# **EFFECTS OF EPISODIC COPPER EXPOSURES ON POPULATION FITNESS**

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# INTRODUCTION

The success of a population is dependent upon the fitness of the individuals. Fitness is defined as the contribution of a genotype to the next generation, thus, referring to the success of a reproductive organism (Campbell, 1996). However, reproduction by an individual is dependent upon growth. Surplus energy not used for maintenance or metabolism is invested in growth (Lawrence, et al., 2003). Eventually, as the organism ages, energy invested in growth will be allocated for reproduction. Although, when exposed to an environmental pollutant, such as copper, the energy allocated for growth and reproduction maybe assigned to maintenance and repair in an effort to restore homeostasis (Heath, 1987; Lawrence, et al., 2003; Brafield and Llewllyn). This has consequences on energy balance in an organism and depending upon the intensity and duration of the copper exposure, growth and reproduction may be affected. Population level effects have been documented at metal contaminated sites, noting reduced growth and development in fish and invertebrates (Peplow and Edmonds, 2005).

Previous work has shown that acute pulsed exposures to copper results in a loss of sodium in larval fish, which may lead to mortality (Zahner, et al., 2006). Further, these organisms have demonstrated an ability to regain sodium homeostasis when given an adequate period of recovery in copper free water (~96-h). However, these organisms may be allocating energy to recovering ion homeostasis rather than growth and Thus, chronic episodic exposures to reproduction. copper over an organisms life-span may have significant consequences on organism growth and reproduction. Researchers have shown that chronic exposures to copper affect growth in larval and juvenile fish (DeBoeck, et al., 1997; Marr, et al., 1996; Woltering, 1984). But growth alone is not a reliable indicator of reproductive success (McCahon and Pascoe, 1990; Handy 1994). Organisms exposed to low levels of pollution may have no notable adverse effects at the larval age; however, reproductive effects may become apparent in later life stages. This may affect the survival of the population.

The current NPDES methodology for assessing the risk of an effluent to the in-stream community does not account for long-term reproductive consequences, or the impacts of episodic exposures occurring over the duration of the organism's life cycle. Effects from pulsed exposures may result in sub-lethal responses to the aquatic community, such as reduced feeding rate, impairment of growth, or reduced reproductive success, which may cause decreased population survival resulting in ecosystem perturbation or degradation (McCahon and Pascoe, 1990; Handy 1994). These latent post-exposure population level responses cannot be assessed using current NPDES testing and this may result in an underestimation or an overestimation of risk to the biological community. However, little research has been completed on the effects of episodic exposures on a Thus, the goal of this research is to population. characterize population responses to episodic copper exposures throughout the life-span of the fathead minnow (Pimephales promelas).

# METHODS

A life-cycle study was used to assess the influence of episodic copper exposures on both individual and population fitness. Life-cvcle reproductive toxicity experiments followed the growth and development of newly hatched larvae (F1 generation) through maturation. P. promelas were maintained in a flow-through tank system using dechlorinated tap water. Water quality measurements were monitored daily. Fish were exposed for 24-h to 50µg Cu/L every 4 or 9-day over their entire life-span. Lethal and sublethal (whole-body sodium, growth, reproduction) endpoints were recorded throughout the organisms life-span and for 30-d following the hatch of the F2 generation. Hatch success and viability of the F2 generation was also monitored.

#### Survival

A significant decrease in survival of the larval fathead minnows in both the 4 and 9-d copper treatment was observed following Exposures 1-3. Mortality continued for up to 72-h following the termination of Exposures 1-3, with the greatest mortality occurring 24-h post exposure termination. Little to no mortality was observed following Exposures 4-39. There are three possible explanations for the lack of mortality following these exposures: 1.) The age specific LC50 has increased; thus, as the organisms ages, the copper concentration is less effective; 2.) The population is tolerantly skewed following the first three exposures; and 3.) The organisms have acclimated to the treatment conditions.

### Growth

Chronic episodic exposures did not significantly affect growth within the larval stage of the fathead minnows. This agrees with previous 21-d episodic exposure studies conducted in our laboratory with larval fathead minnows. However, growth became significantly reduced in copper treatments starting at Exposure 15 (70-d old fish). Adult male and female fish mass was significantly reduced in both copper treatments compared with controls over the 39 exposures (190-d). No significant difference was seen between the 4 and 9-d recovery periods. These results agree with several other researchers who demonstrated that chronic exposures to copper affect the growth of juvenile and adult fish (DeBoeck, et al., 1997; Marr, et al., 1996; Woltering, 1984). However, larval fathead minnows did not have similar growth effects. These results suggest that extrapolation of results from traditional acute larval stage toxicity testing may not be predictive of long-term chronic effects.

### Reproduction

Controls and began copper treatments reproducing at  $\sim$ 4 months (115-d). There was no delay of reproduction for fish exposed to copper. Reproduction, measured by egg counts, egg viability, and hatch success, was followed for 75 days. Both the 4 and 9-d copper treatment egg counts were reduced compared to the controls. Further, egg viability and hatch success was affected in both copper treatments as compared to the controls. Following hatching, 4 broods of larval organisms from each treatment were followed for 30-d to determine any F2 generation affects. All larval organisms survived from all treatments. No developmental or growth effects were noted.

### Whole-body Sodium Recovery

Recovery is defined as the point at which the whole-body sodium of the treatment organism is no longer significantly different (p<0.05) from the controls at that time point. Whole-body sodium measurements were taken until Exposure 24 (116-d), when the fish reached maturation and began reproducing. There was a significant decline in the whole-body sodium concentration of both the copper treated fish from the controls following each exposure. The effect of copper on whole-body sodium was reduced as the organism aged. This may be due to a decreased effect of the copper concentration on older organisms or the organisms may be acclimating to the exposure The response of whole-body sodium to conditions. copper exposures has been shown to be both concentration and duration dependent in fish (Zahner, et al., 2006). Further, researchers have documented the ability of fish to acclimate to chronic copper exposures, resulting in a recovery of plasma sodium (Lauren and McDonald, 1987a: Lauren and McDonald, 1987b).

#### CONCLUSIONS

We hypothesized that if adequate recovery times are used between episodic exposures, no sub-lethal effects should occur. If given an adequate period in copper free water organisms should be able to compensate for sodium loss and acquire needed energy from food for growth and reproduction. However, we found that the energy allocated for recovery following chronic episodic exposures of copper resulted in impairment of growth and reduced reproductive success within the fathead minnow population. This research demonstrates that chronic episodic copper exposures can significantly influence ecological fitness and, hence, has population level consequences, which would have been missed by traditional NPDES acute toxicity test methods.

### LITERATURE CITED

- Brafield, A.E., Llewllyn, M.J. Production. In: *Animal Energetics*, Chapman Hall, London, UK, pp 27-79.
- Campbell, N.A., 1996. Mechanisms of evolution. In: Biology 4<sup>th</sup> ed. The Benjamin/Cummings Publishing Co, Inc, NY, pp 430.
- De Boeck, G., Vlaeminck, A., Blust, R., 1997. Effects of sublethal copper exposure on copper accumulation, food consumption, growth, energy stores, and nucleic acid content in common carp. *Arch Env Contam* 33:415-422.
- Handy, R., 1994. Intermittent exposure to aquatic pollutants: Assessment, toxicity, and sublethal

responses in fish and invertebrates. *Comp Biochem Physiol C* 107:171-184.

- Heath, A.G., 1987. Physiological Energetics. In *Water Pollution and Fish Physiology*, CRC Press Inc: Boca Raton, FL, pp131-163.
- Lauren, D.J., McDonald, D.G., 1987a. Acclimation to copper by rainbow trout, *Salmo gairdneri*: Physiology. *Can J Fish Aquat Sci* 44:99-104.
- Lauren, D.J., McDonald, D.G., 1987b. Acclimation to copper by rainbow trout, *Salmo gairdneri*: Biochemistry. *Can J Fish Aquat Sci* 44:105-111.
- Lawrence, A.J., Arukwe, A., Moore, M., Sayer, M., Thain, J., 2003. Molecular/Cellular Processes and Physiological Response to Pollution. In: *Effects of Pollution on Fish: Molecular Effects* and Population Responses, Lawrence A, Hemingway K (eds), Blackwell Science, Oxford, UK, pp. 83-133.
- Marr, J.C.A., Lipton, J., Cacela, D., Hansen, J.A., Bergman, H.L., Meyer, J.S., Hogstrand, C., 1996. Relationship between copper exposure duration, tissue copper concentration, and rainbow trout growth. *Aquat Toxicol* 36:17-30.
- McCahon, C.P., Pascoe, D., 1990. Episodic pollution: causes, toxicological effects, and ecological significance. *Functional Ecology* 4: 375-383.
- Peplow, D., Edmonds, R., 2005. The effects of mine waste contamination at multiple levels of biological organization. *Ecolog Engineering* 24: 101-119.
- Woltering, D.M., 1984. The growth response in fish chronic and early life-stage toxicity tests: a critical review. *Aquat Toxicol* 5 (1).
- Zahner, H.M., VanGenderen, E.J., Tomasso, J.R., Klaine, S.J., 2006. Whole-body sodium concentration in larval fathead minnows (*Pimephales promelas*) during and following copper exposure. *Environ Toxicol Chem* 25 (6): 1635-1639.