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Maternally derived Cu tolerance in larval fathead minnows: how long does it persist?

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Abstract

Fathead minnow *Pimephales promelas* larvae were produced by either copper (Cu)-exposed or naïve females and then subjected to a 96 h survival test using Cu concentrations of 400 and 800 $\mu\text{g l}^{-1}$. Three survival challenges were conducted: the first survival test featured 0 day-old larvae while the second and third featured 8 and 15 day-old larvae, respectively. The results of this study show that maternally derived Cu tolerance was relatively short-lived as it persisted for <8 days.

Keywords

copper tolerance; fathead minnows; maternal transfer

Maternal transfer is a mechanism by which females can enhance the fitness of their offspring. Through this mechanism females can provide beneficial materials, such as nutrients and antibodies, to their young (Bernardo, 1996). Furthermore, metal tolerance can also be maternally transferred from a female to her offspring. It is unclear, however, how long the enhanced tolerance persists once the larvae hatch. Thus, the objective of this study was to determine how long maternally derived copper (Cu) tolerance persists in larval fathead minnows *Pimephales promelas* Rafinesque.

The fish used in these experiments were produced and maintained by the University of Nebraska, Omaha, fathead minnow colony according to the protocols outlined in Peake *et al.* (2004). Larvae used in this study were produced by either Cu-exposed or naïve females. To accomplish this, adult females were either exposed to 150 $\mu\text{g l}^{-1}$ Cu or sham exposed for 8 days. Exposures occurred in 70 l aquaria separated into three compartments by plastic dividers to keep females from different lineages apart. The water was manually 're-spiked' during the daily one-third static renewal, using a stock solution of CuSO_4 . A total of 34 females from three lineages were exposed to Cu, while 32 from the same three lineages were sham exposed. Two females exposed to Cu died during the 8 day exposure. Cu concentrations were mean $\pm$ s.e. $115 \pm 19 \mu\text{g l}^{-1}$ (76.5% of nominal) in the Cu-exposure tanks and were below the detection limit of $12 \mu\text{g l}^{-1}$ in the sham-exposure aquaria.

Following the maternal exposure, surviving females from the Cu and sham exposures were returned to heated 30 l breeding aquaria (c. 26° C) where breeding pairs were established. Breeding was initiated according to the protocol outlined in Peake *et al.* (2004). When the

required number of larvae hatched within a 24 h period, those individuals were utilized in the larval survival tests.

Three larval survival tests were conducted. The first was initiated when larvae were 0 days old, while the second and third were begun when larvae were 8 and 15 days old, respectively. Newly hatched larvae (<24 h-old larvae) from six females were evenly distributed among 2 l beakers to maintain a density of 45 larvae per beaker. For each trial, larvae were allocated to one of six groups: control 0, maternal transfer (MT) 0, control 400, MT 400, control 800 or MT 800. In each trial, the experimental groups were duplicated. Control larvae were produced by naïve females, while MT larvae were produced by Cu-exposed females. Larvae in the control 0 and MT 0 groups were not exposed to Cu. Larvae in the control 400 and MT 400 groups were exposed to 400 $\mu\text{g l}^{-1}$ Cu, while larvae in the control 800 and MT 800 groups were exposed to 800 $\mu\text{g l}^{-1}$ Cu.

During the survival trials, test chamber solutions had >80% static renewal daily and water samples (125 ml) were collected daily from each test chamber for Cu analysis (USEPA, 1983). Cu concentrations were mean $\pm$ s.e. $308 \pm 44 \mu\text{g l}^{-1}$ (77% of nominal), $593 \pm 80 \mu\text{g l}^{-1}$ (74% of nominal) in 400 and 800 $\mu\text{g l}^{-1}$ Cu test chambers, respectively. In each survival trial, the Cu concentration of water in the 0 $\mu\text{g l}^{-1}$ test chambers, was below the detection limit of 12 $\mu\text{g l}^{-1}$. Following initiation of each 96 h survival challenge, dead larvae were counted and removed at 3, 6, 12, 24, 48 and 96 h.

For each survival trial, survival plots were generated and survival-time analysis was conducted to compare the mortality of larvae produced by Cu-exposed females to that of larvae produced by naïve females according to the methods outlined in Peake *et al.* (2004) and Sellin *et al.* (2005). During each of the three trials, all of the fathead minnows in the control 0 and MT 0 groups survived, thereby excluding them from further analysis. The survival data from the control 400 and control 800 groups were pooled together for the analysis as were the MT 400 and MT 800 survival data (Peake *et al.*, 2004). For the 0 day-old survival challenge, the model assuming a Weibull distribution was selected. At 8 and 15 days old, a model could not be selected because none of the three models fit the data. The models included two covariates: Cu concentration and larval group (those produced by Cu-exposed females or by sham-exposed females). The effects of the covariate 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 χ^2 distribution. Statistical significance throughout the experiments was set at $P \leq 0.05$.

Survivor curves for the survival challenges are shown in Fig. 1. The survival of control 0 and MT 0 larvae were not plotted, as they were not included in the survival time analysis due to the lack of mortality. The mortality of 4 day-old (age at termination of survival test) larvae from naïve females was significantly higher than that of larvae from Cu-exposed females (χ^2 , d.f. = 1, $P < 0.001$; Fig. 1). A significant difference in the mortality of 12 day-old larvae from naïve and Cu-exposed females could not be detected because attempts to fit a model to the data were not successful. Similarly, a significant difference between the survival of 19 day-old larvae from naïve and Cu-exposed females could not be detected.

In addition to the survival trials, newly hatched (<24 h old) larvae were collected from Cu-exposed and naïve females to determine if Cu had been maternally transferred from Cu-exposed females to their offspring. Newly hatched larvae were euthanized and whole-body Cu concentrations were determined. Preliminary data suggested that it was virtually impossible to get consistent wet masses and whole-body ion concentrations on very small larvae (<10 mg). Because of this, newly hatched larvae were gathered into pellets with a minimum mass of 15 mg. Prior to analysis, larval pellets were dried at c. 60° C for a minimum of 24 h (VanGenderen,

2003). After drying, weighed pellets were digested in concentrated nitric acid (Grippo & Dunson, 1991), diluted with nanopure water and analysed for Cu using a graphite furnace (Varian GTA 110). The reported units of Cu concentration ($\mu\text{mol g}^{-1}$ wet mass) were converted from $\mu\text{mol g}^{-1}$ dry mass by using the wet:dry mass ratio specific to each individual pellet. Differences in the whole-body Cu of newly hatched larvae from naïve and Cu-exposed females were tested using a *t*-test. Statistical significance was set at $P \leq 0.05$.

The mean $\pm$ s.e. whole-body Cu of larvae from sham-exposed and Cu-exposed females were 0.015 ± 0.003 ($n = 7$) and 0.015 ± 0.002 ($n = 8$), respectively. There was no significant difference between the whole-body Cu of newly hatched larvae (0 days old) produced by naïve and Cu-exposed females (*t*-test, d.f. = 13, $P > 0.05$).

This study demonstrated that the enhanced Cu tolerance observed in the offspring of Cu-exposed female fathead minnows did not persist once the larvae had reached 8 days old (wet mass of control 0 larvae c. 29 mg). The enhanced tolerance of 4 day-old larvae from Cu-exposed females relative to larvae from naïve females was expected, as this phenomenon has been documented previously. Peake *et al.* (2004) found 0 day-old (age at initiation of survival test) offspring of Cu-exposed female fathead minnows to be more Cu tolerant than offspring of naïve females. Similarly, 0 day-old tilapia *Oreochromis mossambicus* Peters larvae produced by Cd-exposed females are more Cd tolerant than larvae produced by naïve females (Lin *et al.*, 2000).

Enhanced Cu tolerance was not observed in larvae born from Cu-exposed females at 12 or 19 days old indicating that by 12 days maternally derived Cu tolerance had attenuated. The attenuation of maternally derived Cu tolerance as development progresses has previously been reported for white suckers *Catostomus commersoni* (Lacépède). Specifically, Munkittrick & Dixon (1988) showed that white sucker larvae born to females from Cu-contaminated lakes had increased Cu tolerance relative to larvae from naïve females. This enhanced tolerance peaked when larvae were 13 days old, but declined until 21 days old when the observed tolerance could no longer be detected.

This study also determined whether Cu ions are transferred from a female to her offspring. Currently, the identity of the maternally transferred material responsible for the development of enhanced Cu tolerance observed in offspring of Cu-exposed females is unknown. In the literature, it has been suggested that Cu ions are being maternally transferred (Munkittrick & Dixon, 1988; Peake *et al.*, 2004). If maternally transferred, it is possible that Cu ions are acting in the larvae as a signal to induce the expression of acclimatory or detoxifying proteins leading to the enhanced tolerance observed. This hypothesis is plausible considering that Cu has been shown to bind to vitellogenin (Ghosh & Thomas, 1995; Montorzi *et al.*, 1995), and considering that Cu, being an essential micronutrient, must be maternally transferred (at least to some degree) from a mother to her offspring. The results of this study however, do not support this hypothesis as Cu- and sham-exposed females produced larvae with the same whole-body Cu concentrations. Clearly, Cu ions are not the signal responsible for the enhanced tolerance observed in larvae produced by Cu-exposed females.

Acknowledgments

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FIG. 1.

Cumulative survivorship of (a) 4, (b) 12 and (c) 19 day-old larvae of *Pimephales promelas* from Cu-exposed females (----) and from naïve females (—) when exposed to 400 and 800 $\mu\text{g l}^{-1}$ Cu. Control 400 and control 800 survival data were pooled together, as were the MT 400 and MT 800 data. Lines extending beyond 96 h represent larvae that survived the 4 day test. Differences in upper case letters denote significant differences between experimental groups ($\chi^2 = 17.32$, d.f. = 1, $P < 0.001$).