

MATERNAL TRANSFER OF COPPER RESISTANCE IN FATHEAD

MINNOWS

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EXTENDED ABSTRACT ONLY – DO NOT CITE

The overall objective of this research was to determine if differential resistance to copper (Cu) could be transmitted from adult fish to larval offspring. Differential resistance can be transferred from adults to offspring in two different ways. The first is genetic inheritance. This is supported in the literature by several studies that found that differences in Cu resistance were significantly correlated with differences in allozyme genotypes (e.g. Schlueter et al., 1995). The second way is by maternal transfer of non-genetic material (e.g. Lin et al., 2000). We conducted two experiments with fathead minnows (*Pimephales promelas*) to investigate these two potential methods for transfer of Cu resistance from parents to larval offspring.

In our first experiment, we tested the hypothesis that genetic differences in adults were a major influence in determining the resistance of larvae. We used the same methods as detailed in Kolok (1998) to classify 48 male and 48 female adult minnows as being either Cu-susceptible or Cu-resistant. Critical swimming speeds (U_{crit} s) were measured for the 96 minnows before and after an 8-9 d exposure to 150 $\mu\text{g/L}$ Cu. Cu resistance or susceptibility was based on the percent decrease in U_{crit} after Cu exposure. After the minnows were classified, the 12 most Cu-susceptible pairs and 10 most Cu-resistant pairs were bred together.

A 7-d survival test (U.S. EPA, 1994) was conducted on the larvae produced by Cu-resistant, Cu-susceptible, and naïve (previously unexposed) parents. Eighteen 600-ml beakers, each with 250 ml of Cu solution, served as test chambers. Larvae <24 h old from five to seven breeding pairs were distributed

evenly among the test chambers, with about 15 larvae per test chamber. There were triplicates at each of six Cu concentrations (0, 100, 200, 400, 800, and 1600 $\mu\text{g/L}$), with static renewal of $\geq 80\%$ daily with freshly made test solutions. Actual Cu concentrations were analyzed by flame atomic absorption spectrometry. Mortality was recorded daily. Figure 1 shows mortality for the four groups, with replicates pooled.

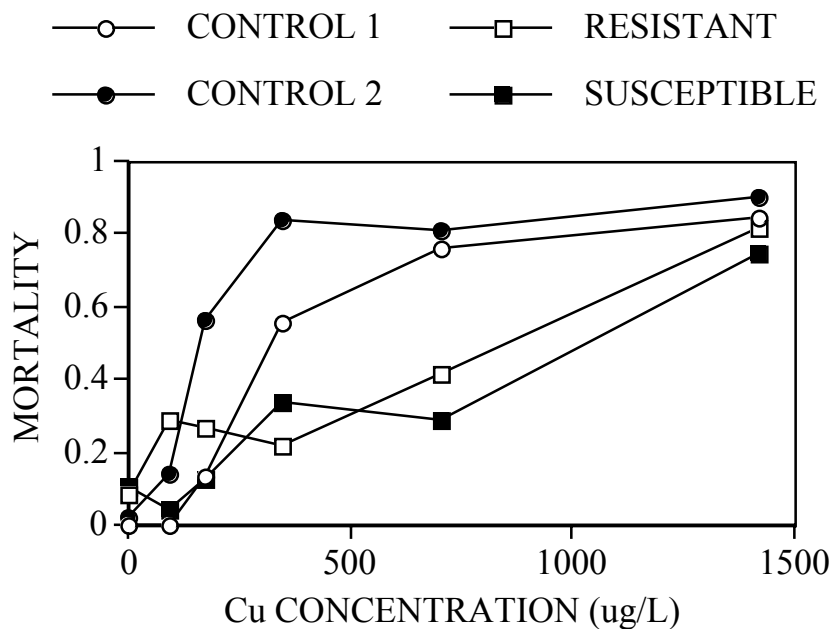


Figure 1. Mortality after 7 days for four larval groups at six actual Cu concentrations, 0-1400 $\mu\text{g/L}$ Cu.

The LC_{50} s of the larvae from Cu-susceptible and Cu-resistant parents were surprisingly similar (922 and 924 $\mu\text{g/L}$, respectively) and significantly greater (t -test, $p=0.02$) than those of controls (412 and 210 $\mu\text{g/L}$). Cu-susceptible parents did not produce Cu-susceptible larvae; therefore the data did not support the hypothesis that the relative Cu tolerance of the larvae would be similar to that of their parents.

In the second experiment, we tested the hypothesis that larvae would be more Cu resistant if their female parent had been previously exposed to Cu 1 week prior to breeding. We bred 12 pairs of minnows and conducted 96-h time-to-death tests on their larvae. About 20 larvae <24 h old from each breeding pair were placed in each of three 600-ml beakers. Each of these test chambers had 350 ml of Cu solution at 0, 400, or 800 $\mu\text{g/L}$ Cu, with daily static renewal of $\geq 80\%$ with freshly made test solutions. Mortality was recorded 3, 6, 12, 24, 48, and 96 h after test initiation. Six females were exposed to 100 $\mu\text{g/L}$ Cu for 5 d, while the remaining females were sham-exposed. Females were then bred with their original partners, and 96-h larval time-to-death tests were again conducted as above. Figure 2 shows cumulative survival for the larval groups.

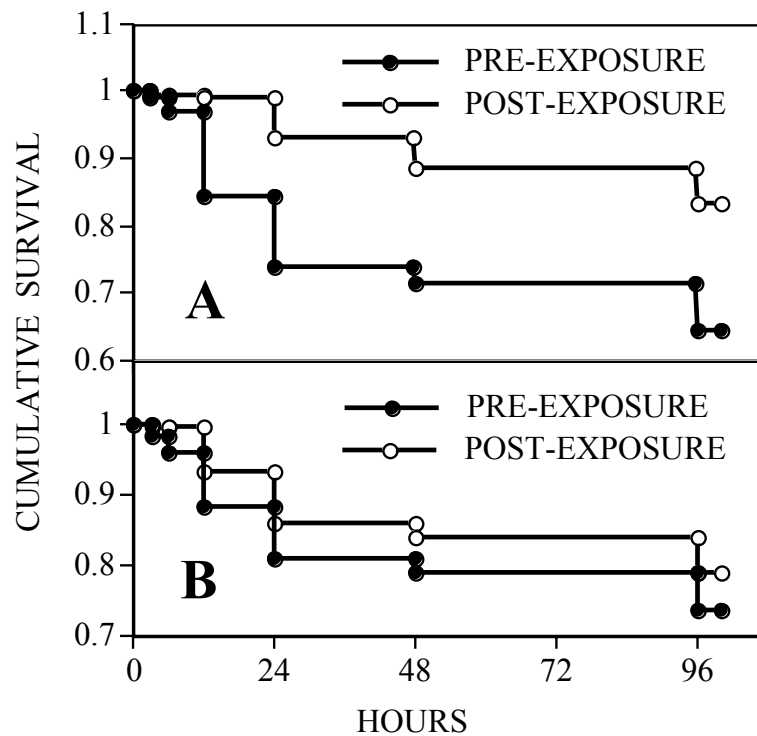


Figure 2. Survival for pre- and post-exposure groups of larvae from female parents exposed to (A) 100 $\mu\text{g/L}$ Cu and (B) 0 $\mu\text{g/L}$ Cu.

For both groups, the effects of each Cu test solution concentration was significantly related to time-to-death, and their inclusion significantly improved the fit of the model to the data. The pre-exposure and post-exposure groups of larvae from sham-exposed females were not significantly different in survivorship. For larvae from Cu-exposed females, however, post-exposure survivorship was significantly higher than pre-exposure survivorship; indicative of maternal transfer.

We were surprised to find that Cu susceptibility in the adults was not directly correlated with Cu susceptibility in their larvae. One reason may be that the mechanism for Cu susceptibility may be different between these two life history stages. Upon closer examination (our second experiment), it became apparent that maternal transfer had a much more important influence on the relative Cu resistance of larvae than we initially anticipated.

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