Factors influencing mercury accumulation in three species of forage fish from Caddo Lake, Texas, USA

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Abstract

Most studies that have examined mercury (Hg) contamination of fish have focused on game species feeding near the top of the food web, while studies that examine forage fish that feed near the base of the food web are rare. We conducted a survey of Hg contamination in three species of forage fish, brook silverside (*Labidesthes sicculus*), threadfin shad (*Dorosoma petenense*) and gizzard shad (*Dorosoma cepedianum*), from Caddo Lake, Texas, USA and found species-specific differences in Hg concentrations. We examined total length, age, trophic position (determined using $\delta^{15}N$), and growth rate of forage fish as factors that could have influenced within- and between-species differences in Hg concentration. Total length and age were the best predictors of within-species differences in Hg concentration. Between-species differences in Hg concentrations were most strongly influenced by trophic position.

Introduction

Mercury (Hg) is a toxic metal that bioaccumulates in fish tissue (NRC, 2000). Much of the within- and between-species variation in Hg concentrations of fish tissue can be explained by biological characteristics of fish. For example, Hg concentrations in fish tissues are positively correlated with fish size and age because Hg binds strongly to muscle tissue and is slowly excreted (the half-life of Hg in fish can be more than one year) (Trudel and Rasmussen, 1997). Trophic position, a continuous measure of vertical food web level, is a strong predictor of Hg concentrations in fish tissues and is one of the most important factors determining between-species differences in Hg concentration (Chumchal and Hambright, 2009). Hg concentrations are often inversely related to growth rate because fish with fast growth rates are thought to experience lower cumulative Hg uptake than those with slow growth rates (Swanson et al., 2003).

Most studies that have examined Hg contamination in fish have focused on game species feeding near the top of the food web while studies that examine forage fish that feed near the base of the food web are rare (but see Swanson et al., 2003, 2006). Here we present a field survey of Hg concentration in three species of low trophic level forage fish from Caddo Lake, Texas, USA. We examined total length (TL), age, trophic position (determined using $\delta^{15}N$), and growth rate of forage fish as factors that could

have influenced within- and between-species differences in Hg concentration.

1 Materials and methods

Caddo Lake is a large (107 km² in surface area), shallow (average depth is 1.4 m) lake (Van Kley and Hine, 1998, Ensminger, 1999) located on the Texas-Louisiana (USA) border. The lake receives inflows from Big Cypress Creek in Texas and Hunts Creek in Louisiana, which enter at the western and northern ends, respectively, and numerous other smaller tributaries (Ensminger, 1999). 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 central portion of Caddo Lake. The area of collection is primarily open-water habitat, even though submerged vegetation may be extensive in summer months. We collected brook silversides ($n = 20$), threadfin shad ($n = 33$), and gizzard shad ($n = 35$) because they are some of the most abundant forage fish species in open-water areas of Caddo Lake. Although each of these species consume plankton, they rely on other food items such as insects and detritus to varying degrees. Brook silversides feed primarily on zooplankton and insects (Mullan et al., 1968, Zimmerman, 1970). Threadfin shad and gizzard shad consume phytoplankton, zooplankton and detritus, with detritus playing a larger role in the diet of gizzard shad than threadfin shad (Baker et al., 1971, Miranda and Gu, 1998). None of the species collected in this study are consumed

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by humans. All species reproduce naturally in the lake and are not stocked. After collection, we placed fish on ice and transported them to a lab where we measured TL and removed otoliths. We then froze fish prior to Hg and stable isotope analyses.

We analyzed total Hg concentrations (ng/g wet weight) in epaxial muscle tissues using a direct Hg analyzer (DMA-80, Milestone Inc., USA) that uses combustion atomic absorption spectrometry (US EPA, 1998). For Hg analyses, we generated a calibration curve using three reference materials from the National Research Council of Canada Institute for National Measurement Standards: MESS-3, PACS-2 and DORM-2. We analyzed reference samples of MESS-3 approximately every 10 samples and the average percent recovery was 101% ± 0.96%, (range = 93%–107%; $n = 34$). We analyzed duplicate samples approximately every 20 samples and the average relative percent difference was 3.8% ± 1.4% (range = 0.1%–9.2%; $n = 13$).

Stable isotope ratios of nitrogen and carbon in fish can be used to infer trophic relationships when interpreted relative to isotope ratios in primary consumers. We collected a gastropod and unionid mussels as representative primary consumers (Post et al., 2000). The gastropod and unionid mussels were collected by hand or with a dip-net ($n = 1$ and 6, respectively) on 29 June 2005. After collection, primary consumers were frozen until processing for stable isotope analyses.

Stable nitrogen and carbon isotope ratios in forage fish tissues and primary consumers were used to estimate forage fish trophic position. 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). ^{13}C and ^{12}C can be used to distinguish between littoral and pelagic sources of carbon which have distinct carbon isotope signatures (Hecky and Hesslein, 1995). Because primary consumers that use littoral sources of carbon are less enriched in ^{15}N than organisms that use pelagic sources of carbon (Post, 2002), we used a gastropod and unionid mussels as representatives of littoral and pelagic primary consumers, respectively (Post et al., 2000; Post, 2002).

Forage fish epaxial muscle subsamples and foot muscle from gastropods and unionid clams were dried in a 60°C oven and homogenized using a ball mill grinder. Tissues were analyzed at the University of California-Davis stable isotope facility using a continuous flow isotope ratio mass spectrometer (Europa Hydra 20/20, PDZ Europa, UK). 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:

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

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

To calculate trophic position of individual fish, $\delta^{15}N$ values in fish were first corrected for differences in basal $\delta^{15}N$ using $\delta^{15}N$ and $\delta^{13}C$ values of gastropods and unionid mussels according to the method of Post (2002). We then calculated trophic position for individual fish from corrected $\delta^{15}N$ values assuming an increase of 3.4‰ per trophic level (Post, 2002). A detailed description of methods used to calculate trophic position values for fish appear in a previous study (Chumchal et al., 2008).

We used otolith annuli to estimate the age of each forage fish. We examined annuli on whole otoliths from brook silversides, threadfin shad and small gizzard shad. For large gizzard shad, we broke otoliths 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 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 instantaneous growth rate as TL divided by age.

We used a series of analysis of covariance (ANCOVA) (SPSS Inc., version 11.5.0, USA) models to explore the relationships between Hg concentration, TL, age, and trophic position. Specifically we examined the effect of species (independent variable) and TL, age, or trophic position (covariates) on Hg concentration, trophic position, or age (dependent variable). In ANCOVA models, regression analysis is used to remove the covariate's effect from the unexplained variability in the dependent variable. The final ANCOVA tests the difference between treatment averages adjusted for the effect of the covariate (Quinn and Keough, 2002). When the slopes of the relationships between the covariate and dependent variable were homogeneous between species (i.e., species x covariate = $p > 0.05$), we removed the interaction term from the model and tested for main effects of fish species and the covariate. If the slopes of the covariate and the dependent variable were not homogenous we tested for main effects of fish species and the covariate with the interaction term included in the model. When the slopes were not homogeneous we also performed the Wilcox procedure (Quinn and Keough, 2002). This test determines the range of the covariate for which there was a significant effect of fish species (WILCOX, version 3.2, Constable, 1989).

We combined data collected in this study with a previous study from Caddo Lake (Chumchal and Hambright, 2009) and used linear regression to determine the relationship between log-transformed Hg concentration, trophic position, and growth rate. We also used ANCOVA to determine if the slope of the relationship between Hg and trophic position for the fish community changed after including data from forage fish examined in this study. The previous study included spotted gar (*Lepisosteus osseus*), gizzard shad, channel catfish (*Ictalurus punctatus*), chain pickerel (*Exos niger*), white bass (*Morone chrysops*), yellow bass (*M. mississippiensis*), bluegill (*Lepomis macrochirus*), redear

sunfish (*L. microlophus*), largemouth bass (*Micropterus salmoides*), and freshwater drum (*Aplodinotus grunniens*). Hg (dependent variable) was log_{10}-transformed in the models that included trophic position as a covariate to meet model assumptions of linearity. The significance level was 0.05 for all analyses.

2 Results and discussion

Average Hg concentrations were highest in brook silversides and lowest in gizzard shad (Table 1). Within each species, Hg concentration was significantly and positively correlated with both total length and age (ANCOVA: $p <$ 0.001) (Fig. 1), a finding that is consistent with studies focused on game fish (e.g., Weiner et al., 2003, Chumchal and Hambright, 2009). Brook silversides had significantly higher length and age-adjusted concentrations of Hg than

both threadfin and gizzard shad, and threadfin shad had significantly higher length and age-adjusted concentrations of Hg than gizzard shad (Wilcox Procedure: $p < 0.05$; Fig. 1).

Brook silversides had the highest average trophic position while gizzard shad had the lowest average trophic position (Table 1). Within each species, trophic position was positively correlated with total length (ANCOVA: $p <$ 0.001) (Fig. 2a). Brook silversides had significantly higher length-adjusted trophic positions than both threadfin shad and gizzard shad and threadfin shad had significantly higher length-adjusted trophic positions than gizzard shad (Wilcox Procedure: $p < 0.05$) (Fig. 2a). Trophic position differences were likely caused by differences in food habits. Although all three species feed on plankton (gizzard shad only as juveniles) they have unique diets (Goldstein and Simon, 1999) with each species utilizing different proportions of animal plankton in their diet. Brook silversides are the most dependent on animal plankton while gizzard shad are the least, instead consuming detritus (diets are reviewed by Hassan-Williams et al., 2009). These differences in food habits are consistent with the variation in trophic position observed in this study in which brook silversides had the highest trophic position and gizzard shad had the lowest trophic position.

log-Transformed Hg concentrations in fish were positively correlated with trophic position (ANCOVA: $p <$ 0.001) (Fig. 2b) but the amount of variance in Hg explained

Table 1 Average (± 95% CI) characteristics of forage fish collected from Caddo Lake

	Brook silverside	Threadfin shad	Gizzard shad
Mercury (ng/g wet weight)	63.0 ± 25.8	40.4 ± 13.8	26.4 ± 4.2
Total length (cm)	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 (cm/yr)	4.4 ± 0.3	5.6 ± 0.3	10.3 ± 1.4
Trophic position	3.4 ± 0.1	3.2 ± 0.1	3.0 ± 0.1
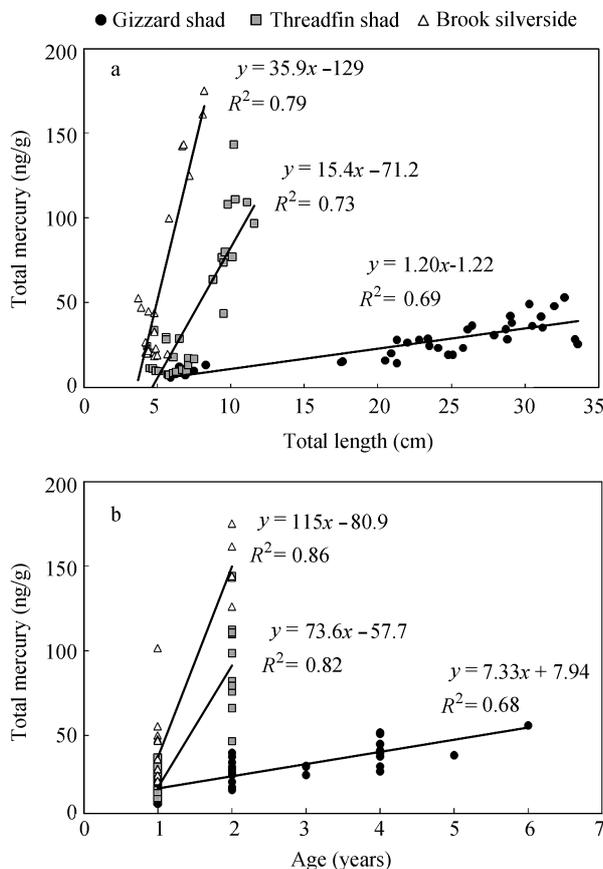
Fig. 1 Relationship between forage fish total length and total Hg concentration in epaxial muscle (a), and age and total Hg concentration (b).
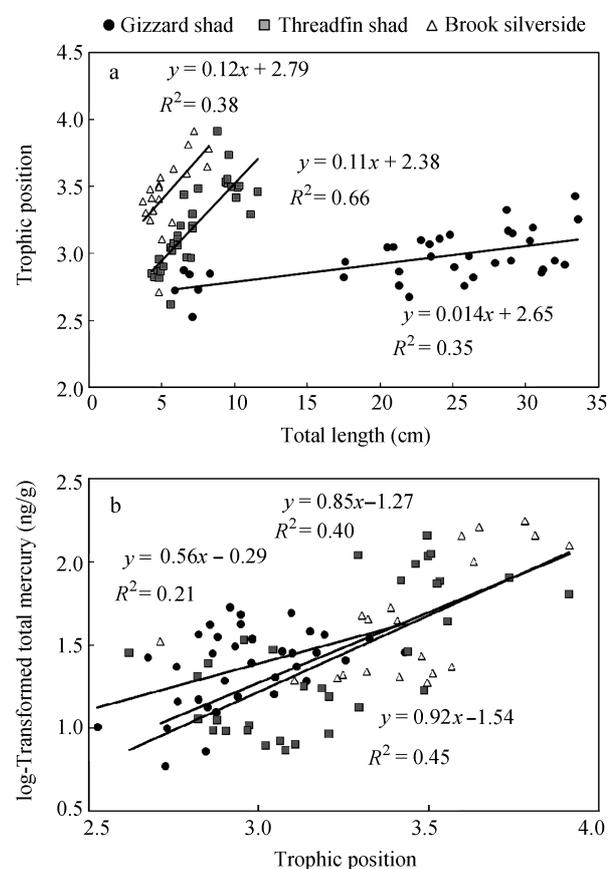
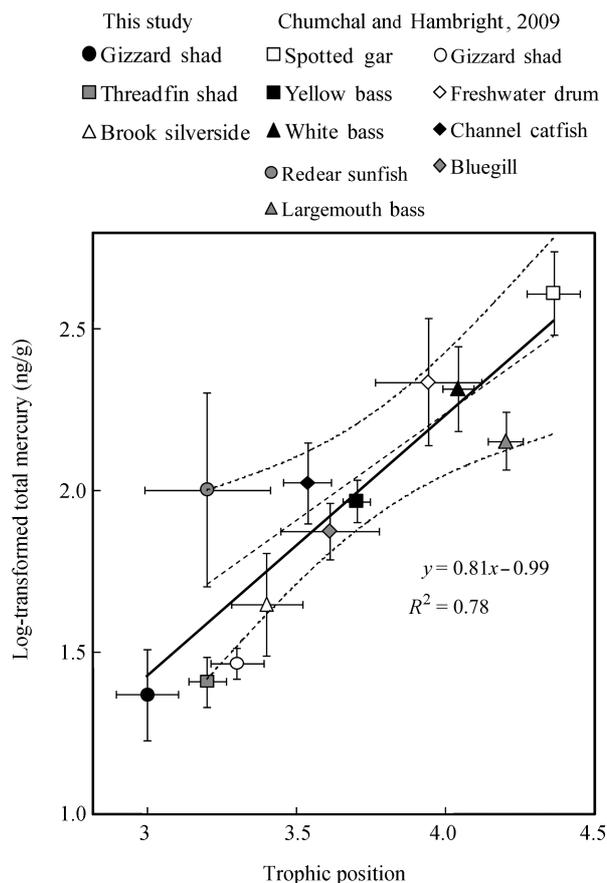**Fig. 2** Relationship between forage fish total length and trophic position (a) and trophic position and log-transformed total mercury concentration in epaxial muscle (b).

by trophic position was lower than that explained by total length or age. To examine the effect of trophic position on Hg concentration relative to total length, we added total length as a second covariate to the model that used trophic position to predict log-transformed Hg concentration in fish. When total length was added to the model, the main effect of trophic position on Hg was no longer significant (ANCOVA: $df = 1{,}81$; $f = 0.29$; $p = 0.60$), indicating that trophic position did not have additional explanatory power after accounting for fish total length. In a previous study at Caddo Lake that included predatory game fish species (Chumchal and Hambright, 2009), we found that age and total length were the best predictors of within-species differences in Hg but that trophic position was generally a poor predictor, as found in this study.

Although trophic position was not a good predictor of within-species variation in Hg concentration, Hg is a biomagnifying contaminant and trophic position is an important determinant of between-species variation in Hg concentration (e.g., Atwell et al., 1998; Campbell et al., 2008; Chumchal and Hambright, 2009). In our study, brook silversides had the highest average trophic position and the highest average Hg concentration while gizzard shad had the lowest average trophic position and lowest average Hg concentration. Thus, trophic position may influence between-species differences in Hg concentrations of fish examined in this study.

To elucidate the potential role of trophic position in regulating Hg concentration between species, we combined data from this study with data from a previous study on Caddo Lake fish (Chumchal and Hambright, 2009) that included game species. We found a strong positive relationship between log-transformed Hg concentration and trophic position, with trophic position explaining 78% of the variation in log-transformed Hg concentration (Fig. 3). Adding data from the forage fish species examined in this study did not have a significant effect on the slope of the relationship between log-transformed Hg concentration and trophic position (ANCOVA: $df = 1{,}17$; $f = 0.42$; $p = 0.53$) (Fig. 3), indicating that the concentration of Hg in forage fish could be predicted using the Hg-trophic position relationship previously established for other species (Chumchal and Hambright, 2009). Swanson et al. (2003, 2006) examined Hg concentrations in forage fish from boreal lakes and hypothesized that trophic differentiation within the forage fish communities may not result in increased contaminant accumulation. Our results do not support the hypothesis by Swanson et al. (2003, 2006); rather, they indicate that the relationship between trophic position and Hg concentration in forage fish from Caddo Lake are similar to that observed in studies that focused on game and predatory species (e.g., Atwell et al., 1998; Campbell et al., 2008; Chumchal and Hambright, 2009).

Growth rate is hypothesized to be an important determinant of Hg concentration in forage fish (Swanson et al., 2003, 2006) with Hg predicted to be inversely correlated with growth rate (i.e., the growth dilution hypothesis, Stafford and Haines, 2001). The average growth rate of brook silverside was 1.3 times slower than the

Fig. 3 Relationship between average trophic position and average log-transformed mercury concentration for forage fish examined in this study and nine species previously studied (Chumchal and Hambright, 2009). Error bars are 95% confidence intervals. The solid line is the average for fish from both studies and the dashed lines are average and 95% confidence limits from the fish collected as part of the previous study only.

average growth rate of threadfin shad and 2.3 times slower than the average growth rate of gizzard shad (Table 1). Brook silverside were significantly smaller than similar-aged threadfin and gizzard shad while threadfin shad were significantly smaller than similar-aged gizzard shad (ANCOVA: $df = 2{,}84$; $f = 81.7$; $p < 0.001$). In this study brook silversides had the slowest average growth rate and the highest average Hg concentration while gizzard shad had the fastest average growth rate and the lowest average Hg concentration; a pattern that is consistent with predictions of the growth dilution hypothesis.

To examine the effect of growth rate on Hg concentration relative to trophic position, we added growth rate as a second covariate to the model that used trophic position to predict log-transformed Hg concentration in fish collected during this study and the previous study. When growth rate was added to this model, it did not have a significant effect on Hg concentration (ANCOVA: $df = 1{,}9$; $f = 0.03$; $p = 0.88$). These data indicate that differences in trophic position are primarily responsible for differences in Hg concentration among fish species in Caddo Lake.

Our finding that trophic position had a larger impact on Hg concentration than growth rate of fish is consistent with modeling studies. Stafford and Haines (2001)

compared the effect of changes in growth rate to changes in dietary Hg levels on Hg concentrations in fish tissue. They determined that Hg concentrations in fish tissue were relatively unresponsive to changes in growth but strongly responsive to changes in dietary Hg levels, a result that was in agreement with a modeling study by Harris and Bodaly (1998). Stafford and Haines (2001) concluded that dietary variability could easily obscure growth dilution of Hg.

The finding that forage fish from Caddo Lake have different average concentrations of Hg has implications for fisheries management. Brook silverside and gizzard and threadfin shad, are frequently introduced, intentionally and unintentionally, into aquatic ecosystems in the southern United States (Lee, 1980; Paller, 2005; Vatland and Budy, 2007; Eagles-Smith et al., 2008). A shift in dominance of forage fish species could lead to a change in Hg concentration of predatory fish via change in Hg concentrations of the forage base. Thus, Hg concentrations in predatory game fish could be affected by the introduction of an exotic forage fish and the Hg concentrations of game fish should be monitored closely following changes in the forage fish community, even if the exotic forage species is considered to be trophically similar to the native species.

3 Conclusions

In general, Hg contamination in forage fish has not been well studied. In this study we found that the same ecological factors that predict Hg concentration in game fish can also predict Hg concentrations in forage fish. Specifically, we found that within-species differences in Hg concentration are best explained by TL or age. Between-species differences in Hg concentrations were most strongly influenced by trophic position. Average Hg concentrations in the three species of forage fish examined in this study differed by a factor of almost three. This has important implications for fisheries management because a shift in the dominance of forage species could result in changes in the Hg concentration in predatory game fish.

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