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Copper or Cadmium Pretreatment Increases the Protection against Cadmium Toxicity in Tilapia Larvae (*Oreochromis mossambicus*)

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Su-Mei Wu and Pung-Pung Hwang (2003) Copper or cadmium pretreatment increases the protection against cadmium toxicity in tilapia larvae (*Oreochromis mossambicus*). *Zoological Studies* **42**(1): 179-185. The purpose of this study was to examine the role of metallothionein (MT) in the acclimation mechanisms in tilapia larvae to environments containing heavy metals. Waterborne Cu^{2+} stimulated MT expression in newly hatched tilapia larvae in dose- and time-dependent patterns. Tilapia larvae, exposed to 35 μg/l $CdCl_2$ or 100 μg/l $CuSO_4$ or normal fresh water for 72 h, respectively, were subsequently transferred to 100 μg/l Cd^{2+} for an additional 48 h. At the end of experiment, whole-body contents of Cd^{2+}, Na^{2+}, Ca^{2+} and MT, as well as mortality in the larvae were examined. The present data indicate that: (1) Cd^{2+}- or Cu^{2+}-pretreated larvae survived much better than did larvae with no pretreatment after the final exposure to 100 μg/l Cd^{2+}; and (2) both pretreatment groups synthesized about 1.8- and 1.6-fold, respectively, more MT than did larvae with no pretreatment. These results suggest the involvement of MT in heavy-metal detoxification in developing tilapia.
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Key words: Metallothionein, Fish larvae, Cd^{2+}, Cu^{2+}, Ca^{2+}.

Cd^{2+} is a toxic metal that may interact metabolically with nutritionally essential metals. For example, Cd^{2+} interacted with Ca^{2+} in the skeletal system to produce osteodystrophies (Goyer 1997). Hepatic Zn^{2+} and Cu^{2+} and renal Zn^{2+} in rat increased after treatment with Cd^{2+} (Tandon et al. 1998). In largemouth bass, dietary Cd^{2+} altered the intestinal Zn^{2+} distribution and raised hepatic Cu^{2+}-binding protein levels but did not alter plasma Zn^{2+} or Cu^{2+} levels (Weber et al. 1992).

Metallothioneins (MTs) are inducible, cysteine-rich, metal-binding, low-molecular-weight, and unique proteins that bind a variety of divalent and trivalent heavy metals, including Cd, Hg, Cu, Zn, Ag, Au, Pb, Pt, etc. MTs are involved in various physiological functions, such as regulation of Cu and Zn storage, regulation of cellular repair, growth, differentiation, and expression of genetic information, as well as detoxification of heavy metals (Kägi and Schaffer 1988, Saito and Kojima 1997). In our recent studies, MT was also indicated to be involved in the detoxification of invading Cd^{2+} in developing fish (Wu et al. 2000).

Pretreatment with Zn^{2+} resulted in less accumulation of Cd^{2+} after challenge with Cd^{2+} in cultured cells (Mishima et al. 1997). Pretreatment with Zn^{2+} also increased the concentration of tissue MT in mice and consequently enhanced the protection against Cd^{2+}-induced toxicity (Liu et al. 1996). In mouse embryos, Zn^{2+} pretreatment induced synthesis of MT which protected against isotretinoin teratogenicity (Blain et al. 1998). Apparently, pretreatment with low doses of bioessential metals can induce the expression of MT and result in enhanced protection against subsequent stressors. This has also been documented in aquatic fishes (Kito et al. 1982, McCarter and Roch 1983, Bradley et al. 1985, Ramo et al. 1992). However, no convincing evidence is available for MT expression during this detoxification

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process.

The present study was aimed at testing whether pretreatment with Cd^{2+} or Cu^{2+} stimulates MT protein expression and enhances protection against toxicity of subsequent Cd^{2+} exposure in tilapia larvae. Tilapia was selected to be the model animal because it has been one of the most popular species for physiological and environment toxicological research, and an ELISA for MT has been established in this species (Wu et al. 1999 2000).

MATERIALS AND METHODS

Fish

Mature adult tilapia (*Oreochromis mossambicus*) from the Tainan Branch of the Taiwan Fisheries Research Institute were reared in 182-l glass aquaria using plastic chips for gravel. Each tank was supplied with dechlorinated, circulated, aerated local tap water at 26-28°C under a photoperiod of 12-14 h. Fish were fed with commercial fish food pellets. Fertilized eggs were collected from the mouth of a brooding female 1 d before hatching and incubated in a gently bubbled 1000-ml container under the same conditions as for adults. Larvae were not fed during the experiments.

ELISA for MT

Twenty larvae were collected as a pooled sample for MT ELISA. Soluble extracts of larvae were prepared by homogenizing whole larvae with homogenization buffer (10 mM Tris-HCl, with 5 mM 2-mercaptal-ethanol, pH 7.0) in a 1: 2.5 (w/v) volume using a plastic homogenizer at 1000-1200 rpm. The homogenates were centrifuged at 12 000 × *g* for 40 min at 4°C. The supernatant was inactivated at 80°C for 10 min then was centrifuged again at 12 000 × *g* for 40 min at 4°C; the final supernatants were subjected to the MT ELISA established by Wu et al. (2000).

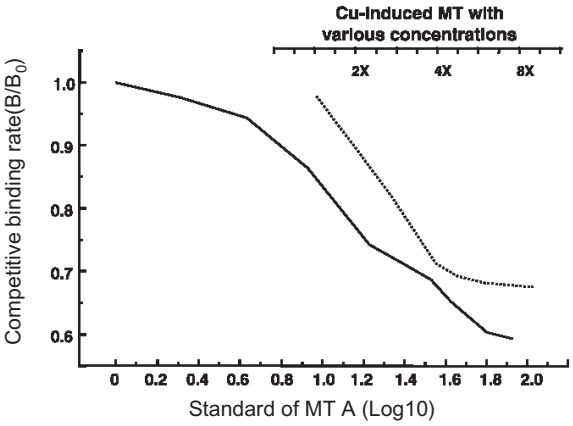

Fig. 1. Competitive binding curves of ELISA for Cu^{2+}-induced MT (dotted line) and standard MT (solid line). Each point represents the average of triplicate determinations.

A synthetic peptide derived from the N-terminal amino-acid sequence of tilapia MT (Wu et al. 1999) was prepared as the standard MT. A typical dose-related standard curve and Cu^{2+}-induced MT from tilapia larval extracts of competitive ELISA are shown in figure 1. The displacement curve for Cu^{2+}-induced MT from larval extracts was parallel to that of the MT standard, indicating that the ELISA is suitable for the measurement of Cu^{2+}-induced MT in tilapia tissues. The line regression coefficient (Microsoft Excel 97 SR-1, 1997; Microsoft Corp.) for the logarithms of MT standard concentrations was -0.99, and that for serial dilutions of larval extracts was -0.92. The coefficients of intra- and inter-assay variations were 5.04% (n = 8) and 15.05% (n = 7), respectively.

Measurements of Ca^{2+}, Na^{+}, $Cu^{2+}$and Cd^{2+}

After being anesthetized with MS222, tilapia larvae were washed in double-deionized water 3 times, and the water left on the body surface was dried with filter paper. After being weighed, the whole larva was dried at 65°C overnight and digested with 200 μl of 13.1 N HNO_3 at 40°C overnight. The digested solutions, as well as water samples from incubation media, were diluted with double-deionized water and subjected to atomic absorption spetrophotometry (Z-8000, Hitachi, Japan), using an air/acetylene flame for Na^{+} and Ca^{2+} analysis, and a graphite furnace for Cd^{2+} and Cu^{2+} analysis. Standard solutions of these ions (Merck, Germany) were used for establishing standard curves. The standard addition method was used for background correction to eliminate the matrix effect.

Preparation of Cu^{2+} and Cd^{2+} media

Completely dried $CdCl_2$ (Sigma, USA) dissolved in 1 ml concentrated HCl was used with double-deionized water to prepare the 10 mg/l Cd^{2+} stock solution. $CuSO_4$ (Riedel, Seelze) dissolved in double-deionized water was used to prepare a 1000 mg/l Cu^{2+} stock solution. These stock

solutions were diluted to desired concentrations with local tap water as described in Hwang et al. (1995). All containers used in these experiments were cleaned with HNO_3 and thoroughly rinsed with double-deionized water before being used. The media in the test containers were changed daily. Deviations of Cd^{2+} and Cu^{2+} concentrations were less than 5%. Other parameters of the exposure media were hardness, 28.1 ± 7.6 mg/l as $CaCO_3$; DO (dissolved oxygen), 7.5 ± 0.5 mg/l; Na^+, 5.6 ± 0.3 mg/l; K^+, 1.4 ± 0.1 mg/l; Ca^{2+}, 9.6 ± 0.3/l; Mg^{2+}, 3.5 ± 0.2 mg/l; and pH 6.9 ± 0.3.

Experiment 1: Dose response of MT to waterborne Cu^{2+}

Newly-hatched (H0) tialpia larvae incubated in 0, 50, 100, 150, and 200 μg/l Cu^{2+} media for 72 h were collected and subjected to MT measurement.

Experiment 2: Time-dependent response of MT to waterborne Cu^{2+}

H0 larvae were incubated in 0 and 100 μg/l Cu^{2+} media for 5 d. Samples were collected 2, 3, and 5 d after treatment and were subjected to MT measurement.

Experiment 3: Exposure to waterborne Cd^{2+} and Cu^{2+}

H0 larvae were incubated in 0 or 35 μg/l Cd^{2+} and 0 or 100 μg/l Cu^{2+}, respectively, for 72 h, and then were transferred to 100 μg/l Cd^{2+} media for an additional 48 h. Survival rates of the larvae were examined; larvae were also collected for MT and ion concentration measurements.

Statistical analysis

Data are presented as the mean ± SE, and were analyzed by one-way ANOVA with Tukey's multiple-comparison analysis or Student's *t*-test. Statistical significance was accepted for $p < 0.05$.

RESULTS

Dose- and time-dependent responses of MT to waterborne Cu^{2+}

Whole-body MT contents in tilapia H0 larvae showed dose-dependent relations with waterborne Cu^{2+} in the range of from 0 to 100 μ/l, and MT content in larvae in 100 μ/l Cu^{2+} showed a 7-fold increase compared with the control in 0 μ/l Cu^{2+}. However, MT contents were not shown to have a positive relation with Cu^{2+} at a level higher than 100 μ/l (Table 1).

MT contents in larvae exhibited a time-dependent pattern after exposure to waterborne 100 μ/l Cu^{2+} for various times; tilapia larvae showed only a 185% increase in MT content after 2 d of treatment while showing a 755% increase after 5 d of treatment (Table 2).

Survival rates in tilapia larvae with different treatments of waterborne Cd^{2+} and Cu^{2+}

Table 2. Metallothionein contents (ng/mg protein) in newly-hatched larvae exposed to 0 (control) and 100 μg/l Cu^{2+} for various times

	Time course (d)		
	2	3	5
Control	1678.9 ± 246.8	1317.6 ± 318.8	325.1 ± 66.3
Treatment	3112.2 ± 182.0*	4042.2 ± 306.5*	2454.2 ± 121.3*
Increase rate[1]	185%	307%	755%

[1] (Treatment ÷ control) × 100%.

*$p < 0.001$ significantly higher than the control (Student *t*-test) for the same time course.

Table 1. Metallothionein contents (ng/mg protein) in newly hatched larvae exposed to different levels of waterborne Cu^{2+} for 72 h

Brood	Concentration of copper (μg/l)					
	Control	50	70	100	150	200
A	915.9 ± 5.3[a]	NA	2538.3 ± 184.4[b]	3562.3 ± 151.4[c]	NA	NA
B	211.7 ± 81.9[a]	504.0 ± 107.7[b]	NA	1496.7 ± 283.4[c]	1372.6 ± 226.4[c]	924.9 ± 335.9[b]

Data represent the Mean ± SD (n = 3-5).

NA: No data available due to an insufficient number of larvae.

Different superscripts for a given brood indicate a significant difference among treatments ($p < 0.05$, ANOVA analysis with Tukey's comparisons).

Tilapia larvae (0→100 Cd) exposed to 100 μ/l Cd^{2+} without pretreatment with Cu^{2+} or Cd^{2+} showed 50% mortality; however mortality rates of larvae pretreated with 35 μ/l Cd^{2+} (35 Cd→100 Cd) or 100 μ/l Cu^{2+} (100 Cu→100 Cd) were 4% and 3%, respectively, when they were exposed to 100 μ/l Cd^{2+} for 48 h.

MT and ion contents in tilapia larvae with different treatments of waterborne Cd^{2+} and Cu^{2+}

Cd^{2+}- and Cu^{2+}-pretreated tilapia larvae (35 Cd→100 Cd and 100 Cu→100 Cd groups, respectively) revealed evident differences in physiological performances from larvae with no pretreatment (0 →100 Cd) after the final exposure to 100 μ/l Cd^{2+} (Tables 3, 4). Upon 100 μ/l Cd^{2+} exposure, the MT content increased 1.8-fold in the 35 Cd→100 Cd group (Table 3), and increased 1.6-fold in the 100 Cu→100 Cd group (Table 4) compared with the 0 →100 Cd group. In the case of body Cd^{2+} content, 35 Cd→100 Cd and 0→100 Cd larvae showed no significant changes, while only 0→100 Cd larvae revealed a significant decrease compared with the control (with no treatment) and 35 Cd→100 Cd larvae.

In the experiment for Cu^{2+} pretreatment, Na+ content showed no significant changes among the different groups. However, Ca^{2+} content differed significantly among the 3 groups of larvae, with that in the 100 Cu→100 Cd was the lowest (Table 4).

In another experiment, body weight and Ca^{2+} content in tilapia larvae with various treatments were compared (Table 5). The treatments revealed similar effects on body weight and Ca^{2+} content in tilapia larvae; both body weight and Ca^{2+} content in the 0→100 Cd and 100 Cu→100 Cd groups were significantly lower than those of the control and 35 Cd→100 Cd groups (Table 5).

Table 3. Changes of metallothionein, Cd^{2+} and Ca^{2+} contents in tilapia larvae with various treatments

Parameter	Pattern of treatment[1]		
	Control	0→100 Cd	35 Cd→100 Cd
MT (ng/mg protein)	114.20 ± 18.92[a]	106.73 ± 18.39[a]	209.20 ± 31.35[b]
Cd^{2+} (ng/mg BW)	0.34 ± 0.03[a]	1.58 ± 0.16[b]	1.62 ± 0.17[b]
Ca^{2+} (μg/mg BW)	0.56 ± 0.03[b]	0.49 ± 0.03[a]	0.53 ± 0.03[b]

[1]Control, with no treatment; 0→100 Cd, pretreated with 0 μg/l of Cd^{2+} for 72 h and then with 100 μg/l Cd^{2+} for 48 h; 35 Cd→ 100 Cd, pretreated with 35 μg/l Cd for 72 h, and then with 100 μg/l Cd for 48 h.

Mean ± SD (n = 4-5). Different superscripts for a given parameter indicate a significant difference among treatments (p < 0.05, ANOVA analysis with Tukey's comparisons).

Table 4. Changes in metallothionein, Cd^{2+}, Ca^{2+}, and Na^{+} contents of tilapia larvae with various treatments

Parameter	Pattern of treatment[1]		
	Control	0→100 Cd	100 Cu→100 Cd
MT (ng/mg protein)	138.42 ± 10.03[a]	134.52 ± 7.29[a]	219.20 ± 61.87[b]
Na (μg/mg BW)	1.27 ± 0.07[a]	1.36 ± 0.07[a]	1.30 ± 0.12[a]
Cd^{2+} (ng/mg BW)	0.01 ± 0.01[a]	1.35 ± 0.01[b]	1.06 ± 0.12[c]
Ca^{2+} (μg/mg BW)	0.60 ± 0.04[c]	0.51 ± 0.02[b]	0.35 ± 0.07[a]

[1]Control, with no treatment; 0 →100 Cd, pretreated with 0 μg/l of Cd for 72 h and then with 100 μg/l Cd^{2+} for 48 h; 100 Cu→ 100 Cd, pretreated with 100 μg/l Cu for 72 h, and then with 100 μg/l Cd for 48 h.

Mean ± SD (n = 4-5). Different superscripts for a given parameter indicate a significant difference among treatments (p < 0.05, ANOVA analysis with Tukey's comparisons).

DISCUSSION

The major findings of the present study are that (1) waterborne Cu^{2+} can induce the protein expression of MT in developing fish with dose- and time-dependent patterns; and that (2) pretreatment with Cd^{2+} or Cu^{2+} enhanced the tolerance of larvae to subsequent Cd^{2+} challenge via induction of additional MT.

In the blue crab *Callinectes sapidus*, Brouwer et al. (1992) purified the MT induced by Cu^{2+} and Zn^{2+} by chromatography and suggested that the metals induced specific MT isoforms. However, based on the N-terminal amino-acid sequencing and mass spectrometry of purified MT, Pedersen

Table 5. Changes in Ca^{2+} concentrationand body weight of tilapia larvae with various treatments

Parameter	Treatment			
	Control	0→100 Cd	35 Cd→100 Cd	100Cu→100Cd
Ca^{2+} (μg/mg BW)	0.49 ± 0.01[b]	0.21 ± 0.02[a]	0.47 ± 0.05[b]	0.25 ± 0.02[a]
Body weight (mg)	8.50 ± 0.30[b]	7.90 ± 0.30[a]	8.30 ± 0.30[b]	7.40 ± 0.40[a]

Control, with no treatment; 0 →100 Cd, pretreated with 0 μg/l of Cd^{2+} for 72 h and then with 100 μg/l Cd^{2+} for 48 h; 35 Cd→100 Cd, pretreated with 35 μg/l Cd for 72 h, and then with 100 μg/l Cd for 48 h; 100 Cu→100 Cd, pretreated with 100 μg/l Cu for 72 h, and then with 100 μg/l Cd for 48 h. Mean ± SD (n = 4-5). Different superscripts for a given parameter indicate a significant difference among treatments (p < 0.05, ANOVA analysis with Tukey's comparisons).

et al. (1998) indicated that Zn^{2+}, Cd^{2+}, and Cu^{2+} induced the identical isoform of MT. The ELISA system used in the present study is suitable for the measurement of the Cu^{2+}-induced MT from the tissues of tilapia larvae, but it is still unknown whether Cu^{2+} induced the same MT isoform as did Cd^{2+}. This will be examined in subsequent studies.

Zn^{2+} or Cu^{2+} was found to be associated with increased expression of the MT gene in liver and kidney of rats (Irato et al. 1996, Tohyama et al. 1996). In astrocyte or neuron cultures, Zn^{2+} or Cd^{2+} induced the protein expression of MT with peaks at 24-96 h (Kramer et al. 1996a b). Similar results have also been reported elsewhere in aquatic animals. There was a strong and positive relationship between hepatic Cu^{2+} concentrations and the level of MT mRNA or protein in rainbow trout (*Oncorhynchus mykiss*) (Dethloff et al. 1999) and channel catfish (*Ictalurus punctatus*) (Perkins et al. 1997). Induction of MT mRNA and protein was rapid and peaked at 1-2 d after Cd^{2+} treatment in gills and kidneys of turbot (*Scophthalmus maximus*) (George et al. 1996). Whole-body MT contents in tilapia larvae (*O. mossambicus*) also showed dose- and time-dependent relations with waterborne Cd^{2+} up to a concentration of 100 μ/l (Wu et al. 2000) or with Cu^{2+} (the present study). Base on these data, MT may also be involved in the detoxification of heavy metals during the early development of fish, as suggested in adults (George 1989, Olsson et al. 1989, Kille et al. 1992, Hogstrand et al. 1994, Schlenk et al. 1995, George et al. 1996).

It has been well documented that pre-exposure of an organism or cells to metals can enhance the tolerance to subsequent metal-induced toxicities. Cultured cells, pretreated with Zn^{2+} or Cd^{2+} for 20-24 h revealed a lower accumulation of Cd^{2+} and/or induction of MT-2 mRNA and total MT protein after subsequent exposure to Cd^{2+} (Koropatnick and Zalups 1997, Mishima et al. 1997). Pretreatment with Zn^{2+} also induced dose-related protein expression of MT in mice embryos and in liver of mice, and consequently decreased isotretinoin-mediated growth retardation, cleft palates, and postpartum mortality, as well as prevented $CdCl_2$ hepatotoxicity (Liu et al. 1996, Blain et al. 1998). Pretreatment of rats with low doses of Cd^{2+} produced adaptive tolerance to a subsequent high-dose Cd^{2+}-induced lethality. This protection was attributable to the 10- to 50-fold induction of hepatic MT by Cd^{2+} pretreatment (Klaassen et al. 1999). Therefore, induction of MT synthesis by pretreatment with metals in organisms or cells appear to increases the tolerance to subsequent metal or other stress factors. A similar phenomenon was also reported in aquatic animals. Pre-exposure to Zn^{2+} caused an increase in metal tolerance in rainbow trout, and it was suggested to be associated with the induced MT (Bradley et al. 1985). Shrimp with Cd^{2+} pretreatment revealed a modification in the Cd^{2+} accumulation rate and an increase in Cd^{2+}-binding ligands (Ramo et al. 1992). However, data about the MT protein in these studies on aquatic animals were no convincing. In the present study, MT protein, detected by ELISA, was found to increase by 1.8- or 1.6-fold in tilapia larvae after respective pretreatment with low-dose Cd^{2+} or Cu^{2+} and subsequent exposure to 100 μ/l Cd^{2+}. Moreover, stimulation in the levels of MT protein was correlated with survival (i.e., tolerance to Cd^{2+}) in the larvae.

Metals diffuse into animals through the epithelia and impact the ion balance in the animals. Waterborne Cd^{2+} caused a significant decrease in Ca^{2+} content in tilapia larvae (Hwang et al. 1995), resulting from the toxic effects of Cd^{2+} on Ca^{2+} influx kinetics (Chang et al. 1997 1998). Cu ions showed only a transient effect on Ca^{2+} homeostasis (Reid and McDonald 1988, Viarengo et al. 1996) but specific inhibition of Na^+ uptake in fish gills (McDonald and Wood 1993) and thus caused significant losses of Na^+ (Reid and McDonald 1988). Tilapia larvae of the 0→100 Cd and 100 Cu→100 Cd groups showed significant decreases in Ca^{2+} contents but no changes in Na^+ contents as compared with the control group (with no treatment). This may be due to inhibited growth in the larvae. The data of body weight, which were positively correlated with levels of Ca^{2+} content (Table 5), provide evidence of growth inhibition caused by the 0→100 Cd and 100 Cu→100 Cd treatments. Ca^{2+} and Na^+ contents in developing tilapia larvae were about 8- and 2-fold higher, respectively, from day 1 to day 5 post-hatching (Hwang et al. 1994, Chou et al. 2002); therefore the inhibited growth by 0→100 Cd and 100 Cu→100 Cd treatments resulted in a decline in the Ca^{2+} content but not the Na^+ content.

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REFERENCES

Blain D, S Kubow, HM Chan. 1998. Zinc pretreatment inhibits isotretinoin teratogenicity and induces embryonic metallothionein in CD-1 mice. J. Nutr. **128:** 1239-1246.

Bradley RW, C DuQuesnay, JB Spragus. 1985. Acclimation of rainbow trout, *Salmo gairdneri* Richardson, to zinc: kinetics and mechanism of enhanced tolerance induction. J. Fish Biol. **27:** 367-379.

Brouwer M, D Schlenk, AH Ringwood, T Brouwer-Hoexum. 1992. Metal-specific induction of metallothionein isoforms in the blue crab *Callinectes sapidus* in response to single- and mixed-metal exposure. Arch. Biochem. Biophys. **294:** 461-468.

Chang MH, HC Lin, PP Hwang. 1997. Effects of cadmium on the kinetics of calcium uptake in developing tilapia larvae, *Oreochromis mossambicus*. Fish Physiol. Biochem. **16:** 459-470.

Chang MH, HC Lin ,PP Hwang. 1998. Ca^{2+} uptake and Cd^{2+} accumulation in larval tilapia (*Oreochromis mossambicus*) acclimated to waterborne Cd^{2+}. Am. J. Physiol. **274:** R1570-R1577.

Dethloff GM, D Schlenk, JT Hamm, HC Bailey. 1999. Alterations in physiological parameters of rainbow trout (*Oncorhynchus mykiss*) with exposure to copper and copper/zinc mixtures. Ecotox. Environ. Safe. **42:** 253-264.

George SG. 1989. Cadmium effects on plaice liver xenobiotic and metal detoxification systems dose-response. Aquat. Toxicol. **15:** 303-310.

George SG, K Todd, J Wright. 1996. Regulation of metallothionein in teleosts: induction of MT mRNA and protein by cadmium in hepatic and extrahepatic tissues of a marine flatfish, the turbot (*Scophthalmus maximus*). Comp. Biochem. Phys. **113C:** 109-115.

Goyer RA. 1997. Toxic and essential metal interactions. Annu. Rev. Nutr. **17:** 37-50.

Hogstrand C, RW Wilson, D Polgar, CM Wood. 1994. Effects of zinc on the kinetics of branchial calcium uptake in freshwater rainbow trout during adaptation to waterborne zinc. J. Exp. Biol. **186:** 55-73.

Hwang PP, SW Lin, HC Lin. 1995. Different sensitivities to cadmium in tilapia larvae (*Oreochromis mossambicus*; Teleostei). Arch. Environ. Con. Tox. **29:** 1-7.

Hwang PP, YN Tsai, YC Tung. 1994. Calcium balance in embryos and larvae of the freshwater-adaptation teleost, *Oreochromis mossambicus*. Fish Physiol. Biochem. **13:** 325-333.

Irato P, GC Sturniolo, G Giacon, A Magro, R D'Inca, C Mestriner, V Albergoni. 1996. Effect of zinc supplementation on metallothionein, copper, and zinc concentration in various tissues of copper-loaded rats. Biol. Trace Elem. Res. **51:** 87-96.

Kägi JHR, A Schaffer. 1988. Biochemistry of metallothionein. Biochemistry **27:** 8509-8515.

Kille P, J Kay, M Leaver, S George. 1992. Induction of piscine metallothionein as a primary response to heavy metal pollutants: applicability of new sensitive molecular probes. Aquat. Toxicol. **22:** 279-286.

Kito H, T Tazawa, Y Ose, T Sato, T Ishikawa. 1982. Protection by metallothionein against cadmium toxicity. Comp. Biochem. Phys. **73C:** 135-139.

Klaassen CD, J Liu, S Choudhuri. 1999. Metallothionein: an intracellular protein to protect against cadmium toxicity. Annu. Rev. Pharmacol. **39:** 267-294.

Koropatnick J, RK Zalups. 1997. Effect of non-toxic mercury, zinc or cadmium pretreatment on the capacity of human monocytes to undergo lipopolysaccharide- induced activation. Brit. J. Pharmacol. **120:** 797-806.

Kramer KK, J Liu, S Choudhuri, CD Klassen. 1996a. Induction of metallothionein mRNA and protein in murine astrocyte cultures. Toxicol. Appl. Pharm. **136:** 94-100.

Kramer KK, JT Zoelle, CD Klaassen. 1996b. Induction of metallothionein mRNA and protein in primary murine neuron cultures. Toxicol. Appl. Pharm. **141:** 1-7.

Liu J, Y Liu, AE Michalska, KH Choo, CD Klaassen. 1996. Metallothionein plays less of a protective role in cadmium-metallothionein-induced nephrotoxicity than in cadmium chloride-induced hepatotoxicity. J. Pharmacol. Exp. Ther. **276:** 1216-1223.

McCarter JA, M Roch. 1983. Hepatic metallothionein and resistance to copper in juvenile coho salmon. Comp. Biochem. Phys. **74C:** 133-137.

McDonald DG, CM Wood. 1993. Branchial mechanisms of acclimation to metals in freshwater fish. *In* JC Rankin, FB Jensen, eds. Fish ecophysiology. London: Chapman and Hall, pp. 297-332.

Mishima A, C Yamamoto, Y Fujiwara, T Kaji. 1997. Tolerance to cadmium cytotoxicity is induced by zinc through non-metallothionein mechanisms as well as metallothionein induction in cultured cells. Toxicology **118:** 85-92.

Olsson PE, A Larsson, A Maage, C Haux, K Bonham. 1989. Induction of metallothionein synthesis in rainbow trout, *Salmo gairdneri*, during long-term exposure to waterborne cadmium. Fish Physiol. Biochem. **6:** 221-229.

Pedersen SN, KL Pedersen, P Hojrup, J Knudsen, MH Depledge. 1998. Induction and identification of cadmium, zinc- and copper- metallothioneins in the shore crab *Carcinus maenas* (L.). Comp. Biochem. Phys. **120C:** 251-259.

Perkins EJ, B Griffin, M Hobbs, J Gollon, L Wolford, D Schlenk. 1997. Sexual differences in mortality and sublethal stress in channel catfish following a 10-week exposure to copper sulfate. Aquat. Toxicol. **37:** 327-339.

Ramo JD, M Martinez, A Pastor, A Torreblanca, J Diaz-Mayans. 1992. Effect of cadmium pre-exposure in cadmium accumulation by brine shrimp *Artemia*: involvement of low-molecular-weight cadmium binding ligands. Mar. Environ. Res. **92:** 29-33.

Reid SD, DG McDonald. 1988. Effects of cadmium, copper and low pH on ion fluxes in the rainbow trout, *Salmo gairdneri*. Can. J. Fish. Aquat. Sci. **45:** 244-253.

Saito S, Y Kojima. 1997. Differential role of metallothionein on Zn, Cd and Cu accumulation in hepatic cytosol of rats. Cell. Mol. Life Sci. **53:** 267-270.

Schlenk D, YS Zhang, J Nix. 1995. Expression of hepatic metallothionein messenger RNA in feral and caged fish species correlates with muscle mercury levels. Ecotox. Environ. Safe. **31:** 282-286.

Tandon SK, S Prasad, S Singh, DK Agarwal. 1998. Efficacy of amphipathic dithiocarbamates in intracellular cadmium mobilization and in modulation of hepatic and renal metallothionein in cadmium pre-exposed rat. Chem. Biol. Interact. **114:** 161-175.

Tohyama C, JS Suzuki, S Homma, M Karasawa, T Kuroki, H Nishimura, N Nishimura. 1996. Testosterone-dependent induction of metallothionein in genital organs of male rats. Biochem. J. **317:** 97-102.

Viarengo A, R Accomando, I Ferrando, F Beltrame, M Fato, G Marcenaro. 1996. Heavy metal effects on cytosolic free

Ca^{2+} level in the marine protozoan *Euplotes crassus* evaluted by confocal laser scanning microscopy. Comp. Biochem. Phys. **113C:** 161-168.

Weber DN, S Eisch, RE Spieler, DH Petering. 1992. Metal redistribution in largemouth bass (*Micropterus salmoides*) in response to restrainment stress and dietary cadmium: role of metallothionein and other metal-binding proteins. Comp. Biochem. Phys. **101C:** 255-262.

Wu SM, CF Weng, MJ Yu, CC Lin, ST Chen, JC Hwang, PP Hwang. 1999. Cadmium-induced Metallothionein from tilapia (*Oreochromis mossambicus*). Bull. Environ. Contamin. Toxicol. **62:** 758-768.

Wu SM, CF Weng, JC Hwang, CH Hwang, PP Hwang. 2000. Metallothionein induction in early larval of tilapia *Oreochromis mossambicus*. Physiol. Biochem. Zool. **73:** 531-537.