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Metal effects on fathead minnows (*Pimephales promelas*) under field and laboratory conditions

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Abstract

The consequences of low-level metal exposure to early life stages of fathead minnows (*Pimephales promelas*) were investigated along a contamination gradient near Sudbury, Canada. Field exposures resulted in elevated hatching time and increased mortality in metal-contaminated lakes, in contrast to laboratory exposures where no effects were observed. Dissolved and ionic Cd and Ni were associated with changes in hatching time and larval mortality under field conditions, though other potential contaminants were not examined and may also have had an influence. The increased biological response of field-exposed fish, relative to fish exposed to the same water in laboratory conditions, may be the result of higher stress in natural environments, which could sensitize fish to contaminants. Analysis also indicated that, as contamination increases, the discrepancies between laboratory and field estimates of effect also increase. A temperature versus hatching time relationship was also quantified for fathead minnows.

Keywords: *Pimephales promelas*; Lab to field extrapolation; Stress; Metals; Cadmium; Nickel; Sudbury; Early life stages; Toxicity testing

1. Introduction

Metals from anthropogenic sources have long been recognized as important contaminants in aquatic ecosystems. Researchers in metal toxicology have focused on how metal toxicity is affected by water chemistry characteristics such as hardness, dissolved organic carbon (DOC), alkalinity, and pH. However, changes in water chemistry do not affect toxicity equally for individual metals (Campbell and Stokes, 1985; Hollis et al., 1997), which has led to a series of investigations that have attempted to quantify the effects of individual water chemistry measures on metal toxicity (Alsop and Wood, 1999; Hollis et al., 2000; McGeer et al., 2002; Pyle et al., 2002a).

In quantifying these relationships, most research has focused on acute toxicity of single metals using simplified water chemistry (e.g., reconstituted water or treated tap water). While important in determining the mechanisms of metal toxicity, these simplified conditions do not represent exposure conditions in natural systems, which typically involve a sublethal mixture of metals in a complex and variable environment. Further, standard methods may involve inappropriate endpoints (Chapman et al., 1996), improperly assess many sublethal endpoints (Fisher and Hook, 2002), and/or ignore dietary uptake (Irving et al., 2003). Diamond and Daley (2000) showed that whole effluent toxicity testing was often (47% of the time) incapable of correctly assessing the response of benthic invertebrate communities to environmental contamination.

This study examined the ability of both field and laboratory testing to measure environmental impacts of metals. Field assessments with caged fish, recommended to increase realism in toxicity testing (La Point and Waller, 2000), were combined with standard ambient water toxicity testing to examine the similarity between the two exposure regimes.

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Ambient water toxicity testing and field exposures were used to examine the biological effects of metals from a contamination gradient to early life stages of fathead minnows (*Pimephales promelas* Rafinesque). Fathead minnows were chosen for this study because they have a wide distribution across North America (Page and Burr, 1991), are present in the industrial region of Sudbury, Ontario (the study area), and have a history of use in metal toxicology. Embryonic mortality, time to hatch (TTH), and larval mortality are recognized as metal-sensitive endpoints in fathead minnows (Eaton, 1974; McKim, 1977; Pyle et al., 2002b) and were used to monitor the effects of metals present in Sudbury lakes (notably Cd, Cu, and Ni). We also provide a baseline for temperature effects on hatching time for the fathead minnow, data which to our knowledge are not currently present in the literature and which improved interpretation of field data where temperature could not be controlled.

2. Materials and methods

2.1. Study design

This study was conceived to examine the responses of embryonic and larval stages of fathead minnows to metal contamination in both field and laboratory environments. Exposure sites were chosen in three reference lakes and four contaminated lakes, in addition to dechlorinated water for laboratory exposures. Laboratory exposures consisted of standardized ambient water toxicity tests ($n = 4$ per test water), which minimized the differences in water chemistry between the two exposure regimes. Field exposures ($n = 6$ per lake) were conducted during periods and in locations where fathead minnows would naturally reproduce.

2.2. Study lakes

Contaminated lakes in the Sudbury area chosen for this study were Hannah, Kelly, Ramsey, and Whitson Lakes. The most contaminated of these lakes was Kelly Lake, which receives effluent inputs from the Copper Cliff smelting operations and associated tailings in addition to inputs from municipal waste water treatment and storm water runoff. Hannah and Whitson Lakes come next in the gradient and represent lakes having elevated metal concentrations in both water and sediments. Ramsey Lake is the least contaminated of the Sudbury lakes in this study but still contains elevated levels of certain metals. Hannah, Kelly, and Ramsey lakes were in close proximity to each other and found near the center of Sudbury. Whitson Lake was located ~4 km north of the other lakes. With the exception of Kelly Lake, which was surrounded by industrial development, all had residential properties on their shores. Kelly Lake was the only lake in the Sudbury area that supported a population of fathead minnows. A more in-depth investigation of the contamination gradient and its effects on fish can be found in Pyle et al. (in press).

Nosbonsing Lake, Talon Lake, and Trout Lake were reference lakes in the North Bay area and had varying degrees of residential shoreline development. All reference lakes supported fish populations typical of Boreal Shield lakes. Although none were known to contain fathead minnows, two lakes, Talon Lake and Trout Lake, contained bluntnose minnows (*Pimephales notatus*), a species of the same genus. The location of each lake is shown in Fig. 1 and the water chemistry and dissolved metal concentrations are reported in Tables 1 and 2.

2.3. Laboratory testing of ambient water samples

Fathead minnows used in ambient water testing and in field exposures were obtained from a culture maintained at Laurentian University. The culture conditions and protocols for ambient water toxicity testing were maintained within guidelines recommended by the Canadian Environmental Protection Service (Environment Canada, 1992).

Test waters consisted of water samples collected from each lake in 4-L high-density polyethylene (HDPE) jugs, with no headspace at the time of collection. Once samples had arrived at the laboratory, they were filtered with a 58-μm Nitex mesh to remove any potential predators or competitors to the fathead minnow embryos and larvae. Samples were stored at 4 °C for a maximum of 4 days after which they were replaced with freshly collected samples. Laboratory control water consisted of dechlorinated Sudbury tap water, stored in the same manner but unfiltered. All water samples were warmed to the temperature used in the study (23 °C) before the test water was replaced.

Eggs were collected daily from the fathead minnow culture for use in the laboratory experiments. Twenty-five eggs were placed into flasks ($n = 4$ per lake) and the flasks (32 in total) were randomly arranged. The flasks were aerated so that water (approx. 90 mL) would gently circulate over the eggs, which clustered in one layer at the bottom of the flask. At least 80% of the water in the flasks was changed daily and exposures lasted until all eggs hatched 4–8 days later.

For larval exposures, 25 newly hatched larvae were randomly assigned to experimental treatments ($n = 4$ replicates per lake) and replicate flasks were arranged randomly. Larval exposures lasted 7 days and more than 80% of the water, and the accumulated debris, were removed and replaced daily with fresh water. Larvae were fed newly hatched *Artemia salina* twice daily *ad*