
TL	Ln age	0.6	<0.001	<0.001	-

[a] Dependent variables and covariates are characteristics of largemouth bass

[b] TL, total length

Figure captions

Figure 1. Map of Caddo Lake. Caddo lake is located on the border of Texas and Louisiana. The western portion of the lake is a 40 km^2 forested wetland with high densities of emergent and submerged aquatic vegetation. The eastern portion of the lake is primarily open water habitat. We collected largemouth bass (circles) and grass shrimp (triangles) from both forested wetland (black symbols) and open water habitats (open symbols).

Figure 2. Relationship between (A) largemouth bass total length and (B) largemouth bass age and total mercury concentration in largemouth bass epaxial muscle ($ng/g_{wet\ weight}$) from forested wetland and open water habitats.

Figure 3. Relationship between largemouth bass total length and largemouth bass age from forested wetland and open water habitats.

Figure 4. Relationship between (A) largemouth bass trophic position and (B) largemouth bass $\delta^{13}C$ and largemouth bass total length from forested wetland and open water habitats.

Figure 1.

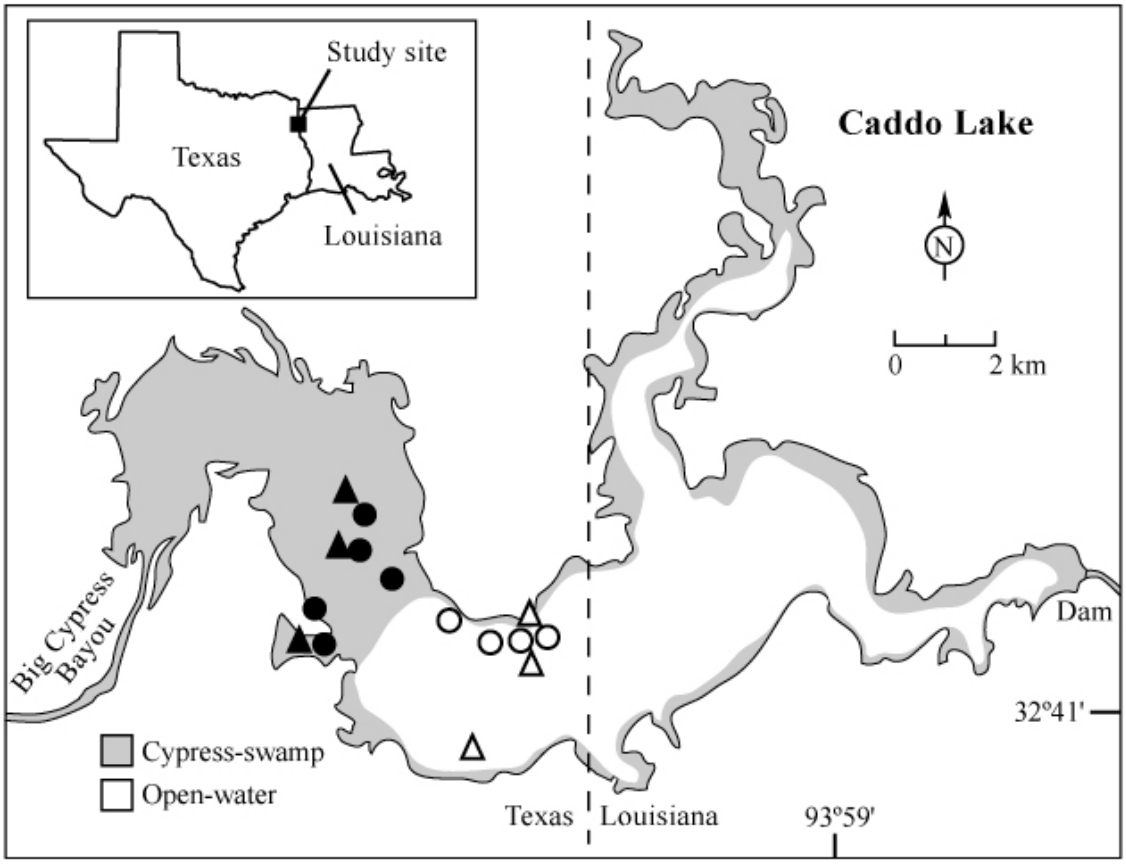

Study site
Texas
Louisiana
Caddo Lake
N
0
2 km
Big Cypress Bayou
Dam
32°41'
Cypress-swamp
Open-water
Texas
Louisiana
93°59'

Figure 2.

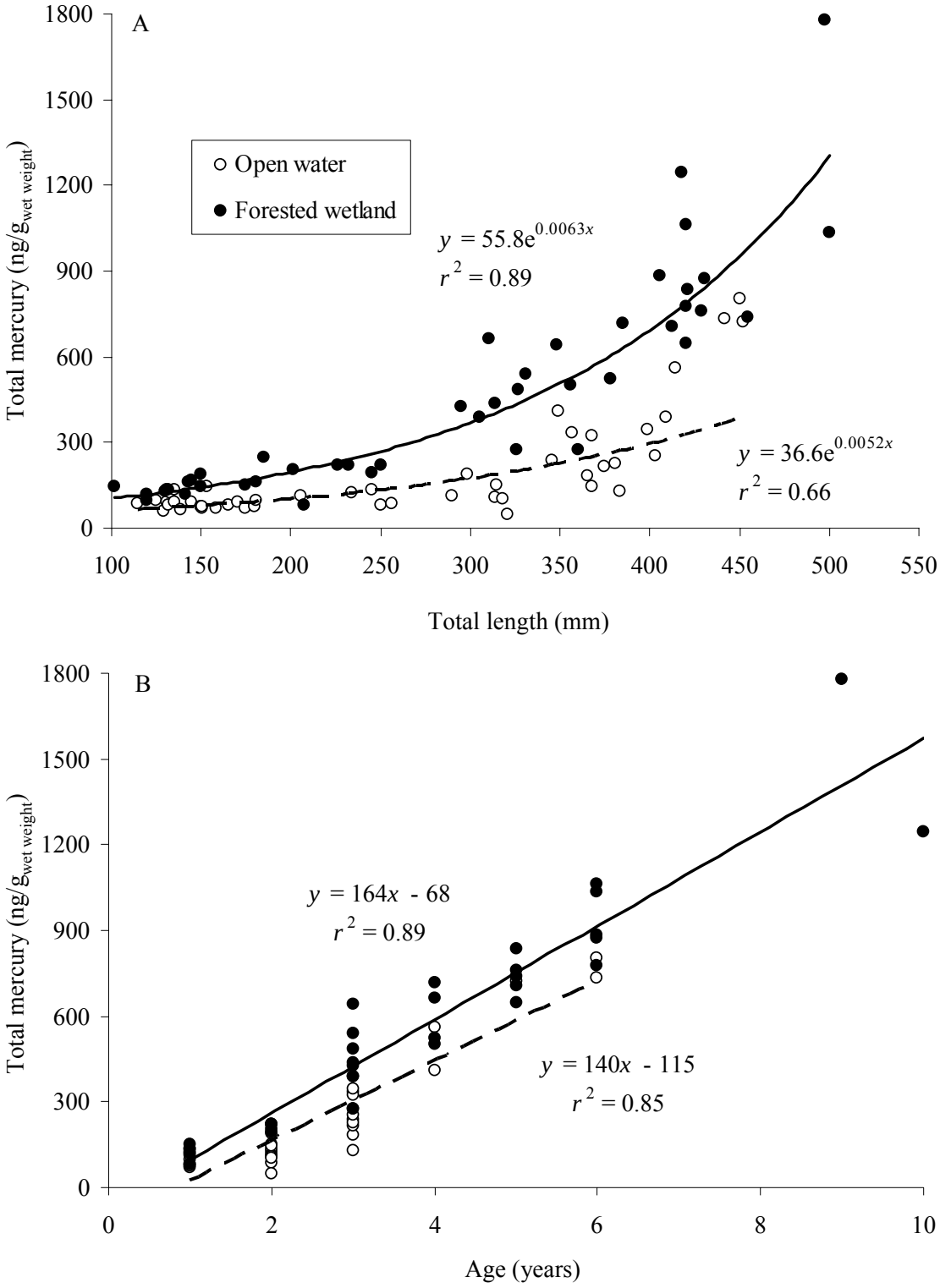

A
Open water
Forested wetland
$y = 55.8e^{0.0063x}$
$r^2 = 0.89$
$y = 36.6e^{0.0052x}$
$r^2 = 0.66$
Total mercury (ng/g$_{wet\ weight}$)
Total length (mm)
B
$y = 164x - 68$
$r^2 = 0.89$
$y = 140x - 115$
$r^2 = 0.85$
Total mercury (ng/g$_{wet\ weight}$)
Age (years)

Figure 3.

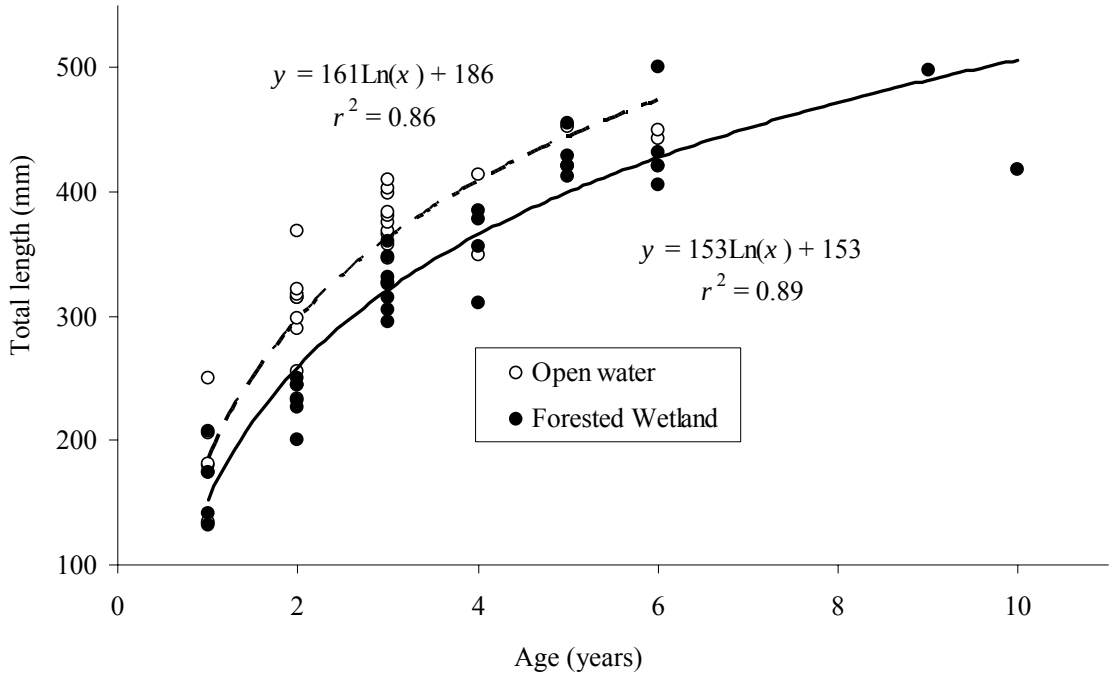
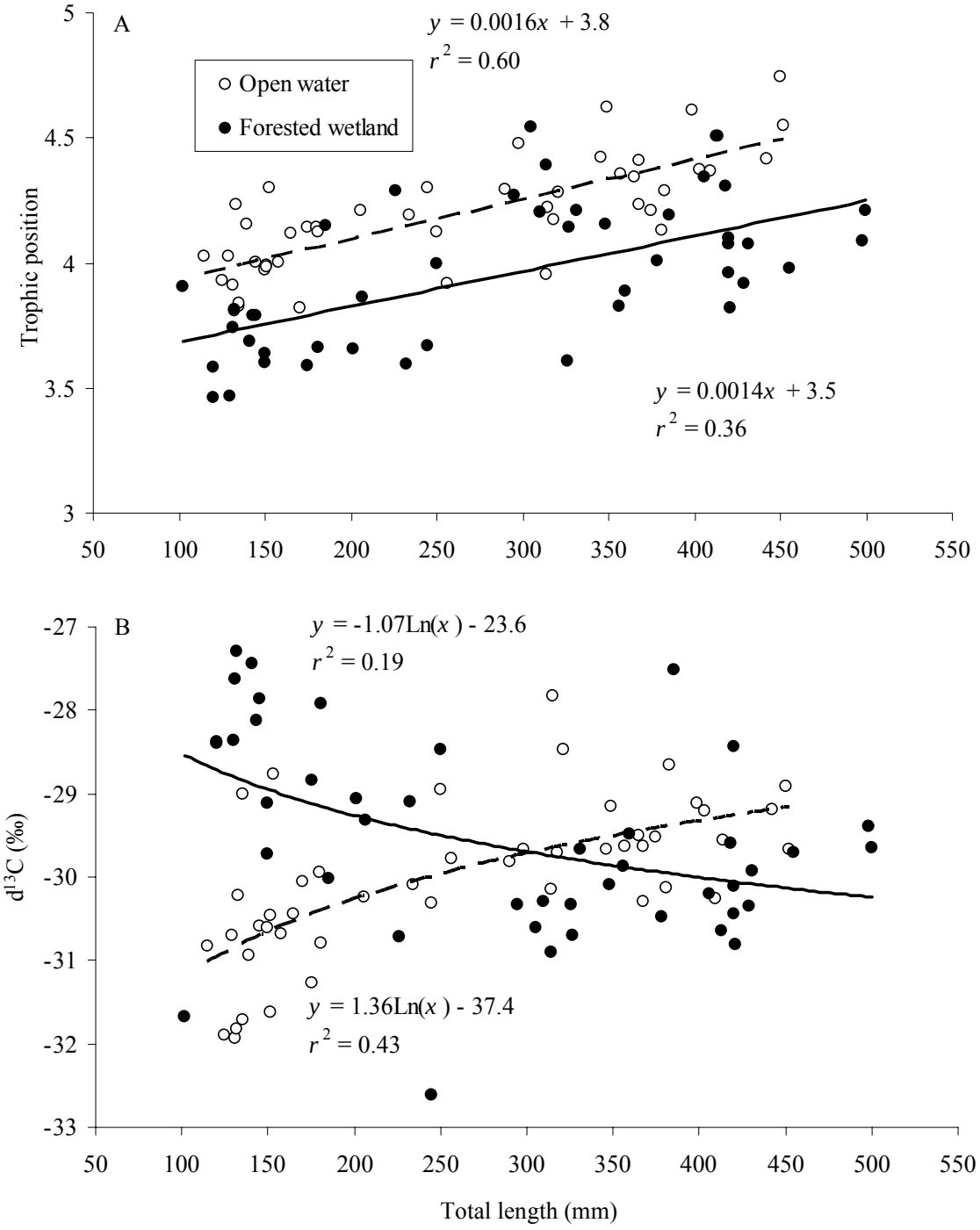

y = 161Ln(x) + 186
r 2 = 0.86
y = 153Ln(x) + 153
r 2 = 0.89
Open water
Forested Wetland
Total length (mm)
Age (years)
100
200
300
400
500
0
2
4
6
8
10

Figure 4.


A
y = 0.0016x + 3.8
r 2 = 0.60
Open water
Forested wetland
y = 0.0014x + 3.5
r 2 = 0.36
Trophic position
5
4.5
4
3.5
3
50
100
150
200
250
300
350
400
450
500
550
B
y = -1.07Ln(x) - 23.6
r 2 = 0.19
y = 1.36Ln(x) - 37.4
r 2 = 0.43
d13C (‰)
-27
-28
-29
-30
-31
-32
-33
Total length (mm)

Chapter 2: Ecological factors regulating mercury contamination of fish in Caddo Lake, Texas

Matthew M. Chumchal and K. David Hambright

Formatted for Environmental Toxicology and Chemistry

Abstract

In this paper, we present the results of a survey of mercury contamination in ten species of fish from Caddo Lake, a reservoir located on the border of Texas and Louisiana. We also examined how size, age, and food web position (estimated using $\delta^{13}C$ and $\delta^{15}N$) were related to mercury concentrations in fish. Concentrations of mercury in the Caddo Lake fish assemblage were high enough to pose a threat to human and wildlife health. Mercury concentrations in fish were elevated in forested wetland habitats relative to open water habitats most likely because there is more mercury entering the base of the food web in wetland habitats relative to open water habitats. Trophic position was the best predictor of mercury concentrations between species and the biomagnification coefficient (the slope of the relationship between trophic position and mercury) was similar to that found in other studies. Age and size were the best predictors of mercury concentration within species. In contrast to previous studies, we did not find a relationship between horizontal food web position and mercury in Caddo Lake fish. This study indicates that there are similarities and differences in the processes governing mercury accumulation in fish that inhabit shallow lakes and reservoirs (common in subtropical regions) relative to large lakes (often found in cold temperate and arctic regions) where most previous studies were conducted.

Introduction

Mercury is a teratogenic neurotoxin that accumulates in food webs and has increased in the environment primarily due to anthropogenic activities (NRC 2000, Pacyna and Pacyna 2005). Anthropogenic activities, primarily the burning of coal, release inorganic mercury into the atmosphere where it resides until it is deposited onto the earth's surface (Jackson, 1997). In aquatic ecosystems, bacteria convert inorganic mercury, the most common form in the environment, to highly toxic methylmercury (Morel et al. 1998, Ullrich et al. 2001). Wetlands have been identified as mercury sensitive ecosystems in which this conversion of inorganic mercury to methylmercury is highly efficient (Wiener et al. 2003, Evers et al. 2007). Organisms at the base of the food web such as phytoplankton and periphyton absorb methylmercury directly from the water (Miles et al. 2001), while consumers, including fish, are primarily exposed to methylmercury through their diet (Hall et al. 1997, Tsui and Wang 2004).

Because mercury contaminated fish are the primary source of mercury to humans and wildlife there is considerable interest in determining the factors that influence the amount of mercury in fish. There is growing recognition that ecological characteristics of fish strongly influence the levels of mercury in their tissues (Stafford et al. 2004). Mercury concentrations in fish are positively correlated with fish size, age, and trophic position (Johnels et al. 1967, Cabana and Rasmussen 1994, McClain et al. 2006). In addition, current evidence suggests that fish that feed in food webs based on phytoplankton primary production are more contaminated with mercury than fish that feed in food webs based on periphyton primary production (Power et al. 2002, Gorski et al. 2003, Kidd et al. 2003). Finally, fish living in areas in which mercury deposition is

high or in mercury sensitive ecosystems have high levels of mercury (Weiner et al. 2003, Hammerschmidt and Fitzgerald 2006, Evers et al. 2007).

Much of our knowledge concerning mercury contamination in fish has come from studies in north temperate ecosystems (e.g. Cabana and Rasmussen 1994, Power et al. 2002, but see Bowles et al. 2001). However the subtropical, southeastern United States also experiences high mercury deposition rates (NADP 2005), contains coal-burning power plants that emit large amounts of mercury into the atmosphere (Miller and Van Atten 2004), and has extensive wetland habitats (Conner and Buford 1998, Dahl 2006). Moreover, all states in the region have issued fish consumption advisories due to high levels of mercury (USEPA 2005). Despite the identification of fish with high levels of mercury and risk factors correlated with mercury contaminated ecosystems, relatively few studies have examined mercury contamination of fish in the southeastern United States (Cleckner et al. 1998, Burger et al. 2001, Burger et al. 2004). More studies are needed to better understand the risks to humans and wildlife that consume fish from this region.

In this paper, we present the results of a survey of mercury contamination in fish from subtropical Caddo Lake, a reservoir located on the border of Texas and Louisiana. Although the lake contains abundant open water habitat, extensive forested wetlands (sensu Cowardin et al. 1979) characterized by the presence of bald cypress (*Taxodium distichum*), shallow depths (approximately 1 m), and submerged and emergent aquatic vegetation characterize the western half of the lake. A previous study indicated that largemouth bass (*Micropterus salmoides*) collected from the forested wetland habitat have elevated concentrations of mercury relative to largemouth bass from the open water

habitats and suggested that mercury availability was higher in the wetland habitats than in the open water habitats in the lake (Chumchal et al. in review). The objectives of this study were to examine how fish size, age, and food web position (measured using $\delta^{15}N$ and $\delta^{13}C$) are related to mercury concentrations in ten species of fish and to determine if habitat-specific variation in mercury occurs in the fish assemblage.

Methods

Study site

Caddo Lake is a large (107 km^2 in surface area), shallow (average and maximum depths of 1.4 m and 8.2 m, respectively) reservoir (Van Kley and Hine 1998, Ensminger 1999) that supports a recreational and subsistence fishery (Ryan and Brice 2001, TXDSHS 2005). The western portion of the lake (approx. 40 km^2, and mostly in Texas) is composed primarily of a forested wetland (hereafter wetland) dominated by bald cypress, water elm (*Planera aquatica*), and other submerged and emergent aquatic vegetation including fanwort (*Cabomba caroliniana*), common waterweed (*Egeria densa*), and yellow pond-lily (*Nuphar luteum*) (Van Kley and Hine 1998). The eastern portion of Caddo Lake (mostly in Louisiana) is primarily open-water habitat, though submerged vegetation can be extensive in summer months (M.M. Chumchal, personal observation).

The primary anthropogenic sources of mercury in the region are coal-burning power plants (Crowe 1996, TDH 1999). Caddo Lake is located within 250 km of five of the 20 highest mercury-emitting power plants in North America (Miller and Van Atten 2004). Fish consumption advisories have been issued for largemouth bass and

freshwater drum (*Aplodinotus grunniens*) in Caddo Lake by the Texas Department of State Health Services (TXDSHS) due to high levels of mercury in their tissues (TXDSHS 1995). The Louisiana Department of Environmental Quality (LADEQ) monitors largemouth bass and freshwater drum on the Louisiana side of Caddo Lake but they have not issued any advisories (LADEQ 2005).

Fish collection

We collected fish with gill nets and a boat-mounted electrofishing unit with assistance from biologists from the Texas Parks and Wildlife Department (TPWD). Gill nets were 38 m long by 2.4 m deep and constructed of monofilament webbing. Each net consisted of 5 panels, 7.6 m in length with bar measures ranging from 25-76 mm. Eight nets (all in open water habitat) were set in the late afternoon on 10 May 2004 and retrieved the following morning. Fish were collected from nine sites (five sites in wetland and four sites in open water habitat) during the early evening of 10 May and the morning of 12 May using a boat-mounted electrofishing unit. After collection, fish were placed on ice and transported to a lab where total length (TL) was measured and otoliths were removed. Fish were then frozen for subsequent mercury and stable isotope analyses.

Mercury analysis

Fillets were dissected from each fish and a small subsample of epaxial muscle was collected from the center of each fillet using a scalpel and forceps and weighed to the nearest 0.1 mg. Total mercury concentrations in fish were analyzed with a direct mercury analyzer (DMA-80, Milestone Inc. Monroe, CT) that uses thermal decomposition, gold amalgamation, and atomic absorption spectrometry (USEPA 1998)

and are reported as ng total mercury/$g_{\text{wet weight}}$ of fish. We used total mercury as a proxy for methylmercury, the predominant form of mercury in fish (Bloom 1992).

For mercury analyses, a calibration curve was generated using three reference materials from the National Research Council of Canada Institute for National Measurement Standards: MESS-3 (marine sediment, certified value = 91 ± 9 ng/$g_{\text{dry weight}}$ total mercury (average ± 95% C.I.)), PACS-2 (marine sediment, certified value = 3040 ± 200 ng/$g_{\text{dry weight}}$ total mercury) and DORM-2 (dogfish muscle, certified value = 4,640 ± 260 ng/$g_{\text{dry weight}}$ total mercury). Quality assurance included reference and duplicate samples. Reference samples of MESS-3 or DORM-2 were analyzed approximately every 10 samples and the mean percent recovery was 100 ± 1% (range = 92–107%; n = 41) and 100 ± 2% (range = 95–104%; n = 11), respectively. Duplicate samples were analyzed approximately every 20 samples and the mean relative percent difference was 3.6 ± 1.3% (range = 0.3–11.4%; n = 28).

Age analyses

We counted otolith annuli to estimate the age of each fish using the methods of Boxrucker (1986) for bluegill (*Lepomis macrochirus*) and redear sunfish (*L. microlophus*), Buckmeier et al. (2002) for channel catfish (*Ictalurus punctatus*), Buckmeier and Howells (2003) for largemouth bass, white bass (*Morone chrysops*), yellow bass (*M. mississippiensis*), chain pickerel (*Exos niger*), and gizzard shad (*Dorosoma cepedianum*), Ferrara (2001) for spotted gar (*Lepisosteus osseous*), and FWRI (2007) for freshwater drum. We examined otolith annuli to estimate the age of a subset of largemouth bass (33 from wetland and 32 from open water habitats). Two readers independently estimated the ages of fish without knowledge of fish length, and

disagreements were resolved by reexamining otoliths and mutually agreeing on age. Growth rates were determined as TL divided by age.

Trophic position and diet analyses

Stable isotopes of nitrogen and carbon in fish and primary consumers (unionid clams and gastropods) were used to estimate the trophic position of fish (i.e., the vertical trophic level at which fish feed) (Post et al. 2000) and determine whether fish were feeding in pelagic (i.e., those based on phytoplankton production) or littoral/benthic food webs (i.e., those based on periphyton production) (sometimes referred to as horizontal food web position, Leibold et al. 1997).

Stable nitrogen isotopes are used differentially in cellular processes (Fry 2006) resulting in a predictable increase in the heavy isotope, ^{15}N, relative to ^{14}N with each increase in vertical trophic level (Minagawa and Wada 1984). Horizontal food web position can be determined using stable carbon isotopes (^{13}C and ^{12}C) because benthic and pelagic primary producers have distinct carbon isotope signatures (Hecky and Hesslein 1995).

Subsamples of fish fillets and foot muscle from gastropods and unionid clams were dried in a 60°C oven and homogenized using a ball-mill grinder (Dentsply, Inc, York, PA). Sixty-one largemouth bass (31 from wetland and 30 from open-water habitats) were analyzed at Louisiana State University (LSU) for isotopic composition using a Thermoquest Finnigan Delta Plus isotope ratio mass spectrometer (IRMS). The remaining largemouth bass (13 from wetland and 17 from open-water habitats) and all other fish and primary consumers were analyzed at the University of California-Davis (UC-Davis) stable isotope facility using a Europa Hydra 20/20 continuous flow IRMS.

Tank nitrogen and carbon dioxide gases calibrated with known standards were used as working reference materials in daily laboratory operation. Carbon and nitrogen isotope results are given as:

(1) $$\delta^{13}C \text{ or } \delta^{15}N = (R_{sample}/R_{standard} - 1) \times 1000$$

where R is $\delta^{13}C/ \delta^{12}C$ for $\delta^{13}C$ and $^{15}N/ ^{14}N$ for $\delta^{15}N$. Standards for $\delta^{13}C$ and $\delta^{15}N$ are Vienna Pee Dee Belemnite (VPDB) and air N_2, respectively. Analysis of replicate samples of dried bovine liver (National Institute of Standards and Technology) indicated good agreement between the results from each lab (mean $\delta^{15}N$ and $\delta^{13}C$ differed by 0.5‰ and 0.2‰, respectively).

To calculate trophic position, $\delta^{15}N$ values in fish were first corrected for habitat-specific differences in basal $\delta^{15}N$ using $\delta^{15}N$ and $\delta^{13}C$ of primary consumers according the method of Vander Zanden and Rasmussen (1999). Primary consumers that utilize littoral sources of carbon are less enriched in ^{15}N than organisms that utilize pelagic sources of carbon (Vander Zanden and Rasmussen 1999) so we collected gastropods and unionid mussels as representatives of littoral and pelagic primary consumers, respectively (Post et al. 2000). We assumed that fish were resident in the habitat in which they were collected. Thus, ^{15}N values of fish collected from wetland and open-water habitats were corrected for differences in ^{15}N at the base of the food web using gastropods and unionid mussels collected from the corresponding habitat. Gastropod $\delta^{15}N$ and $\delta^{13}C$ was 4.32 ± 0.69‰ and -29.4 ± 1.16‰, respectively for the wetland habitat (n=5) and 2.23‰ and -25.8‰, respectively for the open-water habitat (n = 1). Unionid

mussel $\delta^{15}N$ and $\delta^{13}C$ was 6.50 ± 0.66 ‰ and -33.5 ± 0.13‰, respectively for wetland habitat (n = 6) and 4.85 ± 0.22 ‰ and -32.4 ± 0.53‰, respectively for open-water habitat (n = 5). We used the corrected $\delta^{15}N$ ($\delta^{15}N_{corrected}$) values of fish to calculate trophic position (TP_{fish}) as:

$$TP_{fish} = \delta^{15}N_{corrected}/3.4 + 2 \quad (2)$$

We corrected $\delta^{13}C$ of each fish ($\delta^{13}C_{fish}$) for trophic enrichment according to method of Fry (2006) as:

$$\delta^{13}C_{corrected} = \delta^{13}C_{fish} - 0.5 \times (TP_{fish} - 1) \quad (3)$$

Corrected $\delta^{13}C$ values of fish were compared to $\delta^{13}C$ of gastropods and unionid mussels to determine if fish were feeding predominately in either pelagic or littoral food webs.

Statistical analyses

To estimate the risk of fish in Caddo Lake to human health we compared mean mercury concentrations of fish collected in this study to the TXDSHS and USEPA screening values. Screening values (SVs) are derived from a Reference Dose (RfD) determined from epidemiological studies and are predicted to be the level of mercury that can be safely consumed over a lifetime. Screening values are used by states to help make decisions about the issuance of fish consumption advisories. Although USEPA recommends a SV of 300 $ng/g_{wet\ weight}$ (USEPA 2001), each state determines their own

SV to be used when issuing fish consumption advisories. In Texas, TXDSHS uses a screening value of 700 ng/g $_{\text{wet weight}}$ (TXDSHS 2006).

To estimate the risk posed by mercury contaminated fish in Caddo Lake to piscivorous wildlife we compared mean mercury concentration in fish collected in this study to the USEPA wildlife criterion (WC). Similar to the SV, the WC is predicted to represent a safe lifetime dose of mercury for piscivorous wildlife. The WC criterion corresponds to 77 ng/g $_{\text{wet weight}}$ and 346 ng/g $_{\text{wet weight}}$ of mercury for fish that occupy trophic levels 3 and 4, respectively (USEPA 1997). We used these values to construct a linear relationship between mercury and trophic level which is described by the following equation:

(4) $$\text{Total mercury } (ng/g_{\text{wet weight}}) = 269 * \text{trophic position} - 730$$

We considered species of fish whose mean mercury concentration and trophic position corresponded to a point that fell above the line described by the equation (4) to be of potential risk to the health of piscivorous wildlife.

Between species comparisons

To determine which ecological factors were related to mercury contamination in the Caddo Lake fish assemblage (i.e., between species variation) we used a general linear model (GLM) to test for main effects of habitat and a covariate (mean TL, age, trophic position and $\delta^{13}C$) on mean log-transformed mercury concentrations (Quinn and Keough 2002). If the slopes of the relationships between the covariate and dependent variable were homogeneous between habitats (i.e., habitat x covariate = $p > 0.05$), we

removed the interaction term from the model and tested for main effects of habitat and the covariate using analysis of covariance (ANCOVA) (SPSS Inc., version 11.5.0, Chicago, IL).

To further explore habitat-specific differences in mercury contamination we compared mercury concentration and ecological factors that could influence mercury concentration (TL, age, trophic position and $\delta^{13}C$) in fish species that were collected from both habitats for which fish sample size ≥ 5. We do not compare largemouth bass between habitats because similar analyses appear elsewhere (Chumchal et al. in review). Because age and TL are correlated with mercury concentrations in fish (Wiener et al. 2003), we tested for habitat-specific differences in mercury concentrations after controlling for the effects of age and TL using GLM and ANCOVA as described above. If slopes were not homogenous (an assumption of ANCOVA) we tested for an effect of habitat using the Wilcox procedure (Quinn and Keough 2002) that determines the range of the covariate for which the within-group means are significantly different (WILCOX, version 3.2, Constable 1989). We also tested for habitat-specific differences in trophic position and $\delta^{13}C$ (i.e. horizontal food web position) as additional factors that can affect mercury concentrations in fish. Specifically we tested for within-group effects (two levels of habitat) on dependent variables (trophic position or $\delta^{13}C$) after removing the effect of TL as described above for mercury analysis.

Within species comparisons

We used linear regression to determine which factors were correlated with mercury concentration for each fish species (SPSS Inc., version 11.5.0, Chicago, IL). To help explain patterns in mercury bioaccumulation we also used linear regression to

examine the relationships between fish trophic position and TL and between fish $\delta^{13}C$ and TL.

Results

Fish in Caddo Lake exhibited almost 30-fold variation in mean mercury concentration (Fig. 1, Table 1). Spotted gar, freshwater drum, white bass, chain pickerel and largemouth bass had the highest mercury concentrations with individuals collected from both habitats exceeding TXDSHS and EPA SVs for mercury. Bluegill, yellow bass, and gizzard shad had the lowest mercury concentrations with no individuals exceeding the TXDSHS or EPA SVs for mercury.

In general, fish collected from wetland habitats had higher concentrations of mercury and were more likely to exceed EPA and TXDSHS SVs than fish collected from open water habitats (Fig. 1, Table 1). Fifty-one percent and twenty-four percent of fish collected from wetland habitats exceeded the EPA and TXDSHS' SVs for mercury, respectively. While only 20% and 3% of fish collected from open water habitats exceeded the EPA and TXDSHS SVs for mercury, respectively.

In addition to posing risks to human health, fish from the wetland habitats may pose a risk to piscivorous wildlife (Table 1). All fish from the wetland habitats except channel catfish, yellow bass and bluegill exceeded EPA's WC. In the open water habitat, spotted gar was the only species that exceeded EPA's WC.

Ecological variables that are known to affect mercury concentrations in fish exhibited substantial variation within the fish assemblage (Table 1). Mean TL of fish examined in the study ranged from 141 (yellow bass collected from wetland habitats) to 603 mm (spotted gar collected from open water habitats). Mean age ranged from 1.8

(yellow bass collected from wetland habitats) to 8 years (freshwater drum collected from wetland habitats). Fish spanned one trophic level from secondary to tertiary consumers (trophic level 3 and 4 respectively). Most fish had $\delta^{13}C$ values that were similar to those of gastropods or intermediate between gastropods and unionid clams, indicating that they fed in food webs based on littoral primary production or fed on both littoral and pelagic production, respectively.

Between species comparisons

Mean log-transformed mercury concentrations in Caddo Lake fish were significantly and positively related to mean trophic position but not significantly related to mean TL, age, or $\delta^{13}C$ (Table 2). Fish collected from wetland habitats had significantly higher concentrations of mercury than fish collected from open water habitats even after controlling for the effect of trophic position (Figure 2, Table 2).

Comparisons of mercury concentrations and ecological variables that influence mercury between habitats were possible for spotted gar, freshwater drum, yellow bass, bluegill, and redear. Mercury concentrations were significantly higher in fish from the wetland habitat after controlling for TL and age for all species except redear sunfish (Figure 3, Table 3). For all fish species, trophic position and $\delta^{13}C$ values were either lower in fish collected from wetland habitats or not significantly different across habitat type after controlling for TL (Figure 3, Table 3).

Within species comparisons

In many cases, within species variation in mercury concentration was best explained by age or TL, however we also found significant correlations between mercury and trophic position and mercury and $\delta^{13}C$ (Table 4). A positive relationship

between $\delta^{13}C$ and mercury indicates that fish that fed in littoral-based food webs were more contaminated with mercury than fish that fed in pelagic food webs, while a negative relationship indicates that fish that fed in pelagic food webs were more contaminated than fish that fed in littoral food webs. For most species and independent variables, when the relationship between the independent variable and mercury was significant, the independent variable was positively correlated with mercury. Two exceptions were the relationship between mercury and trophic position in gizzard shad and the relationship between mercury and $\delta^{13}C$ in spotted gar collected from wetland habitats.

Trophic position and $\delta^{13}C$ were positively and significantly correlated with TL in several species and $\delta^{13}C$ and TL were negatively and significantly correlated with TL in largemouth bass from wetland habitats. A positive relationship between $\delta^{13}C$ and TL indicates that as fish increased in size their diet became more heavily based on organisms that were part of littoral food webs, while a negative relationship indicates that as fish increased in size their diet became more heavily based on organisms that were part of pelagic food webs.

Discussion

Concentrations of mercury in the Caddo Lake fish assemblage were high enough to pose a threat to human health. This is especially true of fish collected from the wetland habitats. These data should be of concern to public health officials because Caddo Lake is utilized by both recreational and subsistence anglers (Ryan and Brice 2002, TXDSHS 2005). Of particular concern are mercury levels in chain pickerel and

spotted gar. Neither of these species currently have fish consumption advisories yet they have levels of mercury that are similar to freshwater drum and largemouth bass, two species which currently have fish consumption advisories.

In addition to potential negative impacts on human health, elevated mercury concentrations in wetland habitats could be negatively impacting fish and wildlife health. In the wetland habitat most species exceeded EPA's WC for piscivorous wildlife health. Previous studies at Caddo Lake have found that piscivorous snakes captured near wetland habitats had mercury concentrations as high as 8,610 $ng/g_{wet\ weight}$ (Rainwater et al. 2005), confirming that mercury is bioaccumulating to high levels in piscivorous wildlife. In addition to posing a risk to piscivorous wildlife, mercury levels in some Caddo fish are high enough that the fish themselves might be at risk. In laboratory experiments, fish with whole body concentrations of mercury between 440 – 864 $ng/g_{wet\ weight}$ have reduced hormone levels (Drevnick and Sandheinrich 2003), reduced reproductive success (Drevnick and Sandheinrich 2003), reduced weight of offspring (Matta et al. 2001) and altered predator avoidance behavior (Webber and Haines 2003). Therefore, many tertiary consumers (i.e., fish feeding at trophic level four), especially those collected from wetland habitats, could already be suffering from such effects. Piscivorous fish and wildlife that utilize the Caddo Lake wetland should be monitored to determine if they are being negatively impacted by mercury contamination.

This study adds to a growing body of evidence that mercury contamination exhibits habitat and spatial variation in lakes (Munn and Short 1997, Cizdziel et al. 2002, Campbell et al. 2003, Burger et al. 2004, Stafford et al. 2004, Simoneau et al. 2005, Chumchal et al. in review). We found habitat-specific differences in mercury

contamination throughout the fish assemblage, with fish collected from wetland habitats having higher concentrations of mercury than those collected from open water habitats. Relative to the number of studies that have examined mercury contamination of fish in lakes (Drenner et al. 2007) there have been relatively few reports of spatial or habitat variation (refer to references above). It is not clear if spatial or habitat-specific variation in mercury is an uncommon phenomenon or if researchers have simply overlooked it. Future studies should be conducted in a way that would allow for the identification of spatial or habitat variation in mercury contamination of fish.

Several factors could lead to spatial or habitat specific differences in mercury contamination. Ecosystems with high methylmercury availability contain food webs that are highly contaminated with mercury (Wiener et al. 2003); therefore, habitat specific differences in mercury contamination could be caused by differences in methylmercury availability between habitats. However, because fish size, age, and food web position all influence mercury contamination, differences in these ecological factors between habitats could lead to habitat-specific differences in fish mercury contamination. Below we discuss how our data on fish size, age, and food web position can be used to distinguish between these alternative hypotheses.

Using two approaches we conclude that the habitat-specific differences observed in this study were caused by a more contaminated food web in the wetland habitat and not by differences in the ecological characteristics of fish between habitats. First, the relationship between mean trophic position and mean log-transformed mercury concentration in wetland and open water habitats indicates that the wetland food web is more contaminated with mercury. Comparing the slopes of the relationships between

mean log-transformed mercury and trophic position between the two habitats revealed that biomagnification was similar between habitats (i.e., the slopes were not significantly different) (Jardine et al. 2006). However, the y-intercepts of the mean log-transformed mercury-mean trophic position relationships were significantly different between habitats, which indicates that the level of mercury at the base of the food web was elevated in the wetland habitat (Campbell et al. 2003, Jardine et al. 2006).

Second, habitat-specific differences in ecological characteristics known to influence mercury contamination in fish could not explain the habitat-specific pattern in mercury. All species of fish (for those species in which habitat comparisons were possible), except redear sunfish, collected from wetland habitats had significantly higher concentrations of mercury after correcting for TL and age. Further, the trophic positions of all species of fish collected from the wetland habitats were lower or not significantly different from the trophic positions of fish collected from the open water habitats after correcting for TL. Because mercury is a biomagnifying contaminant (Cabana and Rasmussen 1994) it would be expected that the fish with the higher trophic positions (the fish from the open water habitat) would be more contaminated with mercury if differences in trophic position between habitats could explain the pattern in mercury contamination. Therefore, some other factor must have overridden the effect of trophic position. Finally, for all species except bluegill, $\delta^{13}C$ values were not significantly different between habitats, thus differences in horizontal food web position can not explain the differences in mercury between habitats. These results are similar to those previously reported for largemouth bass in Caddo Lake (Chumchal et al. in review).

The wetlands in Caddo Lake have features that may make them conducive to mercury methylation (Zilloux 1994, Ullrich et al. 2001, Wiener et al. 2003). Relative to open water habitats, wetland habitats in Caddo Lake have low pH (Hartung 1983, Darville et al. 1998), low dissolved oxygen concentrations (Hartung 1983, Darville et al. 1998), and high organic carbon concentrations in sediments (Wilson 2003). In contrast to open water habitats, wetland habitats in Caddo Lake have direct connections to seasonally flooded areas (Van Kley and Hine 1998) and sulfate concentrations that are optimal for mercury methylation (i.e., 0.2-0.5 mM SO_4^{2-}) (Hartung 1983). In addition, in a previous study we found higher levels of mercury in grass shrimp (*Palaemonetes kadiakensis*), a primary consumer, collected from wetland habitats relative to open water habitats (Chumchal et al. in review). Therefore we conclude that there is more mercury entering the base of the food web in the wetland habitats than in the open water habitats.

The strong relationship between mercury and TL and mercury and age observed in this study reinforces the importance of taking age or length of fish into account when issuing fish consumption advisories (McClain et al. 2006). Despite the large number of studies that have found positive relationships between mercury and TL and mercury and age (e.g., Johnels et al. 1967, McClain et al. 2006), many states still do not consider these factors when issuing fish consumption advisories (AFS 1999).

We observed biomagnification within the Caddo Lake fish assemblage. Tertiary consumers (e.g., spotted gar) had average mercury concentrations that were three times higher than secondary consumers (e.g., bluegill). The slope of the relationship between mean mercury and mean trophic position (measured using $\delta^{15}N$) is a measure of biomagnification and can be used to compare transfer efficiencies between mercury and

biomass in food webs (Rolff et al. 1993, Campbell et al. 2003, Jardine et al. 2006). A slope greater than 0 indicates that mercury is transferred more efficiently than biomass through the food web, in other words that biomagnification is occurring (Rolff et al. 1993). In this study the biomagnification coefficients for the wetland and open water habitat were 0.82 and 0.68 which are equivalent to 0.24 and 0.2 if $\delta^{15}N$ values are substituted for trophic position (refer to equation 2). These slopes are similar to those observed in other studies conducted in marine and freshwater ecosystems (Table 5). This is the only study that has examined biomagnification coefficients in the subtropical US and one of the few studies that have examined biomagnification coefficients outside of the temperate or subarctic. The similarities between the biomagnification coefficients observed in this study and those observed in other studies suggest that biomagnification coefficients are similar across all regions and aquatic ecosystems.

We did not always detect biomagnification within species. Trophic position was significantly and positively correlated with mercury concentration in only four of the ten species examined. Similarly, Power et al. (2002) found that within species, $\delta^{15}N$ was a significant predictor of mercury for only three of eight species examined. Our results and previous studies indicate that trophic position can be used to successfully predict which species within a community will have high concentrations of mercury (Table 5) but that it is less successful at predicting the mercury concentrations of individuals within a species.

These results imply that biomagnification is not the primary process responsible for mercury contamination in all fish species or at all life stages; rather the commonly observed increase in mercury concentration with fish TL and age may be due simply to

time-related bioaccumulation (Fagerstrom 1991). Once assimilated mercury is slowly excreted (Trudel and Rasmussen 1998) and can result in positive mercury-TL relationships and mercury-age relationships (Fagerstrom 1991). We collected several fish species whose trophic position did not increase with TL, yet they exhibited a significant mercury-TL relationship. Because age, TL, and trophic position are intercorrelated an experimental and/or modeling approach would be necessary to fully assess the role that time-related bioaccumulation and biomagnification play in the mercury contamination of Caddo Lake fish. However, we believe it is logical to assume that if, for a given species, mercury concentration but not trophic position increases with TL that biomagnification is not occurring.

Unlike previous studies (Power et al. 2002, Gorski et al. 2003, Kidd et al. 2003) we found no relationship between $\delta^{13}C$ and mercury within the fish assemblage. The lack of a relationship between $\delta^{13}C$ and mercury in this study was surprising and may result from the lack of variation in $\delta^{13}C$. The fish assemblage of Caddo Lake was relatively homogenous in terms $\delta^{13}C$ (Table 1) and intermediate between primary consumers of littoral and pelagic carbon, indicating a high degree of omnivory. Vegetation-dominated shallow lakes are characterized by high levels of omnivory (Gu et al. 1996, Jones and Waldron 2003, Herwig et al. 2004) and the horizontal food web structure exhibited in large, deep lakes (Vanderzanden and Rasmussen 1999) is less apparent. Our data may indicate that unlike in deep lakes, horizontal food web structure is not an important predictor of mercury in fish assemblages in shallow lakes and reservoirs that are common in subtropical and tropical regions.

Within species, the δ^{13}C-mercury relationships observed in this study were also different than those found in previous studies. For all species except spotted gar collected from wetland habitats, we found positive relationships between δ^{13}C and mercury in fish, indicating that individuals of a given species that were more connected to littoral food webs were more contaminated with mercury than fish connected to pelagic food webs. These findings are in contrast to previous studies that have found negative relationships between δ^{13}C and mercury levels in fish (i.e., fish connected to pelagic food webs were more contaminated with mercury). However previous studies were conducted in large deep lakes with extensive pelagic zones (Power et al. 2002, Gorski et al. 2003, Kidd et al. 2003). In shallow ecosystems, littoral zones are important sites of mercury methlyation (Cleckner et al. 1999); therefore fish that are dependent on periphtyon-based food webs may be more contaminated with mercury.

This study indicates that there are important similarities and differences in the processes governing mercury accumulation in fish inhabiting shallow lakes and reservoirs common in subtropical climates of the southeastern US relative to large lakes, often found in cold temperate and arctic regions where most previous studies were conducted. The biomagnification coefficients observed in this study are similar to those observed in similar studies. Important differences include habitat and spatial variation caused by prominent wetland habitats associated with southern lakes and reservoirs and differences in the relationship between δ^{13}C and mercury in fish. More studies are needed to understand the mechanisms underlying the δ^{13}C-mercury relationship observed in this study and to understand if sampling programs designed for the issuance

of fish advisories should place a larger emphasis on sampling multiple habitats in southern lakes and reservoirs.

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Table 1. Descriptive statistics for species examined in this study including mean and 95% CI for independent variables and mercury concentration. N = total number of fish collected.

Species	N	Total Length (mm)	Age (years)	Trophic position	$\delta^{13}C$	Hg ($ng/g_{wet\ weight}$)
Forested wetland						
Spotted gar	5	542 ± 70	5.2 ± 1.1	4.3 ± 0.1	-29.7 ± 0.5	833 ± 136
Chain pickerel	6	403 ± 79	2.7 ± 1.1	3.9 ± 0.2	-29.6 ± 0.8	500 ± 216
Channel catfish	3	352 ± 59	4.3 ± 1.7	3.2 ± 0.5	-29.1 ± 0.4	105 ± 27
Freshwater drum	6	439 ± 24	8.0 ± 0	3.8 ± 0.2	-29.5 ± 0.9	600 ± 177
Yellow bass	5	141 ± 29	1.8 ± 1.1	3.3 ± 0.2	-30.5 ± 0.5	61.5 ± 36
Largemouth bass	44[a]	287 ± 37	3.9 ± 0.7	3.9 ± 0.1	-29.5 ± 0.4	465 ± 113
Bluegill	6	164 ± 17	3.7 ± 0.7	3.8 ± 0.2	-30.8 ± 1.2	180 ± 52
Redear sunfish	5	193 ± 15	4.2 ± 0.7	3.2 ± 0.2	-30.6 ± 0.7	234 ± 54
Open water						
Spotted gar	19	603 ± 48	5.6 ± 0.6	4.4 ± 0.1	-29.7 ± 0.2	474 ± 91

Gizzard shad	29	345 ± 19	5.1 ± 0.5	3.3 ± 0.1	-29.6 ± 0.5	30.7 ± 3.7
Channel catfish	27	385 ± 35	4.4 ± 0.5	3.5 ± 0.1	-29.7 ± 0.3	139 ± 40
Freshwater drum	18	409 ± 25	7.1 ± 1.2	4.0 ± 0.2	-29.4 ± 0.4	319 ± 120
White bass	21	347 ± 23	3.2 ± 0.7	4.1 ± 0.1	-28.7 ± 0.3	262 ± 78
Yellow bass	39	217 ± 11	3.2 ± 0.2	3.7 ± 0.0	-29.8 ± 0.2	104 ± 15
Largemouth bass	47[a]	260 ± 33	2.6 ± 0.5	4.2 ± 0.1	-30.0 ± 0.3	193 ± 54
Bluegill	14	150 ± 11	2.6 ± 0.4	3.6 ± 0.2	-29.4 ± 0.3	81.4 ± 20
Redear sunfish	5	170 ± 34	2.6 ± 0.5	3.2 ± 0.2	-30.1 ± 0.2	127 ± 76

[a] Age was determined for a subset of largemouth bass (33 from wetland and 32 from open water habitats).

Table 2. Significance values associated with analysis of covariance (ANCOVA).

Covariate[a]	Covariate x habitat *p*	ANCOVA	
		Habitat *p*	Covariate *p*
Total length	0.54	0.07	0.006
Age	0.62	0.17	0.09
Trophic position	0.62	0.009	<0.001
$\delta^{13}C$	0.84	0.11	0.31

[a] Covariates are species averages

Dependent variable was mean log-transformed mercury concentration.

Table 3. Significance values associated with analysis of covariance (ANCOVA) used to compare mercury and other ecological factors in species of fish collected from both habitats after collecting for TL or age.

Species	Total mercury vs TL		Total mercury vs age		Trophic position vs TL		$\delta^{13}C$ vs TL	
	Habitat *p*	Covariate *p*	Habitat *p*	Covariate *p*	Habitat *p*	Covariate *p*	Habitat *p*	Covariate *p*
Spotted gar	<0.001	<0.001	<0.001	0.02	0.9	0.006	0.7	0.6
Freshwater drum	0.03	<0.001	-	-	0.01	<0.001	0.9	0.6
Yellow bass	0.007	<0.001	[a]		0.001	0.04	0.07	<0.001
Redear	0.2	0.008	0.9	<0.001	0.8	0.8	0.3	0.2
Bluegill	<0.001	0.01	0.007	<0.001	0.2	0.4	0.002	0.2

[a] The assumption of homogeneity of slopes was violated therefore we looked for habitat specific differences in mercury after accounting for age using the Wilcox procedure and found no significant differences ($p > 0.05$) between habitats.

Table 4. Pearson's correlation coefficients (r). Bold text indicates a significant correlation ($P \leq 0.05$).

Species	Hg vs. Total length	Hg vs. age	Hg vs. trophic position	Hg vs. $\delta^{13}C$	Trophic position vs. total length	$\delta^{13}C$ vs. total length
Forested wetland						
Spotted gar	0.43	-0.11	0.18	**-0.99**	-0.50	-0.36
Chain pickerel	**0.87**	**0.94**	0.19	**0.84**	0.59	0.69
Channel catfish	0.44	-0.05	0.91	-0.98	0.02	-0.61
Freshwater drum	0.37	-	0.78	0.30	0.61	0.16
Yellow bass	**0.97**	**0.91**	0.20	**0.90**	0.34	**0.91**
Largemouth bass	**0.87**	**0.89**	**0.54**	-0.23	**0.60**	**-0.40**
Bluegill	0.69	0.74	0.59	0.11	0.55	0.40
Redear sunfish	**0.56**	**0.89**	-0.03	-0.17	0.04	0.34
Open water						
Spotted gar	**0.71**	**0.59**	**0.76**	0.13	**0.66**	0.06

Gizzard shad	-0.25	-0.32	**-0.37**	-0.36	**0.73**	**0.72**
Channel catfish	**0.66**	**0.67**	0.38	0.28	0.21	**0.57**
Freshwater drum	**0.92**	**0.65**	**0.84**	-0.01	**0.80**	-0.12
White bass	**0.68**	**0.93**	**0.50**	0.31	**0.90**	**0.67**
Yellow bass	**0.81**	**0.91**	0.22	**0.45**	0.31	**0.76**
Largemouth bass	**0.74**	**0.85**	**0.74**	**0.36**	**0.78**	**0.63**
Bluegill	0.52	**0.92**	0.18	0.13	0.30	0.34
Redear sunfish	0.83	0.83	0.15	**0.88**	0.14	0.70

Table 5. Biomagnification coefficients from studies that used $\delta^{15}N$ to estimate trophic position.

Study	Region	Ecosystem	Biomagnification coefficient
Yoshinga et al. 1992	Subtropical	Lake/estuary	0.21
Kidd et al. 1995 - Orange	Temperate	Lake	0.29
Kidd et al. 1995 - Linge	Temperate	Lake	0.23
Kidd et al. 1995 - Sydney	Temperate	Lake	0.17
Kidd et al. 1995 - Trout	Temperate	Lake	0.21
Kidd et al. 1995 - Musclow	Temperate	Lake	0.21
Kidd et al. 1995 - Green	Temperate	Lake	0.48
Jarman et al. 1996	Temperate	Marine	0.32[a]
Atwell et al. 1998	Arctic	Marine	0.20
Bowles et al. 2001	Subtropical	Lake	0.28
Power et al. 2002	Subarctic	Lake	0.19
Campbell et al. 2003 – Napoleon Gulf	Tropical	Lake	0.16
Campbell et al. 2003 – Winam Gulf	Tropical	Lake	0.17
Kidd et al. 2003	Tropical	Lake	0.20
Campbell et al. 2004	Tropical	Lake	0.28
Campbell et al. 2005	Arctic	Marine	0.20
Garcia and Carignan 2005 – Reference lakes	Temperate	Lake	0.20
Garcia and Carignan 2005 – Cut lakes	Temperate	Lake	0.22
Garcia and Carignan 2005 – Burnt lakes	Temperate	Lake	0.26
Sampaio Da Silva et al. 2005	Tropical	Lake	0.24[b]
Campbell et al. 2006 – Saka	Tropical	Lake	0.14

Campbell et al. 2006 - Nkuruba	Tropical	Lake	0.14
McIntyre and Beauchamp 2007	Temperate	Lake	0.19[c]
This study – open water	Subtropical	Reservoir	0.20
This study – forested wetland	Subtropical	Reservoir	0.24

[a]Calculated using data from their Tables 1 and 2

[b]Calculated using data from their Tables 1 and 3, all lakes and seasons combined.

[c]Calculated using Data from their Table 1

Figure captions

Fig 1. Mercury contamination in Caddo Lake fish. Boxes, whiskers, and dark horizontal bars signify the 1st and 3rd quartile, range, and median mercury concentration of each fish species, respectively.

Fig 2. Relationship between mean log-transformed mercury and mean trophic position. Markers with square borders and a solid trend line represent fish collected from wetland habitats. Markers with oval borders and dashed trend line represent fish collected from open water habitats. SG = spotted gar, GS = gizzard shad, CP = chain pickerel, CC = channel catfish, FD = freshwater drum, WB = white bass, YB = yellow bass, LMB = largemouth bass, BG = bluegill, RE = redear sunfish

Fig 3. Relationships between total length (TL) and total mercury, age and total mercury, and trophic position and TL in spotted gar, freshwater drum, yellow bass, bluegill, and redear sunfish collected from forested wetland (black points and solid line) and open water (open points and dashed line) habitats.

Figure 1.

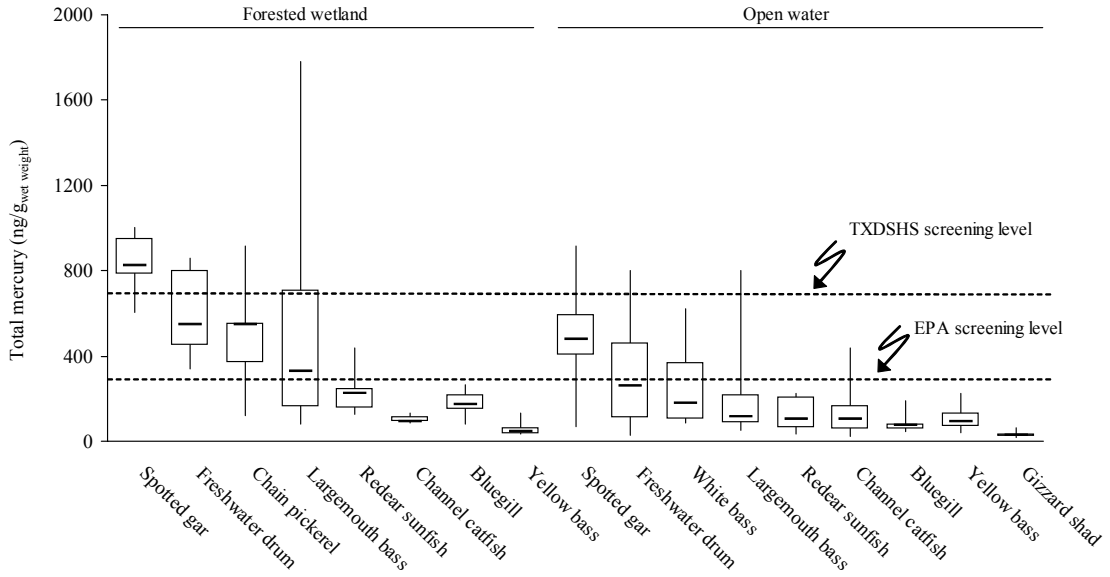

Forested wetland
Open water
2000
1600
1200
800
400
0
Total mercury (ng/g wet weight)
TXDSHS screening level
EPA screening level
Spotted gar
Freshwater drum
Chain pickerel
Largemouth bass
Redear sunfish
Channel catfish
Bluegill
Yellow bass
Spotted gar
Freshwater drum
White bass
Largemouth bass
Redear sunfish
Channel catfish
Bluegill
Yellow bass
Gizzard shad

Figure 2.

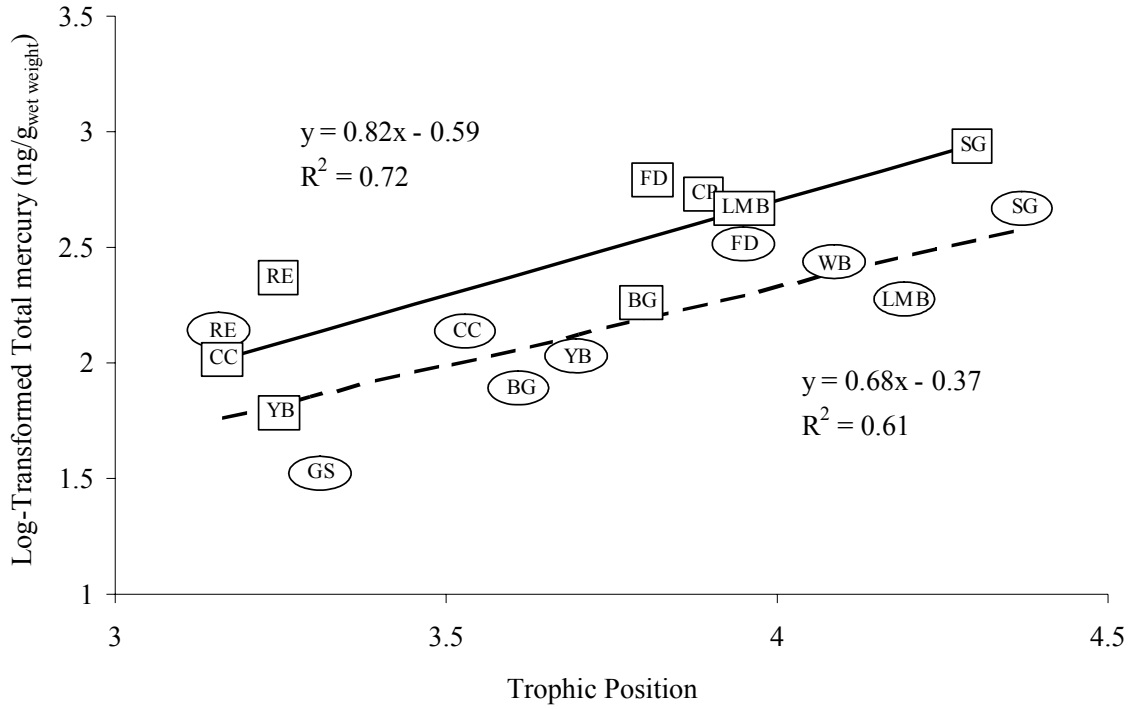
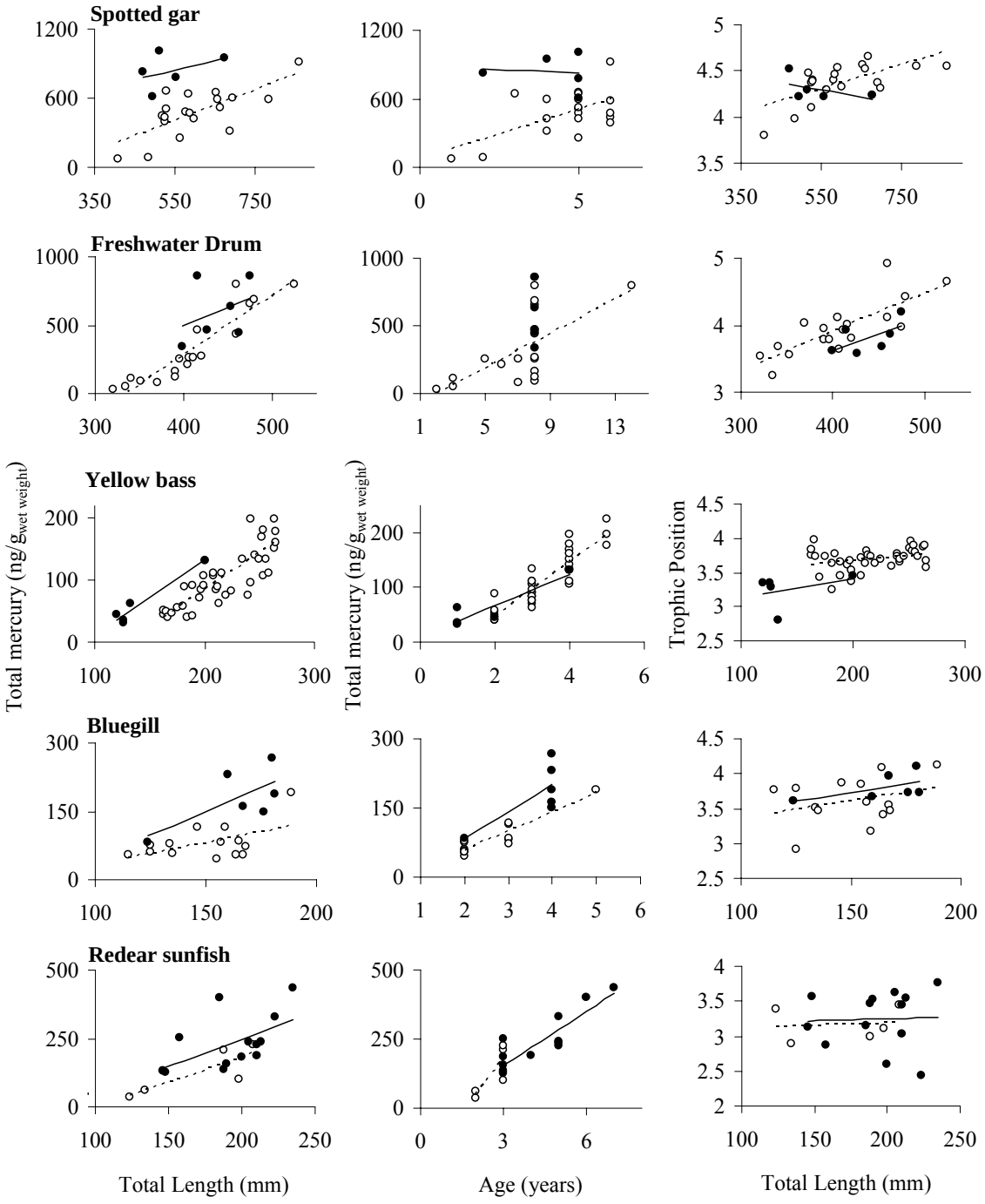

y = 0.82x - 0.59
R2 = 0.72
y = 0.68x - 0.37
R2 = 0.61
Log-Transformed Total mercury (ng/gwet weight)
Trophic Position
3.5
3
2.5
2
1.5
1
3
3.5
4
4.5
SG
FD
CP
LMB
SG
FD
WB
LMB
BG
RE
RE
CC
CC
YB
BG
YB
GS

Figure 3.


Spotted gar
Freshwater Drum
Yellow bass
Bluegill
Redear sunfish
Total mercury (ng/g wet weight)
Trophic Position
Total Length (mm)
Age (years)
Total Length (mm)

Chapter 3: Species-specific differences in mercury concentrations of planktivorous fish are related to fine-scale differences in trophic position

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Abstract

We conducted a survey of mercury contamination in three species of planktivorous fish, brook silverside (*Labidesthes sicculus*), threadfin shad (*Dorosoma petenense*) and gizzard shad (*Dorosoma cepedianum*), from Caddo Lake, Texas and identified species-specific differences in mercury contamination. We also examined trophic position (determined using $\delta^{15}N$), growth rate, and horizontal food web position (determined using $\delta^{13}C$) of planktivorous fish as factors that could have led to species-specific differences in mercury contamination. Mean trophic position differed by less than one trophic level between planktivorous fish species but this difference was likely responsible for species-specific differences in mercury concentration. We observed biomagnification between the species and the biomagnification coefficient (the slope of the relationship between trophic position and mercury) was similar to a previous study that examined the biomagnification in the Caddo Lake fish assemblage. Species-specific differences in growth rate and horizontal food web position could not explain differences in mercury concentrations between planktivorous fish. This implies that trophic position is one of the most important predictors of interspecific mercury concentration in fish, even among species within a trophic guild that only exhibit fine scale differences in trophic position.

Introduction

Mercury is a toxic metal that accumulates in food webs and has increased in the environment primarily due to anthropogenic activities (NRC 2000, Pacyna and Pacyna 2005). The burning of coal releases inorganic mercury into the atmosphere where it resides until it is deposited onto the earth's surface (Jackson, 1997). In aquatic ecosystems, bacteria convert inorganic mercury, the most common form in the environment, to highly toxic methylmercury (Morel et al. 1998; Ullrich et al. 2001). Organisms at the base of the food web such as phytoplankton and periphyton absorb methylmercury directly from the water (Miles et al. 2001), while consumers, including fish, are exposed to methylmercury primarily through their diet (Hall et al. 1997; Tsui and Wang 2004).

Mercury is a biomagnifying contaminant (i.e., it is found at higher concentrations at each successive vertical trophic level in a food web) and trophic position (a continuous measure of vertical food web position) is consistently identified as the best interspecific predictor of mercury levels within fish assemblages (Cabana and Rasmussen 1994, Power et al. 2002, Chumchal and Hambright in review). Most studies that have quantified biomagnification have examined mercury concentration in fish assemblages composed of species that represent more than one trophic level (i.e., whose mean trophic positions differ by more than one trophic level) (Cabana and Rasmussen 1994, Power et al. 2002, Chumchal and Hambright in review). Swanson et al. (2003, 2006) hypothesized that fine-scale trophic differences (i.e., a difference of one-fourth to one trophic level) among species, particularly species within a trophic guild, do not result in differences in mercury contamination. Rather, within a trophic guild, a fish's

mean growth rate is the best predictor of mercury concentrations between species, with higher growth rates leading to lower mercury concentrations.

We conducted a survey of mercury contamination in three species of fish, brook silverside (*Labidesthes sicculus*), threadfin shad (*Dorosoma petenense*) and gizzard shad (*Dorosoma cepedianum*), belonging to a single trophic guild, in Caddo Lake, Texas and identified species-specific differences in mercury contamination. All three species are planktivorous, although gizzard shad become increasingly dependent on detritus and sediment-associated organisms as they age (Robnson and Buchanan 1988). We tested Swanson et al.'s (2003, 2006) hypotheses that differences in growth rate and not trophic position between species within a trophic guild explain differences in mercury concentration by examining trophic position (determined using $\delta^{15}N$) and growth rate in these species. We also examined horizontal food web position (determined using $\delta^{13}C$) of the planktivores in this study as an alternative explanation for the observed species-specific differences in mercury.

Methods

Study site

Caddo Lake is located on the Texas-Louisiana border within Jefferson and Marion counties in Texas and Caddo Parish in Louisiana. Caddo Lake is a large (107 km^2 in surface area), shallow (mean and maximum depths of 1.4 m and 8.2 m, respectively) reservoir (Van Kley and Hine 1998, Ensminger 1999) that supports a recreational and subsistence fishery (Ryan and Brice 2001, TXDSHS 2005). Caddo Lake contains organisms with high levels of mercury (as shown in recent survey of

fishes and snakes (Rainwater et al. 2004, Chumchal et al. in review, Chumchal and Hambright in review). There are no point-sources of mercury to Caddo Lake and the most important regional sources of mercury are coal burning power plants (Crowe 1996; TDH 1999, Miller and Van Atten 2004, Slattery et al. in review).

Fish collection

We collected fish along four transects (approximately 0.8-2.8 km in length) on 28 and 29 June 2005 using a boat-mounted electrofishing unit in the eastern portion of Caddo Lake. This area is primarily open water habitat, though submerged vegetation can be extensive in summer months (Chumchal et al. in review). We targeted brook silversides (n = 20), threadfin shad (n = 33), and gizzard shad (n = 35) because they are the most abundant species in open water areas of Caddo Lake (M.M. Chumchal personal observation) and feed primarily on plankton (throughout life for brook silversides and threadfin shad, up to ~ 10 cm TL for gizzard shad after which they can become heavily dependent on detritus and sediment-associated organisms; Robison and Buchanan 1988). After collection, we placed fish on ice and transported them to a lab where we measured total length (TL) and removed otoliths. We then froze fish for subsequent mercury and stable isotope analyses.

Mercury analysis

We dissected fillets from each fish and collected a small subsample of epaxial muscle from the center of each fillet using a scalpel and forceps. We analyzed concentrations of total mercury (ng $g_{\text{wet weight}}^{-1}$), a proxy for methylmercury, the predominant form of mercury in fish (Bloom 1992) with a direct mercury analyzer

(DMA-80, Milestone Inc. Monroe, CT) that uses thermal decomposition, gold amalgamation, and atomic absorption spectrometry (USEPA 1998).

For mercury analyses, we generated a calibration curve using three reference materials from the National Research Council of Canada Institute for National Measurement Standards: MESS-3 (marine sediment, certified value = 91 ± 9 ng $g_{dry\ weight}^{-1}$ total mercury (mean ± 95% C.I.)), PACS-2 (marine sediment, certified value = 3040 ± 200 ng $g_{dry\ weight}^{-1}$ total mercury) and DORM-2 (dogfish muscle, certified value = 4,640 ± 260 ng $g_{dry\ weight}^{-1}$ total mercury). Quality assurance included reference and duplicate samples. We analyzed reference samples of MESS-3 approximately every 10 samples and the mean percent recovery was 101 ± 0.96% (range = 93–107%; n = 34). We analyzed duplicate samples approximately every 20 samples and the mean relative percent difference was 3.8 ± 1.4% (range = 0.1–9.2%; n = 13).

Planktivore trophic position and diet

We used stable isotopes of nitrogen in fish and primary consumers (unionid clams and gastropods) to estimate the trophic position of fish (i.e., the vertical trophic level at which fish feed). Some studies have concluded that fish that feed in food webs based on phytoplankton primary production are more contaminated with mercury than fish that feed in food webs based on periphyton primary production (Power et al. 2002; Gorski et al. 2003; Kidd et al. 2003; but see, Chumchal et al. in review, Chumchal and Hambright in review). Therefore we used stable isotopes of carbon to determine whether fish were feeding in pelagic (i.e., those based on phytoplankton production) or littoral/benthic food webs (i.e., those based on periphyton production) (sometimes referred to as horizontal food web position, Leibold et al. 1997).

Stable nitrogen isotopes are used differentially in cellular processes (Fry 2006) resulting in a predictable increase in the heavy isotope, ^{15}N, relative to ^{14}N with each increase in vertical trophic level (Minagawa and Wada 1984). Horizontal food web position can be determined using stable carbon isotopes (^{13}C and ^{12}C) because benthic and pelagic primary producers have distinct carbon isotope signatures (Hecky and Hesslein 1995).

We dried planktivorous fish epaxial muscle subsamples and foot muscle from gastropods and unionid clams in a 60°C oven and then homogenized them using a ball-mill grinder (Dentsply, Inc, York, PA). Samples were analyzed at the University of California-Davis (UC-Davis) stable isotope facility using a Europa Hydra 20/20 continuous flow IRMS. Tank nitrogen and carbon dioxide gases calibrated with known standards were used as working reference materials in daily laboratory operation. Carbon and nitrogen isotope results are given as:

$$\delta^{13}C \text{ or } \delta^{15}N = (R_{sample}/R_{standard} - 1) \times 1000 \quad (1)$$

where R is $\delta^{13}C/\ \delta^{12}C$ for $\delta^{13}C$ and $^{15}N/\ ^{14}N$ for $\delta^{15}N$. Standards for $\delta^{13}C$ and $\delta^{15}N$ are Vienna Pee Dee Belemnite (VPDB) and air N_2, respectively.

To calculate trophic position, we first corrected $\delta^{15}N$ values in planktivores for differences in basal $\delta^{15}N$ using $\delta^{15}N$ and $\delta^{13}C$ of primary consumers according to the method of Vander Zanden and Rasmussen (1999). Primary consumers that utilize littoral sources of carbon are less enriched in ^{15}N than organisms that utilize pelagic sources of carbon (Vander Zanden and Rasmussen 1999) so we collected gastropods and

unionid mussels as representatives of littoral and pelagic primary consumers, respectively (Post et al. 2000). Gastropod $\delta^{15}N$ and $\delta^{13}C$ was 1.94 ‰ and -25.12‰, respectively ($n = 1$). Unionid mussel $\delta^{15}N$ and $\delta^{13}C$ was 5.67 ± 0.04 ‰ and -33.2 ± 0.18‰, respectively ($n = 6$). We used the corrected $\delta^{15}N$ ($\delta^{15}N_{corrected}$) values of planktivorous fish to calculate trophic position (TP_{fish}) as:

$$TP_{fish} = \delta^{15}N_{corrected} / 3.4 + 2 \quad (2)$$

We corrected $\delta^{13}C$ of each planktivorous fish ($\delta^{13}C_{bass}$) for trophic enrichment according to method of Fry (2006) as:

$$\delta^{13}C_{corrected} = \delta^{13}C_{fish} - 0.5 \times (TP_{fish} - 1) \quad (3)$$

We compared corrected $\delta^{13}C$ values of planktivorous fish to $\delta^{13}C$ of gastropods and unionid mussels to determine if planktivorous fish were feeding predominately in pelagic or littoral food webs.

Planktivore age and growth rates

We used otolith annuli to estimate the age of each planktivorous fish. We broke otoliths from large gizzard shad perpendicular to their longest axis through the nucleus and then polished them using 400 and 600-grit sandpaper (Buckmeier and Howells 2003, Clayton and Maceina 1999). We examined annuli on whole otoliths from small gizzard shad and other planktivores. We counted annuli at 8-40× magnification under a dissecting microscope. Two independent readers estimated the ages of fish without

knowledge of fish length, and resolved disagreements by reexamining otoliths and mutually agreeing on age. To account for growth that occurred prior to the formation of annuli, we added one year to the number of visible annuli. We determined growth rate as TL divided by age.

Statistical analyses

Because age and TL are correlated with mercury concentrations in fish (McClain et al. 2006), we tested for species-specific differences in planktivorous fish mercury concentrations after controlling for the effects of age and TL. Specifically, we tested for within-group effects (three levels of species) on planktivorous fish mercury concentrations, after removing the effect of a covariate (age or TL). We also tested for species-specific differences in growth rate, trophic position, and $\delta^{13}C$ (i.e. horizontal food web position) as additional factors that can affect mercury concentrations in fish. Specifically, we tested for within-group effects on dependent variables (trophic position, $\delta^{13}C$, TL, or mercury concentrations) after removing the effect of a covariate (age, TL, trophic position, or $\delta^{13}C$). We used a general linear model to determine if the slopes of the relationships between the covariate and dependent variable were homogeneous between species. If the slopes were homogeneous between species (i.e., habitat x covariate = $P > 0.05$), we removed the interaction term from the model and tested for main effects of habitat and the covariate using analysis of covariance (ANCOVA) (SPSS Inc., version 11.5.0, Chicago, IL). When we detected a significant within-group effect, we used SPSS' Compare Means procedure to test for pairwise differences in least squares means after applying a Bonferroni adjustment. If slopes were not homogenous (an assumption of ANCOVA) we tested for species differences using the Wilcox

procedure (Quinn and Keough 2002) that determines the range of the covariate for which the within-group means are significantly different (WILCOX, version 3.2, Constable 1989). We transformed some variables to increase linearity. Finally we used linear regression to determine the relationship between mean log-mercury and trophic position.

Results

Mercury concentrations were highest in brook silversides and lowest in gizzard shad (Table 1). Within species, both age and TL were significantly and positively correlated to mercury concentration (Table 2; Figure 1). However, after controlling for TL and age, brook silversides still had significantly higher concentrations of mercury than both threadfin and gizzard shad and threadfin shad had significantly higher concentrations of mercury than gizzard shad (Table 2; Figure 1).

Brook silversides had the highest mean trophic position while gizzard shad had the lowest mean trophic position (Table 1), but it is worth noting that mean trophic positions differed by less than one trophic level. Within species trophic position was positively correlated with TL (Table 2; Figure 2A). After controlling for TL brook silversides had the highest trophic position followed by threadfin shad followed by gizzard shad (Table 2; Figure 2A).

For each species the relationship between trophic position and log-transformed mercury was positive, indicating that biomagnification was occurring within each species (Table 2, Fig 2B). After controlling for the effect of trophic position we found no difference in mercury concentrations between species, which indicates that differences in mercury concentrations between species were caused by differences in

trophic position between species (Table 2, Fig 2B). We also found a significant and positive relationship between mean log-transformed mercury and mean trophic position ($P = 0.008$, $y = 0.63x - 0.41$) which indicates that biomagnification was occurring between species.

Brook silverside mean growth rate was 1.3 times slower than threadfin shad mean growth rate and 2.3 times slower than gizzard shad mean growth rate (Table 1). Brook silversides were significantly smaller than similar-aged threadfin and gizzard shad and threadfin shad were significantly smaller than similar-aged gizzard shad (Table 2; Figure 3).

Mean horizontal food web position ($\delta^{13}C$) exhibited species-specific differences with brook silversides being the least enriched in ^{13}C followed by threadfin shad and gizzard shad (Table 1). However, after controlling for TL there was not a significant difference in the $\delta^{13}C$ values of brook silversides and threadfin shad or threadfin shad and gizzard shad (Table 1; Figure 4A). There were significant differences in $\delta^{13}C$ values between brook silversides and gizzard shad even after controlling for TL. Comparison of planktivorous fish $\delta^{13}C$ values with $\delta^{13}C$ values of primary consumers indicated that brook silversides and threadfin shad were likely feeding in food webs based more on littoral primary production (i.e., $\delta^{13}C$ values in these species were more similar to those in the gastropod) than gizzard shad (i.e., $\delta^{13}C$ values in gizzard shad were more similar to those in unionids). However most brook silversides and threadfin shad had $\delta^{13}C$ values between the $\delta^{13}C$ values of unionid mussels and the gastropod indicating that they likely relied on both littoral and pelagic primary production. In addition, as each species

of planktivorous fish increased in size they became more dependent on pelagic primary production (i.e. their $\delta^{13}C$ values were inversely related to length).

Within each species, individuals exhibited an inverse relationship between $\delta^{13}C$ and log-transformed mercury indicating that fish feeding in pelagic food webs were more enriched in mercury than those feeding in littoral food webs (Table 2, Fig 4B). We also found a significant main effect of species on log total mercury after controlling for $\delta^{13}C$ values. Pairwise comparisons indicated that brook silversides had significantly higher concentrations of mercury than threadfin shad ($P < 0.001$) and gizzard shad ($P < 0.001$) and that threadfin shad had significantly higher concentrations of mercury than gizzard shad ($P = 0.015$). The detection of species-specific differences in log-mercury concentration even after correcting for $\delta^{13}C$ indicates that differences in horizontal food web position between species can not explain differences in mercury concentration.

Discussion

The primary objective of this study was to test Swanson et al.'s (2003, 2006) hypotheses that differences in mercury concentrations between species within a trophic guild are caused by differences in growth rate and not trophic position. Although both growth rate and trophic position differed between species our data suggest that, in contrast to Swanson et al.'s (2003, 2006) predictions, the primary factor responsible for species-specific differences in planktivorous fish mercury levels were differences in trophic position.

Two lines of evidence support the conclusion that variation in planktivorous fish mercury concentration was likely caused by differences in trophic position. First, we

found no species-specific differences after mercury concentrations were corrected for trophic position. This indicates that the relationship between mercury and trophic position was similar in each species and that differences in mercury concentration can be explained by differences in mean trophic position. Second, we found a positive relationship between mean mercury concentration and mean trophic position. The slope of the relationship between mean mercury and mean trophic position (measured using $\delta^{15}N$) is a measure of biomagnification and can be used to compare transfer efficiencies between mercury and biomass in food webs (Rolff et al. 1993, Campbell et al. 2003, Jardine et al. 2006). A slope greater than 0 indicates that mercury is transferred more efficiently than biomass through the food web, in other words that biomagnification is occurring (Rolff et al. 1993). The biomagnification coefficient found in this study (0.63) was remarkably similar to the biomagnification coefficient found in a previous study that examined 9 species of Caddo Lake fish whose mean trophic positions varied by >1 trophic level (0.68, Chumchal and Hambright in review). These slopes are similar to those observed in other studies conducted in both marine and freshwater ecosystems (Chumchal and Hambright in review).

Despite differences between species, growth rates do not appear to explain the pattern of mercury contamination observed in this study. Consistent with the hypothesis that fish with slow growth rates have elevated concentrations of mercury (Rodgers 1996; Stafford and Haines 2001; Simoneau et al. 2005), brook silversides had slower growth rates and higher concentrations of mercury than threadfin shad and threadfin shad had slower growth rates and higher concentrations of mercury than gizzard shad. However, scrutiny of our data reveals that growth rate is not a sufficient explanation for species-

specific differences in mercury concentrations. Using a series of models, Rodgers (1996) examined the effects of changes in dietary mercury levels and growth rate on mercury concentrations in fish tissue. Populations of fish with different levels of mercury in their diets exhibited size- and age-specific concentrations of mercury that were distinct from populations of fish with different growth rates. Fish with high levels of mercury in their diets had higher size- and age-specific concentrations of mercury in their tissues than fish with low levels of mercury in their diet. Fish with slow growth rates had higher size-specific concentrations of mercury in their tissues but *lower* age-specific concentrations of mercury in their tissues relative to fish with fast growth rates. In this study, brook silversides had higher length-specific and age-specific concentrations of mercury in their tissues relative to threadfin shad and threadfin shad had higher length-specific and age-specific concentrations of mercury in their tissues relative to gizzard shad. Therefore, differences in growth rate can not explain the species-specific differences in mercury concentration we observed. Rather, TL- and age-specific patterns in mercury contamination of planktivorous fish indicate that their diets differed in mercury concentration.

Variation in mercury concentration does not appear to be related to horizontal food web position which we examined as an alternative explanation for species-specific differences in mercury. Fish feeding in food webs based predominantly on pelagic primary production have elevated concentrations of mercury (Lindqvist et al. 1991; Power et al. 2002; Gorski et al. 2003; Kidd et al. 2003). In this study we found a similar pattern with individuals that relied more heavily on pelagic food webs having higher concentrations of mercury. However after correcting $\delta^{13}C$ values for TL, we found no

differences in $\delta^{13}C$ values between brook silversides and threadfin shad or threadfin shad and gizzard shad.

In this study we present evidence that even small differences in mean trophic position (< 1 trophic level) can lead to differences in mercury concentration. These results are in contrast to the conclusions of Swanson et al. (2003, 2006) who state that trophic position will not be important in determining mercury concentrations within guilds. Swanson et al. (2003) present convincing evidence that differences in mass-adjusted mercury concentration between spottail shiner and three other "forage" fish species (which did not have significantly different mass-adjusted mercury concentrations) were not caused by trophic position. They later present evidence that spottail shiner likely had elevated mercury concentrations due to slow growth rates (Swanson et al. 2006). In light of our data and previous studies by Swanson et al. (2003, 2006) we feel that the most logical conclusion is that differences in either growth rate *or* trophic position can lead to differences in mercury concentrations even within trophic guilds. Which factor is more important will be species-specific and dependent on the magnitude of differences in trophic position and growth rate between species under consideration.

This is one of the few studies to examine ecological factors that affect mercury concentrations in planktivorous fish (Swanson et al. 2003, 2006). These studies are critical because fish in this trophic guild are important prey items and therefore important sources of mercury to piscivorous fish, which often contain levels of mercury that are dangerous to fish and wildlife health. In this study we identified species-specific differences in mercury concentrations but mean mercury concentrations of all three

species were below the USEPA wildlife criterion (WC). The WC is predicted to represent a safe lifetime dose of mercury for piscivorous wildlife and corresponds to 77 ng/g ww of mercury for fish that occupy trophic level 3 (USEPA 1997). These data corroborate previous research from Caddo Lake that indicates that most species of fish from open water habitats, the habitats from which the fish in this study were collected, do not pose a health risk to piscivorous wildlife (Chumchal and Hambright in review).

In this study, we found that differences in mercury concentrations between planktivorous fish species were related to differences in trophic position. Biomagnification of mercury has been consistently reported at multiple scales of ecological organization (within species, trophic guilds, communities, ecosystems) (Cabana and Rasmussen 1994, Atwell et al. 1998, Bowles et al. 2001, Power et al. 2002, Chumchal and Hambright in review, this study). Further trophic position predicts mercury levels between species when other variables related to mercury concentrations (e.g. age or size) do not (Chumchal and Hambright in review, this study). This implies that, although not all differences in mercury concentration can be explained by trophic position (i.e. Swanson et al. 2003, 2006), trophic position is one of the most important predictors of interspecific mercury concentration in fish, even among species within a trophic guild that only exhibit fine-scale differences in trophic position.

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Table 1.–Mean (± CI) characteristics of planktivorous fish collected from Caddo Lake.

	Brook Silverside	Threadfin Shad	Gizzard Shad
Mercury (ng $g_{wet\ weight}^{-1}$)	63.0 ± 25.8	40.4 ± 13.8	26.4 ± 4.2
Total length (mm)	5.3 ± 0.6	7.3 ± 0.8	23.0 ± 2.9
Age (years)	1.3 ± 0.2	1.3 ± 0.3	2.5 ± 0.5
Growth rate (mm/year)	4.4 ± 0.3	5.6 ± 0.3	10.3 ± 1.4
Trophic position	3.5 ± 0.1	3.2 ± 0.1	2.9 ± 0.1
$\delta^{13}C$ (‰)	-28.6 ± 0.6	-29.8 ± 0.6	-31.2 ± 0.6

Table 3. Significance values associated with analysis of covariance (ANCOVA) used to compare mercury and other ecological factors in different species of planktivorous fish after correcting for TL or age.

Dependent variable[a]	Covariate[a]	Covariate x species[a] *P*	ANCOVA		Wilcox procedure results
			Species *P*	Covariate *P*	
Mercury	TL[b]	<0.001	-	-	All pairwise comparisons *P* <0.05
Mercury	Age	<0.001	-	-	All pairwise comparisons *P* <0.05
Trophic position	TL[b]	0.04	-	-	All pairwise comparisons *P* <0.05
Log-Mercury	Trophic position	0.32	0.15	<0.001	-
TL[b]	ln age	<0.001	-	-	All pairwise comparisons *P* <0.05
$\delta^{13}C$	TL[b]	0.001	-	-	BS vs GS *P* <0.05; BS vs TS & TS vs GS *P* >0.05[c]
Log-Mercury	$\delta^{13}C$	0.58	<0.001	<0.001	-

[a] Fixed factor in the ANCOVA model was species (three levels: brook silverside, threadfin shad and gizzard shad)

[b] TL, total length

[c] BS = brook silverside, TS = threadfin shad, GS = gizzard shad

Figure captions

Figure 1 – Relationship between (A) planktivorous fish total length and (B) planktivorous fish age and total mercury concentration in epaxial muscle (ng $g_{wet\ weight}^{-1}$).

Figure 2 – Relationship between (A) planktivorous fish trophic position and total length and (B) and planktivorous fish trophic position and total mercury concentration in epaxial muscle (ng $g_{wet\ weight}^{-1}$).

Figure 3 – Relationship between planktivorous fish total length and planktivorous fish age.

Figure 4 – Relationship between (A) planktivorous fish $\delta^{13}C$ and total length and (B) planktivorous fish $\delta^{13}C$ values and total mercury concentration in epaxial muscle (ng $g_{wet\ weight}^{-1}$).

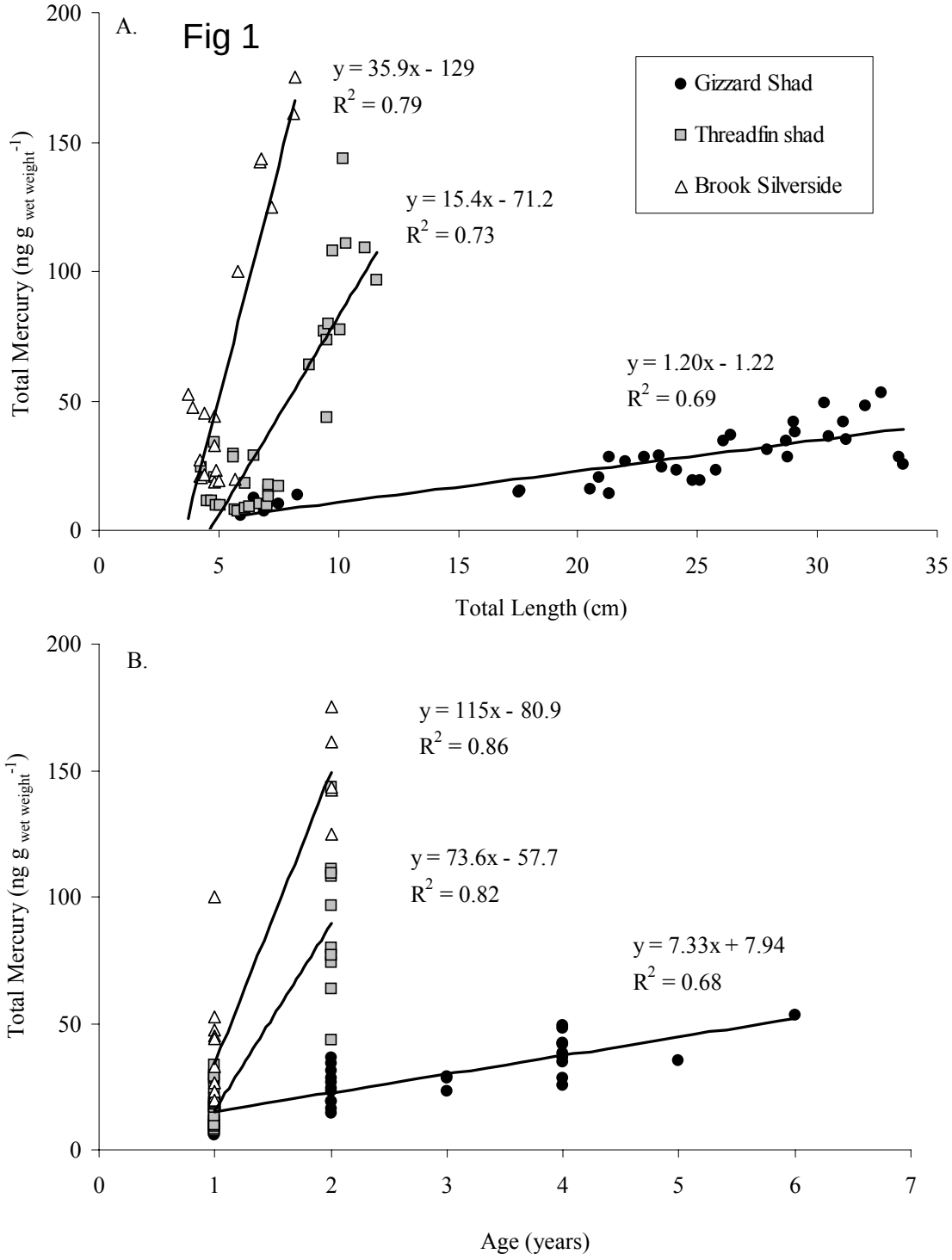

A.
Fig 1
y = 35.9x - 129
R2 = 0.79
y = 15.4x - 71.2
R2 = 0.73
y = 1.20x - 1.22
R2 = 0.69
Gizzard Shad
Threadfin shad
Brook Silverside
Total Mercury (ng g wet weight-1)
Total Length (cm)
B.
y = 115x - 80.9
R2 = 0.86
y = 73.6x - 57.7
R2 = 0.82
y = 7.33x + 7.94
R2 = 0.68
Total Mercury (ng g wet weight-1)
Age (years)

Fig 2

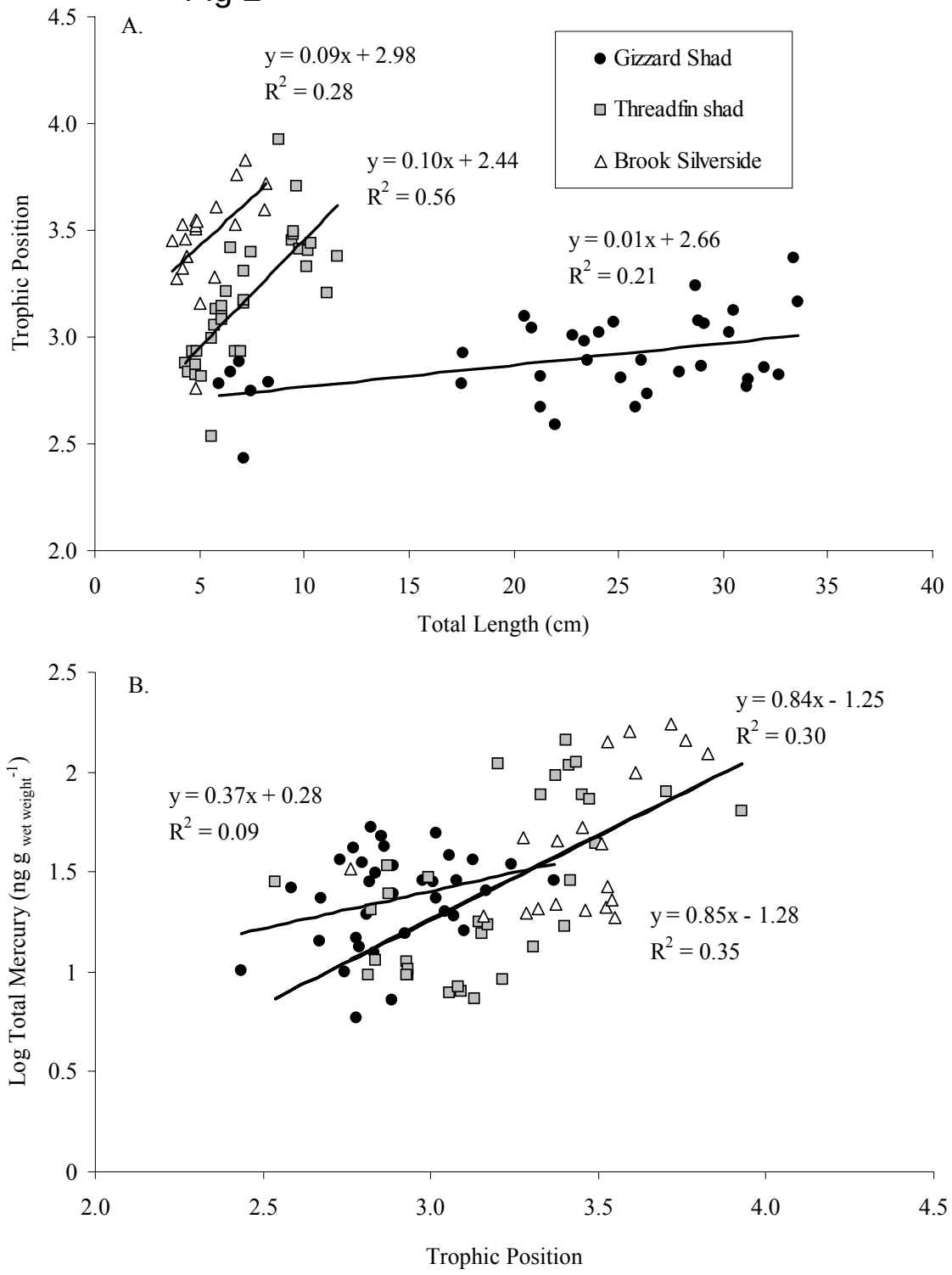

A.
y = 0.09x + 2.98
R2 = 0.28
y = 0.10x + 2.44
R2 = 0.56
y = 0.01x + 2.66
R2 = 0.21
Gizzard Shad
Threadfin shad
Brook Silverside
Trophic Position
Total Length (cm)
B.
y = 0.84x - 1.25
R2 = 0.30
y = 0.37x + 0.28
R2 = 0.09
y = 0.85x - 1.28
R2 = 0.35
Log Total Mercury (ng g wet weight-1)
Trophic Position

Fig 3

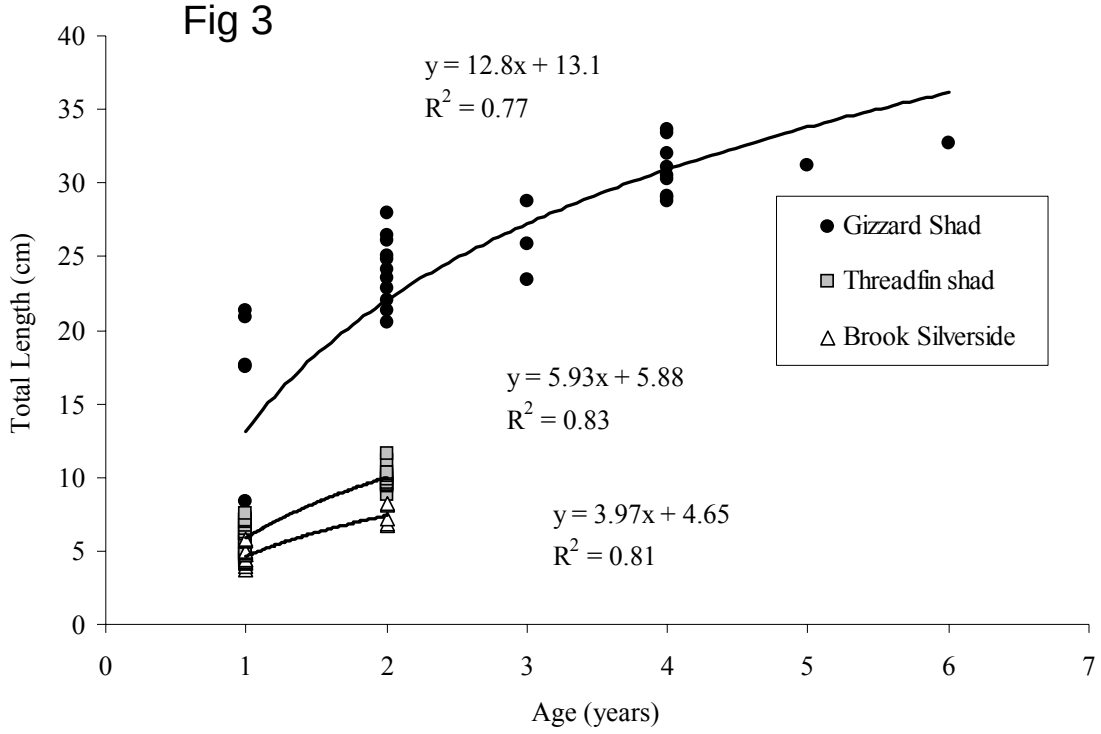
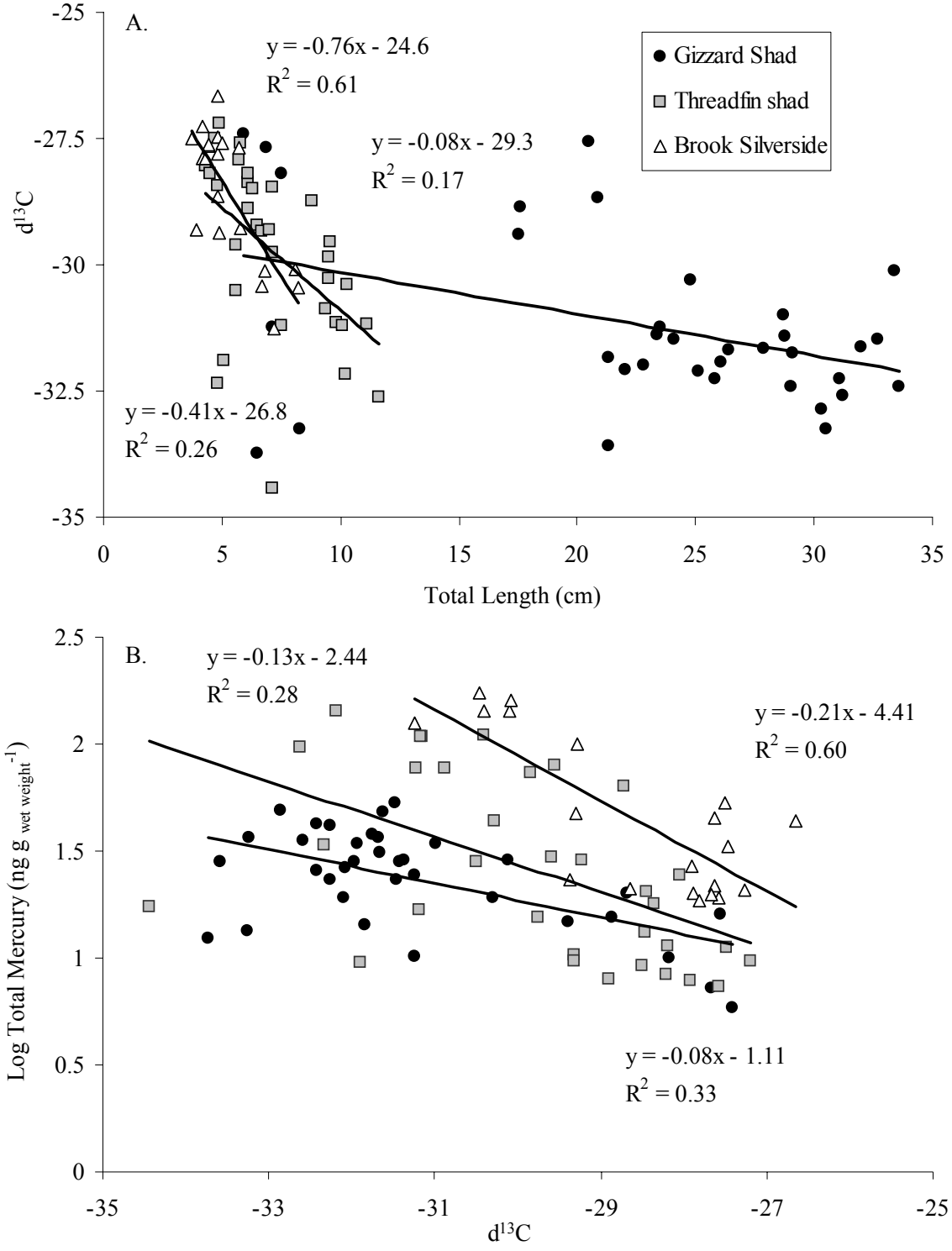

y = 12.8x + 13.1
R2 = 0.77
y = 5.93x + 5.88
R2 = 0.83
y = 3.97x + 4.65
R2 = 0.81
Gizzard Shad
Threadfin shad
Brook Silverside
Total Length (cm)
Age (years)
0
5
10
15
20
25
30
35
40
0
1
2
3
4
5
6
7

Fig 4


A.
y = -0.76x - 24.6
R2 = 0.61
y = -0.08x - 29.3
R2 = 0.17
y = -0.41x - 26.8
R2 = 0.26
Gizzard Shad
Threadfin shad
Brook Silverside
d13C
Total Length (cm)
B.
y = -0.13x - 2.44
R2 = 0.28
y = -0.21x - 4.41
R2 = 0.60
y = -0.08x - 1.11
R2 = 0.33
Log Total Mercury (ng g wet weight-1)
d13C

Chapter 4: Comparison of total mercury estimates from wet and dry fish tissues

Matthew M. Chumchal and Brian Fry

Formatted for Water, Air, and Soil Pollution

Abstract

United States Environmental Protection Agency protocols for analyzing total mercury concentration in fish tissue recommend using wet tissues, but in many cases, it would be advantageous to use dried tissues. This study compared total mercury concentration from wet and dry tissues, using 30 individual fish representing 11 freshwater and estuarine species. After correcting for water content, estimates of mercury concentrations from dry tissue were not significantly different from estimates of mercury concentrations from wet tissue. Variation in estimates of mercury concentration from wet and dry tissues was random and was not related to the level of contamination of the tissue. Our data indicate that dried fish tissues are suitable for estimating mercury concentration, and give results equivalent to those of wet tissues.

Introduction

Methylmercury is a highly toxic pollutant (NRC, 2001) that bioaccumulates in fish (Bowles *et al*., 2001; Power *et al*., 2002). Total mercury concentration is used as a proxy for methylmercury concentration in fish because 95-99% of mercury in fish tissue is in the form methylmercury (Bloom, 1992). The United States Environmental Protection Agency (USEPA) recommends using wet tissue to determine total mercury concentration in fish (USEPA, 2000). Cizdziel *et al*. (2002) hypothesized that that deviating from USEPA recommendations by drying tissues may lead to a loss of total mercury and therefore to an underestimation of mercury concentration. However, the analysis of total mercury concentration in dried tissues may be more desirable because water content of wet tissues can be variable due to excess external water or dehydration (Busacker *et al.*, 1990). In addition, unlike wet tissue, dry tissue samples can be stored for long periods of time without decomposition or weight changes. The purpose of this study was to compare the estimated mercury concentration of fish using wet and dry tissues and to determine if dry tissues can be used to accurately estimate mercury concentration in fish.

Materials and methods

Fish collection and analyses

Freshwater and estuarine fish were collected with a boat-mounted electrofishing unit on 10-12 April 2005 from three sites within the Mississippi River and adjacent drainages near New Orleans, LA. We collected 30 individual fish representing 11 species (Table 1). Fish total length was measured to the nearest mm in the field.

Muscle tissue was dissected in the field with a clean stainless-steel knife. A sample of epaxial muscle tissue was dissected from the right and left side of each fish. One sample was used for the wet tissue analysis and the other sample was used for the dry tissue analysis. Tissue samples were placed individually into plastic bags and stored on ice until being transported to the lab and frozen. Tissue samples used in the dry tissue analysis were dried to a constant weight in a 60° C oven (48 hours) and homogenized with a ball-mill grinder (Dentsply International, York, PA).

Total mercury analyses were performed with a direct mercury analyzer (DMA-80, Milestone Inc. Monroe, CT USA) that uses thermal decomposition, gold amalgamation and atomic absorption spectrometry (USEPA, 1998). A calibration curve was generated using three reference materials from the National Research Council of Canada (MESS-3, PACS-2 and DORM-2). Reference samples (National Research Council of Canada, TORT-2) were analyzed each time the mercury analyzer processed 10 consecutive samples (percent recovery =100.4 ± 0.05% (mean ± 95% C.I.)), n = 5). We analyzed 111.9 – 198.5 mg (wet weight [ww]) and 33.3 – 41.4 mg (dry weight [dw]) of wet and dry tissues, respectively.

Estimates of mercury concentrations from dry tissues were corrected for loss of water (i.e., made equivalent to estimates from wet tissues) by multiplying by the dw:ww ratio. The ww of each tissue was determined by immediately weighing the tissue after it was removed from its plastic bag. Water-weight of each tissue was estimated by subtracting dw from ww.

Statistical analyses

To determine whether differences between estimates of mercury concentration from wet and dry tissues ($[Hg]_{wet}$ and $[Hg]_{dry}$, respectively) could be explained by loss of water upon drying, we compared the mercury concentration of water-weight corrected dry tissues ($[Hg]_{dry\text{-}corrected}$) to $[Hg]_{wet}$. We used linear regression analysis to test the null hypothesis that $[Hg]_{wet}$ and $[Hg]_{dry\text{-}corrected}$ were not statistically different (i.e., the relationship between $[Hg]_{wet}$ and $[Hg]_{dry\text{-}corrected}$ would be a line with slope and y-intercept equal to 1 and 0, respectively) (SPSS Inc., version 11.5.0, Chicago, IL).

To determine if the variation between the $[Hg]_{wet}$ and $[Hg]_{dry\text{-}corrected}$ was higher than would be expected based on laboratory analytical error, we compared the average level of disagreement found between $[Hg]_{wet}$ and $[Hg]_{dry\text{-}corrected}$ to the average level of disagreement found between laboratory replicates. Level of disagreement was quantified by taking the absolute difference between two paired replicate samples and dividing by the mean of the pair to obtain a normalized difference or relative percent difference (RPD), where $RPD = |([Hg]_{wet} - [Hg]_{dry\text{-}corrected})|/(([Hg]_{wet} + [Hg]_{dry\text{-}corrected})/2)*100$. We used mean RPDs to compare differences among groups. Specifically, we compared the mean RPD from paired wet and dry-corrected samples to the mean RPD from 30 paired replicate samples of wet tissues and 30 paired replicate samples of dry tissue using independent samples t-tests. These comparisons were made after averaging absolute values of the RPDs in each group. The 60 replicate samples (30 wet and 30 dry) were analyzed as part of routine quality assurance protocols in our lab and are not the same individual fish examined in the other analyses described in this study (i.e., the fish in Table 1). For both wet and dry tissues we chose the 30 most recently analyzed replicate samples. These tissues were collected as part of other studies

but were handled as described above. Mercury concentrations in laboratory replicates ranged from 21.2-1,413 ng·g ww^{-1} for wet tissues and 34.5-2,307 ng·g dw^{-1} for dry tissues.

Finally we tested the null hypothesis that variation between $[Hg]_{dry\text{-}corrected}$ and $[Hg]_{wet}$ was related to the level of mercury contamination of the tissue. To test this hypothesis we regressed the residuals from the relationship between $[Hg]_{dry\text{-}corrected}$ and $[Hg]_{wet}$ against $[Hg]_{wet}$. We used linear regression to test the hypothesis that the slope and y-intercept were equal to 0.

Results and discussion

Estimated mercury concentrations from wet and dry tissues

Estimates of total mercury concentration in muscle tissue ranged from 9.52-568 ng·g ww^{-1} in wet tissues and 41.7-2,698 ng·g dw^{-1} in dry tissues, with 4.78 ± 0.36 times higher concentrations observed in dry tissues. This difference was expected because drying reduces the weight of the tissue through water loss, effectively concentrating the mercury per unit remaining weight. The water content of fish tissues, as estimated from water loss upon drying, was 78.6 ± 0.56% and was relatively uniform between samples (Table 1). To compare estimates of total mercury concentration from wet and dry tissues, we corrected the mercury concentration of dry tissue for loss of water. When we corrected for differences in water weight, estimates of mercury from wet and dry tissues showed 1-to-1 correspondence (Fig. 1) (i.e,. the slope was not significantly different than 1, $t = 0.42$, $p = 0.68$ and the y-intercept was not significantly different than 0, $t = -0.24$, $p = 0.81$). These data indicate that dried fish tissues are suitable for estimating mercury concentration, and give results equivalent to wet tissues.

Disagreement between $[Hg]_{dry\text{-}corrected}$ and $[Hg]_{wet}$

Despite of the good overall agreement between estimates of mercury concentration from wet and dry tissues, there was considerable residual variation around the regression line (Fig. 1). The RPD between $[Hg]_{dry\text{-}corrected}$ and $[Hg]_{wet}$ was approximately 7 times higher than the RPD between wet tissue and dry tissue laboratory replicates (Table 2, $t = 4.79$, $p < 0.001$ and $t = 4.88$, $p < 0.001$, respectively). The RPD between replicate wet tissue samples was not significantly different from the RPD between replicate dry tissue samples (Table II, $t = -0.57$, $p = 0.51$). These data indicate that the variation between $[Hg]_{dry\text{-}corrected}$ and $[Hg]_{wet}$ is greater than the variation that would be expected due to laboratory error alone.

Effect of level of mercury contamination on estimation of mercury concentration

We determined if the residual variation from Fig 1 (residuals from $[Hg]_{dry\text{-}corrected}$ vs $[Hg]_{wet}$) was related to the level of contamination of the sample measured as $[Hg]_{wet}$. The slope and y-intercept of the relationship between the residual variation from Fig 1 and $[Hg]_{wet}$ were not statistically different from 0 (slope: $t \leq 0.001$, $p = 1$; y-intercept: $t \leq 0.001$, $p = 1$). On a percentage basis, with $[Hg]_{wet}$ set at 100%, the $[Hg]_{dry\text{-}corrected}$ was both higher and lower than the $[Hg]_{wet}$ in a random fashion (Table I). These data indicate that the disagreement between $[Hg]_{dry\text{-}corrected}$ and $[Hg]_{wet}$ was not related to the concentration of total mercury in the samples.

The random disagreement between estimates of mercury concentration from wet and dry tissues may be due to variability in mercury concentrations within individual fish. Cizdziel *et al.* (2002) found relatively high levels of within-fillet variation in mercury concentration with relative standard deviations (RSD = [Standard deviation /

mean] *100) of 7.2, 7.6, 11 and 6.3 from samples of striped bass, largemouth bass, bluegill, and rainbow trout, respectively (data from their Table I). Our samples were dissected from different sides of the same fish, and disagreements between $[Hg]_{dry\text{-}corrected}$ and $[Hg]_{wet}$ (RSD = 8.56 ± 3.23) were similar to those found by Cizdziel et al. (2002) and may reflect fillet-to-fillet variation in fish mercury concentrations. These findings suggest that in future studies estimates of fillet mercury concentrations could be improved by combining tissues from several pieces of fillet tissue, rather than just sampling a small amount of tissue from a large fillet.

Acknowledgements

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Table 1. Mercury (Hg) concentration (expressed as $ng \cdot g^{-1}$), % water weight of wet tissues, and % difference between mercury concentrations estimated from wet and water corrected dry tissues (wet tissue set as 100%). TL = total length of fish

Species	TL(mm)	% water weight	[Hg] wet tissue	Water corrected [Hg] in dry tissue	% difference
Bigmouth buffalo (*Ictiobus cyprinellus*)	660	79.1	128	164	-28.1
Blue catfish (*Ictalurus furcatus*)	740	77.5	18.7	19.5	-4.28
	845	79.6	70.6	70.9	-0.42
Bluegill (*Lepomis macrochirus*)	157	78.9	101	107	-5.94
	181	77.4	231	211	8.66
Common carp (*Cyprinus carpio*)	436	77.8	26.3	23.7	9.89
	595	77.5	30.0	30.8	-2.67
	618	80.1	41.0	32.5	20.7
Freshwater drum (*Aplodinotus grunniens*)	356	80.0	38.9	71.5	-83.8
	468	81.5	79.9	96.3	-20.5

Gizzard shad (*Dorosoma cepedianum*)	368	72.9	22.5	15.7	30.2
Largemouth bass (*Micropterus salmoides*)	197	78.9	227	164	27.8
	207	78.6	178	169	5.06
	229	78.4	199	175	12.1
	238	76.7	192	173	9.90
	283	78.4	414	394	4.83
	292	80.2	280	283	-1.07
	314	78.7	396	442	-11.6
	322	79.2	378	451	-19.3
Striped mullet (*Mugil cephalus*)	186	79.5	10.1	9.02	10.7
	350	76.7	9.52	11.0	-15.5
Red drum (*Sciaenops ocellatus*)	585	77.5	158	157	0.63
	592	80.2	273	285	-4.40
Smallmouth buffalo (*Ictiobus*	510	80.5	30.2	33.9	-12.3

bubalus)					
Spotted gar (*Lepisosteus*	380	79.2	122	126	-3.28
oculatus)					
	503	79.1	314	263	16.2
	504	79.7	326	306	6.13
	554	78.3	373	383	-2.68
	579	78.3	378	378	0
	808	78.1	568	586	-3.17

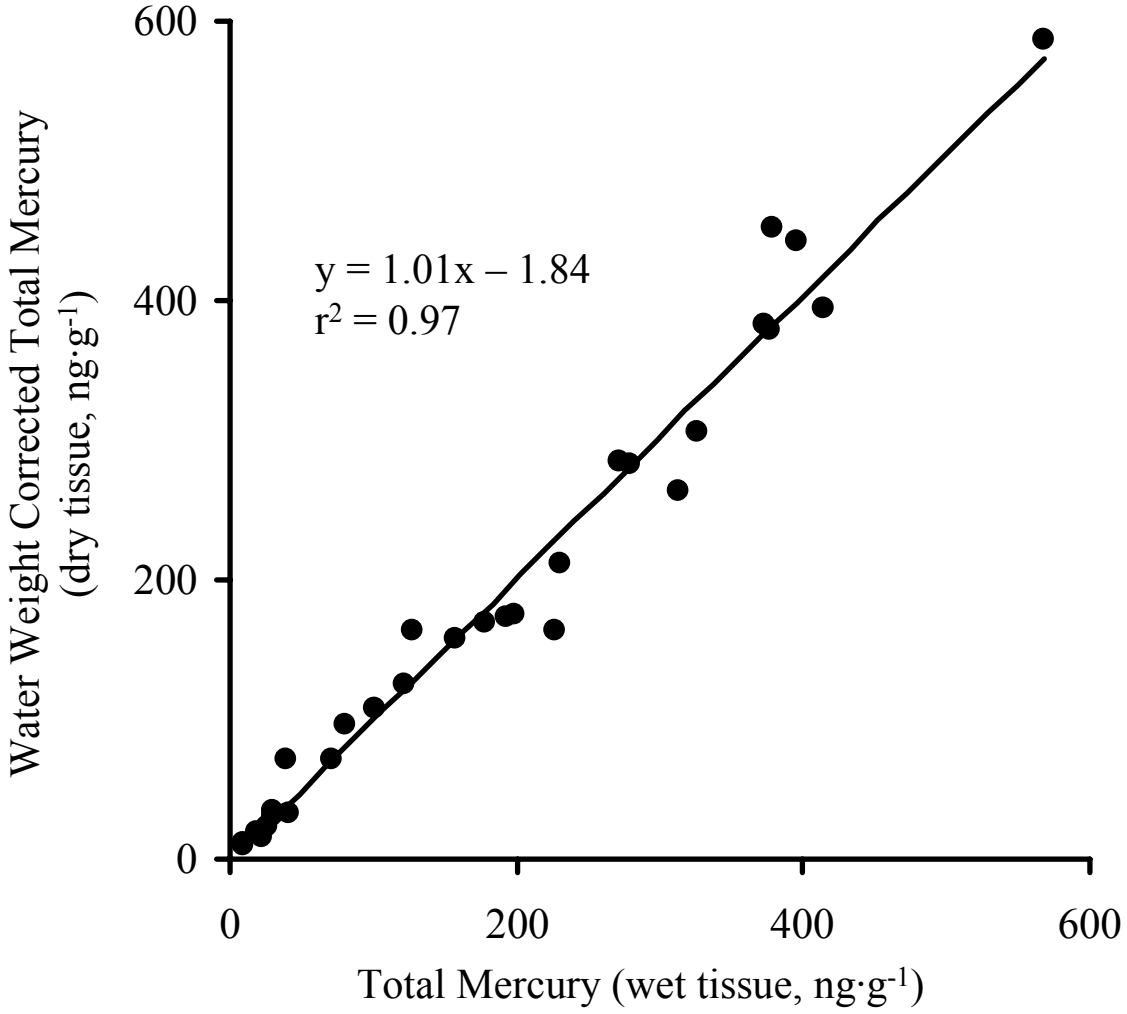
Table 2. Relative percent difference (RPD) between mercury analyses. CI = 95% confidence interval, * denotes values that were not statistically different.

Comparison	**RPD ± CI**
$[Hg]_{dry\text{-}corrected}$ vs $[Hg]_{wet}$	21.9 ± 7.89
[Hg] lab $replicates_{dry}$	2.91 ± 3.25*
[Hg]lab $replicates_{wet}$	3.21 ± 3.01*

Figure captions

Figure 1. Relationship between estimates of mercury concentration from wet tissue and water weight corrected dry tissues.

Figure 1.


600
400
200
0
0
200
400
600
y = 1.01x – 1.84
r2 = 0.97
Water Weight Corrected Total Mercury
(dry tissue, ng·g-1)
Total Mercury (wet tissue, ng·g-1)