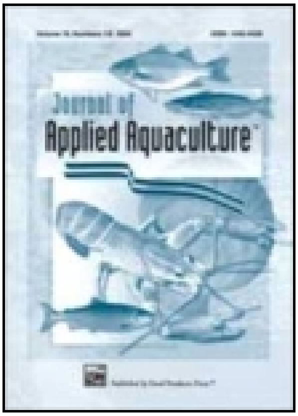

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Acute Toxicity of Copper to Juvenile Freshwater Prawns, Macrobrachium rosenbergii

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Acute Toxicity of Copper to Juvenile Freshwater Prawns, *Macrobrachium rosenbergii*

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ABSTRACT. Copper sulfate is an algicide that is commonly used for phytoplankton and filamentous algae control and has been used as a therapeutant in aquaculture. The objectives of this study were to determine the acute toxicity of copper sulfate and the safe level for use in freshwater prawn, *Macrobrachium rosenbergii*, production ponds in a high calcium and alkalinity environment. Six concentrations of copper sulfate (0, 0.2, 0.4, 0.6, 0.8, 1.0 mg/L) were tested in 8-L glass aquaria for 48 hours with three replicate aquaria per treatment. Concentrations of calcium hardness and alkalinity were set at 100 mg/L using calcium chloride and sodium bicarbonate, respectively. After 48 hours, survival of the control treatment (0% $CuSO_4$) averaged 97%, which was significantly higher ($P < 0.05$) than that of all other treatments. The survival in the 0.2 mg/L and 0.4 mg/L (70% and 73%, respectively) concentrations of $CuSO_4$ were significantly greater ($P < 0.05$) than higher dose treatments; but were not significantly different from each other ($P > 0.05$). Treatments containing 0.6, 0.8, and 1.0-mg/L copper sulfate demonstrated a dramatic decrease in prawn survival, which averaged 30, 7, and 0%, respectively. Regression analysis of the data predicted 48-hour LC_{50} for copper sulfate to be 0.46 mg/L. Since recommended application rates for use of copper sulfate as an algicide are 1.0 mg/L or more for

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water with alkalinities of 100 mg/L, copper sulfate treatments are not recommended for prawn production ponds.

KEYWORDS. Freshwater prawn, *Macrobrachium rosenbergii*, toxicity, copper

INTRODUCTION

Copper sulfate is often used as an algicide in commercial and recreational fishponds to control growth of filamentous algae, inhibit phytoplankton growth, control certain fish diseases, and control organisms responsible for off-flavor in fish and shrimp (Boyd 1990; Tucker and Robinson 1990). Boyd (1990) stated that the concentrations of copper sulfate used for phytoplankton control are seldom directly toxic to fish, but do kill large numbers of invertebrate food organisms, such as rotifers, cladocerans, and copepods. Therefore, cultured crustaceans would presumably be more sensitive to copper based treatments than most finfish species.

As a result, treatment recommendations for the use of copper sulfate in crustacean culture are generally lower (0.1-0.5 mg/L) than for most finfish (0.5-1.0 mg/L) (Lightner 1983; Boyd 1990). Therefore, caution in the use of copper sulfate in production ponds of freshwater prawns, *Macrobrachium rosenbergii*, is recommended (Boyd and Zimmerman 2000). In pond production of the freshwater prawn, grow-out ponds are managed to provide high natural productivity. As a result, a relatively high abundance of phytoplankton, and blue-green algae often occurs, often producing high pH (> 9.5) that can lead to prawn mortality. In addition, poor management of ponds can result in the proliferation of filamentous algae and aquatic macrophyte growth, producing problems with feed distribution and harvest. Determination of recommendations for the safe use of copper sulfate in prawn grow-out ponds would assist in the establishment of management recommendations.

Copper toxicity is known to be regulated by alkalinity, hardness, and pH of water (Masuda and Boyd 1993). Copper sulfate has also been shown to be more toxic to algae in soft, acidic water rather than alkaline water, suggesting that the cupric ion is the toxic agent. Therefore, recommendations for safe use of copper sulfate have been based on hardness (Sawyer et al. 1989; Perschbacher and Wurts 1998), total alkalinity concentrations of the water (Boyd 1990, Reardon and Harrell 1990, Wurts and Perschbacher 1994), and pH (Masuda and Boyd 1993). Recommended application rates in aquaculture ponds vary from 0.025 to 2 mg/L, depending on water pH and alkalinity (Boyd 1990). The recommended application rate of copper sulfate as an algicide or therapeutant in ponds with total alkalinity and hardness equal to or greater than 100 mg/L is 1.0 mg/L or more (Huner and Dupree 1984).

Little research has been conducted to determine safe levels for use of copper sulfate or other herbicides in prawn ponds. A study performed by Liao and Guo (1990) with juvenile freshwater prawn indicated that copper sulfate is toxic above 0.39 mg/L. However, Natarajan et al. (1992) reported a 96-h LC_{50} of 0.012 mg/L in water having a total alkalinity of 200 mg/L, and suggested 0.00012 mg/L as a "safe level." Given the great differences in reported rates relative to prawns, and the complexity of water quality parameters that may affect copper sulfate toxicity, examination of the relative toxicity relative to regional water quality parameters is recommended before pond management recommendations are established for farmers.

The objective of the present study was to determine the acute toxicity of copper to juvenile freshwater prawns. The study was conducted in water with a calcium content and alkalinity that is characteristic of production ponds in the region.

MATERIALS AND METHODS

A preliminary bioassay/range finding study was conducted to evaluate the mortality response of juvenile prawns to different copper concentrations and the suitability of the test system. These experiments were not intended as an examination of species' resistance to copper or to establish specific recommendations for copper use.

Using the preliminary data, a dose-response experiment was conducted to evaluate the mortality of prawns exposed to different concentrations of $CuSO_4$ for a 48-hour period. To simulate water quality conditions prevalent in central Kentucky, a stock solution for all test units was prepared to contain 100 mg/L calcium hardness and 100 mg/L bicarbonate alkalinity using distilled water, calcium chloride (Aquatic Eco-System, Inc., Apopka, Florida[1]), and sodium bicarbonate (baking

1. Use of trade or manufacturer's name does not imply endorsement.

soda). Reagent grade potassium chloride was also added to the test water at a concentration of 5 mg/L as recommended by the EPA (1975) to provide the necessary ion concentration for survival (Perschbacher and Wurts 1998).

The experimental prawns were not fed 24 hours prior to stocking and were maintained in a holding tank with water at the same temperature, pH, alkalinity, and calcium concentrations. One hundred prawns were individually weighed to the nearest 0.01 g. The weights of the prawns ranged from 0.2-0.7 g with a mean (±standard deviation) of 0.32±0.16 g. Prior to stocking aquaria, a water quality test was conducted on the stock water solution to determine baseline values of temperature, pH, dissolved oxygen, nitrite, ammonia, alkalinity, and hardness to ensure that the test water was suitable for juvenile freshwater prawns. After 48 hours, all water quality parameters were again measured for each aquarium within a 30-minute period.

Six concentrations of copper sulfate were tested in increments of 0.2 mg/L from 0.0 (control) to 1.0 mg/L. Treatment 1 (control) contained 0 mg/L of copper sulfate. Treatments 2-6 contained 0.2, 0.4, 0.6, 0.8, and 1.0 mg/L of copper sulfate, respectively. The treatments were replicated in three, aerated, 8-L glass aquaria. Test concentrations were produced through the addition of the appropriate volume of a stock solution containing 0.6 g of $CuSO_4$/L of distilled water. The mixture was slightly heated and was stirred continuously to dissolve the $CuSO_4$.

Each aquarium was randomly assigned a treatment concentration. Before the stocking of experimental prawns, a 1-mL syringe was filled with the proper volume of copper sulfate stock solution and then added to each aquarium according to the assigned treatment. After thirty minutes, each aquarium was stocked with 10 juvenile prawn/aquarium. Prawns were observed and mortalities removed every 12 hours. Cumulative mortalities were recorded at 24 and 48 hours post-treatment.

Analysis of variance using ANOVA was performed on mortality data. Data were arc sine transformed prior to analysis and are presented untransformed to facilitate interpretation. Significance was tested at the $P = 0.05$ level. Regression analysis was used to estimate the lethal concentration of copper at 50% mortality at 48 hours post-treatment.

RESULT AND DISCUSSION

There were no significant differences ($P > 0.05$) in water quality variables among treatments. Overall means (±S.D.) for water quality variables for the duration of the study were: combined morning/afternoon temperature 25.7±0.5°C; combined morning/afternoon dissolved oxygen, 6.7±0.2 mg/L; combined morning/afternoon pH 7.6±0.1; total ammonia-nitrogen, 1.5±0.1 mg/L; and nitrite-nitrogen 0.036±0.001 mg/L. These values are within the acceptable range for prawn growth and survival (Boyd and Zimmerman 2000).

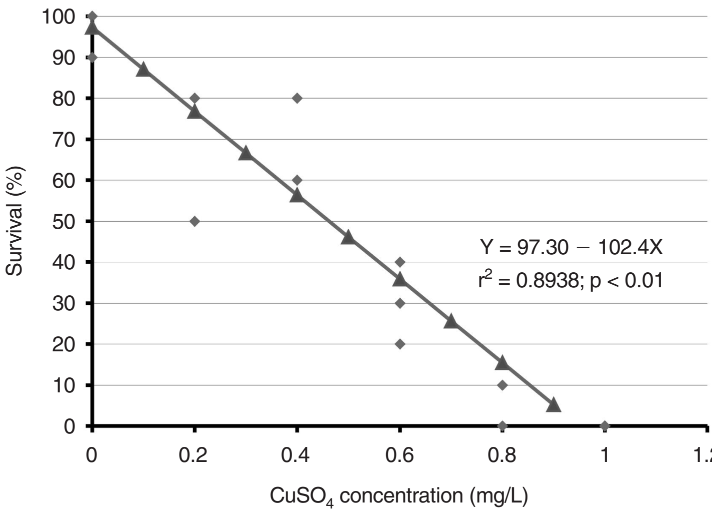
After 48 hours, mean survival of prawns in control tanks (0% $CuSO_4$) was 97% and significantly higher ($P < 0.05$) than that of prawns in all other treatments (Table 1). At 24 and 48 hours, survival of prawns exposed to 0.2 mg/L and 0.4 mg/L concentrations of copper sulfate were significantly greater ($P < 0.05$) than for prawns exposed to higher doses, but were not significantly different from each other ($P > 0.05$). Survival of prawns exposed to 0.6, 0.8, and 1.0 mg/L of copper sulfate was significantly lower ($P < 0.05$), averaging 53, 20, and 10%, respectively after 24 hours; and 30, 7 and 0%, respectively, after 48 hours (Table 1). A regression analysis of prawn survival (%) on $CuSO_4$ concentration was highly significant ($P < 0.01$; $r^2 = 0.89$ $r^2 = 0.89$; Figure 1). Using the resulting regression equation, the 48-hour LC50 for copper sulfate was calculated to be 0.46 mg/L, which is equivalent to 0.18 mg/L copper.

Freshwater prawns appear to be more sensitive to copper than most other species of crustaceans that have been studied. The majority of published work has been conducted with marine species that appear to have greater tolerance in more saline environments. The 96-hour LC_{50} of copper increased for juvenile kuruma shrimp, *Penaeus japonicus*, as

TABLE 1. Percent survival (mean±S.D.) of juvenile freshwater prawns, *Macrobrachium rosenbergii*, exposed to copper sulfate at different concentrations for 24 and 48 hours post-treatment. Values within a column followed by different letters are significantly different ($P < 0.05$).

Copper (mg/L) as $CuSO_4$	% Survival 24 hours	% Survival 48 hours
0	96.7±5a	96.7±5.8a
0.08	76.7±15.3a	70.0±17.3b
0.16	80.0±10a	73.3±11.5b
0.24	53.3±11.5b	30.0±10.0c
0.32	20.0±20c	6.7±5.8d
0.40	10.0±10c	0.0±0.0d

FIGURE 1. The relationship of survival of juvenile freshwater prawns to different concentrations of Cu^{++}.



salinity increased from 1.20 to 2.05 mg/L at 17 ppt and 37 ppt, respectively (Bambang et al. 1995). The tolerance of tiger shrimp, *P. monodon*, juveniles also increased as salinity increased, with 96-hour LC_{50} of 3.13 and 7.73 mg/L at 15 ppt and 25 ppt, respectively (Chen and Lin 2001). In this study, the 48 hour LC_{50} for juvenile freshwater prawns was determined to be 0.18 mg/L copper in freshwater. However, prawn of the family Palaemonidae, are indigenous to tropical fresh and brackish waters of the Indo-Pacific region and therefore inhabit a wide range of salinities from 0 ppt to 18 ppt during its life cycle. The tolerance of prawn to copper may also increase as salinity increases.

The toxicity of copper to crustaceans is also affected by temperature and size. Ronny et al. (1994) demonstrated that copper toxicity in brine shrimp, *Artemia franciscana*, increased as temperatures increased. The authors stated that copper uptake is a facilitated diffusion process that increases as temperature increases and transport of the free cupric ion across the solution/body interface increases. The tolerance of kuruma shrimp to copper sulfate was shown to increase with developmental stage and size (Bambang et al. 1995). As prawns grow and mature they may become more tolerant to copper. Future research should be devoted to the evaluation of the effect of salinity, temperature, and developmental stage on copper toxicity in freshwater prawn.

Copper toxicity for fish is primarily related to structural damage to the gills (Wilson and Taylor 1993) but in crustaceans the physiological effects of copper toxicity are not as clearly understood. MacFarlane et al. (2000) reported that copper was accumulated and regulated in the hepatopancreas of the Semaphore crab, *Heloecius cordiformis*. In the shore crab, *Carcinus maenas*, the toxicity of copper was reduced when the hemolymph protein in the hepatopancreas, particularly hemocyanin, was reduced (Rtal et al. 1996). Hemocyanin concentrations in the hemolymph of crustaceans have been reported to vary according to nutritional state (Hagerman 1983), the molt cycle (Chen and Cheng 1993), and size (Cheng et al. 2001). Gunter and Quinitio (1994) demonstrated the ability of white shrimp to detoxify copper by granule formation in the hepatopancreas tubules and excretion through the feces. The levels of hemocyanin found in prawns compared to these marine crustaceans are not known. Cheng et al. (2001) indicated that hymolymph protein and hemocyanin levels were lower during the post-molt than during the pre-molt stage in prawns due to water and Ca^{+2} uptake during the molt. Crustaceans that have recently molted may be more sensitive to copper due to changes in hemolymph osmolality.

According to Huner and Dupree (1984), copper sulfate concentrations of 1.0 mg/L or more are needed to kill most algae in water with alkalinities higher than 100 mg/L. In the current study, the 1.0 mg/L copper sulfate treatment in water with alkalinity of 100 mg/L resulted in 100% prawn mortality. According to these data, the maximum "safe" concentration of copper sulfate for use in ponds containing juvenile prawns is 0.03 mg/L. Copper sulfate is not a suitable compound for use as an algicide in prawn-production ponds. Copper sulfate is also commonly used to control species of blue-green algae that are responsible for off-flavor in fish and marine shrimp (Chen and Lin 2001). Boyd (1990) reported that copper sulfate is effective at a rate of 0.084 mg/L for use in controlling blooms of *Microcystis* and other blue-green algae responsible for "off-flavor" in ponds. Toxicity of copper sulfate on advanced juvenile sizes and adult freshwater prawns needs to be determined so that the potential for using copper sulfate for controlling blue green algae in ponds can be established.

Knowledge of the acute toxicity of copper to prawns will be helpful relative to water management. Based on these data, the addition of copper through the use of copper sulfate in prawn ponds should be avoided. Caution should be exercised regarding: water source contamination, trophic transfer of contaminants, exposure to chromated-copper-arsenate (CAA) treated wood, and the use of copper heating coils in recycle systems. Additional research is needed to evaluate the singular and interactive effect of salinity, temperature, developmental stage, and the molt cycle on copper toxicity in freshwater prawns.

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