BIOCHEMICAL AND POPULATION EFFECTS OF LONG-TERM MERCURY EXPOSURE IN LARGEMOUTH BASS (*MICROPTERIS SALMOIDES*) AND CHANNEL CATFISH (*ICTALURUS PUNCTATUS*)

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INTRODUCTION

The occurrence of mercury in edible fish throughout the United States has caused widespread concern about potential hazardous effects to humans consuming mercury-laden fish. In recent years, fishery surveys of the lower Ouachita River have discovered levels of mercury exceeding the US FDA advisory limit of 1 mg/g in large predatory fish such as the largemouth bass and channel catfish (Nix et al. 1992). Fillets of mature largemouth bass collected from Woodard Lake, an ox-bow lake of the lower Ouachita river (3 mi NE of Camden, AR), possessed residues of mercury up to 1.5 ppm. The source of mercury and the effect of these residual levels in fish is unknown. The impact to human health has been the predominant endpoint in studies designed to examine the effects of mercury. However, the impact on the environment , or specifically, on fishery populations has not been adequately addressed.

One way to measure the sublethal effects of metals such as mercury on aquatic organisms such as fish is to determine whether fishery populations with excessive mercury residues were experiencing biochemical stress. Metallothioneins (MTs) are low molecular weight proteins found in various tissues that are induced by exposure to various heavy metals and cellular stress. The function of these proteins appears to be primarily related to metal detoxification and homeostasis (Kagi and Schaffer 1988). Induction by heavy metals and stress may allow this protein to serve as a biochemical marker in organisms exposed chronically to heavy metals such as mercury. Several groups have utilized MTs and other heavy metal binding proteins as a biochemical marker for exposure to heavy metals (Benson et al. 1990; Hogstrand et al. 1991; Gagne and Blaise 1993).

Consequently, the purpose of this study was to examine the effects of a chronic laboratory exposure of methylmercury to channel catfish and compare these results to those obtained from field studies where catfish and large-mouth bass had excessive mercury residues.

METHODS

Ouachita River Study

Feral Sampling and Cage Location

Feral samples of mature female largemouth bass (*Micropterus salmoides*) (0.8-1.0 kg) (n = 4) were obtained from near-shore habitats of Lake Ouachita and Woodard Lake (Figure 1) by electro-shocking from a moving boat. After netting fish, livers were immediately excised, frozen in dry ice and stored at -80°C until analyzed. Fillets of largemouth bass were frozen for subsequent mercury analysis.

Ten 75 g juvenile channel catfish (Stuttgart Strain) were placed in a 0.125 cubic meter wire cage and lowered to a depth of approximately 2 meters at the same sites where feral samples were obtained. After a two week exposure, the caged fish were removed with immediate dissection of the liver from each animal. Each biological sample was frozen in dry ice and stored at -80°C until analyzed for mercury and MT mRNA. Preliminary studies with channel catfish have indicated that induction of MT mRNA occurs following a 2 wk exposure of 10 ppb cadmium chloride (Zhang and Schlenk 1995).

Sampling and cage placement was carried out in January of 1994. January was chosen for collection and cage placement in order to reduce the potential for cage tampering. Lake Ouachita was formed by impoundment of the Ouachita River in 1953. Generally, the water of Lake Ouachita is low in specific conductance (20-40 mS), slightly acidic, and very weakly buffered. Total organic carbon generally ranges from 3-5 mg/L (Nix et al. 1992). Woodard Lake is an Ox Bow lake within the flood plain of the Ouachita River.

Mercury Analysis

Frozen tissue was thawed and 0.25 g was added to 9 ml of a 1:1 (v/v) mixture of sulfuric and nitric acids. After adding 5 ml of 5 % potassium persulfate, the samples were digested in a heating block for 30 min at 50-60°C. Following the addition of 2.5 ml of 5 % Potassium permanganate, the samples were heated again for 30 min at 50-60°C. After a second addition of Potassium permanganate and subsequent digestion, samples were allowed to cool at room temperature for 1 h. To clear the permanganate, 2.5 ml of hydroxylamine sulfate was added with a subsequent dilution of the sample to 100 ml of water. Mercury analyses were carried out on a LDC Analytical Mercury Monitor 3200 (Thermoinstrumental Systems, Inc., Rivera Beach, FL).

Ribonuclease Protection Assay

Approximately 100 mg of hepatic tissue from feral fish and caged catfish were placed in a 2.0 ml sterile plastic tube and total RNA was isolated using RNA Trireagent (Molecular Research Center, Cincinnati, OH). Prior to RPA analysis, total RNA (10 mg) was separated on a 1.5 % agarose gel and assessed for purity and degradation. Loading of RNA was normalized by the densitometric measurement of 18S bands from each sample. Winter Flounder MT cDNA, graciously provided by Dr. King Ming Chan (Chan et al. 1989), was transcribed to the corresponding cRNA with ^{32}P-a-UTP using Maxiscript Transcription Kit (Ambion Inc. Austin, TX). The radiolabeled cRNA probe added to 10 mg of total RNA from each sample and allowed to hybridize for 16 h at 45°C. The ribonuclease protection assay was performed using the RPAII Kit(Ambion Inc., Austin, TX). RNAse digested samples were heated to 90°C, ran on an 8% acrylamide urea gel, and exposed to XAR X-ray film for 12-96 h. Hepatic RNA from cadmium treated (500 mg/l) channel catfish (Zhang and Schlenk 1995) served as a positive control, while samples containing yeast RNA were used as negative controls. Densitometric measurements were carried out using a BioRad GS670 imaging densitometer (Biorad Inc. Hercules, CA) and values of each group of individuals compared using a paired students t-test.

Bayou Bartholomew Study

Female largemouth bass *Micropteris salmoides* (n = 9) were sampled using electrofishing techniques and seining procedures from 7 locations in an Arkansas Bayou (Bartholomew) which is a low gradient stream arising in central Arkansas and flows in a sourtherly directions to the Louisiana border. Livers were immediately dissected at the site of collection, placed in labeled plastic bags, frozen between two blocks of dry ice and subsequently stored at -80°C until used for analyses. A modification of the method of Goede and Barton (1990) used to numerically quantitate 14 variables of feral centracid health. In this approach, index variables were assigned numerical values based on the degree of severity or damage caused by environmental stressors. Organs that were grossly examined include: thymus, fins, spleen, hindgut, kidney, skin, liver, eye, gill, and pseudobranchs. Other variables included the presence of parasites, as well as the levels of hemocrit, leukocrit, and plasma proteins. All values derived from the observations were equally weighted and ranged from 0 (no effect) to 30 (severely affected). This Health Assessment Index (HAI) has been used in a wide range of waterways and river basins throughout the United States (North Carolina, Tennessee, Alabama, Kentucky) as well as reservoirs in Georgia and South Carolina (Adams et al. 1993). With the exception of the plasma protein, hematocrit and leukocrit measurements, every other variable was obtained by a field necropsy performed on the feral samples. MT analyses and hepatic concentrations of mercury were analyzed as above.

Laboratory Exposure

Mature female channel catfish (n =3) were daily fed a single Japanese medaka (*Oryzia latipes*) injected with a 0.1 mg/kg dose of methylmercury for 30 days. Fish were housed individually in flow-through aquaria and also fed ARKAT catfish food at 2% of their body weight. Following exposure, the lengths and weights of the fish were measured. The livers were dissected, weighed, and frozen for MT analysis as described above.

RESULTS AND DISCUSSION

Ouachita River Study

Total residual mercury in bass fillets varied from < 1 ppb to 1 ppm from each site. When comparing mercury residues with MT mRNA expression, the coefficient of determination (r^2) was 0.756 while the coefficient of correlation was 0.87 (Figure 1). The equation that fit the line was $y = 3.3084 + 5.0167x$. To determine whether Woodard Lake was a point source of mercury contamination, caged catfish were placed in each location where feral samples were obtained. There was no significant ($p \geq .375$) difference between two protected

MT mRNA bands or muscle mercury residues in animals of each site (Figure 2).

A significant correlation of MT expression with muscle mercury was observed in largemouth bass sampled from the Ouachita river system and is the first such report showing a correlation of mercury residues and hepatic MT in fish. Food-chain contamination by mercury has been observed in many waterways around the world without a point source of inorganic mercury (Gilmour and Henry 1991). Although mercury appears to enter pristene waterways as inorganic mercury, methylation of the inorganic form allows rapid bioaccumulation in aquatic biota (Spry and Wiener 1991). Methylmercury is primarily (Bouquegneau 1986) stored in the muscle bound as an acto-myosin complex (Barghigiani et al. 1989). However, upon chronic lifetime exposures, as much as 50% of hepatic methylmercury has been shown to be demethylated to inorganic mercury (Barghigiani et al. 1989) which is capable of MT induction . It is unclear from our study whether inorganic mercury derived from methylmercury or stress related to mercury toxicity is responsible for initiating MT transcription in bass. Single doses of methylmercury in a laboratory setting have never been shown to induce MT in any species (Olson et al. 1978). The difficulty in showing a relationship between MT induction and methylmercury exposure in the laboratory appears to be due to the inability to duplicate lifetime dietary exposures of methylmercury in the laboratory.Other metals demonstrating MT induction in the laboratory have also been shown to induce MT in the field. A positive correlation between hepatic MT of feral fish and metal exposure has been previously observed in the Campbell River system of British Columbia, Canada, where the drainage water from a mine contained a mixture of zinc, copper, and cadmium (mixture approximately 400:20:1) (Roch et al. 1982). In another study, a positive correlation was observed between copper residues and hepatic MT from feral fish taken from a site down-stream from a brassworks plant in Sweden (Hogstrand et al. 1991). It is interesting to note that in each of these studies, water and/or sediment levels of heavy metals were also significantly higher than the control sites. However, in the present study, although muscle residue levels of mercury were elevated over those of a control site, water and sediment analyses of Woodard Lake failed to show elevated levels of mercury or any other heavy metal (Nix et al. 1992). In addition, Woodard Lake does not receive direct industrial effluent of any type suggesting that exposure to mercury in largemouth bass may be occuring through the food web. MT measurement verified this known phenomenon in the caged catfish (*Ictalurus punctatus*) exposures. The lack of significant induction demonstrated that exposure to water or sediment from these sites was unable to induce MT expression.

Bayou Bartholmew Studies

Lengths of the bass ranged from 18.7-38.5 cm and HAI values ranged from 50 to 130 between the seven sites. Linear regression analysis failed to show a correlation between MT expression and HAI ($r^2 = .009$; $p = .800$). Recent work has also shown that MT expression also failed to correlate with numbers of largemouth bass collected as well as other population measurements such as species diversity, richness, and community metrics such as the Shannon Weaver index (Schlenk et al. 1996). It is interesting to note that fish obtained from the Ouachita River study above also failed to show overt signs of toxicity. Since the muscle appears to be the primary site for methylmercury storage, critical organs such as those examined in this assay may avoid insult based on a dispositional protection. To determine whether methylmercury affects organal function or causes changes in population, population metrics such as length, weight, liver-somatic index, and condition factor were examined in a laboratory exposure mimicing the trophic transfer of mercury from prey to predator.

Chronic Exposure Study

As observed in field studies above, channel catfish chronically exposed to methylmercury through a dietary route failed to show any overt signs of population effects such as reduced growth, length, or somatic indices. Mercury concentrations in the liver and muscle of methylmercury treated fish were significantly elevated compared to controls ($p \leq 0.002$) and ranged from 1.9 to 2.8 mg/g in liver and 1.2 to 1.8 mg/g in muscle (Figure 3). MT protein concentrations were unaltered as were whole-animal endpoints such as condition factor and liver somatic indices (Figure 4). Consequently, it appears that fish chronically exposed to methylmercury for 30 days are not undergoing biochemical stress in the liver or being significantly altered at the whole-animal level. It is possible that other organs may be affected by exposure such as gonad or kidney. In addition, since earlier studies did not show a relationship between mercury residues and MT, it is possible that even a 30 day exposure does not successfully mimic a life-time exposure. It also indicates that demethylation of mercury is a relatively slow process because only demethylated mercury is able to induce MT.

SUMMARY

In summary, although biochemical detoxification pathways were induced in feral large mouth bass which contained mercury residues exceeding 1 mg/g, overall health of bass did not appear to change. When a centrarid health assessment index of feral bass or population indices were measured and compared to biochemical detoxification proteins in bass, no correlation was observed. These data indicated that animals with elevated mercury had elevated biochemical defenses, but did not appear to show whole-animal or population abnormalities suggesting adaptative change to mercury. To test this hypothesis, adult channel catfish were exposed to methylmercury via the diet for 30 days with biochemical and whole-animal endpoints determined. As observed in the field, animals with elevated mercury residues did not appear to be affected at the whole-animal or population level. In addition, although animals had elevated hepatic and muscle residues of mercury, biochemical defenses were not induced indicating exposed animals were not under hepatic stress. Obviously, effects at other organs need to be examined, especially at the reproductive and immunologic levels. In order to carry out these studies in the laboratory to mimic the feral situtaion, life-time exposures to methylmercury are necessary with subsequent measurements of reproductive and immunologic endpoints. Such data will provide evidence whether feral populations of predatory fish are susceptible and adversely affected by methylmercury.

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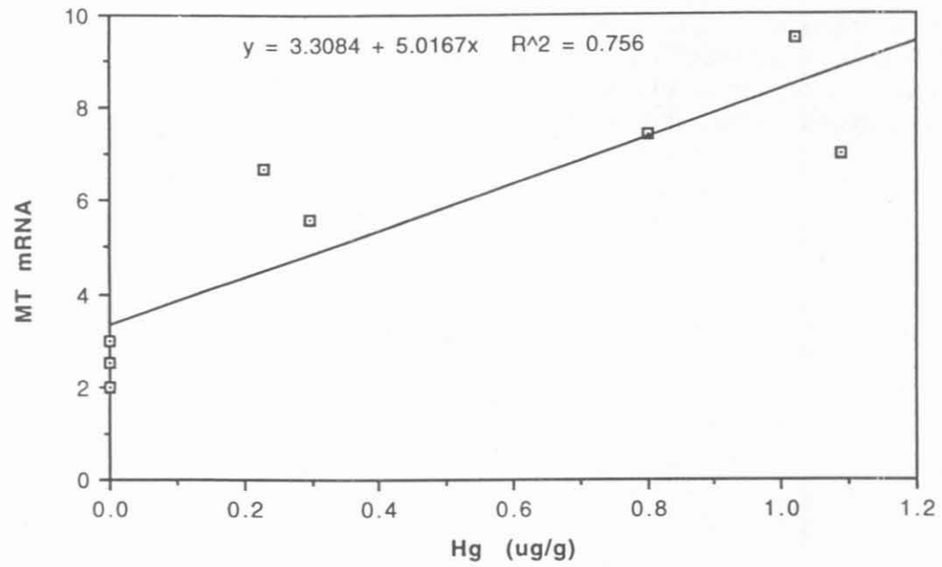

Figure 1. Correlation of Muscle Mercury Residues and Hepatic MT mRNA Induction.

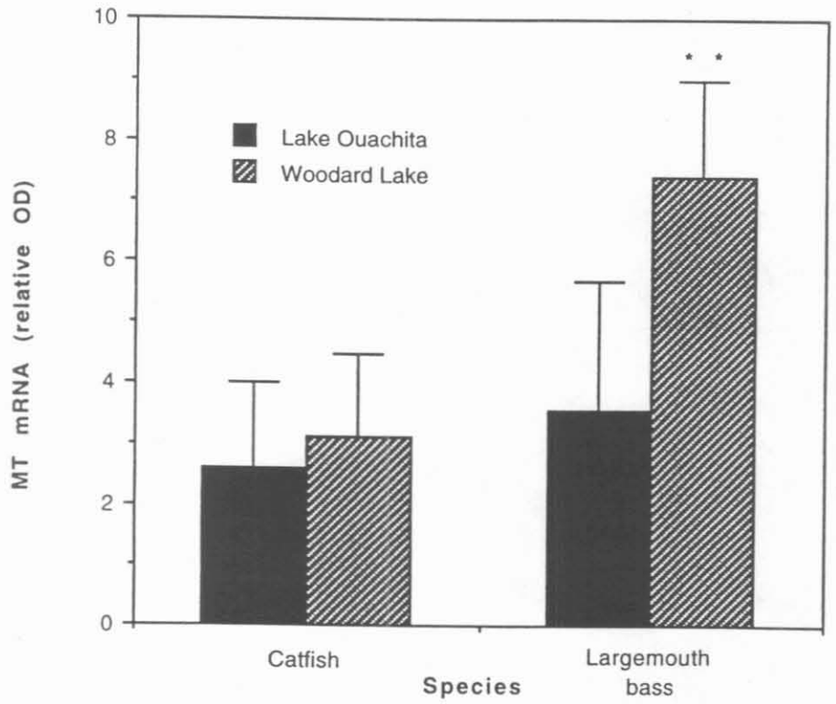

Figure 2. Metallothionein mRNA levels in Caged Catfish and Feral Largemouth Bass from Lake Ouachita and Woodard Lake.

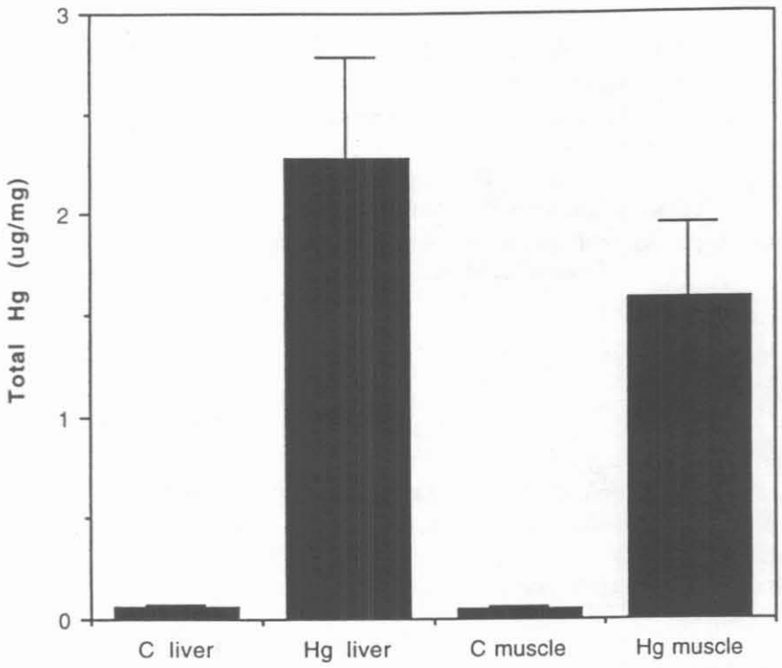

Figure 3. Liver and Muscle Residues of Mercury in Channel Catfish Following a 30 day Trophic Exposure to Methylmercury

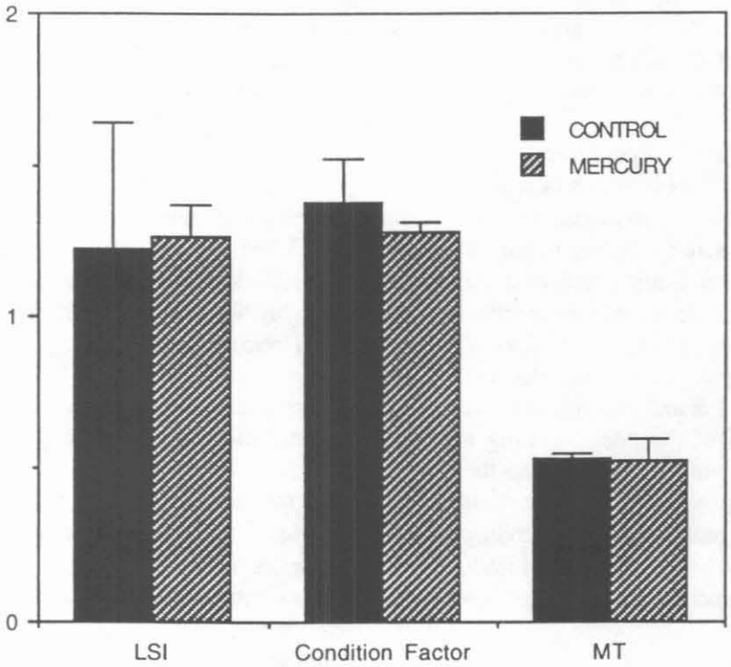

Figure 4. Effect of 30 day Methylmercury Exposure on Whole-Animal and Biochemical Endpoints in Channel Catfish