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Use of preserved museum fish to evaluate historical and current mercury contamination in fish from two rivers in Oklahoma, USA

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Abstract We examined the effects of a commonly used preservation technique on mercury concentration in fish tissue. After fixing fish muscle tissue in formalin followed by preservation in isopropanol, we found that mercury concentration in fish muscle tissue increased by 18%, reaching an asymptote after 40 days. We used formalin–isopropanol-preserved longear sunfish (*Lepomis megalotis*) from the Sam Noble Oklahoma Museum of Natural History to examine historical changes and predict current mercury concentrations in fish from two rivers in southeastern Oklahoma. Glover River was free-flowing, while Mountain Fork River was impounded in 1970 and a coldwater trout fishery was established upstream from the collection site in 1989. Mercury concentrations in longear sunfish from Glover River showed no historical changes from 1963 to 2001. Mercury concentrations in longear sunfish from Mountain Fork River showed no change from 1925 to 1993 but declined significantly from 1993 to 2003. We also compared mercury concentrations of the most recently collected longear sunfish in the museum to mercury concentrations of unpreserved fish collected from the rivers in 2006. Concentrations of mercury in museum fish were not significantly different from mercury concentrations in unpreserved fish we collected from the rivers. Our study indicates that preserved museum fish specimens can be used to evaluate historical changes and predict current levels of mercury contamination in fish.

Keywords Preserved fish · Museum specimens · Total mercury · River · Longear sunfish

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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, such as the burning of coal for power generation, 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 bioaccumulative methylmercury by bacteria in aquatic ecosystems (Morel et al. 1998; Ullrich et al. 2001). Algae at the base of aquatic food webs 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). Methylmercury bioaccumulates in aquatic organisms and is found at highest

concentrations in top consumers in aquatic food webs (Wiener et al. 2003). Most of the mercury in carnivorous fish is methylmercury (Bloom 1992). This results in potentially dangerous concentrations of mercury in fish, but it also means that fish can be important indicator organisms of mercury contamination in aquatic ecosystems.

Although ichthyology collections in museums are potentially an invaluable resource to examine historical changes and efficiently assess current mercury contamination of aquatic ecosystems (Suarez and Tsutsui 2004), only a few studies have used museum fish for this purpose (Barber et al. 1972; Evans et al. 1972; Miller et al. 1972; Gibbs et al. 1974; Kelly et al. 1975; Swain and Helwig 1989; Martins et al. 2006). Investigators using preserved museum fish assume that preservatives do not affect mercury in fish tissues, but this assumption has been controversial (Gibbs et al. 1974; Martins et al. 2006). Gibbs et al. (1974) concluded that until the effects of preservation are properly understood, fluid-preserved museum specimens cannot be used for meaningful comparisons of metal concentrations. Martins et al. (2006) stated that the results of their study of preserved glacier lantern fish (*Benthosema glaciale*) indicated that museum myctophids may be suitable for the assessment of historical changes in mercury contamination of marine ecosystems, but they did not establish the relationship between mercury in preserved and unpreserved fish. In this study, we had three objectives: (1) to examine the effects of the commonly used formalin–isopropanol fish preservation technique on mercury concentrations in fish; (2) to assess historical changes in mercury concentrations in preserved museum fish from two Oklahoma rivers; and (3) to examine whether preserved museum fish could be used to predict current mercury concentrations in fish from the two rivers.

Methods

Preservative study

The effects of preservation on mercury concentrations in fish tissue were studied using 22 largemouth bass (*Micropterus salmoides*). We used largemouth bass because they are a member of the sunfish family (Centrarchidae) and their large size allowed multiple tissue samples to be taken from an individual fish, enabling us to analyze time-related preservative effects. We collected largemouth bass by electrofishing in fall 2005 from White Rock Lake, Dallas, TX, USA with assistance of biologists of the Texas Parks and Wildlife Department. Fish were frozen for later study of preservation effects on mercury concentration.

To obtain tissue samples for analysis of mercury, skin-on fillets of largemouth bass epaxial muscle were dissected from each fish and an approximately 0.15-g subsample of muscle tissue was collected from the center of each fillet using a scalpel and forceps. The subsample was dried at 60°C for at least 48 h and analyzed to determine initial total mercury concentration. We observed unpreserved fish tissue to reach constant weight within 48 h in a 60°C drying oven**.** The remaining fillet was used to study the effects of formalin–isopropanol preservation on mercury concentration.

Fillets were preserved using the preservation technique employed by the Sam Noble Oklahoma Museum of Natural History (University of Oklahoma, Norman, Oklahoma; S. Cartwright, personal communication). Fillets were fixed in 10% formalin for 7 days, soaked in deionized water for 2 days to remove formalin (water changed each day), placed in 50% isopropanol for 7 days, and transferred to fresh 50% isopropanol for preservation. Preserved tissue samples were then analyzed for total mercury concentration at 40-day intervals for a total of 160 days using the same procedure we used for unpreserved tissues.

Museum collection

We used preserved fish from the Sam Noble Oklahoma Museum of Natural History to examine historical changes in mercury concentrations of fish. We searched museum records for fish collected from southeastern Oklahoma, a region with high atmospheric mercury deposition (NADP 2005). Inspection of the museum records revealed that two rivers in this region, Glover and Mountain Fork (Fig. 1), had been repeatedly

sampled over several decades and had large fish collections maintained in the museum. Fish from Glover River were collected near the intersection of the river and Oklahoma State Highway 3 (34° 05′ 51.48″ N, 94°54′ 07.93″ W), approximately 17 km northwest of the city of Broken Bow in McCurtain County, Oklahoma. Fish from Mountain Fork River were collected near the intersection of the river and US Highway 70 (34° 02′ 30.96″ N, 94° 37′ 16.37″ W), approximately 11 km east of the city of Broken Bow. Glover River was free-flowing while Mountain Fork River was impounded to form Broken Bow Reservoir 18.6 river kilometer above the collection site. The reservoir filled in 1970 and metalimnetic discharge from the dam was used to develop and maintain a coldwater trout fishery beginning in 1989 (P. Balkenbush, personal communication).

We focused on longear sunfish (*Lepomis megalotis*) because they had been collected from the two rivers over several decades and were available in the museum for studies of mercury contamination. In rivers, longear sunfish feed on aquatic and terrestrial invertebrates and an occasional small fish (Robison and Buchanan 1988). Because longear sunfish are carnivorous, they would be expected to have detectable mercury concentrations if the river watersheds were contaminated with mercury.

We obtained 188 longear sunfish from the museum collection. Fish ranged from 40 to 145 mm total length (TL) and had been collected from 1963 to 2001 and 1925 to 2003 from Glover River and Mountain Fork River, respectively. For Glover River, the dates and number of individual fish collected were: 1963, $n = 14$; 1972, $n = 28$; 1980, $n = 23$; 1990, $n = 24$; 2001, $n = 17$. For Mountain Fork River, the dates and number of individual fish collected were: 1925, $n = 12$; 1955, $n = 24$; 1957, $n = 6$; 1960, $n = 5$; 1961, $n = 6$; 1963, $n = 5$; 1993, $n = 12$; 2003, $n = 8$. We dissected tissue samples of epaxial muscle from the museum fish and dried them prior to mercury analysis.

Field study

To investigate whether mercury concentrations in museum specimens could be used to predict

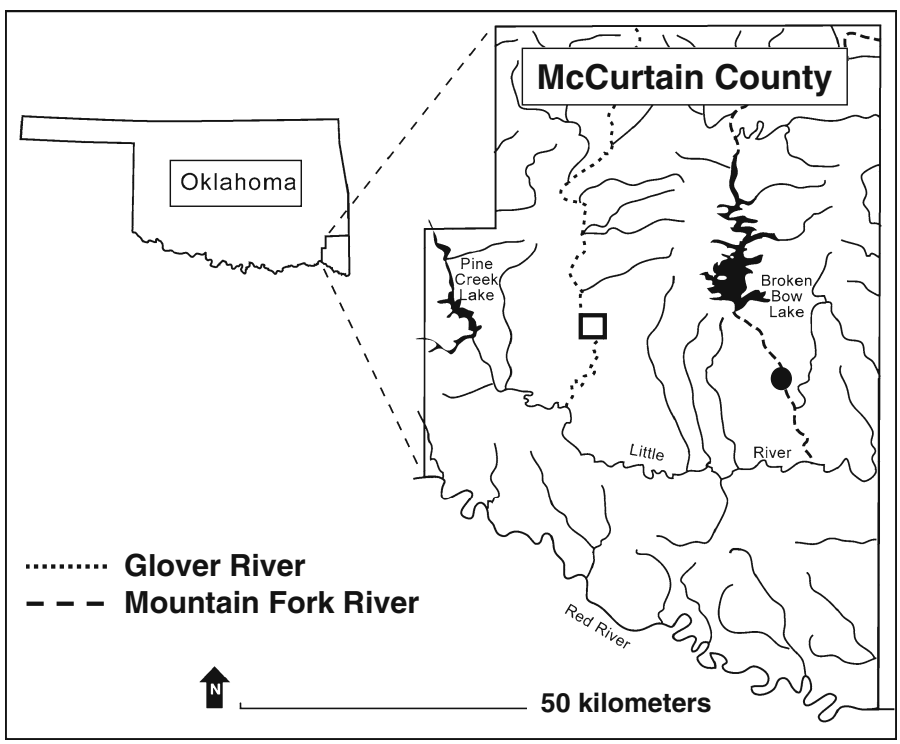

Fig. 1 Map of McCurtain County, OK, USA, showing collection sites on Glover River (*open square*) and Mountain Fork River (*solid circle*)

current concentrations of mercury in fish, we collected longear sunfish from the same river sites sampled for the museum collections. In August 2006, we collected 28 and 13 longear sunfish from Glover River and Mountain Fork River, respectively. Longear sunfish in Glover River were collected using a backpack electrofishing unit (LR-24 Electrofisher, Smith-Root, Inc., Vancouver, WA, USA). Because of greater channel depth at the Mountain Fork River site, longear sunfish were collected using a seine. Fish were placed on ice, transported to the laboratory, and frozen for 2 days. Fish were thawed and measured for TL (40–85 mm). We dissected tissue samples of epaxial muscle from the field-collected fish and dried them prior to mercury analysis.

Mercury analyses

The concentrations of total mercury in dried fish tissue samples were 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^{-1} [mean ± 95% confidence interval] total mercury dry weight [dw]), PACS-2 (marine sediment, certified value = 3,040 ± 200 ng g^{-1} total mercury dw), and DORM-2 (dogfish muscle, certified value = 4,640 ± 260 ng g^{-1} total mercury dw). TORT-2 (lobster hepatopancreas, certified value = 270 ± 60 ng g^{-1} total mercury dw) was a laboratory standard analyzed during runs as a reference. Due to the large variance around the certified value of TORT-2, after every calibration, five samples of TORT-2 were run to determine a mean value that was then used as the reference value. Quality assurance included reference and duplicate samples. Reference samples (MESS-3 or TORT-2) were analyzed approximately every ten samples and the mean percent recovery was 98.1± 0.6% (range = 93–105%, $n = 73$). Duplicate samples were analyzed approximately every 20 samples and the mean relative percent difference was 2.1 ± 2.2% (range = 0.1–13.9%, $n = 29$).

Statistics

The effects of preservation on mercury concentrations in largemouth bass muscle tissue were analyzed using a randomized complete-block experimental design (Milliken and Johnson 1984) with fish as a random block factor. To adjust for mercury variation among fish, changes in mercury concentrations across time were expressed as percent changes from the initial concentrations in fresh tissue. Estimates of the ultimate percent change and the rate at which it reached a constant level were obtained by fitting the asymptotic function

$$P(t) = A \times (1 - \mathrm{EXP}(-b \times t))$$

to the data where $P(t)$ is the percent change occurring by day t; A is the estimated asymptotic change, and b is the percent rate of change in fraction per day. The asymptotic function described above was fitted to the data using PROC NLIN of the Statistical Analysis System (Der and Everitt 2001).

To examine historical changes in mercury concentrations of longear sunfish, we used separate one-way analyses of variance (ANOVA) for each river to test for differences among years in mean mercury concentration. Although sample sizes and variances differed among years, these differences were not large enough to invalidate the use of ANOVA (Kohr and Games 1974; Zar 1999). We tested for differences in mercury concentrations among years using Gabriel's multiple comparisons of means procedure which adjusts for differences in sample sizes among means (Field 2005). Statistical significance was set at $P < 0.05$ for all analyses.

Results and discussion

Preservation Study

Mercury concentrations in formalin–isopropanol-preserved largemouth bass tissue increased relative to unpreserved tissue (Fig. 2). The mean percent increase was significantly greater than 0 for all time periods. The data indicated an estimated asymptotic increase of 18 ± 1.5%

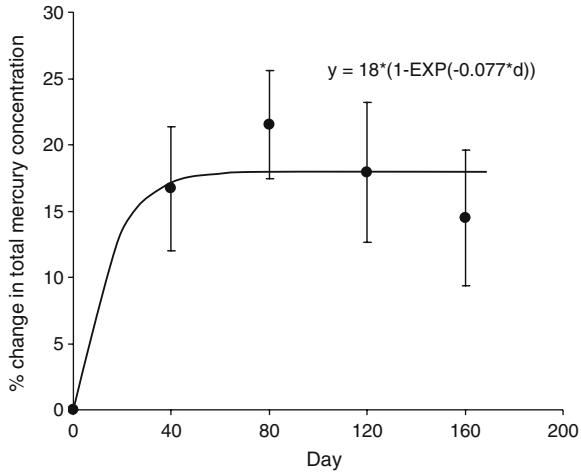

Fig. 2 Mean percent change in total mercury concentration in largemouth bass tissues preserved in formalin–isopropanol. *Error bars* are 95% confidence intervals

(mean ± standard error), reaching 96% of this level within 40 days of preservation. It is because the mercury concentrations in fish tissues reach an asymptote that we can use preserved museum fish for mercury studies.

The increase in concentrations of mercury in largemouth bass muscle tissue may be due, in part, to lipid removal by the isopropanol (Hara and Radin 1978). Mercury is bound to the protein of muscle (Wiener and Spry 1996) and the extraction of lipids from the muscle tissue by isopropanol may have reduced the weight of the dried tissue thereby increasing the concentration of mercury. Meek (1972) suggested that old museum fish specimens had lost several percent of their dry weight due to loss of fats into solution. Future studies need to assess how the extraction of lipids and consequent increases in mercury concentrations may vary with type of preservative, fat content of the fish species, and different preservation techniques (i.e., fillet vs whole body). Because our preservation study was conducted with fillets of muscle tissue from largemouth bass, we did not apply a correction factor for mercury concentrations in whole-body preserved longear sunfish.

Museum collection

Longear sunfish in the two rivers had different concentrations of mercury (Fig. 3). Fish in Glover River had mean mercury concentrations ranging from 1,152 ± 48.1 to 1,674 ± 43.7 ng g^{-1} total mercury dw (mean ± 95% confidence interval), while fish in Mountain Fork River had mean mercury concentrations ranging from 369 ± 133 to 1,188 ± 162 ng g^{-1} total mercury dw.

Although year of collection had a significant effect on mercury concentration in longear sunfish from Glover River (ANOVA, $F = 19.6$; $df = 5, 127$, $P < 0.001$), there was no discernible time-related trend in mercury concentrations from 1963 to 2001 (Fig. 3). The mean concentration of mercury in preserved fish collected in 1963 did not differ significantly (Gabriel's test, $P > 0.05$) from

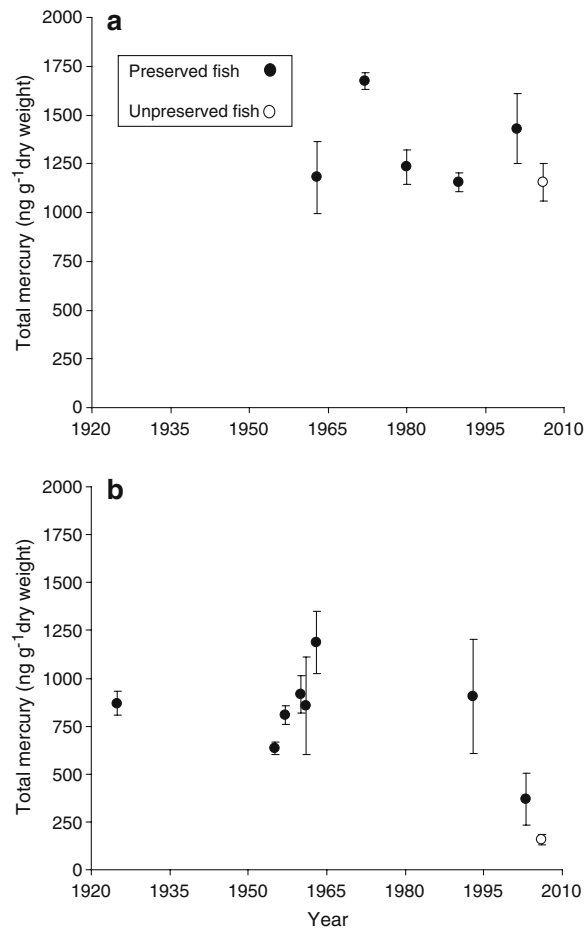

Fig. 3 Mean total mercury concentrations of preserved and unpreserved tissue of longear sunfish in Glover (**a**) and Mountain Fork (**b**) rivers. *Error bars* are 95% confidence intervals

preserved fish collected in 1980, 1990, and 2001 or the unpreserved fish collected in 2006. The mean concentration of mercury in longear sunfish collected in 1972 was greater than those in all other years.

Year of collection also had a significant effect on mercury concentrations of longear sunfish from Mountain Fork River (ANOVA, $F = 17.0$, $df = 8, 81$, $P < 0.001$). From 1925 to 1993, there was no discernable time-related trend in mercury concentrations of longear sunfish. The mean concentration in 1925 did not differ significantly from those in any year from 1955 through 1993 (Gabriel's test, $P > 0.05$). There was a large reduction in mercury concentrations of longear sunfish after 1993 (Fig. 3). The concentration in 2003 was significantly less than all years from 1925 through 1993 except for 1955. The concentration in 2006 was significantly less than those in all years except 2003. The 2003 and 2006 concentrations did not differ significantly ($P > 0.10$).

The decline in mercury concentrations in fish from Mountain Fork River from 1993 to 2003 may be related to impoundment and the operation of the coldwater trout fishery in the river immediately above the collection site. These alterations could reduce mercury contamination of fish by entrapment of mercury-laden sediments above the dam (Waldron et al. 2000), reduction of methylation rates in the river by the coldwater discharge (Hammerschmidt and Fitzgerald 2004), and stabilization of river flow (Leitch et al. 2007). The lack of change in mercury concentration in longear sunfish in nearby Glover River suggests that the reduction in mercury in longear sunfish from Mountain Fork River is not due to a reduction in atmospheric deposition of mercury.

Field study

To assess whether preserved museum fish could be used to predict current mercury concentrations in fish from the two rivers, we compared the concentrations of mercury in the most recently collected museum fish to the concentrations of mercury in unpreserved fish we collected in 2006. For Glover River, mean concentration of mercury in fish collected in 2006 was 1,155 ± 95.0 ng g^{-1} total mercury dw which was not statistically different (Gabriel's test, $P < 0.05$) than the concentrations of mercury in museum fish collected in 2001 (1,430 ± 177 ng g^{-1} total mercury dw). For Mountain Fork River, the mean concentration of mercury in fish collected in 2006 was 156 ± 28.8 ng g^{-1} total mercury dw which was not statistically different (Gabriel's test, $P < 0.05$) than the concentration of mercury in museum fish collected in 2003 (369 ± 133 ng g^{-1} total mercury dw). These results suggest that preserved fish in a museum could be used as a screening tool to predict current mercury concentrations in fish. Collecting fish from the field often requires permits, personnel, time, money, and equipment that are not needed to sample tissues from preserved fish in museums. Therefore, sampling tissues from museum fish that have been recently collected may be a more efficient method to assess current mercury levels in fish, but the mercury concentrations should be confirmed by fresh field samples.

Conclusion

One of the important functions of biological museum collections has been to provide specimens that can be used to monitor environmental change (Suarez and Tsutsui 2004). By examining museum specimens, researchers can estimate historical levels of contamination and construct a baseline against which current levels can be compared (Suarez and Tsutsui 2004). Museum collections of fish have been used to examine the accumulation of toxic compounds, such as mercury, in fish (Barber et al. 1972; Evans et al. 1972; Miller et al. 1972; Gibbs et al. 1974; Kelly et al. 1975; Swain and Helwig 1989; Martins et al. 2006). Gibbs et al. (1974) questioned the use of preserved museum fish and pointed out the need for more comparisons of concentrations of metals between preserved and unpreserved fish. Our study indicates that preserved museum fish can be used to evaluate historical changes and predict current levels of mercury contamination in fish.

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