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Effects of Sublethal Copper Exposure on Behavior and Growth of *Rana pipiens* Tadpoles

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Amphibians are considered keystone members of ecosystems and often constitute the largest amount of vertebrate biomass due to sheer densities (McDiarmid and Mitchell 2000). Various roles amphibians play in an ecosystem range from top carnivores-consuming invertebrates, to the main source of food for birds, mammals and aquatic insects (Blaustein and Wake 1995). Because they act as both predator and prey, amphibians are vital links in the food chain. Due to the vital role amphibians play in an ecosystem, their decline or loss may conceivably cause serious ramifications throughout entire ecosystems.

Amphibians also function as valuable biological indicators of environmental stresses and are therefore sentinels for environmental change. Since the early 1980s, several species have become extinct locally and some globally. That numerous anuran populations have declined and disappeared throughout much of the world (Halliday 1998) is reason for alarm.

The decrease in amphibian numbers has prompted investigations to identify biotic and abiotic factors responsible for these declines either alone or in combination with other factors Although they have been poorly studied, negative influences may leave affected animals less fit for survival, which may have significant consequence on population densities (McDiarmid and Mitchell 2000). Common tadpole responses to sublethal environmental pollution, especially heavy metal and pesticide exposure, include tail kinking, erratic swimming and loss of appetite, all of which may leave individual organisms more susceptible to secondary stressors such as predation or disease (Lefcort et al. 1998).

The primary focus of this study was to determine if exposure to sublethal concentrations of copper would cause behavioral changes that may leave tadpoles more susceptible to secondary stressors. Secondarily, we wanted to determine recovery times after sublethal exposures with regard to length of tadpole.

MATERIALS AND METHODS

Rana pipiens eggs were purchased from Carolina Biological Supply. On arrival in the laboratory, the eggs were transferred to a high-density polyurethane

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container and allowed to acclimate to room temperature. Hardness of the original water was measured so embryos could be properly acclimated to dechlorinated tap water at 20° C in the laboratory. Dechlorinated tap water was softer (104 total hardness as $CaCO_3$) than the water in which the tadpoles arrived (156 total hardness as $CaCO_3$) so 500 ml dechlorinated tap water was added twice daily until toxicity testing began (about 7 days). A 16:8 full spectrum light dark cycle was maintained throughout the experiment. Water was aerated gently to avoid disturbing the egg clutch.

Acute 96 hour range finding experiments indicate lethality is caused by exposure to Cu concentrations between 0.14 mg/L and 0.04 mg/L. To determine sublethal toxicity, 3 replicates of 10 *Rana* larvae were exposed to one of 5 Cu concentrations (0.1, 0.08, 0.06, 0.04, 0.02, mg/L, respectively) plus controls in 300 ml solution for 7 days beginning the day after hatch. Cu concentrations were analyzed at the beginning and end of the experiment by atomic absorption spectrophotometry. Nominal concentrations were within the 95% confidence interval for the actual concentrations. Tadpoles were fed crushed alfalfa pellets every other day for 2 hours before 100% of the solution was changed.

To determine aberrant behavior, tadpoles were placed in a flat-bottomed jar over graph paper. Tadpoles were prodded once with a blunt tip and observed. The inability to travel in a straight line or swim at least 2cm was considered abnormal.

After the toxicity portion of the study, it was apparent tadpoles exposed to higher sublethal concentrations were smaller than controls. To monitor growth of tadpoles exposed to copper, three replicates of 8 tadpoles exposed to 0, 0.04, 0.08, and 0.1 mg/L were maintained until the appearance of front leg buds. This allowed us to examine post-exposure growth and survivorship.

These tadpoles were maintained in eight 38 L aquaria, each divided into four equal compartments with polyurethane screen and filled with dechlorinated tap water. Tadpoles from the same clutch were housed in the same aquarium. Water temperature was maintained at 20°C with a 16:8 light dark cycle throughout the experiment. Tadpoles were fed crushed alfalfa pellets and waste was siphoned off the bottom weekly. Tadpoles were transferred to a flat-bottomed specimen jar and total length (nose to the end of tail) measured weekly using graph paper.

Toxicity data were analyzed using Probit analysis by Toxstat (Gulley 1994) to determine LC 50 concentrations. Growth recovery data were found to be non-normal using Shapiro-Wilk tests and therefore analyzed by Kruskal Wallis one way, multiple sample test followed by Dunn's post hoc test using SAS (SAS Institute Inc. 1989).

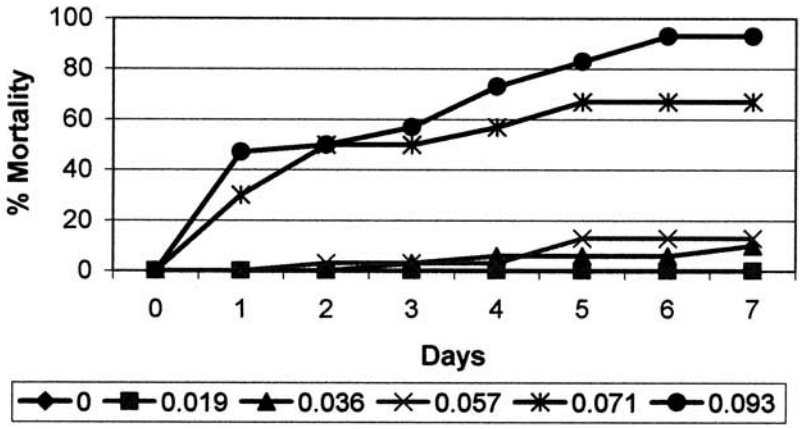

Figure 1. Percent mortality of *Rana pipiens* exposed to copper in solution. Sample size is 3 for each concentration with 10 observations per replicate.

RESULTS AND DISCUSSION

Calculated LC50s for the 7-d exposure was 0.067 mg/L Cu, although mortality was induced at concentrations as low as 0.036 mg/L (Figure 1). This is typical for a 96-hour exposure (Gottschalk, 1995); however, there is no 7-d exposure information to compare to the values generated during this experiment. Longer exposure periods were designed to reveal behavioral or morphological changes that had previously been observed prior to mortality in the 96-hour exposure without actually causing mortality.

Behavioral changes throughout the 7-d exposure included lethargy, loss of equilibrium and apparent loss of appetite. These effects were detected in a portion of individuals at Cu concentrations of 0.036 mg/L, nearly 50% lower than the calculated LC50 for a 7-d exposure. At Cu concentrations above 0.071 mg/L, all tadpoles exhibited struggling movement (Figure 2). When prodded, affected tadpoles would jerk sporadically before settling again on the bottom of the chamber.

At times, tadpoles exposed to Cu solutions exceeding 0.036 mg/L were observed to lie motionless on their side or back with no attempts to right themselves. Control tadpoles swam about the chamber periodically or anchored themselves motionless against sides of the exposure chamber. After aberrant behavior was apparent, the response continued until tadpoles were removed from solution or mortality occurred. Affected tadpoles appeared to display normal behavior within one week after transition to uncontaminated solution

After addition of food, unaffected tadpoles appeared to consume alfalfa pellet particles voraciously, attaching themselves to particles of the pellet. Lethargic

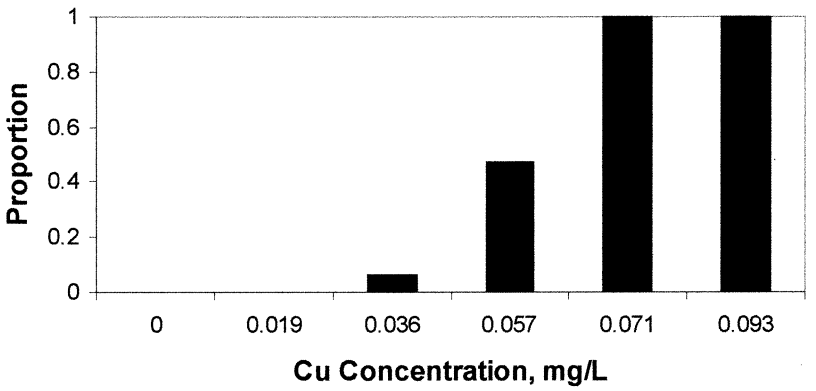

Figure 2. Proportion of tadpoles affected by Cu in solution. Aberrant behavior is defined as twitchy, or struggling movement and the inability to swim 2 cm after a single prod with a blunt tip. Sample size is 3 for each concentrations with 10 observations per replicate.

tadpoles affected by Cu exposure didn't move after food was introduced to the exposure chamber. Little if any food consumption occurred in concentrations nearing and above the LC50 for the 7-d exposure.

Copper exposure did not cause hyperactivity but rather lethargy, which is a common response to heavy metal exposure (Lefcort et al.1998). Tadpoles in exposure groups higher than 0.071mg/L only moved when prodded or relocated from one chamber to another after a solution change. Control tadpoles swam around the tank continuously after relocation.

After the 7-d toxicity experiment, tadpoles exposed to Cu above 0.071 mg/L were clearly smaller than control tadpoles ($p = 0.0001$ Kruskal Wallis). After 8 and 15 days of recovery, tadpoles in Cu concentrations greater than 0.071 mg/L remained significantly smaller than controls ($p = 0.0051$ and $p = 0.043$ Kruskal Wallis respectively). After 19 days of recovery, all treatment groups were similar in size to controls (Figure 3).

Nineteen days after the tadpoles were removed from Cu treatments, there was no difference in body length between exposed and control tadpoles. However, length was the only parameter measured. Blood and tissue samples were not analyzed for changes in enzyme or protein levels that may result from Cu exposure.

This study suggests that brief exposure to Cu may not have lasting effects because *R. pipiens* exposed to sublethal Cu concentrations, recovered once the stressor was removed. Because most aquatic predators are visually oriented and only strike moving prey (O'Brien et al. 1976; Skelly 1994), copper may be protective, inducing lethargy, which lessens their visibility to predators and may lead to lower predation rates.

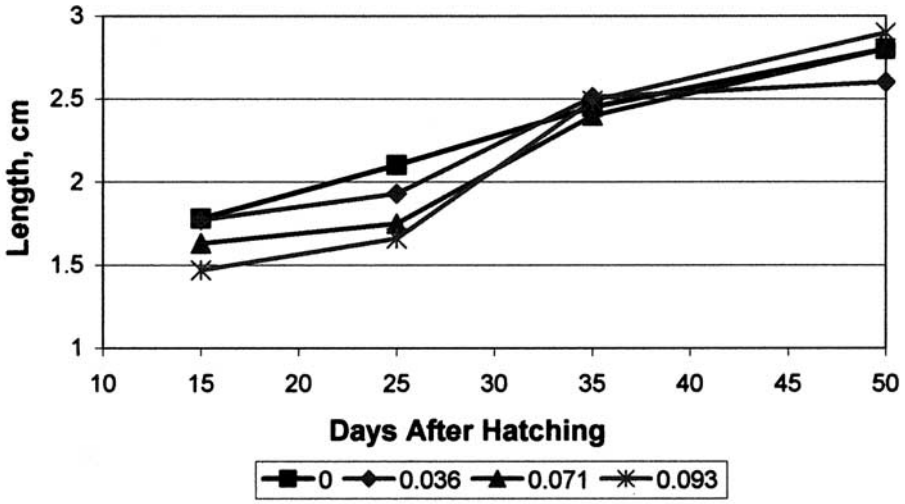

Figure 3. Differences in body length beginning the day tadpoles were removed from solution. 19 days after tadpoles were removed from Cu solutions, there were no differences in body length. Sample size is 3 replicates each with 8 observations.

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