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Short communication

Effects of calcium and magnesium hardness on acute copper toxicity to juvenile channel catfish, *Ictalurus punctatus*

Peter W. Perschbacher [a], William A. Wurts [b,*]

[a] *Aquaculture and Fisheries Center, Box 4912, University of Arkansas at Pine Bluff, Pine Bluff, AR 71611, USA*

[b] *Cooperative Extension Program, Kentucky State University, P.O. Box 469, Princeton, KY 42445-0469, USA*

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Abstract

Two experiments were conducted to evaluate the effects of calcium or magnesium hardness on the acute toxicity of copper sulfate to juvenile channel catfish (*Ictalurus punctatus*) in low alkalinity environments. A preliminary bioassay determined the 48-h LC_{50} of copper sulfate to be 1.25 mg l^{-1} for juvenile catfish placed in water with calcium hardness and total alkalinity set at 20 mg l^{-1} $CaCO_3$. In the first experiment, catfish were exposed to 1.25 mg l^{-1} copper sulfate in environments where calcium hardness was varied from 10–400 mg l^{-1} $CaCO_3$. Total alkalinity was 20 mg l^{-1} $CaCO_3$. As calcium hardness increased, copper-induced catfish mortalities decreased significantly from 90% at 10 mg l^{-1} $CaCO_3$ to 5% at 400 mg l^{-1} $CaCO_3$. In the second experiment, catfish were exposed to 1.25 mg l^{-1} copper sulfate in environments containing either calcium or magnesium hardness, 20 and 400 mg l^{-1} $CaCO_3$, with total alkalinity set at 20 mg l^{-1} $CaCO_3$. Survival rates in calcium hardness treatments were consistent with those in the first experiment. However, 100% mortality was observed in both treatments containing magnesium-based hardness. These data suggest a calcium-specific mechanism with respect to acute copper toxicity in channel catfish.

Keywords: Channel catfish; Toxicity; Copper; Calcium

1. Introduction

Copper sulfate is routinely used as an algicide in commercial and recreational fish ponds. It has also been used as an effective treatment for pathogenic protozoan parasites

* Corresponding author

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of fish. It is generally recognized that copper can be highly toxic to teleosts. However, several studies have reported that either calcium hardness or alkalinity concentrations have significant effects on copper toxicity. Therefore, recommendations for safe use of copper sulfate have been based on hardness (Inglis and Davis, 1972; Post, 1983; Sawyer et al., 1989) and total alkalinity concentrations of water (MacMillan, 1985; Wellborn, 1985; Reardon and Harrell, 1990).

Straus and Tucker (1993) reported that total alkalinity and total hardness had significant effects on acute copper toxicity to juvenile channel catfish (*Ictalurus punctatus*). Wurts and Perschbacher (1994) observed that alkalinity concentration had the most pronounced effect on acute copper toxicity to juvenile channel catfish when calcium hardness and alkalinity concentrations were treated as independent variables. Wurts and Perschbacher (1994) also reported a calcium hardness effect, which could affect channel catfish tolerance to copper toxicity in low alkalinity environments. Miller and Mackay (1980) believed calcium hardness was more important than alkalinity in protecting fish from copper toxicity, based on experiments with juvenile rainbow trout (*Oncorhynchus mykiss*). Research with fathead minnows (*Pimephales promelas*) and rainbow trout, however, found no significant calcium effect on copper uptake sites (Lauren and McDonald, 1987a; Playle et al., 1993a; Zia and McDonald, 1994). Furthermore, it has been proposed that magnesium hardness also competes with copper for binding sites on the gills (Playle et al., 1993b).

The present study determined whether acute copper toxicity to juvenile channel catfish was affected by increasing calcium hardness concentrations in low alkalinity waters. Then by substituting magnesium for calcium at equal hardness concentrations, it was possible to compare the effects of magnesium versus calcium on the acute toxicity of copper to juvenile channel catfish.

2. Methods

Two bioassays were conducted to facilitate evaluations about calcium and magnesium effects on acute copper toxicity. The first bioassay determined the amount of copper sulfate needed to effect a 48-h LC_{50} for 7–10 g juvenile channel catfish in water with calcium hardness and total alkalinity concentrations set at 20 mg l^{-1} $CaCO_3$. The second bioassay examined whether 48-h survival would be adversely affected if juvenile channel catfish were placed in calcium-free water with high magnesium concentrations and no copper added. Techniques followed EPA guidelines (U.S. EPA, 1975).

Experiments were conducted to evaluate the mortality response of juvenile channel catfish exposed to a potentially toxic concentration of copper sulfate in waters with differing concentrations of calcium or magnesium hardness and a constant low alkalinity concentration. Two trials were conducted: one varied calcium hardness and the other varied calcium or magnesium hardness. Each combination of hardness and alkalinity was replicated in four, aerated, 7.6-l aquaria. Each aquarium was stocked with seven juvenile channel catfish. Length and weight averages for catfish were 102 $\pm$ 5.2 mm and 8.2 $\pm$ 0.9 g.

Trial 1 involved exposing fish to 1.25 mg l^{-1} copper sulfate in environments with five different concentrations of calcium hardness, ranging from 10 to 400 mg l^{-1}. Total alkalinity was held constant at 20 mg l^{-1}. Catfish were also observed in a control environment where calcium hardness was 400 mg l^{-1} $CaCO_3$ and total alkalinity was 20 mg l^{-1} $CaCO_3$, and no copper was added.

Trial 2 examined the relative effects on copper toxicity (1.25 mg l^{-1} copper sulfate) of magnesium versus calcium hardness at concentrations of 20 and 400 mg l^{-1} $CaCO_3$. Total alkalinity was held constant at 20 mg l^{-1} $CaCO_3$.

Methods used to create and test water treatments, copper toxicity and water quality were the same as those reported by Wurts and Perschbacher (1994). Magnesium hardness was adjusted to desired concentrations with reagent grade magnesium sulfate.

Fish were not fed 48 h prior to or during each experiment. Catfish were held for 24 h preceding each experiment in a holding tank with water containing calcium hardness and total alkalinity, set at 20 mg l^{-1} $CaCO_3$. Water temperature, dissolved oxygen, ammonia–nitrogen (NH_3–N) and pH were measured to monitor water quality. Mortalities were removed and totalled at regular intervals.

Survival data were analyzed using PROC GLM and Fischer's LSD (Ott, 1977; SAS, 1989). Percentile data were transformed using the arc-sine method suggested by Mostellar and Youtz (1961). Significance was tested at the 0.05 level.

3. Results and discussion

A copper sulfate concentration of 1.25 mg l^{-1} was required to effect a 48-h LC_{50} for juvenile channel catfish placed in water containing total alkalinity and calcium hardness set at 20 mg l^{-1} $CaCO_3$. Water temperature was 21.5°C.

After 48 h, survival was 100% for juvenile catfish placed in aquaria containing calcium-free water with a magnesium hardness of 400 mg l^{-1} $CaCO_3$.

It is interesting to note that the copper concentration producing 48-h LC_{50} in this study, 1.25 mg l^{-1} copper sulfate at low alkalinity (20 mg l^{-1} $CaCO_3$), was substantially lower than that reported by Wurts and Perschbacher (1994) for water of moderate alkalinity (i.e., 28 mg l^{-1} $CuSO_4$, at 75 mg l^{-1} $CaCO_3$). At a low alkalinity concentration, much less copper was required to produce acute toxicity.

In general, water quality was poorest in aquaria with the highest survivals (Tables 1 and 2). Water temperatures ranged from 22.6–23.8°C in trial 1 and 21.7–22.3°C in trial 2. Mean total NH_3–N concentrations ranged from 1.4–1.6 mg l^{-1} at 2 h and 2.9–4.0 mg l^{-1} at 42 h in the first experiment. Mean pH ranged from 6.6–7.0 in trial 1 and 6.5–6.9 in trial 2. Mean dissolved oxygen concentrations ranged from 4.8–6.4 mg l^{-1} in trial 1 and 5.0–7.5 mg l^{-1} in trial 2.

In one aquarium each, from trial 1 and trial 2, disruption of aeration occurred for several hours; and two fish jumped from one aquarium in trial 1. Survival data from these aquaria were treated as missing data in the statistical analyses.

In trial 1, there were significant differences among experimental groups with respect to survival and calcium hardness concentrations. As calcium hardness increased, catfish survival improved significantly from 10% at 10 mg l^{-1} $CaCO_3$ to 95% at 400 mg l^{-1}

Table 1
Mean 48-h survivals and water quality data for juvenile channel catfish exposed to 1.25 mg l^{-1} copper sulfate at varying calcium hardness concentrations with total alkalinity held constant at 20 mg l^{-1} $CaCO_3$

Hardness (mg l^{-1})	42-h pH	42-h NH_3–N (mg l^{-1})	DO		Survival[a] (%)
			2-h (mg l^{-1})	18-h (mg l^{-1})	
10[b]	7.0	2.9	5.5	5.8	10[w]
20	6.8	3.2	5.3	5.7	32[w]
50	6.8	3.7	5.6	6.1	71[x]
200	6.8	3.9	5.1	5.1	93[x,y]
400[b]	6.9	3.9	5.7	5.6	95[y]
400[c] (control)	6.7	4.0	5.3	4.8	100[y]

[a] Values followed by the same superscript were not significantly different at the 0.05 level.
[b] Means for survival, pH and NH_3–N within these rows were based on three values rather than four because fish either jumped from or aeration was disrupted in one tank after 18-h.
[c] The control was not exposed to copper sulfate.

$CaCO_3$ (Table 1). Survival was 100% in the control. Mean survivals (93 and 95%) at 200 and 400 mg l calcium hardness were not significantly different from one another or from 100% survival in the control. The data indicate a calcium hardness between 50 and 200 mg l^{-1} would reduce toxicity and mortality for juvenile channel catfish exposed to a copper sulfate concentration of 1.25 mg l^{-1}, where total alkalinity is 20 mg l^{-1} $CaCO_3$.

In trial 2, there was 100% mortality in both treatments containing magnesium-based hardness, 20 and 400 mg l^{-1} $CaCO_3$. Survivals were 48 and 100% in 20 and 400 mg l^{-1} calcium hardness treatments, respectively, and were consistent with those in trial 1 (Tables 1 and 2).

These data suggest a calcium-specific mechanism with respect to acute copper toxicity in juvenile channel catfish. There is convincing evidence to suggest that copper

Table 2
Mean 48-h survivals and water quality data for juvenile channel catfish exposed to 1.25 mg l^{-1} copper sulfate at varying calcium or magnesium hardness concentrations with total alkalinity held constant at 20 mg l^{-1} $CaCO_3$

Hardness (mg l^{-1})	pH	DO		Survival[a] (%)
		2-h (mg l^{-1})	18-h (mg l^{-1})	
Calcium				
20[b]	6.5	6.3	5.0	48[x]
400	6.7	6.4	5.3	100[w]
Magnesium				
20	6.7	6.4	5.7	0[y]
400	6.8	6.5	6.6	0[y]

[a] Values followed by the same superscript were not significantly different at the 0.05 level.
[b] Mean survival within this row was based on three values rather than four because aeration was disrupted after 18-h in one tank.

disrupts ion homeostasis (Lewis and Lewis, 1971; Lauren and McDonald, 1986, 1987b; Reid and McDonald, 1988) and that environmental calcium directly affects osmoregulation in teleosts (Potts and Fleming, 1971; Bournancin et al., 1972; Flemming et al., 1974; Eddy, 1975; Evans, 1975; Isaia and Masoni, 1976; McWilliams and Potts, 1978; Pic and Maetz, 1981). Indeed, it seems plausible that copper competitively inhibits calcium binding sites, such as those associated with calcium-activated channels for monovalent ions (Perez et al., 1994; Vambutas et al., 1994; Levitan and Rogowski, 1996). Inhibition or suppression of osmoregulatory mechanisms would result in critical losses of serum electrolytes; which in turn could cause tetany, cardiovascular failure and death. As observed in this study, a high ratio of the concentrations of calcium to copper ions would minimize the toxic effects of copper (by reducing or preventing competitive inhibition).

The present research substantiates reports that indicate calcium hardness affects copper toxicity in teleosts. Calcium hardness significantly affected survival of juvenile channel catfish exposed to a toxic concentration of copper sulfate in low alkalinity water. But, magnesium hardness provided no protection from copper toxicity. This study emphasizes the importance of measuring calcium hardness before using copper sulfate in waters with low alkalinity concentrations.

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