ELSEVIER

Aquaculture 125 (1994) 73-79

Aquaculture

Effects of bicarbonate alkalinity and calcium on the acute toxicity of copper to juvenile channel catfish (Ictalurus punctatus)

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

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

[b] *University of Arkansas at Pine Bluff, Agriculture Experiment Station Center, Box 40005, Pine Bluff, AR 71601, USA*

Accepted 1 March 1994

Abstract

Three experiments were conducted to evaluate the relative importance of calcium hardness and bicarbonate alkalinity to the acute response of juvenile channel catfish (*Ictalurus punctatus*) exposed to a toxic concentration of copper sulfate. A preliminary bioassay revealed 28 $mg \cdot l^{-1}$ copper sulfate caused 50% mortality within 48 h (48-h LC_{50}) in juvenile channel catfish placed in water with calcium hardness and bicarbonate alkalinity, set at 75 $mg \cdot l^{-1}$ $CaC0_3$. Catfish were then exposed to 28 $mg \cdot l^{-1}$ copper sulfate concentrations in environments where hardness or alkalinity concentrations were varied. Bicarbonate alkalinities above 75 $mg \cdot l^{-1}$ $CaC0_3$, with calcium hardness held at 20 $mg \cdot l^{-1}$ $CaC0_3$, significantly reduced catfish mortalities from 97-100% to 63-70%. Copper-induced mortalities were 100% for all fish placed in calcium hardness treatments (20-250 $mg \cdot l^{-1}$ $CaC0_3$) in which bicarbonate alkalinity was held at 20 $mg \cdot l^{-1}$ $CaC0_3$. When bicarbonate alkalinity was held constant at 75 $mg \cdot l^{-1}$ $CaC0_3$ and calcium hardness was varied from 20 to 250 $mg \cdot l^{-1}$ $CaC0_3$, copper related catfish mortalities displayed high variability and means ranged from 6.7 to 60%. Mortalities decreased as calcium concentrations increased. Although differences in mortalities were not statistically significant, the latter hardness findings appear to suggest a biologically significant calcium effect on copper toxicity in the presence of sufficient alkalinity concentrations.

1. Introduction

Copper sulfate has been used to control protozoan diseases in fish and is used extensively in ponds as an algicide. However, above a specific concentration, copper is toxic to fish including such cultured species as salmonids, cyprinids and catfish. Recommendations for

*Corresponding author.

SSDI 0044-8486(94)00101-S

safe use have been based on water hardness (Inglis and Davis, 1972; Sawyer et al., 1989) and, more recently, on alkalinity (Post, 1983; MacMillan, 1985; Reardon and Harrell 1990; Wurts,1990).

Alkalinity is generally recognized as influencing copper toxicity to fish through the formation of less toxic copper-base complexes (Stiff, 1971; Pagenkopf et al., 1974). High concentrations of calcium, a major component of hardness, are also thought to limit copper toxicity by protecting the ion-regulating mechanisms at the gills from the disruptive effects of copper (Pagenkopf, 1983). Experiments evaluating the effects of hardness and alkalinity on copper toxicity have been conducted with salmonids in soft water. Studies with rainbow trout *(Salmo gairdneri)* demonstrated that a threshold level of calcium hardness must be present before alkalinity will influence copper toxicity (Miller and Mackay, 1980).

Information about the relative effects of bicarbonate alkalinity and calcium hardness on copper toxicity to channel catfish *(Ictalurus punctatus)* is inconclusive. The purpose of our research was to determine whether alkalinity, calcium concentration or an interrelationship of the two had the most pronounced effects on the acute toxicity of copper sulfate to channel catfish fingerlings.

2. Methods

A preliminary bioassay revealed that 28 $mg \bullet l^{-1}$ copper sulfate was required to effect 50% mortality in 3-6 g juvenile channel catfish within 48 h (48-h LC_{50}) in water with predetermined concentrations of bicarbonate alkalinity and calcium hardness. Increments of copper concentrations used to determine this 48-h LC_{50} followed EPA recommendations (US Environmental Protection Agency, 1975). Test water contained 75 $mg \bullet l^{-1}$ calcium hardness and 75 $mg \bullet l^{-1}$ bicarbonate alkalinity. Water temperature was 20°C.

The preliminary bioassay was conducted to facilitate comparisons of the relative importance of calcium hardness versus bicarbonate alkalinity on acute copper toxicity to juvenile channel catfish. These experiments were not intended as an examination of species resistance to copper or to establish specific recommendations for copper use.

Controlled, laboratory experiments were conducted to evaluate the mortality response of juvenile channel catfish exposed to a 28 $mg \bullet l^{-1}$ concentration of copper sulfate in waters with differing concentrations of bicarbonate alkalinity and calcium hardness. Three independent trials were conducted: one which varied alkalinity and two which varied hardness. Each combination of hardness and alkalinity was replicated in three, aerated, 7.6-liter aquaria.

Each aquarium was stocked with 10 juvenile channel catfish ranging in size from 3.0-5.6 g and 55-70 mm TL. Averages were 4.4 ± 0.8 g and 64 ±4.4 mm. Fish were not fed for 48 h before experiments were begun. Catfish were maintained for 24 h preceding each experiment in a holding tank with water at the same temperature and containing the median concentrations of alkalinity and hardness for the subsequent trial. After stocking, juvenile catfish were held in test aquaria environments for 2 h before adding copper sulfate. Water temperature, dissolved oxygen, and pH were measured in all aquaria before the addition of copper and at 24-h intervals. Mortalities were removed regularly and totaled at 18, 24 and 48 h.

In all experiments, calcium hardness and bicarbonate alkalinity were adjusted to desired levels with reagent-grade calcium sulfate dihydrate and food-grade sodium bicarbonate. Copper concentrations for each experiment were added using a 3000 $mg \cdot l^{-1}$, stock solution of reagent-grade cupric sulfate pentahydrate. Reagent grade potassium chloride was added to all experimental treatments to create a concentration of 5 $mg \cdot l^{-1}$ as recommended by EPA guidelines (US Environmental Protection Agency, 1975). Water treatments and stock solutions were made with distilled water.

Copper sulfate concentrations in test environments were verified by measuring free or non-complexed, dissolved cuprous and cupric ions using a bicinchoninate photometric technique (Hach Co., Loveland, CO). Bicarbonate alkalinity and calcium concentrations were measured as $mg \cdot l^{-1}$ calcium carbonate ($mg \cdot l^{-1}$ $CaCO_3$) with bromcresol green-methyl red and EDTA titration techniques, respectively.

Trial 1 involved exposing fish to copper in environments with 5 concentrations of bicarbonate alkalinity ranging from 20 to 250 $mg \cdot l^{-1}$. Calcium hardness was held at 20 $mg \cdot l^{-1}$. This experiment was conducted to determine the relative importance of bicarbonate alkalinity to copper toxicity.

Trial 2 examined the effects of copper on fish in environments with 5 levels of calcium hardness ranging from 20 to 250 $mg \cdot l^{-1}$. Bicarbonate alkalinity was held constant at 20 $mg \cdot l^{-1}$. The objective of this experiment was to ascertain the relative importance of calcium to copper toxicity.

Trial 3 subjected fish to copper in environments with 5 calcium hardness concentrations ranging from 20 to 250 $mg \cdot l^{-1}$. Bicarbonate alkalinity was held constant at 75 $mg \cdot l^{-1}$. The purpose of the experiment was to expand the findings of trial 2.

Analysis of variance and Fischer's LSD (Ott, 1977) were performed on mortality data. Percentile data were transformed using the arc-sine method suggested by Mostellar and Youtz (1961). Significance was tested at the 0.05 level. Regression analyses examined mortality as a function of alkalinity and hardness.

3. Results

Dissolved oxygen concentrations ranged from 6.6 to 8.2 $mg \cdot l^{-1}$ and were above 75% of saturation in each aquarium for all experiments. In all water treatments, pH ranged from 6.95 to 7.8. The water temperature reported for each trial was stable for the duration of that experiment.

In trial 1, there were significant differences among experimental groups with respect to mortality and bicarbonate alkalinity concentrations (Table 1). At 48 h, mortality was significantly higher for fish exposed to 28 $mg \cdot l^{-1}$ copper sulfate in environments with alkalinity concentrations of 75 $mg \cdot l^{-1}$ or less. Water temperature was 24°C.

In trial 2, all experimental groups exhibited 100% mortality at 48 h (Table 2). Calcium hardness concentrations from 20 to 250 $mg \cdot l^{-1}$ did not protect catfish fingerlings from the toxic effects of copper with bicarbonate alkalinity held constant at 20 $mg \cdot l^{-1}$. Water temperature was 21°C.

In trial 3, no significant differences were found among experimental groups with respect to mortality and calcium hardness (20-250 $mg \cdot l^{-1}$) with bicarbonate alkalinity held at 75

Table 1
Mean mortalities of juvenile channel catfish exposed to 28 mg•l^{-1} copper sulfate at varying bicarbonate alkalinity concentrations and a fixed, low concentration of calcium hardness

Alkalinity (mg•l^{-1})	Hardness (mg•l^{-1})	pH	DO (mg•l^{-1})	Mortality[1]		
				18-h	24-h (%)	48-h
20	20	7.15	6.6	100.0^{w}	100.0^{w}	100.0^{w}
50	20	7.15	7.1	100.0^{w}	100.0^{w}	100.0^{w}
75	20	7.22	7.2	90.0^{w}	96.7^{w}	96.7^{w}
125	20	7.48	7.5	36.7^{x}	53.3^{x}	70.0^{x}
250	20	7.81	7.3	16.7^{x}	20.0^{x}	63.3^{x}

[1]Values within a specific time period (18-, 24- or 48-h) and followed by the same superscript were not significantly different at the 0.05 level.

Table 2
Mean mortalities of juvenile channel catfish exposed to 28 mg•l^{-1} copper sulfate at varying calcium hardness concentrations and a fixed, low concentration of bicarbonate alkalinity

Alkalinity (mg•l^{-1})	Hardness (mg•l^{-1})	pH	DO (mg•l^{-1})	Mortality[1]		
				18-h	24-h (%)	48-h
20	20	7.14	7.2	100.0^{w}	100.0^{w}	100.0^{w}
20	50	7.12	7.1	100.0^{w}	100.0^{w}	100.0^{w}
20	75	7.00	7.4	100.0^{w}	100.0^{w}	100.0^{w}
20	125	7.00	7.2	100.0^{w}	100.0^{w}	100.0^{w}
20	250	6.95	6.9	93.3^{w}	100.0^{w}	100.0^{w}

[1]Values within a specific time period (18-, 24- or 48-h) and followed by the same superscript were not significantly different at the 0.05 level.

Table 3
Mean mortalities of juvenile channel catfish exposed to 28 mg•l^{-1} copper sulfate at varying calcium hardness concentrations and a fixed, moderate concentration of bicarbonate alkalinity

Alkalinity (mg•l^{-1})	Hardness (mg•l^{-1})	pH	DO (mg•l^{-1})	Mortality[1]		
				18-h	24-h (%)	48-h
75	20	7.24	8.0	30.0^{w}	53.3^{w}	60.0^{w}
75	50	7.27	8.1	6.7^{w}	16.7^{w}	20.0^{w}
75	75	7.45	8.1	10.0^{w}	10.0^{w}	13.3^{w}
75	125	7.48	8.2	10.0^{w}	17.7^{w}	20.0^{w}
75	250	7.53	7.9	6.7^{w}	6.7^{w}	6.7^{w}

[1]Values within a specific time period (18-, 24- or 48-h) and followed by the same superscript were not significantly different at the 0.05 level.

Table 4
Correlation coefficients comparing 18-, 24-, and 48-h mortality and concentrations of alkalinity (Trial 1) or hardness (Trial 3) for juvenile channel catfish exposed to 28 mg•l^{-1} copper sulfate

Trial	*n*	*r*		
		18-h	24-h	48-h
1. Alkalinity	15	-0.88	-0.92	-0.74
3. Hardness	15	-0.30	-0.44	-0.47

mg•l^{-1} (Table 3). Although percent mortality was highly variable, 48-h mortality was the lowest (mean, 6.7%; range, 0-20%) in environments with the highest hardness (250 mg•l^{-1}). Mortality was the highest (mean, 60%; range, 20-90%) in replicates with the lowest hardness concentrations (20 mg•l^{-1}). Temperature was 16°C.

Correlations between mortality and alkalinity or hardness are presented in Table 4. A significant ($P < 0.01$) linear relationship was demonstrated between increasing alkalinity and decreasing mortality in trial 1 at all time intervals. However, no statistically significant relationship could be established between mortality and calcium concentration.

4. Discussion

The close inverse relationship between alkalinity and mortality observed in trial 1 appeared to have resulted from the increased bases, associated with elevated alkalinity concentrations, which combined with copper to form less toxic compounds (Pagenkopf et al., 1974; Boyd, 1979). While an increase in alkalinity diminishes the toxicity of copper, an increase in water temperature generally exacerbates the toxic effects (Sorensen, 1991). It is likely that the lower temperature (16°C) in Trial 3 contributed to the reduction in mortalities as well as the high variability observed.

Some researchers have noted that a minimum concentration of free calcium was necessary for alkalinity to have an effect on copper or zinc toxicity. For example, Jones (1938) found that a calcium hardness of 25 mg•l^{-1} was necessary to increase survival of sticklebacks (*Gasterosteus aculeatus*) exposed to toxic levels of zinc. Miller and Mackay (1980) observed the incipient LC_{50} (ILC_{50}) of copper for juvenile rainbow trout (*Salmo gairdneri*) did not change when alkalinity concentrations were increased from 10 to 50 mg•l^{-1} and hardness was held at 12 mg•l^{-1}. However, the ILC_{50} increased 3-fold when hardness was increased from 12 to 100 mg•l^{-1} and alkalinity was held at 10 mg•l^{-1}.

In trial 3, mortality decreased as calcium hardness levels increased from 20 to 250 mg•l^{-1}, when bicarbonate alkalinity was held at 75 mg•l^{-1} (Table 1). The statistical analysis for trial 2 and the analysis for trial 3 did not detect significant differences among mean mortalities for either individual experiment. However, a relative comparison of mean, 48-h mortalities between the two studies (100 vs. 6.7-60.0%, respectively) suggests calcium was biologically important when sufficient alkalinity (20 vs. 75 mg•l^{-1}, respectively) was present. Miller and Mackay (1980) reported the ILC_{50} copper concentration for juvenile rainbow trout was significantly greater at 100 mg/l hardness when alkalinity was increased

from 10 to 50 mg•l^{-1}. The present findings suggest that a calcium hardness between 20 and 250 mg•l^{-1} may minimize mortality in juvenile channel catfish exposed to toxic concentrations of copper at specific alkalinity concentrations. These observations with channel catfish are consistent with those for trout.

Juvenile channel catfish were remarkably resistant to a 48-h exposure to copper, requiring a concentration of 28 mg•l^{-1} copper sulfate to effect a LC_{50} at 20°C (hardness and alkalinity, 75 mg•l^{-1}). Miller and Mackay (1980) calculated the ILC_{50} at 13°C for juvenile rainbow trout to be 80 µg•l^{-1} copper at 50 mg•l^{-1} alkalinity and 75 mg•l^{-1} hardness—a considerably lower copper concentration. However, it would be inappropriate to make direct comparisons between these findings with channel catfish and those for juvenile trout. Experimental designs were dissimilar (acute vs. chronic exposure) and there appear to be species-related differences in sensitivity to copper.

While alkalinity reduces toxicity by combining chemically with copper, high calcium concentrations apparently block or minimize the effects of copper at the sites of toxic action. Copper, a divalent cation, would have chemical activity and ionic form similar to the calcium ion (and possibly magnesium). It has been theorized that calcium-activated proteins control the passive and energy-dependent processes regulating ion metabolism at the gills (Evans, 1975; Wurts and Stickney, 1989). Pic and Maetz (1981) observed that an environmental calcium concentration of 40 mg•l^{-1} was necessary to sustain maximum function of the mechanisms associated with the energy-dependent exchange of sodium and potassium ions in mullet *(Mugil capito).* It is likely that copper competes directly with calcium for the same binding sites on ion regulating proteins. Therefore, high concentrations of calcium (i.e., a high ratio of calcium to copper ions) would keep binding sites maximally saturated, preventing copper from attaching and interfering with normal protein functions (i.e., ion metabolism). Sorensen (1991) indicated that both calcium and magnesium may confer similar protection. Further research in this area is warranted.

The present study supports recommendations and research which indicate that alkalinity is the primary factor affecting acute copper toxicity in aquatic environments. A minimum calcium hardness concentration between 20 and 250 mg/1 may be important to the maintenance of normal ion metabolism in juvenile channel catfish exposed to toxic concentrations of copper. These experiments emphasize the importance of controlling alkalinity concentration, as an independent variable, before attempting to evaluate a calcium effect on acute copper toxicity to fish. The acceptance of alkalinity-based recommendations (Wellborn, 1985; MacMillan, 1985) for the use of copper in fish ponds seems prudent.

References

Boyd, C.E., 1979. Water quality in warmwater fish ponds. Auburn University Agricultural Experiment Station, pp. 44-47.

Evans, D.H., 1975. Ionic exchange mechanisms in fish gills. Comp. Biochem. Physiol, 51A: 491-495.

Inglis, A. and Davis, E.L., 1972. Effects of water hardness on the toxicity of several organic and inorganic herbicides to fish. U.S. Fish and Wildlife Service Technical Paper 67.

Jones, J.R.E., 1938. The relative toxicity of salts of lead, zinc and copper to the stickleback *(Gasterosteus aculeatus* L.) and the effect of calcium on the toxicity of lead and zinc salts. J. Exp. Biol, 4: 394-407.

MacMillan, J.R., 1985. Infectious diseases. In: C.S. Tucker (Editor), Channel Catfish Culture. Elsevier, Amsterdam, pp. 405-496.

Miller, T.G. and Mackay, W.C., 1980. The effects of hardness, alkalinity and pH of test water on the toxicity of copper to rainbow trout (*Salmo gairdneri*). Water Res. 14: 129-133.

Mostellar, F. and Youtz, C., 1961. Tables of the Freeman-Tukey transformations for the binomial and Poisson distributions. Biometrika,48: 433-440.

Ott, L. 1977. An introduction to statistical methods and data analysis. Wadsworth Publishing Company, Inc., Belmont, CA, 730 pp.

Pagenkopf, G.K., 1983. Gill surface interaction model for trace metal toxicity to fishes: role of complexation, pH and water hardness. Environ. Sci. Technol., 17: 342-347.

Pagenkopf, G.K., Russo, R.C. and Thurston, R.V., 1974. Effect of complexation on toxicity of copper to fishes. J. Fish. Res. Board Can., 31: 462-465.

Pic, P. and Maetz, J., 1981. Role of external calcium in sodium and chloride transport in the gills of seawater-adapted *Mugil capito*. J. Comp. Physiol., 141: 511-521.

Post, G.W., 1983. Textbook of Fish Health. T.F.H. Publications, Neptune City, NJ, p. 241

Reardon, I.S. and Harrell, R.M., 1990. Acute toxicity of formalin and copper sulfate to striped bass fingerlings held in varying salinities. Aquaculture, 87: 255-270.

Sawyer, M.D.J., Reader, J.P. and Morris, R., 1989. The effect of calcium concentration on the toxicity of copper, lead and zinc to yolk-sac fry of brown trout, *Salmo trutta* L., in soft, acid water. J. Fish Biol., 35: 323-332.

Sorensen, E.M., 1991. Metal Poisoning in Fish. CRC Press, Boca Raton, FL, pp. 235-283.

Stiff, M.J., 1971. Copper/bicarbonate equilibrium in solution of bicarbonate ion at concentrations similar to those found in natural water. Water Res. 5: 171-176.

US Environmental Protection Agency, 1975. Methods for acute toxicity tests with fish, macroinvertebrates, and amphibians. EPA-660/3-75-009, Nat. Tech. Info. Ser., Washington, DC.

Wellborn, T., 1985. Control and therapy. South. Coop. Ser. Bull., 225: 50-67.

Wurts, W.A. and Stickney R.R., 1989. Responses of red drum (*Sciaenops ocellatus*) to calcium and magnesium concentrations in fresh and salt water. Aquaculture, 76: 21-35.

Wurts, W.A. 1990. Update: use of bluestone (copper sulfate) for algae control. Kentucky State University Cooperative Extension Program, WKY-193.