Characterization of hepatic metallothionein expression in channel catfish (*Ictalurus punctatus*) by reverse-transcriptase polymerase chain reaction

Daniel Schlenk, Marisa Chelius, Laurie Wolford, Shabana Khan and King Ming Chan

Measurements of metallothioneins (MTs) can be labour intensive and require expensive probes and/or analytical instrumentation. In contrast, the reverse-transcriptase polymerase chain reaction (RT-PCR) has provided a relatively sensitive and simple mechanism to measure expression of MT in sentinel species in aquatic environments. The objectives of this study were to characterize expression of hepatic MT in a commonly occurring fish species of the southeastern United States, the channel catfish *(Ictalurus punctatus)*. Expression of hepatic MT was assessed following exposure to several metals found naturally and in excess due to anthropogenic inputs. MT expression by RT-PCR was confirmed by Southern blot with a cDNA probe (552 bp) encoding MT and the complete 3′ untranslated region from the common carp (*Cyprinus carpio*) and a 388 bp cDNA probe encoding 49 bp of the 5′ untranslated region, the entire MT protein, and the complete 3′ untranslated region of winter flounder (*Pleuronectes americanus*). Each probe recognized a single band of approximately 550 bp which corresponded to the RT-PCR product from hepatic RNA of channel catfish 24 h after an intraperitoneal injection of cadmium chloride (10 mg kg^{-1}). Catfish were also injected with intraperitoneal administrations of zinc sulphate (10 mg kg^{-1}) and copper sulphate (2 mg kg^{-1}). After 24 h, zinc increased MT expression 16-fold over controls whereas cadmium or copper increased levels 13- and nearly 6-fold, respectively. A 1-week exposure to water-borne 1 mg l^{-1} sodium arsenite or monosodium methylarsonate (MSMA) induced hepatic MT expression nearly two-fold, whereas water-borne exposure to the same dose of sodium arsenate failed to increase MT levels. To examine the effect of dietary sub-chronic methylmercury exposure on hepatic MT expression, Japanese medaka (*Oryzias latipes*), were lethally injected with solutions of methylmercuric chloride to provide a 0.1 mg kg^{-1} daily dose to catfish. After 30 days, hepatic MT expression, condition factor and liver somatic indices were unchanged, although hepatic and muscle residues of total mercury were significantly increased following treatment. The results of this study show that MT expression can vary in aquatic organisms in response to different metal treatments and may be utilized as a biomarker of exposure and possibly effect in the channel catfish.

Keywords: *metallothionein, RT-PCR, cadmium, zinc, arsenic, copper, methylmercury, channel catfish,* Ictalurus punctatus, *liver.*

Abbreviations: *MSMA, monosodium methylarsonate; MT, metallothionein, RT-PCR, reverse-transcriptase polymerase chain reaction.*

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Introduction

Metallothioneins (MTs) are low-molecular weight cytosolic proteins that contain highly conserved cysteinyl residues allowing the binding, transport and storage of various transition metals (Hamer 1986, Andrews 1990). Expression of MTs are induced in many terrestrial and aquatic organisms not only by exposure to various transition metals, but also endogenous constituents released by cellular stress, such as cytokines and hormones (Hamer, 1986, Andrews 1990). The association of MTs with detoxification and metal exposure as well as their presence during acute cellular stress indicates a potential role as a bioindicator of metal exposure and/or cellular stress. Indeed, recent studies have shown a direct correlation between MT induction and lipid peroxidative damage and physiological alterations in feral fish populations (Farag *et al.* 1995). However, due to alterations caused by presumably secondary homeostatic functions, it is imperative that MT expression be adequately characterized in any animal used as a sentinel species prior to its use as a bioindicator of effect.

One of the major disadvantages of using MT as a biomarker has been the difficulty in obtaining consistent measurements of its expression. Initially, purification with subsequent quantitation of individual isoforms by metal content was performed to measure MT protein (Olafson and Thompson 1974). Other methods of protein measurement have relied on indirect saturation assays with radiolabelled metal or analysis of metal–MT complexes by graphite furnace atomic absorption spectroscopy (Hamilton and Mehrle 1987). Although these methods are relatively simple to perform, they are extremely sensitive to the dilution of the protein used and isoforms also must be partially purified to remove glutathione or other sulphhydral–metal complexes which may lead to overestimation of MT (Bienengraber *et al.* 1995). In addition, the likelihood of underestimated protein is enhanced due to dimerization which readily occurs in aerated buffers used to homogenize tissue samples (Palumaa and Vasak 1992). Recently, the development of antibodies to isoforms in fish has allowed a fairly rapid assessment of MT levels (Hylland *et al.* 1995). However, antibodies are difficult to obtain and require purification and characterization studies prior to their usage in field studies (George *et al.* 1996). Another approach that has shown promise is the measurement of MT mRNA by Northern blot or ribonuclease protection analyses (Kille *et al.* 1992

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Roesijadi *et al.* 1995, Schlenk *et al.* 1995, Zhang and Schlenk 1995, Unger and Roesijadi 1996). These sophisticated methods are very sensitive, but often require specific cDNA or cRNA probes and radiolabelled materials for detection and can be labour intensive. An alternative method for maintaining sensitivity, but reducing the need for radiolabel and laboratory preparation was recently suggested by Jessen-Eller (Jessen-Eller *et al.* 1994) utilizing the reverse-transcriptase polymerase chain reaction (RT-PCR). RT-PCR has been used to measure MT expression in field and laboratory studies with *Fundulus heteroclitus* exposed to cadmium (Kaplan *et al.* 1995). A modification of this procedure, Rapid Amplification of cDNA Ends (RACE), has allowed establishment of a relatively inexpensive but sensitive method for determining levels of MT transcripts in fish (Chan 1994).

The channel catfish (*Ictalurus punctatus*) has been observed in waterways throughout the United States, but is predominantly found and commercially cultured in the southeastern states (Douglas 1974). It is an opportunistic omnivore ranging throughout the water column but is considered a bottom-dwelling species (Tucker 1985). Because of its relative abundance and natural history, the channel catfish has the potential to be used as a sentinel species for biomarker studies in aquatic systems, especially in the southeastern United States. In previous studies in *I. punctatus*, the induction of hepatic MT in response to cadmium was characterized (Zhang and Schlenk 1995). However, the effects of other metals on MT expression in this species have not been examined. Consequently, the purpose of this study was to demonstrate that RT-PCR can be used as a method measuring MT induction by multiple metal species and to characterize MT induction following exposure to different metals by varied routes of exposure. Exposure routes included intraperitoneal injection (cadmium, zinc and copper), water-borne exposure (arsenicals), and trophic transfer (methylmercury) with subsequent measurement of hepatic MT expression.

METHODS

Chemicals

Cadmium chloride, zinc chloride, copper sulphate, sodium arsenate, and sodium arsenite were purchased from Sigma Chemical Co. (St Louis, Mo). Monosodium methylarsonate (MSMA) H.C. was obtained from Cleveland Chemical Co. (Cleveland, MS). Methyl mercuric chloride was obtained from Aldrich Chemical Co. (Milwaukee, WI).

Animal treatments

Juvenile channel catfish, *Ictalurus punctatus* (12–15 cm), weighing about 80–100 g were obtained from the US Department of Agriculture National Aquaculture Laboratory in Stuttgart, Arkansas. Fish were acclimated and maintained in a flow-through aquarium using charcoal-filtered, dechlorinated water (18–22°C). Fish were daily fed ARKAT catfish chow (Dumas, AR) at 2% of their body weight.

To compare the efficacy of MT expression by metals, fish ($n = 4$) received intraperitoneal injections of 10 mg kg^{-1} of copper sulphate, cadmium chloride, and zinc sulphate. Controls received a saline injection. After 24 h, blood was obtained from the caudal vein, the fish was euthanized, and the liver excised and frozen between two slabs of dry ice. Tissues were stored at –80°C until RNA isolation. To examine the effects of various arsenical agents, catfish ($n = 6$) were exposed for 1 week by 24 h static renewal to 1 mg l^{-1} sodium arsenate, sodium arsenite and MSMA (as the pure ingredient). Untreated animals were maintained concurrently with arsenic-treated animals for controls. Since exposure to mercury in predatory and omnivorous fish is predominantly through the food chain (Stein *et al.* 1996), the effect of dietary sub-chronic methylmercury exposure on hepatic MT expression was examined. Japanese medaka (*Oryzias latipes*) (approximately 0.1 g) were lethally injected with solutions of methylmercuric chloride to provide a 0.1 mg kg^{-1} daily dose to catfish. Dose and duration were determined from preliminary range-finding studies. Channel catfish were fed the methylmercury-laden medaka daily for 30 days in flow-through tanks receiving filtered, dechlorinated water. After the medaka was consumed, fish were then fed diets of ARKAT catfish food at 2% of their body weight. Following the 30-day exposure, catfish were euthanized, weighed and the liver dissected. The livers were frozen and stored as above. An aliquot of the liver was used for RT-PCR and the remaining portion for total mercury measurement. Total mercury in axial muscle and liver was analysed using an LDC Analytical Mercury Monitor 3200 (Thermoinstrumental Systems, Inc., Rivera Beach, Fl) after acid digestion and oxidation with potassium permanganate as described previously (Schlenk *et al.* 1995). Condition factors [100 (body weight, g)/standard length, $cm)^3$] and liver somatic indices (LSI) (percent body weight represented by the liver) were calculated in untreated and mercury-treated fish.

Isolation of RNA from the livers

Total RNA was extracted from approximately 75 mg of frozen fish liver using TRI reagent (Molecular Research Center Inc., Cincinnati, OH). The final RNA pellet was dissolved in 40 μl of DEPC-treated water and the concentration was measured using a Hitachi U-2000 spectrophotometer at 260 nm (Sambrook *et al.* 1989). Agarose gel electrophoresis was used to check the integrity of the RNA (presense of 18S and 28S ribosomal bands) and the density of the band corresponding to the 18S subunit was measured by image analysis using the software NIH Image 1.5.2 on a scanned polaroid photograph of the gel. The density of the 18S ribosomal RNA was used to verify spectrophotometric determination of RNA concentrations.

Synthesis of cDNA and PCR (RT-PCR)

RT-PCR was carried out using the 1st Strand cDNA Synthesis Kit and PCR master kit (Boehringer Manheim, Indianapolis, IN) as previously described (Chan 1994). The Universal RACE-T primer (5′CCGAA TTCTC GAGAT CGATT TTTTT TTTTT TT-3′) for the RT reaction as well as the 5′-(5′-ATGGA TCCNT GCGAA TG-3′) and 3′-adaptor primer (5′CCGAA TTCTC GAGAT CGA-3′) were designed based on the initial six amino acid residues of piscine MT (Chan 1994) and obtained from National Biosciences Inc. (Plymouth, MN). For the RT reaction, 1.0 μg of RNA was used in a total reaction volume of 50 μl containing 1X reaction buffer, 5 mM $MgCl_2$, 1 mM DNTP mix, primer 1 μM, 10 units of RNAse inhibitor, 0.8 μl of AMV reverse transcriptase, and 10 ng of RACE-T primer. The reaction mixture was incubated at 42°C for 1 h and then diluted with 150 μl water to a total volume of 200 μl. PCR was carried out on a 2 μl aliquot of the single stranded cDNA solution resulting from RT. The PCR master mix contained 25 units of *Taq* DNA polymerase in 20 mM Tris–HCl, 100 mM KCl, 3 mM $MgCl_2$, 0.01% Brij^r 35 (v/v), dNTP mix (dATP, dCTP, dGTP, dTTP) each 0.4 mM, pH 8.3 (20°C) in a final volume of 0.1 ml. A set of specific primers for metallothionein expression was used as mentioned above. The PCR was performed using 25 cycles comprised of three segments of 94°C for 1 min; 50°C for 2 min; and 72°C for 3 min and then a final extension at 70°C for 5 min. Amplification of an 800 bp fragment of β-actin was used to normalize quantities of the amplified transcript. The primers for actin were 5′-ACTCACATCTGCTGGAAGGT-3′ and 5′-TCACCAACTCCCATCACATC-3′. Studies

carried out with coincubation of β-actin primers and MT primers led to reduced MT signals (see Figure 4). Consequently, separate amplification reactions were performed with equal volumes of single stranded cDNA from the RT reaction. Each PCR product was resolved on 1.0% agarose gels (Sambrook *et al.* 1989), photographed, and analysed by image analysis as above. Southern blot analysis was performed using standard methods (Sambrook *et al.* 1989) with cDNAs to *Pleuronectes americanus* (388 bp) and *Cyprinus carpio* (522 bp). Hybridization of transferred PCR products to the probes was carried out at 50°C and the cDNAs were labelled by random primer extension to a specific activity of > 10^8 cpm μg^{-1} with [α-^{32}P]-dCTP (Sambrook *et al.* 1989). Following an 18 h hybridization, the membranes were washed three times at 47°C with 0.5×SSC containing 0.1 SDS and exposed to Kodak XAR film overnight at –80°C.

Statistics

To compare animals injected with metals and water-borne exposures, a one-way ANOVA was performed in order to assess variability and significance was tested by Dunnets *t*-test. For mercury experiments, a Student's *t*-test was used to compare untreated and mercury-treated animals.

Results

Utilizing the RACE-T method, primers were designed to reverse transcribe the coding region of MT beginning at the amino-terminus and include the complete 3′ untranslated region. A single product was obtained and was 550 bp in length. Southern blot analysis of the PCR product using cDNAs encoding winter flounder or carp MTs revealed a single band corresponding to the 550 bp product confirming its identity as the MT cDNA for channel catfish (Figure 1).

Expression of the hepatic MT cDNA for catfish was dependent upon the amount of total RNA present (Figure 2). Comparing the induction capacities of injected zinc, cadmium, and copper, zinc was the strongest MT-inducing agent followed by cadmium, copper and saline-treated controls when samples were normalized on a mg of metal basis (Figure 3 and Table 1). When compared on a molar basis, MT expression by zinc was twice (24.8 Absorbance Units [AU]) that of cadmium (11.5 AU) and more than two-fold higher than copper-induced MT expression (9.6 AU). Following aqueous exposures to 1 mg l^{-1} sodium arsenite, arsenate and the herbicide MSMA, arsenite and MSMA were stronger inducers than arsenate, but relatively weak compared with cadmium or zinc (Figure 4 and Table 2).

Treatment of catfish for 30 days with methylmercury-laced medaka (0.1 mg kg^{-1}) failed to induce hepatic MT expression (Figure 5 and Table 3). However, total mercury

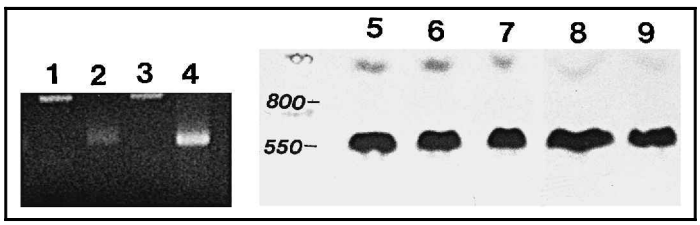

Figure 1. RT-PCR products from hepatic RNA of channel catfish (*I. punctatus*) probed with ^{32}P-labelled winter flounder (*P. americanus*) and common carp (*C. carpio*) MT cDNAs. Lanes 1–4 ethidium bromide stained agarose gel; lane 1: 800 bp RT-PCR β-actin fragment from hepatic RNA of untreated catfish; lane 2: 550 bp RT-PCR-product from hepatic RNA of untreated catfish; lane 3: 800 bp RT-PCR β-actin fragment from hepatic RNA of cadmium-treated (10 mg kg^{-1}) catfish; lane 4: 550 bp RT-PCR product from hepatic RNA of cadmium-treated (10 mg kg^{-1}) catfish. Lanes 5–7: Southern blot of RT-PCR products from cadmium-treated catfish probed with ^{32}P-labelled winter flounder MT cDNA; Lanes 8–9: Southern blot of RT-PCR products from cadmium-treated catfish probed with ^{32}P-labelled common carp MT cDNA.

	Control	Cadmium	Zinc	Copper
MT expression	104±55	1300±350[a]	1621±222[a]	588±101[ab]

Table 1. Effect of the intraperitoneal injection of cadmium chloride (10 mg kg^{-1}), zinc sulphate (10 mg kg^{-1}), and copper sulphate (2 mg kg^{-1}) on the expression of hepatic MT after 24 h in channel catfish (*Ictalurus punctatus*). Each value represents the mean absorbance of four animals normalized against the absorbance of β-actin±SD.

[a] Statistically significant ($p < 0.05$) from control.

[b] Statistically significant from a ($p < 0.05$).

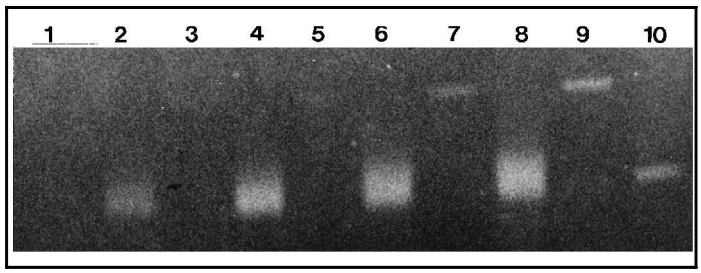

Figure 2. Effect of hepatic RNA content on hepatic MT expression from cadmium-treated catfish (*I. punctatus*) by RT-PCR. Lane 1: MT expression from 0 μg of RNA; Lane 2: MT expression from 0.5 μg of RNA; Lane 3: β-actin expression from 0.5 μg of RNA; Lane 4: MT expression from 1.0 μg of RNA; Lane 5: β-actin expression from 1.0 μg of RNA; Lane 6: MT expression from 2.5 μg of RNA; Lane 7: β-actin expression from 2.5 μg of RNA; Lane 8: MT expression from 5.0 μg of RNA; Lane 9: β-actin expression from 5.0 μg of RNA. Lane 10: 564 bp DNA standard from Hind III-digested λ phage (Biorad).

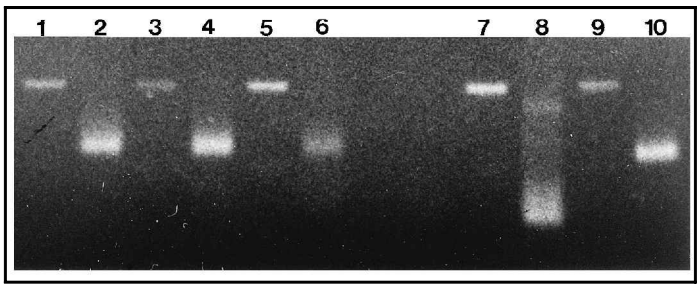

Figure 3. Representative ethidium bromide-stained gel of RT-PCR products of hepatic RNA from channel catfish (*I. punctatus*) and rainbow trout (*O. mykiss*) following various metal treatments. Lanes 1 and 9: β-actin expression from cadmium-treated catfish; Lanes 2 and 20: MT expression from cadmium-treated catfish; Lane 3: β-actin expression from zinc-treated catfish; Lane 4: MT expression from cadmium-treated catfish; Lane 5: β-actin expression from copper-treated catfish; Lane 6: MT expression from copper-treated catfish; Lane 7: β-actin expression from zinc-treated rainbow trout; Lane 8: MT expression from zinc-treated rainbow trout.

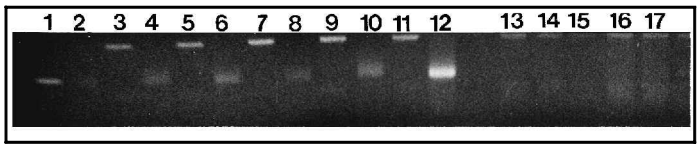

Figure 4. Representative ethidium bromide-stained gel of RT-PCR products of hepatic RNA from channel catfish (*I. punctatus*) exposed to various arsenical agents (1 mg l^{-1}) for 1 week. Lane 1: 564 bp DNA standard from Hind III-digested λ phage (Biorad); Lane 2: no template control; Lane 3: β-actin expression from untreated catfish; Lane 4: MT expression from untreated catfish; Lane 5: β-actin expression from arsenite-treated catfish; Lane 6: MT expression from arsenite-treated catfish; Lane 7: β-actin expression from arsenate-treated catfish; Lane 8: MT expression from arsenate-treated catfish; Lane 9: β-actin expression from MSMA-treated catfish; Lane 10: MT expression from MSMA-treated catfish; Lane 11: β-actin expression from cadmium-treated catfish; Lane 12: MT expression from cadmium-treated catfish; Lanes 13–17: co-amplification of β-actin and MT from reversed transcribed hepatic RNA of cadmium-treated catfish.

	Control	Arsenite	Arsenate	MSMA
MT expression	120±46	224±33[a]	127±57	25±55[a]

Table 2. Effect of the 7-day waterborne exposure (1 mg l^{-1}) to various arsenical agents on the expression of hepatic MT in channel catfish (*Ictalurus punctatus*). Each value represents the mean absorbance of four animals normalized against the absorbance of β-actin±SD.

[a] Statistically significant ($p < 0.05$) from control.

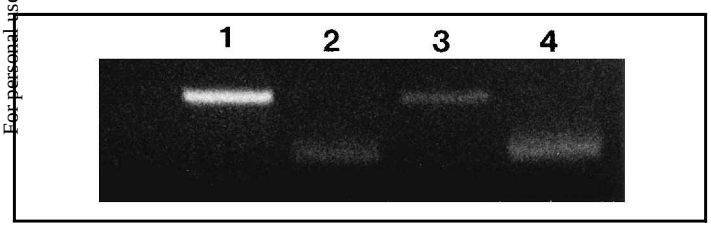

Figure 5. Representative ethidium bromide-stained gel for RT-PCR products of hepatic RNA from channel catfish exposed to dietary methylmercury for 30 days. Lane 1: β-actin expression from untreated catfish; Lane 2: MT expression from untreated catfish; Lane 3: β-actin expression from methylmercury-treated catfish; Lane 4: MT expression from methylmercury-treated catfish.

	Condition factor	Liver Somatic Index	MT expression
Control	1.38±0.14	1.22±0.41	135±62
Methyl-mercury	1.28±0.03	1.27±0.10	125±77

Table 3. Effect of dietary exposure for 30 days to 0.1 mg kg^{-1} of methylmercury on condition factor, Liver Somatic Indices, and hepatic MT expression in channel catfish (*Ictalurus punctatus*).

Each value represents the mean of four animals±S.D.

concentrations in the liver and muscle of methylmercury-treated fish were significantly elevated compared with controls ($p < 0.002$) and ranged from 1.9 to 2.8 μg g^{-1} in liver and 1.2 to 1.8 μg g^{-1} in muscle. In addition, fish were not significantly affected physiologically, since there were no apparent changes in condition factors or Liver Somatic Indices (LSIs) (Table 3).

Discussion

MT expression has been shown to be an effective biomarker of exposure to metals in various laboratory and field studies with fish (Hamilton and Mehrle 1986, Hogstrand *et al.* 1991, Roesijadi 1992). However, many groups have argued that MT expression does not significantly contribute to measuring the adverse effects of these compounds and may be less cost-effective than performing ordinary metal residue analysis (Petering *et al.* 1990). Because the endogenous function of MT is unclear and expression may be altered through normal physiological processes (i.e. spawning in female fish), it can be very difficult to determine whether MT is an effective biomarker of effect in uncharacterized animal systems (Roesijadi 1992, Olsson *et al.* 1995b). Recently, several studies have shown that MT expression can be quickly measured in laboratory and field studies, and that expression was significantly related to altered cellular as well as physiological effects (Farag *et al.* 1995, Perkins *et al.* 1996). In order to identify relationships between cellular, physiological or even reproductive stresses and MT expression, characterization of a model system must be performed with an evaluation of several inducers.

Expression of hepatic MT in the channel catfish has been previously characterized following cadmium exposure (Zhang and Schlenk 1995). However, it was unclear what effect other metals might have on MT expression in this model system. Original studies with channel catfish indicated the presence of, at least, two isoforms of hepatic MT that appeared to be encoded from two mRNAs (Zhang and Schlenk 1995). However, only a single PCR product of 550 bp was observed. Similar findings were also observed utilizing the same RACE-T, 5′ and 3′ primers with rainbow trout (Figure 3) which also possess two isoforms of MT. Since both isoforms of the trout have the same initial ten N-terminal amino acid residues (George and Hodgson 1995), it is not surprising that a single PCR product was observed. Thus, as in trout, it is quite likely the measurements in the present study represent the expression of both MT isoforms observed in channel catfish livers. Studies are currently under way to sequence the 3′ untranslated region of the amplified transcript in hopes of possibly separating the mRNAs for each isoform for future studies. The extremely long 3′ untranslated region of the MT product of channel catfish is similar to the 522 bp MT-transcript of the *C. carpio* (K.M. Chan, unpublished data). The lengths of the catfish and carp transcripts were considerably longer than other RT-PCR products using the N-terminal and polyT primers such as rainbow trout (*Oncorhyncus mykiss*), tilapia (*Oreochromis niloticus*) and goldfish (*Carassius auratus*) which were approximately 350–380 bp (Chan 1994). The significance of these differences in the 3′ untranslated region is unclear but warrants further study.

Zinc was slightly more efficient than cadmium and each of these were significantly more efficient than copper in enhancing the expression of hepatic MT in channel catfish. In juvenile rainbow trout, an intraperitoneal injection of cadmium caused a 10-fold greater induction in hepatic MT mRNA than an equi-molar intraperitoneal injection of zinc

(Olsson *et al.* 1995a). When comparing expression of one of the two MT genes in *O. mykiss* (MTa) by zinc and copper in transfected RTH cells, the authors concluded that zinc was 'a good inducer' whereas copper was 'a poor inducer' (Olsson *et al.* 1995a). However, when the levels of MTa induction are normalized for equi-molar doses of metal, zinc induction was actually only 25% higher than copper (Olsson *et al.* 1995a). Although numerical values were not reported in studies with winter flounder, the authors concluded that cadmium was the most potent inducer of hepatic MT mRNA when compared with zinc, copper, lead or inorganic mercury (Chan *et al.* 1989). Reasons for differences in MT transcription by various metals are primarily related to the numbers of metal response elements (MREs) located in the 5′ untranslated region of the MT gene (Kille and Olsson 1994). However, other factors such as metal disposition and transcription factor activation may also be differentially affected by specific metals.

Arsenic is a naturally occurring element in soils and waterways with average mean concentrations in soil of 2 mg kg^{-1} and surface water concentrations of 3 μg l^{-1} in the United States (ATSDR 1993). In addition, arsenical herbicides are widely used throughout the southeastern United States in cotton agriculture (Snipes and Byrd 1994). Earlier studies in our laboratory demonstrated a dose-dependent increase of hepatic MT protein and mRNA following aqueous treatment to arsenite, arsenate and the herbicide, MSMA (Schlenk *et al.* 1997). MT protein was measured by the cadmium–haemoglobin saturation assay (Eaton and Toal 1982) and mRNA was measured by Northern blot analysis using a cDNA encoding for winter flounder MT (Zhang and Schlenk 1995). In the current study, only the highest doses of the arsenicals (1 mg l^{-1}) were repeated and similar levels of MT expression were observed by RT-PCR as those measured by saturation and Northern analysis. Arsenite exposure led to higher concentrations of MT expression than exposures to MSMA and arsenate, respectively. Although MT expression was induced by arsenic, arsenic-induced MT expression was significantly lower than levels of MT expression induced by cadmium. Although the effects of arsenic on MT expression have not been thoroughly examined in fish previously, studies in mice indicate a similar pattern of weak induction by arsenicals when compared with transition metals (Kreppel *et al.* 1993). In addition, *in vivo* studies in rats and mice suggest an indirect mechanism of MT induction because *in vitro* arsenic treatments in cell lines have not been shown to induce MT (Albores *et al.* 1992, Kreppel *et al.* 1993).

Reports of mercury-contaminated fishery populations have been continuing to rise across the United States (Gilmour and Henry 1991, Nix *et al.* 1992). In previous studies involving feral large mouth bass (*Micropterus salmoides*) collected from sites under USFDA advisory for excessive mercury in food-fish, a direct correlation was observed between MT expression and total mercury content in muscle (Schlenk *et al.* 1995). However, the general appearance of the fish did not indicate obvious stress (i.e. tissue coloration, fin erosion, etc.). In a separate study performed with feral populations of largemouth bass collected from mercury advisory sites as well as sites not under advisory, no correlation was observed between hepatic metallothionein and fish health (Schlenk *et al.* 1996b). Moreover, associations were not observed between hepatic MT of several fish species and population or species diversity endpoints from the same waterway (Schlenk *et al.* 1996a). Consequently, to further examine the effect of mercury contamination on fish and determine whether MTs might be used as indicators of health and/or exposure, channel catfish were fed Japanese medaka injected with methylmercury to achieve a dose of 0.1 mg kg^{-1} for 30 days. Methylmercury was chosen because it is the predominant form of mercury which is accumulated in feral animals by trophic transfer (Stein *et al.* 1996). Of the few laboratory studies examining the effects of methylmercury on fish, most have focused on acute intraperitoneal injections or low concentration water-borne exposures (Olson *et al.* 1978, Barghigiani *et al.* 1989). Neither of these exposure routes mimic typical uptake of methylmercury by fish which is by long-term dietary intake (Driscoll *et al.* 1994). Consequently, unlike the other metals used in MT induction studies, to adequately evaluate the effects of methylmercury on fish, a low-level chronic exposure through the diet was necessary.

As observed in previous field studies, 30-day exposure to mercury did not change condition factor or LSI values, although hepatic and axial muscle concentrations were significantly elevated. Moreover, hepatic MT expression was unaltered by the 30-day methylmercury treatment contrasting studies in feral large mouth bass which showed a direct correlation between MT mRNA and total mercury in axial muscle (Schlenk *et al.* 1995) and liver (D. Schlenk, unpublished data). Studies in mammals have shown that methylmercury must undergo demethylation to inorganic mercury to initiate MT transcription (Leyshon-Sorland *et al.* 1994, Kramer *et al.* 1996). The lack of MT induction by methylmercury in the present study may be explained by species differences in rates of methylmercury demethylation between feral large mouth bass and hatchery-raised channel catfish. Other possibilites may be related to an inaccurate modelling of exposure such that a feral organism may not eat mercury-laden fish every day at 0.1 mg kg^{-1}. Although hepatic and axial muscle residues of mercury approached concentrations observed in previous feral studies, exposure studies at even lower doses for longer duration may be more representative of feral exposures.

Since the amino-terminal sequence of MT appears to be conserved in fish, RT-PCR could theoretically be used to measure MT expression in various tissues of most if not all fish species. Indeed, the RT-PCR has been used in our laboratory to measure MT expression in the gills of Japanese medaka, in cultured *Poeciliopsis* hepatocytes, and in catfish erythrocytes. Studies are currently under way to further characterize the use of RT-PCR to measure biomarkers of exposure as well as expression of other biochemical defense mechanisms that may serve as bioindicators of effect.

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