HABITAT-SPECIFIC DIFFERENCES IN MERCURY CONCENTRATIONS OF MISSISSIPPI GRASS SHRIMP FROM CADDO LAKE, TEXAS

by

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Thanks to Katie Burgess for drawing the map of Caddo Lake (Fig. 1).

None of this would have been possible without the loving support of my wife April. This is dedicated to her and to our daughter Alara Rose.

TABLE OF CONTENTS

LIST OF FIGURES

Introduction

Methylmercury is a potent neurotoxin that concentrates in the tissues of aquatic organisms (Wiener 2003). In the United States, atmospheric mercury (Hg) deposition is 3-4 times higher than it was prior to the Industrial Revolution (Swain et al. 1992). The primary source of inorganic Hg emissions to the atmosphere is coal-burning power plants (Pacyna et al. 2002). Inorganic Hg in the atmosphere is eventually deposited onto the surface of the earth and subsequently converted to highly toxic methylmercury (MeHg) in aquatic ecosystems by sulfate-reducing bacteria (Morel et al. 1998; Ullrich 2001). At a regional scale some habitats, such as wetlands, have been identified as hotspots of MeHg availability because they have conditions that favor MeHg production (Driscoll et al. 2007). At the base of the food web, phytoplankton and periphyton directly absorb MeHg from the water (Miles 2001). Consumers are exposed to MeHg through their diet (Hall 1997).

Caddo Lake, located on the border of northern Texas and Louisiana (Fig. 1), is a shallow reservoir that contains multiple habitat types and is contaminated with Hg. Water flows from a river, Big Cypress Bayou, into a forested-wetland (hereafter wetland) which forms the western end of Caddo Lake before flowing into an open-water zone on the eastern end of the lake. Fish, snakes and other aquatic biota from Caddo Lake have concentrations of Hg high enough that they may be adversely affected or put higher level consumers, including humans at risk (TDSHS 1995; Rainwater et al. 2005; Chumchal et al. 2008). Mercury contamination in Caddo Lake is of particular concern because the lake supports high biodiversity, including rare and threatened species, and provides important habitat for migratory birds (Caddo Lake Institute, 2008). Chumchal et al. (2008)

examined mercury concentrations in largemouth bass (*Micropterus salmoides*) and Mississippi grass shrimp (*Palaemonetes kadiakensis*) from a small area in the southeastern portion of the wetlands and found that these organisms had higher concentrations of Hg than organisms collected from the open-water.

Spatial variation in Hg contamination at the ecosystem scale, like that found in Caddo Lake (Chumchal et al., 2008), has rarely been examined (Munn and Short 1997; Cizdziel et al. 2002a; Campbell et al. 2003; Burger et al. 2004; Stafford et al. 2004; Simoneau et al. 2005). Assessing spatial variation in the Hg levels of a particular area requires an appropriate bioindicator species. Short-lived, low trophic level consumers are ideal indicators of MeHg availability (Lindqvist et al., 1991) because they are less affected by biomagnification and time-related bioaccumulation than longer lived, higher trophic level consumers. Consumers that exhibit site-fidelity reflect the level of Hg contamination in a given location (Bank et al. 2007). Grass shrimp are common in Caddo Lake and may by ideally suited for assessing spatial variation in MeHg levels because they feed near the base of the food web and exhibit limited mobility (Key et al. 2006). Because grass shrimp are short lived, they also can provide insight into annual variation in Hg concentrations (Key et al. 2006).

Methylmercury bioaccumulates in the tissues of organisms. However, total Hg (organic and inorganic forms of Hg) is often measured as a proxy for MeHg (e.g. Chumchal et al 2008) because most of the Hg in higher level consumers, like fish, is MeHg (> 95%, Bloom 1992) and some methods of total Hg (THg) analyses are less labor intensive than MeHg analysis (Cizdziel et al. 2002b). In invertebrates, the percentage of MeHg ranges from 30 - 100% of THg (Tremblay et al. 1998, Hothem et al. 2007). If grass shrimp have a high MeHg:THg ratio that is consistent between habitats then they could be used in biomonitoring studies without the need for MeHg analyses.

In this study I used grass shrimp in Caddo Lake to address the following questions: 1) Did grass shrimp exhibit spatial variation in Hg concentration? 2) If spatial variation existed, was it consistent from 2006 to 2007? 3) What was the MeHg:THg ratio in grass shrimp and was it consistent between habitats?

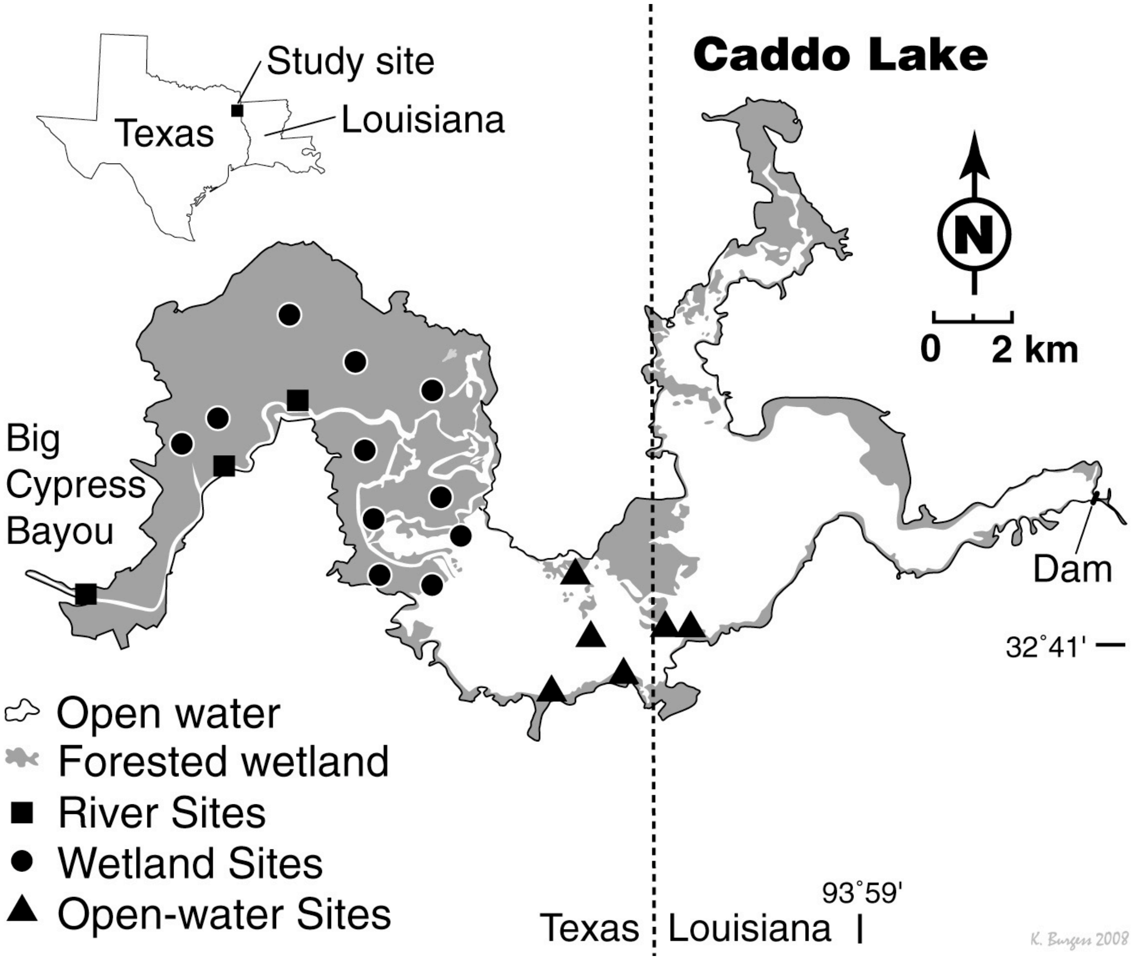

Figure 1. Sampling locations at Caddo Lake.

Methods

Study site

Caddo Lake is approximately 107 km^2 in area (Van Kley and Hines 1998). Big Cypress Bayou, Caddo Lake's primary tributary, is relatively deep in the main channel (6m), and there is high consistent flow (mean flow = 29 m^3/s; max flow ~ 85 m^3/s) (USGS 2008). The western portion of the lake (approx. 40 km^2, and mostly in Texas) is shallow (average depth < 1 m) composed primarily of a forested wetland dominated by bald cypress *Taxodium distichum*, 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 Hines 1998). The wetland is one of the largest in the southern U.S. and has been designated as a Wetland of International Importance by the Ramsar Convention (Van Kley and Hines 1998). The eastern portion of Caddo Lake (mostly in Louisiana) is primarily open-water habitat (average and maximum depths = 1.4 m and 8.2 m, respectively; Ensminger 1999). The eastern portion of the lake is impacted by a small tributary, James' Bayou, which has seasonal flows but is not thought to contribute significant quantities of water to the sites examined in this study (Darville - personal communication). The primary anthropogenic sources of Hg in the region are coal-burning power plants (Crowe 1996; TDSH 1999). Caddo Lake is located within 250 km of five of the 20 highest mercury-emitting power plants in North America (Miller 2004).

Grass Shrimp collection

I collected grass shrimp from 3 sites in the river, 11 in the wetland, and 6 in the open-water habitats of Caddo Lake (total of 20) from May 14-16, 2007 (Fig. 1). I collected replicate samples at each sample site with the exception of one site in the river where only one sample was collected. Each sample consisted of approximately 50 grass shrimp. Grass shrimp were collected with a dip net from vegetation < 1 m below the surface of the water and placed on ice prior to being transported to a lab and frozen. Grass shrimp were later thawed, identified to species and measured for total length (TL) under a dissecting microscope. Whole grass shrimp from each sample site were composited, dried at 65°C for 72 hours, and homogenized with a ball-mill grinder (Dentsply, Inc, York, PA). All lab-ware was rinsed with deionized water and 95% ethanol between samples.

Mercury analysis

Total Hg concentrations in grass shrimp homogenates 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). For THg analysis, a calibration curve was generated using reference materials from the National Research Council of Canada Institute for National Measurement Standards: MESS-3 (marine sediment, certified value = 91 ± 9 $ng/g_{dry\ weight}$ THg (average ± 95% C.I.). Quality assurance included reference and duplicate samples. Reference samples of MESS-3 were analyzed approximately every 10 samples and the mean percent recovery was 94.7 ± 1.4% (range = 93 – 97%; n = 8). Duplicate samples were analyzed approximately every 20 samples and the mean relative percent difference was 1.8 ± 0.9% (range = 0.8 – 2.6%; n = 4). Total Hg is reported as ng THg/$g_{\text{dry weight}}$ of shrimp tissue.

Methylmercury concentrations were analyzed with a Model III Cold Vapor Atomic Fluorescence Spectrophotometer (Brooks Rand, LLC., Seattle, WA) that uses thermal desorption, gas chromatographic (GC) separation, and pyrolytic reduction (USEPA 2001). Approximately 0.5g homogenized dried shrimp was digested in methanolic potassium hydroxide (KOH) (DeWild et al. 2004). Thirty microliters of the digested tissue was ethylated with sodium tetraethyl borate ($NaBEt_4$), and purged with nitrogen gas (N_2) for 20 minutes depositing the extracted Hg on Tenax™ traps. These traps were thermally desorbed using a gas chromatography (GC) column, reduced by pyrolytic column, and detected with cold vapor atomic fluorescence spectrometry (CVAFS) (Bloom 1992; USEPA 2001; DeWild et al. 2004). For MeHg analysis, a calibration curve was generated using dilutions of a certified standard, methylmercury (II) hydroxide (1.0μg/mL, Brooks-Rand, LLC, Seattle). Quality assurance included reference and duplicate samples. Reference samples of DORM-2 (dogfish muscle, certified value = 4,470 ± 320 ng/$g_{\text{dry weight}}$ MeHg) were analyzed approximately every 10 samples and the mean percent recovery was 96.1 ± 15.5% (range = 83 – 114%; n = 11). Duplicate samples were analyzed approximately every 20 samples and the mean relative percent difference was 12.9 ± 14.8% (range = 0.5 – 30%; n = 6). Methylmercury is reported as ng MeHg/$g_{\text{dry weight}}$ of shrimp tissue.

Data analysis

I used a univariate analysis of variance (ANOVA) to determine if there was a main effect of habitat type (three levels: river, wetland, open-water) on THg, MeHg, and the MeHg:THg ratio in grass shrimp (dependent variables). I averaged replicate samples to obtain a single data measure for each site because a nested ANOVA using replicate samples from each site produced the same outcome as the univariate ANOVA. I tested for differences in dependent variables between habitat types using Gabriel's multiple comparisons of means procedure which adjusts for differences in sample sizes among means (Field 2005). I used a linear regression and t-tests to determine if there was annual variation in grass shrimp THg concentrations. Mean THg concentrations from grass shrimp collected from three sites in the open and wetland habitat (total of six sites) in May 2006 (Chumchal et al. 2008) were compared to mean THg concentrations in grass shrimp collected from the same six sites in 2007. To determine if there was a 1:1 relationship between samples collected in 2006 and 2007 I used t-tests to test the null hypotheses that the slope = 1 (H_0:b_1 = 1) and y-intercept = 0 (H_0:b_0 = 0). I also used linear regression to determine the relationship between a sample sites distance from the flow path of Big Cypress Bayou and the THg concentration of grass shrimp collected from that site. Distance to flow path was determined using Google Earth's ruler tool (Google Inc., Mountain View, CA) and shrimp collected from sites in the river (n=3) were not included in this analysis. Finally, I also used linear regression analysis to explore the relationship between THg and MeHg concentration. Variables were tested for normality using Kolmogorov-Smirnov's test and found to meet the assumptions for normality. I used SPSS (v. 14; SPSS Inc., Chicago, IL) for all analyses. Statistical significance was determined at $P < 0.05$ for all analyses.

Although some studies have found a positive correlation between fish length and Hg concentration (e.g. McClain et al. 2006) this has not been true of invertebrates (Hothem et al. 2008; Allen et al. 2005; Chumchal et al. 2008). I found no correlation between dependent variables and TL of grass shrimp, therefore data were not adjusted for TL.

Results and Discussion

I detected a significant main effect of habitat on both THg and MeHg in grass shrimp (ANOVA; df = 2, 17; THg: $F = 8.73$, $P < 0.01$; MeHg: $F = 7.85$, $P < 0.01$). Both THg and MeHg concentrations in Mississippi grass shrimp were significantly ($P < 0.05$) higher in samples collected from the river habitat than samples collected from either the wetland or open-water habitats (Fig. 2). Sample size from the river was low (n=3), so this result should be interpreted with caution. However, given the low variance these three samples are likely to be representative of Hg concentrations of grass shrimp collected in other areas of the river. The concentrations of THg and MeHg were not significantly different in shrimp collected from the wetland and open-water habitats ($P > 0.05$). This result is in contrast to a previous study in which THg concentrations in grass shrimp from the wetland were significantly higher than THg concentrations in grass shrimp from the open-water (Chumchal et al. 2008) and was unexpected because wetlands are hypothesized to be more conducive to Hg methylation than other habitat types (Driscoll 2007). Mercury methylation is high in wetlands because conditions favoring sulfate-reducing bacteria such as low pH, high organic carbon levels, and low dissolved oxygen typically are present (Watras 2005). Habitats where these conditions are not present, such as open-water and riverine, would be expected to have lower methylation potential and thus lower levels of MeHg present in the organisms found there. Below I present two hypotheses to explain why Hg concentrations in grass shrimp were not different between wetland and open water habitats like they were in the previous study.

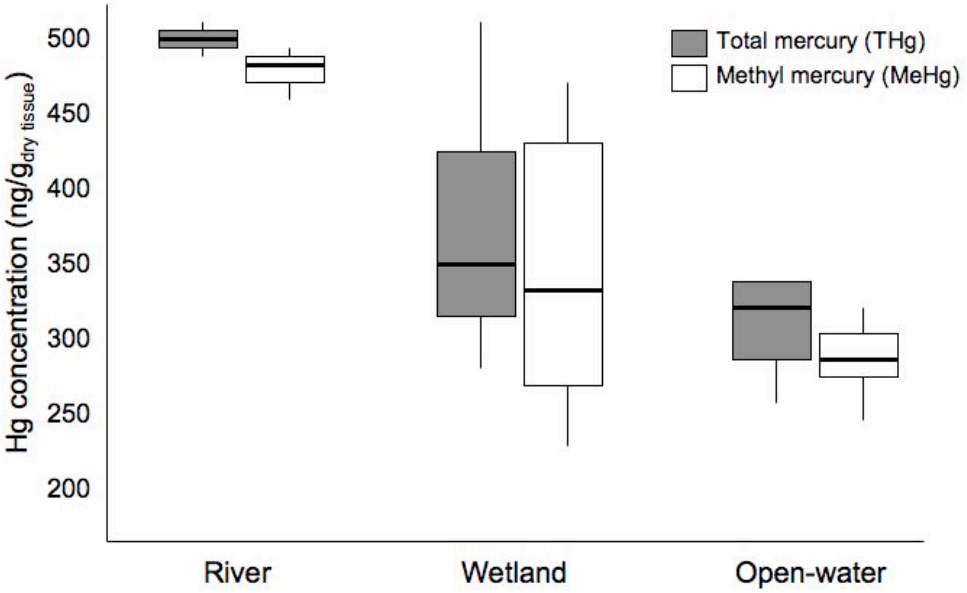

Figure 2. Box Plots of total mercury (THg) and methylmercury (MeHg) concentrations in grass shrimp. Dark bar is median, shaded area is middle 1/2 of values for THg. White area is middle 1/2 of values for MeHg. Lines are minimum and maximum Hg concentrations.

My first hypothesis is that there was annual variability in grass shrimp Hg concentrations and the pattern observed in 2006 was no longer present in 2007. During 2006 Caddo Lake had low water levels due to a drought. In January 2007 there was a large flood and in May 2007 water levels were above average (USGS 2008). I used linear regression to test the hypothesis that Hg concentrations changed between 2006 and 2007 and found a 1:1 relationship between THg in grass shrimp collected in 2006 and 2007 (linear regression: $R^2 = 0.84$; t-test: $H_0{:}b_0 = 0$, $t = 0.34$, $P = 0.75$; $H_0{:}b_1 = 1$, $t = 0.36$, $P = 0.26$) indicating that no change in THg concentration had occurred (Fig. 3).

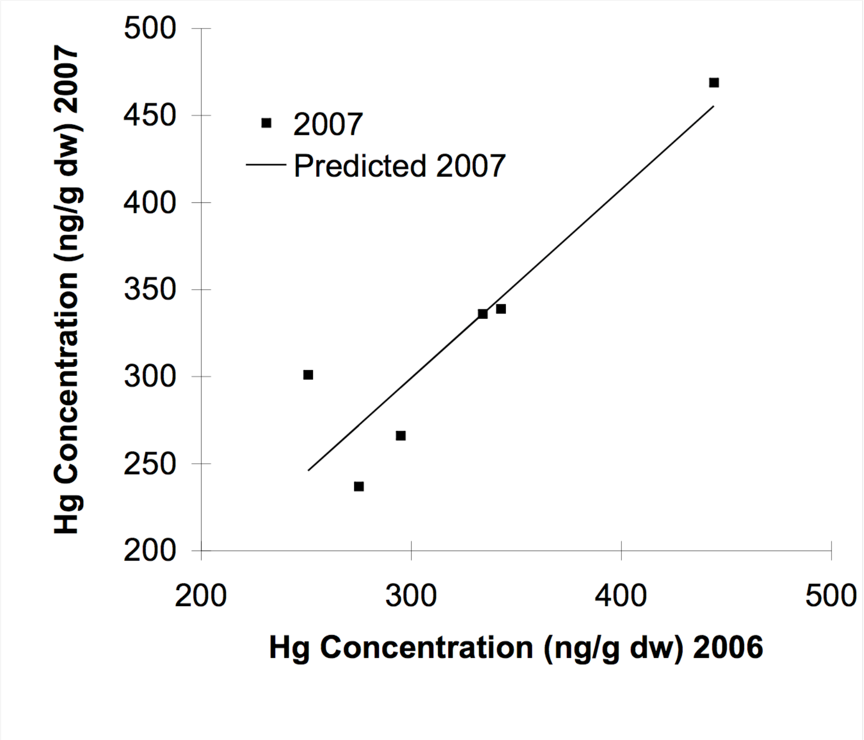

Figure 3. Relationship between total mercury (THg) concentrations of grass shrimp collected from the same sites in 2006 and 2007.

My second hypothesis to explain the lack of difference in Hg concentrations of grass shrimp between wetland and open-water habitats was that large variability in MeHg availability in the wetland habitat obscured a habitat-scale pattern. The river and open-water habitats in Caddo Lake are relatively homogenous compared to the wetland which is a mosaic of six different vegetation community types. The Caddo Lake wetland community types correspond to a flooding and elevation gradient that range from mesic, rarely flooded terraces and levees to nearly continuously flooded cypress swamps (Van Kley and Hines 1998). Further, some sites in the wetland are in close proximity to the flow path of Big Cypress Bayou, which contained shrimp with the highest concentrations of Hg, while some wetland sites are separated from the river by more than 6 km. I used linear regression to determine if distance from Big Cypress Bayou was related to Hg concentration in Caddo Lake grass shrimp and found a negative relationship (linear regression; df = 16, $R^2 = 0.25$, $P < 0.05$) (Fig. 4). Distance from the river flow path explained 25% of the variation in grass shrimp THg. The remaining variation is most likely explained by differences in Hg availability between samples sites.

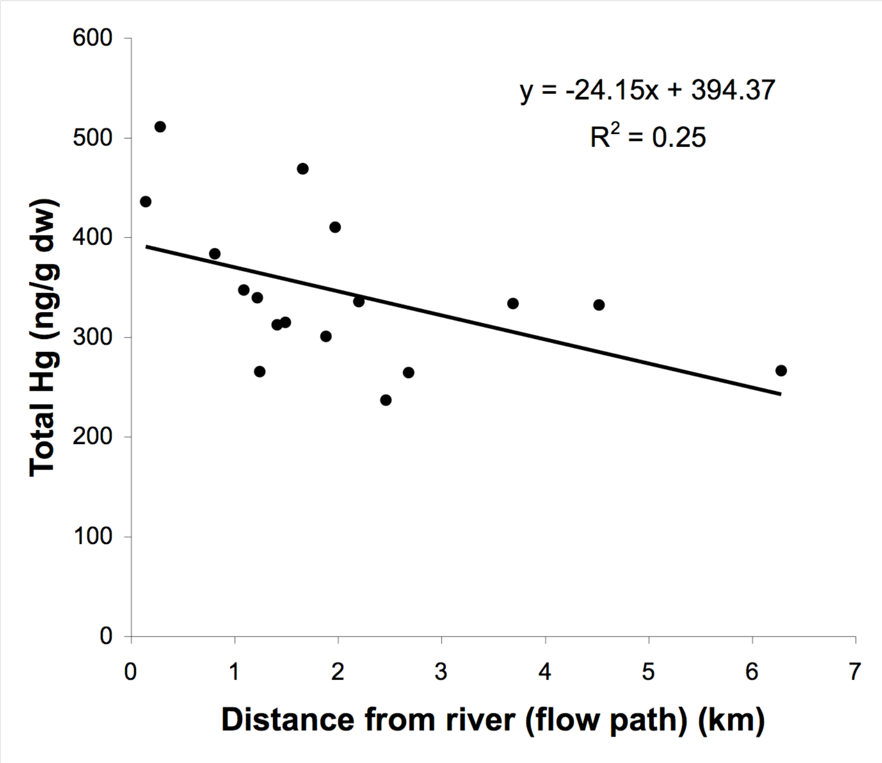

Figure 4. Distance from the main flow path of the river to all wetland and open-water sample sites.

There are several possible reasons why the THg concentrations in Caddo Lake shrimp may be related to distance from the river. First MeHg may be produced in wetlands located in the Big Cypress Bayou watershed and transported to the river. Big Cypress Bayou may be a source of MeHg to Caddo Lake. Rivers in Wisconsin whose watersheds included wetlands had elevated concentrations of MeHg in the water compared to watersheds that did not contain wetlands (Hurley et al. 1995). Paller et al. (2004) found that Asian clams (*Corbicula fluminea*) collected from tributaries adjacent to wetlands had significantly higher concentrations of MeHg than Asian clams collected from tributaries that drained uplands or from the Savannah River. An alternative but not mutually exclusive explanation for the relationship between distance to the river and Hg in grass shrimp is that the river is delivering sulfate to Caddo Lake. Sulfate levels in Big Cypress Bayou are elevated relative to levels in Caddo Lake (Darville - personal communication) and sulfate concentration is positively correlated with Hg methylation (Drevnick et al. 2007). Finally, the river may be a source of inorganic Hg, carbon or nutrients, all factors which can limit methylation (Wiener 2003), however data on these factors are not available.

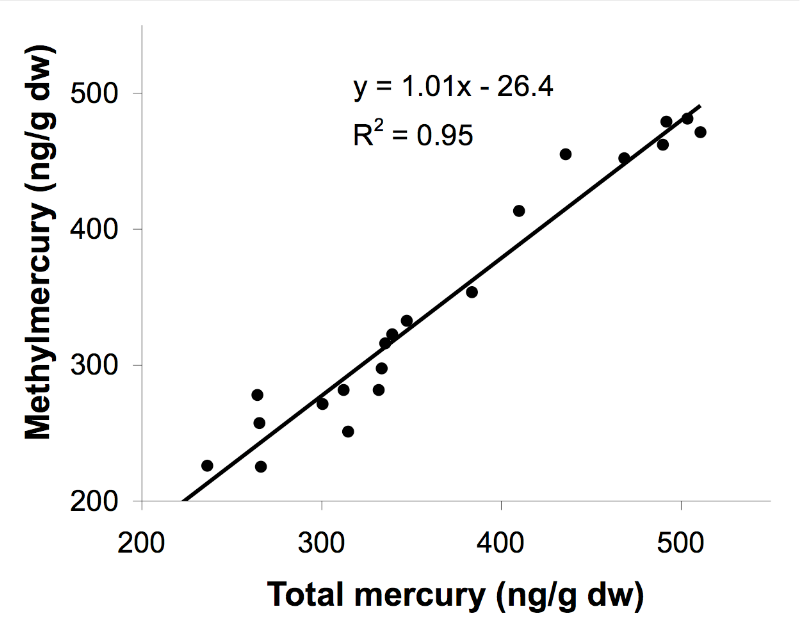

Figure 5. Regression of methylmercury (MeHg) to total mercury (THg).

Methylmercury comprised 94% (SD = 0.064) of the THg found in grass shrimp at Caddo Lake and was highly correlated with THg (linear regression: $R^2 = 0.95$, $P < 0.001$) (Fig. 5). The proportion of MeHg to THg did not vary significantly between habitats (ANOVA: df = 2, 17; F = 0.219; $P > 0.05$). The high MeHg:THg ratio allows for analysis of THg which is easier and less expensive to analyze than MeHg (Cizdziel et al.

2002b). Only one previous study has examined MeHg:THg in grass shrimp of the genus *Palaemonetes* (Cleckner et al. 1998). Cleckner et al. (1998) indicated that the ratio of MeHg:THg was high but did not report the proportion. Because grass shrimp 1) have a high MeHg:THg ratio (this study, Cleckner et al. 1998), 2) are abundant and ubiquitous throughout the southeastern U.S. (Key et al. 2006) and 3) exhibit site fidelity (Key et al. 2006), they may be useful bioindicator of MeHg availability.

Conclusion

Wetlands have been identified as hot spots of Hg availability on a regional scale (Driscoll 2007), but I found significant variation in Hg concentrations in grass shrimp within the wetland habitat, suggesting variation in Hg availability in different parts of the wetland at Caddo Lake. This is a novel result and may not have been previously reported because most studies of spatial variation of Hg contamination in aquatic ecosystems use fish as bioindicator species. Fish are unlikely to reflect variation within a habitat because they are long lived and can move large distances thus integrating Hg concentration over space and time and obscuring variation. The Hg concentrations of grass shrimp collected in the wetlands covered nearly the entire range of Hg concentrations found in all three habitats. This variability indicates there are factors affecting Hg levels in wetlands at Caddo Lake that are more complex and localized than previously reported.

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ABSTRACT

HABITAT-SPECIFIC DIFFERENCES IN MERCURY CONCENTRATIONS OF MISSISSIPPI GRASS SHRIMP FROM CADDO LAKE, TEXAS

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Total and methylmercury concentrations were measured in Mississippi grass shrimp (*Palaemonetes kadiakensis*) that were collected from river, wetland, and open-water habitats in Caddo Lake, Texas. Because grass shrimp are short-lived and relatively immobile, their mercury concentrations are representative of the mercury levels at the location from which they are collected. The highest concentrations of mercury are in grass shrimp collected from the river and the lowest were from open-water habitats. Mercury levels in shrimp collected in the wetland are intermediate between river and open-water habitats but exhibit a large amount of variation suggesting the wetlands of Caddo Lake are quite heterogeneous and require further study to understand their spatial variation. Methylmercury is strongly correlated to total mercury and constitutes 94% of the total mercury regardless of the habitat from which they were collected. This suggests total mercury concentrations are a useful proxy for predicting methylmercury concentrations.