USE OF PRESERVED MUSEUM FISH TO EVALUATE MERCURY CONTAMINATION IN TWO OKLAHOMA RIVERS

by

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Introduction

Mercury is an environmental contaminant that adversely affects fish, wildlife, and human health (NRC, 2000; Wiener et al., 2003). Anthropogenic activities release inorganic mercury into the atmosphere where it resides until it is deposited onto the earth's surface (Driscoll et al., 2007). Inorganic mercury is converted to toxic methylmercury by bacteria in aquatic ecosystems (Morel et al., 1998; Ullrich et al., 2001). Methylmercury tends to be found at higher levels in aquatic food webs relative to terrestrial food webs (Wiener et al., 2003).

Icthyological museum collections potentially provide an invaluable resource to examine temporal and spatial changes of mercury contamination in aquatic ecosystems. In fish, the majority of methylmercury is concentrated in skeletal muscle tissue (Giblin and Massaro, 1973; Boudou and Ribeyre, 1983; Harrison et al., 1990). A few studies have examined mercury in muscle tissue of preserved fish to examine temporal and spatial changes (Barber et al., 1972; Evans et al., 1972; Miller et al., 1972; Gibbs et al., 1974; Martins et al., 2006). However, use of preserved museum fish assumes that preservatives do not affect mercury levels in fish tissues, but this has been little studied (Gibbs et al., 1974). In this paper, I show that preservation does not affect mercury concentration of fish. I then use preserved fish from a museum collection to examine temporal changes in mercury concentrations of fish from two rivers in southeastern Oklahoma.

Methods

Preservative Study

The effects of preservation on mercury concentrations in fish tissue were studied using a centrarchid, largemouth bass (*Micropterus salmoides*). In the fall of 2005, 158 largemouth bass were collected using an electrofishing boat by Texas Parks and Wildlife Department (TPWD) from reservoirs in the Dallas-Fort Worth area. Fish were euthanized on ice, transported to the TPWD lab for collection of standard fisheries data, and frozen.

For analyses of mercury, each largemouth bass was thawed and a fillet of dorsal muscle tissue (skin intact) was dissected. A small skinless tissue sample from the fillet was removed using a scalpel and forceps rinsed with deionized (DI) water. The tissue was then dried at 60°C for at least 48 hours and analyzed to determine initial mercury concentration. Unpreserved fish tissue of similar size was observed in a pilot study to reach constant mass within 48 hours in a drying oven. The remaining fillet was used to study the effects of two techniques commonly used to preserve fish in museum collections (formalin-ethanol and formalin-isopropanol) on mercury concentration. Fillets were first fixed in 10% formalin for seven days, and then soaked in DI water for two days (water changed each day) to remove the formalin. Fillets were then placed in either 70% ethanol (n= 158) or 50% isopropanol (n= 23) for seven days, after which the alcohol was discarded and fillets were placed in fresh alcohol. Tissue samples were then analyzed for total mercury concentration at 40-day intervals for a total of 160 days.

To sample a preserved fillet for mercury analysis, I removed a small skinless tissue sample using a scalpel and forceps rinsed with DI water, and then dried the tissue

in an oven at 60°C for at least 24 hours. Preserved fish tissue of similar size was observed in a pilot study to reach constant mass within 24 hours in a drying oven. The dried sample removed from the fillet was then placed into a 20-ml scintillation vial until analysis of total mercury.

Mercury Analyses

Total mercury was analyzed 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 Institute for National Measurement Standards: MESS-3 (marine sediment, certified value = 91 ± 9 ng/g total mercury [dry weight]), PACS-2 (marine sediment, certified value = 3,040 ± 200 ng/g total mercury [dry weight]), and DORM-2 (dogfish muscle, certified value = 4,640 ± 260 ng/g total mercury [dry weight]). TORT-2 (lobster hepatopancreas, certified value = 270 ± 60 ng/g total mercury [dry weight]) was a laboratory standard analyzed during runs as a reference. TORT-2 has a published mean of 270 ppb (National Research Council of Canada Institute for National Measurement Standards), and the variance around the mean is ± 22% of the published value. Due to the large variance around the reference mean, after every calibration, five samples of TORT-2 were run to determine a mean that was then used as the reference value. Quality assurance included reference and duplicate samples. Reference samples (MESS-3 or TORT-2) were analyzed every 10 samples and the mean percent recovery was 99.3 (SD = 3.25). Duplicate samples were analyzed every

20 samples and the mean relative percent difference was 4.46 (SD = 3.45). Fish tissue analyzed ranged from 3.9 to 104.5 mg.

Museum Collection

One hundred and eighty-eight longear sunfish (*Lepomis megalotis*), with a total length range of 40 to 145 mm, were obtained from the Sam Noble Oklahoma Museum of Natural History during the summer of 2006. Longear sunfish had been collected from 1963 to 2001 and 1925 to 2003 in Glover River and Mountain Fork River respectively, in McCurtain County, Oklahoma (Figure 1). McCurtain County was chosen based on its proximity to coal-burning power plants that are located in northeast Texas. Longear sunfish in the museum had been preserved using 10 % formalin as a fixative and stored in 50% isopropanol. For mercury analysis, a skinless dorsal muscle fillet was removed using a scalpel and forceps that had been rinsed with DI water. Fillets were then dried at 60ºC for at least 24 hours and stored in 20-ml scintillation vials until analysis of total mercury. Dried fillets ranged from 3.9 to 33.9 mg.

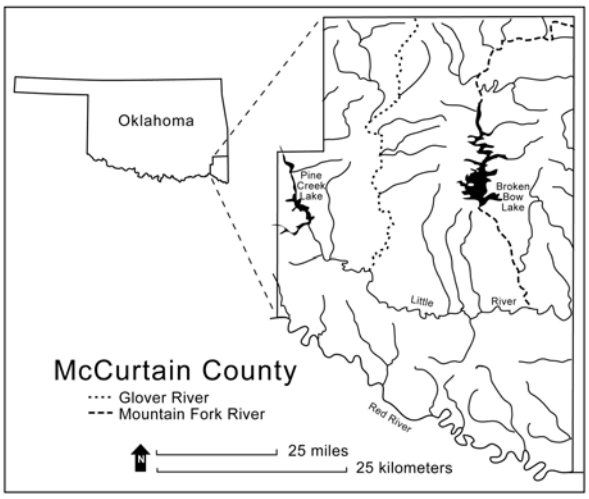

Figure 1. Map of McCurtain County, Oklahoma showing the locations of Glover River and Mountain Fork River.

Field Collection

Twenty eight and thirteen longear sunfish, with a total length range of 40 to 85 mm, were collected in August 2006 from Glover River and Mountain Fork River in McCurtain County, Oklahoma, respectively. Glover River was sampled at the intersection of the river and Oklahoma State Highway 3, approximately 17 km northwest of the city of Broken Bow in McCurtain County, Oklahoma (34° 5'51.00"N, 94°54'7.00"W). Mountain Fork River was sampled at the intersection of the river and U.S. Highway 70, approximately 11 km east of the city of Broken Bow in McCurtain County, Oklahoma (34° 2'30.00"N, 94°37'11.00"W). Some museum specimens of longear were also collected from these two sites.

Longear sunfish in Glover River were collected using a backpack Smith-Root, Inc. LR-24 Electrofisher and euthanized on ice. Because of greater channel depth at the Mountain Fork site, longear sunfish were collected using a seine and euthanized on ice. Fish were stored in a freezer for two days. Fish were thawed and measured for total length and a dorsal muscle fillet was removed and dried at 60ºC for at least 48 hours and stored in a 20-ml scintillation vial until analysis of total mercury.

Statistics

The effects of formalin-ethanol on changes in mercury concentrations among lakes and preservation times of 0, 40, 80, 120 and 160 days in 158 preserved largemouth bass collected from 6 different lakes were tested using a split-plot univariate analysis of variance (Milliken and Johnson, 1984). The F-test of the effect of time was computed by dividing the Mean Square for time by the Mean Square for the time*lake interaction. The

effects of formalin-isopropanol on changes in mercury concentrations were tested using 22 largemouth bass collected from the same lake in a randomized complete block experimental design (Milliken and Johnson, 1984) with fish as a random factor. Tests of significant differences due to the effects of preservation method and time were performed by dividing the appropriate mean squares for method and time by the mean square for the method*time interaction.

For the museum fish, because mercury concentrations increased with increasing length in longear sunfish, an analysis of covariance (Milliken and Johnson, 2002) followed by the Tukey-Kramer multiple comparison procedure on adjusted means (Milliken and Johnson, 2002) was used to test for differences in mean concentrations among years. Separate analyses were conducted for each river system.

Results

I detected no significant change in mercury concentrations of formalin-ethanol preserved largemouth bass tissue with time ($F = 0.57$; $df = 4, 20$; $P > 0.10$) (Figure 2A). I also detected no significant change in mercury concentrations of formalin-isopropanol preserved largemouth bass tissue with time ($F = 3.29$; $df = 4, 4$; $P > 0.10$) (Figure 2B). No significant difference in the mean mercury concentrations was found between the two preservative treatments ($F = 0.002$; $df = 1, 4$; $P > 0.10$). The mean change in mercury concentrations from day 0 to day 160 for largemouth bass from the two preservative treatments was +21.1 ppb (Standard Error = 23.26). The mean percent change in mercury from day 0 to day 160 for largemouth bass from the two preservation treatments was +11.0 % (Standard Error = 46.52) with the larger difference occurring in the

formalin-isopropanol preserved fish. Neither of these differences is statistically significant and, moreover, both are < 15 % change of the initial mercury concentrations in the muscle samples.

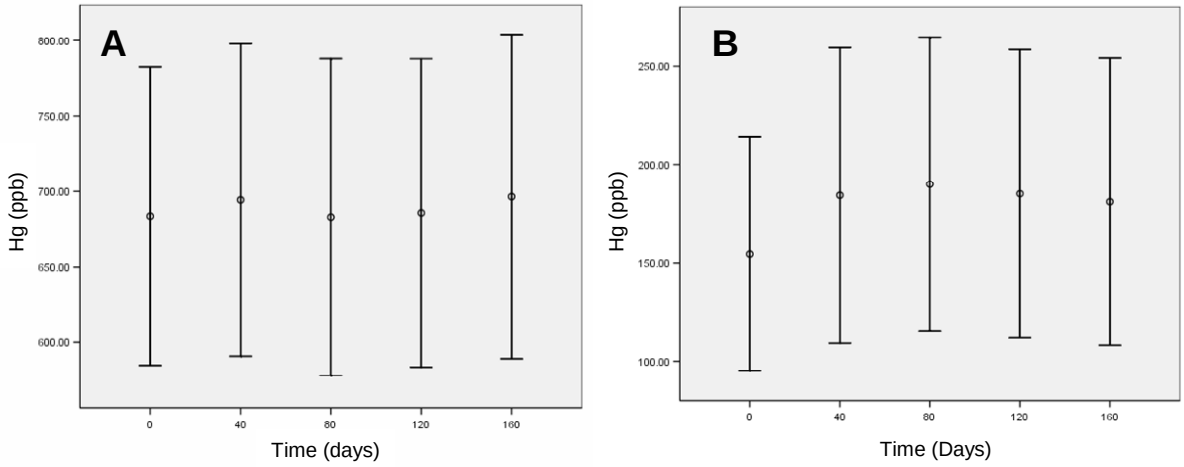

Figure 2. Mean mercury concentrations in unpreserved (day 0) and preserved largemouth bass tissue (days 40-160). A) Tissue preserved in formalin-ethanol. B) Tissue preserved in formalin-isopropanol. Bars indicate 95% confidence intervals.

Longear sunfish in Glover River from 1963 to 2006 had a significant difference among their mean mercury concentrations after adjusting for differences in fish length among times ($F= 21.16$; $df= 5, 128$; $p <0.001$) (Figure 3A), but there was no discernable time-related trend. Longear sunfish from 1972 were the only group that had a significantly greater mean mercury concentration than fish from all other years ($p <0.05$) (Table 1).

Longear sunfish in Mountain Fork River from 1925 to 2006 also had a significant difference among their mean mercury concentrations after adjusting for differences in fish length among times ($F=17.44$; $df= 9, 84$; $p <0.001$) (Figure 3B), but in this case there was a time-related trend. Mercury concentrations appeared to be relatively constant from 1925 to 1993 and then declined. Longear sunfish from 2003 and 2006 had mean mercury

concentrations that were significantly less than mean mercury concentrations in all previous years ($p < 0.05$) (Table 2).

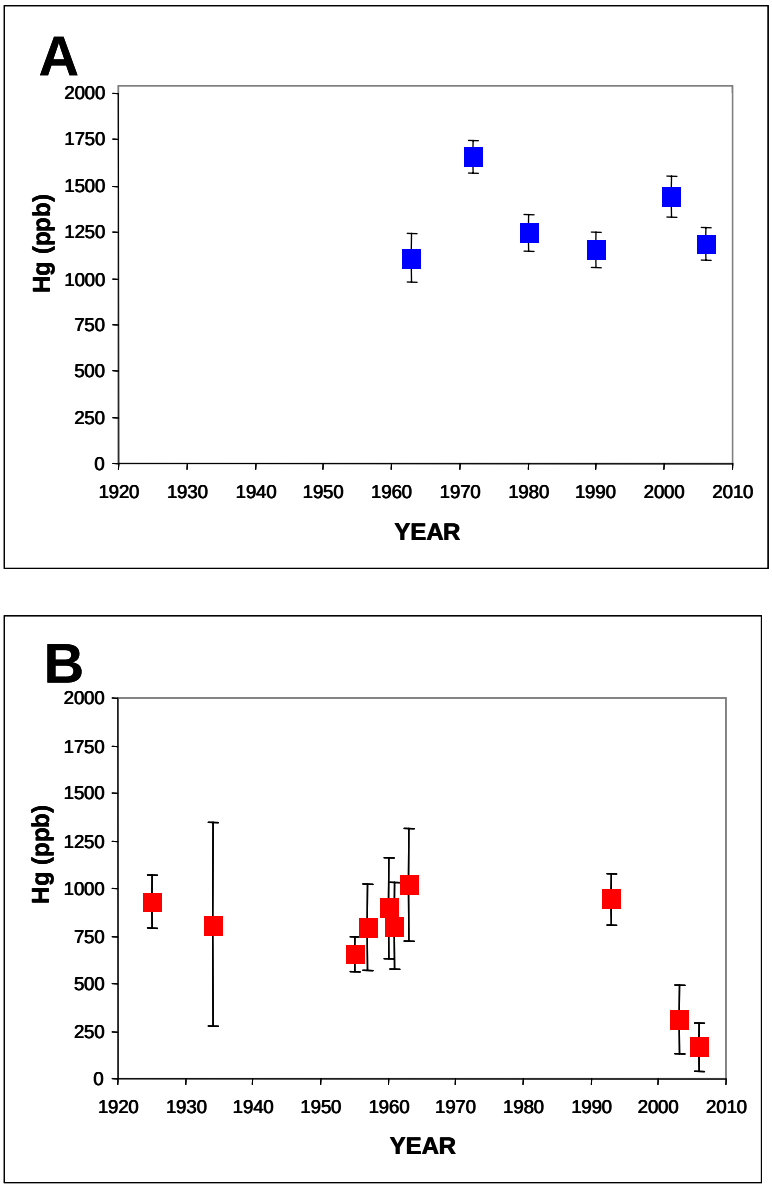

Figure 3. Mean mercury concentrations in preserved museum longear sunfish and unpreserved longear sunfish caught in 2006 from Glover River (A) and Mountain Fork River (B) in McCurtain County, Oklahoma. Bars indicate 95% confidence intervals. See Table 3 for number of longear sunfish sampled.

Year	1972	1980	1990	2001	2006
1963	<.0001*	0.4873	0.9928	0.0015*	0.9326
1972		<.0001*	<.0001*	0.0291*	<.0001*
1980			0.6941	0.0930	0.8979
1990				0.0012*	0.9973
2001					0.0035*

Table 1. P-values from mean mercury concentration comparisons from each year in Glover River with * indicating a significant difference ($p < 0.05$).

Year	1934	1955	1957	1960	1961	1963	1993	2003	2006
1925	0.9967	0.0169*	0.9645	1.0000	0.9776	0.9994	1.0000	<.0001*	<.0001*
1934		0.9721	1.0000	0.9999	1.0000	0.9593	0.9933	0.0384*	0.0005*
1955			0.9108	0.3782	0.8885	0.0644	0.0089*	0.0097*	<.0001*
1957				0.9983	1.0000	0.7978	0.9344	0.0028*	<.0001*
1960					0.9991	0.9969	1.0000	0.0003*	<.0001*
1961						0.8172	0.9551	0.0022*	<.0001*
1963							0.9998	<.0001*	<.0001*
1993								<.0001*	<.0001*
2003									0.8899

Table 2. P-values from mean mercury concentration comparisons from each year in Mountain Fork River with * indicating a significant difference ($p < 0.05$).

Glover River		Mountain Fork River	
Year	**Number of Fish**	**Year**	**Number of Fish**
1963	14	1925	12
1972	28	1934	3
1980	23	1955	24
1990	24	1957	6
2001	17	1960	5
2006	28	1961	6
		1963	5
		1993	12
		2003	8
		2006	13

Table 3. Longear sunfish sample sizes for different years in Glover River and Mountain Fork River. The sample size for 1957 includes five fish collected in 1957 and a single fish collected in 1956.

Discussion

Preservative Study

Preservation effects on mercury and the use of preserved museum fish to study mercury contamination have been controversial. Gibbs et al. (1974) stated that "until the effects are properly understood, fluid preserved museum specimens cannot be used for meaningful comparisons of metal concentrations." Conversely, Martins et al. (2006) concluded that their study indicated "that museum myctophids may be suitable for the assessment of historical changes in mercury contamination of marine ecosystems."

For museum collections to be used for mercury analyses, we must understand preservation effects on fish. Gibbs et al. (1974) conducted the only study of the effects of preservation on mercury concentrations in fish. They compared mercury concentrations in frozen fish to fish that had been preserved in formalin-isopropanol or formalin-ethanol for 30 days. Preserved fish were reported to have lower concentrations of mercury but no information about sample sizes or statistical analyses were provided. Results from my study determined no time-related trends in mercury concentration were observed for formalin-ethanol or formalin-isopropanol preserved fish at 40 day intervals for 160 days.

Because preservation is known to affect fish weight (DiStefano et al. 1994), it might be hypothesized that preservation would also affect mercury concentration in fish. DiStefano et al. (1994) found an increase in whole weight of fish that were preserved in 10% formalin and a decrease in whole weight of fish that were preserved in 75% ethanol and 50% isopropanol over a 90-day period. They did not examine weight change of fish that had first been preserved in formalin and then preserved in alcohol. In such, fish weight gain associated with formalin preservation may offset weight loss associated with alcohol preservation. Mercury in muscle tissue is bound to sulfhydryl groups in protein

(Giblin and Massaro, 1973; Boudou and Ribeyre, 1983; Harrison et al., 1990) and I suggest net weight change of the preservation techniques is limited, thus probably not significantly changing the mercury concentration of the tissue.

Study Using Museum Fish

A number of studies have used museum fish to examine temporal trends of mercury in the environment (Barber et al., 1972; Evans et al., 1972; Miller et al., 1972; Gibbs et al., 1974; Martins et al., 2006). I observed no pattern of consistent change through time in Glover River although there was a significant difference in the mean mercury values among some dates. I also observed no pattern of consistent change through time in Mountain Fork River from 1925 to 1993, however there is a precipitous decline in mean mercury concentrations after 1993. Mean mercury concentrations from both 2003 and 2006 fish in Mountain Fork were significantly less those observed from all dates 1925 to 1993. I suggest the most likely explanation for the recent decline in mercury contamination observed in fish from Mountain Fork River includes impoundment effects and/or air pollution controls. Mountain Fork River was impounded upstream of all sampling sites of longear sunfish obtained from the museum to construct Broken Bow Reservoir. The reservoir started to flood in 1968 and was filled in 1970. Studies have shown a pulse of mercury to the aquatic ecosystem when reservoirs are flooded, and it may take up to 30 years before a decline to preimpoundment levels (Verdon et al., 1991; Kelly et al., 1997; Paterson et al., 1998; Porvari, 1998; Therriault and Schneider, 1998; St. Louis et al., 2004; Boudou et al., 2005; Hall et al., 2005). Reservoirs trap large masses of sediment (Nilsson and Berggren, 2000; Vorosmarty,

2003), which decreases suspended sediment downstream and may decrease total mercury in the water column of streams (Whyte and Kirchner, 2000; Balogh et al., 2003; Wall et al., 2005). Finally, Congress passed air-quality standards with amendments to the Clean Air Act in 1970, 1977, and 1990 that decreased emissions of mercury (Engstrom and Swain, 1997). Changes in air quality standards should have affected both rivers, but we observed no change in the mercury concentrations in longear sunfish from Glover River (~27 km west of Mountain Fork River) during the same time period that mercury concentrations in longear sunfish declined in Mountain Fork River.

Because museum collections house specimens from numerous sampling sites, they would seem to be an ideal resource to examine temporal and spatial patterns of mercury contamination (Martins et al., 2006). To determine whether museum specimens for Glover and Mountain River could predict location-specific differences in mercury, I collected fish in 2006. Longear sunfish from both Glover River and Mountain Fork River, sampled in the summer of 2006, had mercury concentrations that were not different than the mercury concentrations observed in the museum fish.

My results suggest that preserved fish from museum collections are a potential predictor of the mercury contamination observed in the environment and can be used to examine temporal and spatial trends. Museum collections could be used to assess potential mercury hotspots across wide geographic areas, and could also be used as baselines to assess the impact of future mercury regulation.

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In August, 2005, he enrolled in graduate study at Texas Christian University, where he received his Master of Science in 2007. While working on his masters in Environmental Science, he held a Teaching Assistantship in 2005-2007. He was awarded both Graduate Student of the Year and Graduate Teaching Assistant of the Year by the Biology Department in 2007. He is a student member of the American Association for the Advancement of Science, the American Society of Limnology and Oceanography, and the American Fisheries Society Texas Chapter.

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ABSTRACT

USE OF PRESERVED MUSEUM FISH TO EVALUATE MERCURY CONTAMINATION IN TWO OKLAHOMA RIVERS

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To determine if preserved museum fish specimens could be used evaluate mercury contamination through time, we preserved largemouth bass (*Micropterus salmoides*) in formalin-ethanol and formalin-isopropanol and found no significant change in mercury concentration over 160 days. To evaluate how mercury contamination of two rivers in southeastern Oklahoma may have changed through time, we determined mercury concentrations in preserved longear sunfish (*Lepomis megalotis*) from the Sam Noble Oklahoma Museum of Natural History. Longear sunfish had been collected over 41- and 79-year periods from Glover and Mountain Fork Rivers, respectively. Glover River is unimpounded, whereas Mountain Fork River was impounded upstream from the sampling sites in 1968. Mercury concentrations in longear sunfish from Glover River showed no temporal trend from 1963 to 2004. Mercury concentrations in longear sunfish from Mountain Fork River showed no temporal trend from 1925 to 1993 but then declined from 1993 to 2003.