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COPPER TOLERANCE IN FATHEAD MINNOWS: II. MATERNAL TRANSFER

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Abstract

Female fathead minnows (*Pimephales promelas*) exposed to copper (Cu) maternally transfer Cu tolerance to their larval offspring. Larvae produced after female parents received a sublethal 5-d, 100 µg/L Cu exposure had significantly greater survivorship in potentially lethal Cu solutions than larvae produced before those females were exposed to Cu.

Keywords

Copper; Copper tolerance; Maternal transfer; Fathead minnow

INTRODUCTION

A variety of toxic substances have been found to be maternally transferred into developing fish oocytes, including anthracene [1], organochlorines [2], selenium [3], and metals such as cadmium (Cd) [4] and mercury [5]. Maternal transfer of toxic compounds can be deleterious; for example, Hall and Oris [1] found that maternal transfer of anthracene in fathead minnows (*Pimephales promelas*) was associated with reductions in egg production, hatching success, and survival of larvae or fry. More recently, Lin et al. [6] found that female tilapia (*Oreochromis mossambicus*) injected with Cd maternally transferred metallothionein messenger ribonucleic acid (mRNA) to their offspring. In this case, the maternal transfer appeared to be beneficial, as these larvae had significantly greater Cd tolerance than controls.

The results of our previous study [7] suggested that maternal transfer could have increased the Cu tolerance of larval fathead minnows. Adults classified as Cu-tolerant were bred to each other, as were adults that were classified as Cu-susceptible. Larvae produced by these two groups and by two groups of control minnows were subjected to 7-d survival and growth tests. The median lethal concentration (LC50) for larvae from Cu-susceptible parents was not significantly different from the LC50 for larvae from Cu-resistant parents; however, both groups' LC50s were significantly higher than the LC50s for larvae from control parents. While the LC50 results suggested maternal transfer, other aspects of the study (failure-time analysis and body mass of surviving larvae) did not support maternal transfer of Cu tolerance as strongly. Therefore, the objective of this study was to determine whether fathead minnows can maternally transfer Cu tolerance to their larval offspring. To do this, the experimental design was modified to reduce potential genetic variation and to delineate the shape of the failure-time curve within the first 24 h of Cu exposure.

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MATERIALS AND METHODS

Adult fish maintenance and breeding

Sixteen-week-old fathead minnows were obtained from a commercial fish supplier (Environmental Consulting and Testing, Superior, WI, USA). The fish were allowed to acclimate to laboratory conditions (aerated, dechlorinated tap water; 20°C; 16:8-h light:dark photoperiod). After 14 d, males and females could be differentiated and were placed in two large single-sex holding aquaria until transferred to breeding aquaria. All fish were fed to satiation daily with high-protein flake food (Aquatic Ecosystems, Apopka, FL, USA). One-third static renewals were conducted daily in all aquaria throughout the experiments.

A male and female fish, randomly chosen from the holding aquaria, were placed in each of 20 breeding aquaria, 40-L glass tanks with 30 L of aerated, dechlorinated water. Each breeding aquarium was divided in half by a perforated plastic screen, which separated the male from the female. Spawning substrates (12-cm-long sections of polyvinyl chloride tubing 8 cm in diameter, split in half lengthwise) were placed in with each fish, and their feeding was supplemented with a daily feeding of *Artemia* nauplii. Water temperature was gradually increased to $25.9 \pm 0.1^\circ\text{C}$ (mean $\pm$ standard error [SE]). After 7 d, the screen and one of the spawning substrates were removed from each aquarium to initiate breeding. Eggs adhered to the underside of the spawning substrates, which were removed for incubation each morning.

Larval survival tests

Each spawning substrate with eggs was placed in a 1-L glass beaker filled with aerated, dechlorinated water. The water in the beakers was maintained at 25°C in a circulating warm water bath. Unfertilized eggs or eggs with fungus were removed twice a day, and the eggs hatched in 3 to 5 d. When more than 60 larvae from a spawn hatched within 24 h (between two consecutive mornings), those individuals were used in a 96-h survival test.

Twelve sets of preexposure larvae (from the first 12 parent pairs to produce larvae) were subjected to 96-h survival tests conducted from February 26 to March 6, 2002, following standard protocols [8] except as noted below. The tests were initiated over a 5-d period, with test initiation dates dependent on larval hatching dates. Sixty larvae from a single parent pair were evenly distributed among three test chambers, for a total of 36 test chambers. Test chambers were 600-ml glass beakers containing 350 ml of test solution at one of three nominal Cu concentrations: 0, 400, or 800 µg/L. Preliminary range-finding tests indicated that substantial, but not total, mortality would occur in at least one of these Cu concentrations. Larvae were fed 1-d-old *Artemia* nauplii twice per day. Dead larvae were counted and removed 3, 6, 12, 24, 48, and 96 h after initial larval transfer. Test chamber solutions had $\geq 80\%$ static renewal daily. After 96 h, surviving larvae were sacrificed by placement of test chambers on ice until larvae were immobile and unresponsive to prodding, and then were counted to determine survivorship.

After the 96-h larval survival tests, six of the twelve female parents were exposed for 5 d to 100 µg Cu/L in a 70-L aquarium that was divided into six equal sections by perforated plastic screens. The water was manually respiked during the daily one-third static renewal, using a stock solution of CuSO₄. The other six females were sham-exposed in an identical aquarium. Water was dechlorinated, aerated, and maintained at (mean $\pm$ SE) $25.8 \pm 0.05^\circ\text{C}$.

One female exposed to 100 µg/L Cu died during the 5-d exposure. The eleven surviving females were returned to their respective breeding aquaria and were separated from the males by screens for 7 d. The screens were then removed, and the pairs of fish were allowed to breed. Nine sets of postexposure larvae (four from Cu-exposed females and five from sham-exposed

females) were subjected to larval survival tests conducted from March 24 to April 3, 2002, using procedures detailed previously.

Water analyses

Laboratory water quality was evaluated weekly and had the following characteristics (mean $\pm$ SE): alkalinity (expressed as mg/L CaCO_3), 104 ± 3 ; hardness (expressed as mg/L CaCO_3), 200 ± 1 ; pH, $8.26 \pm .03$; and conductivity, $689 \pm 10 \mu\text{S}$.

A water sample (125 ml) was collected daily for Cu analysis, according to U.S. Environmental Protection Agency Guidelines [9], from each exposure aquarium; from each daily Cu-renewal solution; and from all end-of-test solutions in larval test chambers. Actual Cu concentration was determined for all water samples by flame atomic absorption spectrometry according to previously established methods [9]. The actual Cu concentrations averaged 98.5 ± 2.5 (SE) $\mu\text{g/L}$ in the Cu-exposure aquarium and were below the detection limit of $12 \mu\text{g/L}$ in the sham-exposure aquarium and in the renewal and end solutions that contained a nominal $0 \mu\text{g/L}$ Cu. For solutions with nominal Cu concentrations of 400 or 800 $\mu\text{g/L}$, measured Cu concentrations in renewal solutions ranged between 92 and 110% of nominal, while end solutions ranged between 90 and 106% of nominal.

Temperature, dissolved oxygen, and pH were recorded for the solutions in the test chambers on day 0, after the larvae were introduced; on days 1 to 3 just prior to static renewal; and at the end of day 4. Dissolved oxygen remained above 4.0 mg/L, consistent with U.S. Environmental Protection Agency requirements [9]. Temperatures were $25.5 \pm 0.03^\circ\text{C}$ (SE). The pH ranged from 7.1 to 8.2. For all larval groups, pH values for $0 \mu\text{g/L}$ Cu solutions were found to be significantly lower by an analysis of variance ($p < 0.0001$ in all cases) than pH for the 400 or 800 $\mu\text{g/L}$ Cu solutions. Furthermore, pH decreased over time in all Cu concentrations.

Statistical analysis

Survival-time analysis [10] was performed using JMP Version 5.0 [11] to evaluate larval mortality. The times at which individual deaths were recorded (time-to-death) were fit to models describing the relationship between mortality and time of exposure to Cu. Larvae alive at the end of each experiment were recorded as right-censored. Survival-time data for the larvae of the Cu-exposed females and sham-exposed females were analyzed separately. The models included two covariates: Cu concentration and larval group (preexposure or postexposure). The best-fitting model was the one with the greatest log-likelihood value. The effects of covariates on survival time were tested by likelihood ratio tests, in which log-likelihoods of the model with and without the covariate were computed and the results were compared against a chi-square distribution. Statistical significance throughout the experiments was assumed at $p \leq 0.05$.

RESULTS

Percent hatch was determined for eggs produced by the sham-exposed and Cu-exposed fish, before and after exposure. All nonfungused, fertilized eggs were hatchable, i.e., produced larvae. The four larval groups did not differ significantly in the percentage of total eggs that were hatchable (analysis of variance, $p > 0.05$; Fig. 1).

Larval mortality increased with increases in Cu concentrations and time of exposure for all four larval groups. It was determined by survival analysis [10] that models assuming a Weibull distribution of time-to-death (Table 1 and Table 2) fit the data significantly better than models incorporating log-normal or exponential distributions.

Inclusion of the different Cu concentrations, without differentiating between larval groups, significantly improved the fit of the model to the survivorship data for the larvae from Cu-exposed females and from sham-exposed females ($p < 0.0001$; Table 1 and Table 2). Including covariates for the preexposure and postexposure larval groups without differentiating Cu concentrations significantly improved the fit of the model to survival data for the larval groups from Cu-exposed females ($p = 0.0002$; Table 1), but not for the larval groups from shamexposed females ($p > 0.05$; Table 2). Therefore, the survivability of preexposure and postexposure larvae produced by sham-exposed females did not differ significantly. In contrast, the survivability of larvae produced by Cu-exposed females was significantly greater postexposure than it was preexposure (Fig. 2).

DISCUSSION

The objective of this research was to determine whether Cu tolerance was maternally transferred from Cu-exposed female fathead minnows to their larval offspring. The significantly greater survivorship of postexposure larvae compared to preexposure larvae produced by the Cu-exposed females, coupled with the lack of significant differences in survivorship between the preexposure and postexposure larvae produced by the sham-exposed females, support the occurrence of maternal transfer of Cu tolerance in fathead minnows. Our results agree with those of Lin et al. [6] who found that Cd tolerance was maternally transferred in tilapia.

One alternative explanation for our results might be that the Cu exposure of the adult females led to the death of oocytes or eggs, leaving only the most Cu-tolerant offspring to hatch. Although we were not able to monitor oocyte survival within the ovary, we did monitor egg survivorship from deposition to hatch. The fact that there were no significant differences in egg mortality among the four larval groups (Fig. 1) supports the hypothesis that Cu tolerance had been maternally transferred to the larval fathead minnows.

Although this study was not designed to determine the substance(s) that may have been maternally transferred, there are a few likely candidates. The copper ion itself may have been transferred, perhaps associated with vitellogenin [4,12]. An alternative candidate for maternal transfer is mRNA for acclimatory proteins. One such protein is the metal-binding protein metallothionein, and indeed Lin et al. [6] found maternal transfer of metallothionein mRNA. A third possibility for maternal transfer is the acclimatory proteins themselves. Maternal transfer of immunoglobulins has been reported for tilapia [13,14], giving support to the hypothesis that acclimatory proteins could also be maternally transferred. Clearly, more research is needed to elucidate exactly what substances are being maternally transferred into fathead minnow oocytes.

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REFERENCES

1. Hall AT, Oris JT. Anthracene reduces reproductive potential and is maternally transferred during long-term exposure in fathead minnows. *Aquat Toxicol* 1991;19:249–264.
2. Ankley GT, Tillitt DE, Giesy JP. Maternal transfer of bioactive polychlorinated aromatic hydrocarbons in spawning chinook salmon (*Oncorhynchus tshawytscha*). *Mar Environ Res* 1989;28:231–234.

3. Gillespie RB, Baumann PC. Effects of high tissue concentrations of selenium on reproduction by bluegills. *Trans Am Fish Soc* 1986;115:208–213.
4. Ghosh P, Thomas P. Binding of metals to red drum vitellogenin and incorporation into oocytes. *Mar Environ Res* 1995;39:165–168.
5. Johnston TA, Bodaly RA, Latif MA, Fudge RJP, Strange NE. Intra- and interpopulation variability in maternal transfer of mercury to eggs of walleye (*Stizostedion vitreum*). *Aquat Toxicol* 2001;52:73–85. [PubMed: 11163432]
6. Lin HC, Hsu SC, Hwang PP. Maternal transfer of cadmium tolerance in larval *Oreochromis mossambicus*. *J Fish Biol* 2000;57:239–249.
7. Kolok AS, Peake EB, Tierney LL, Roark SA, Noble RB, See K, Guttman SI. Copper tolerance in fathead minnows: I. The role of genetic and nongenetic factors. *Environ Toxicol Chem* 2004;23:200–207. [PubMed: 14768886]
8. Lewis, PA.; Klemm, DJ.; Lazorchak, JM.; Norberg-King, TJ.; Peltier, WH.; Heber, MA., editors. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Fresh-water Organisms. Vol. 3rd ed.. Cincinnati, OH: U.S. Environmental Protection Agency; 1994. EPA/600/4–91/002
9. U.S. Environmental Protection Agency. Methods for chemical analysis of water and wastes. Cincinnati, OH: 1983. EPA/600/4–79/20
10. Dixon, PM.; Newman, MC. Analyzing toxicity data using statistical models for time-to-death: An introduction. In: Newman, MC.; McIntosh, AW., editors. *Metal Ecotoxicology, Concepts and Applications*. Chelsea, MI, USA: Lewis; 1991. p. 207-242.
11. SAS Institute. JMP, Ver 5.0. Cary, NC, USA: 2002.
12. Montorzi M, Falchuk KH, Vallee BL. Vitellogenin and lipovitellin: Zinc proteins of *Xenopus laevis* oocytes. *Biochemistry* 1995;34:10851–10858. [PubMed: 7662665]
13. Mor A, Avtalion RR. Transfer of antibody activity from immunized mother to embryo in tilapias. *J Fish Biol* 1990;37:249–255.
14. Takemura A, Takano K. Transfer of maternally-derived immunoglobulin (IgM) to larvae in tilapia, *Oreochromis mossambicus*. *Fish Shellfish Immunol* 1997;7:355–363.

Fig. 1. Egg survivorship, or percentage of eggs that survived to hatch (mean $\pm$ standard error), for the four larval groups. Numbers in parentheses denote the number of replicates in each larval group.

Fig. 2. Cumulative survivorship for preexposure (solid circles) and postexposure (open circles) groups of larvae from females exposed to (A) 100 µg/L Cu and (B) 0 µg/L Cu (controls). Lines extending beyond 96 h represent the larvae that survived the 4-d test.

Table 1

Summary of survival-time analysis for the larvae hatched before and after their female parents were exposed to 100 $\mu\text{g/L}$ Cu for 5 d. Chi-square (χ^2) values are from likelihood ratio tests of the effect of each covariate, and associated probabilities are based on one degree of freedom each

Parameter	Coefficient	Standard error	χ^2	<i>p</i> value
Intercept	5.775	0.207	774.974	<0.0001
Cu concn.			51.730	<0.0001
0 $\mu\text{g/L}$	2.112	0.325	42.313	<0.0001
400 $\mu\text{g/L}$	1.124	0.220	26.022	<0.0001
800 $\mu\text{g/L}$	0	0		
Larval group				
Preexposure	-0.784	0.209	14.104	0.0002
Postexposure	0	0		

Table 2

Summary of survival-time analysis for the larvae hatched before and after their female parents were sham-exposed. Chi-square (χ^2) values are from likelihood ratio tests of the effect of each covariate, and associated probabilities are based on one degree of freedom each

Parameter	Coefficient	Standard error	χ^2	<i>p</i> value
Intercept	5.391	0.171	996.274	<0.0001
Cu concn.			53.757	<0.0001
0 µg/L	3.057	0.454	45.359	<0.0001
400 µg/L	0.972	0.207	22.108	<0.0001
800 µg/L	0	0		
Larval group				
Preexposure	−0.099	0.183	0.294	0.5876
Postexposure	0	0		