CHAPTER 5

Toxicity of Copper Sulfate Pentahydrate to Rainbow Trout *Oncorhynchus mykiss*

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Abstract

The effect of copper sulfate on survival of 230- to 250-mm rainbow trout *Oncorhynchus mykiss* was investigated in indoor tanks for seven days. Rainbow trout were exposed to three copper concentrations (0.5, 1.0 and 2.0 mg Cu/L) and a control (0 mg Cu/L). In a 3 X 4 study design (replicates X treatments), 12 2,271-L circular tanks were stocked at five fish per tank. Mean survival of rainbow trout in copper sulfate treated tanks was 74% for 2 mg/L, 86% for 1 mg/L, 94% for 0.5mg /L treatments, and 100% for the untreated controls. The differences in survival were not significant among treatment and control groups. Survival declined progressively with increasing copper concentration, and the onset of fish mortality was sooner for the highest copper concentration treatment and later for the lowest (0.5 mg Cu/L) treatment. No mortality occurred in the control tanks. Although the differences in survival were not statistically significant, the observed differences could be biologically or economically important. Because of the small sample size, the effect of the copper concentrations on survival of rainbow trout was unclear.

Introduction

In February 2001, the presence of the toxic alga *Prymnesium parvum* was confirmed in ponds at the Dundee State Fish Hatchery (DSFH), located in Archer County, Texas. Among the species adversely affected was rainbow trout *Oncorhynchus mykiss*, with approximately 7,000 (mean length = 229 mm) lost to the toxic alga. Methods used to control *P. parvum* in Israeli fish farm ponds involve the use of ammonium sulfate (Shilo and Shilo 1953) or copper sulfate (Sarig 1971). Both chemicals were used successfully to control *P. parvum* at the DSFH during summer 2001. Cold water temperatures (8-10 ^{o}C) typically encountered when holding trout at the DSFH limit treatment options to copper sulfate(Sarig 1971), but rainbow trout is known to be sensitive to copper toxicity (Anderson and Mayer 1993).

Because the toxicity of copper is influenced by water quality, especially alkalinity (Boyd 1990), we suspected that the high alkalinity of the DSFH water might reduce copper toxicity to rainbow trout. The characteristics of the DSFH water are: total hardness, 959 mg/L as $CaCO_3$; calcium hardness, 255 mg/L as $CaCO_3$; and total alkalinity, 88 mg/L as $CaCO_3$. The objective of this study was to compare the effects of three concentrations of copper (0.5, 1.0, and 2.0 mg Cu/L) on the survival of rainbow trout in the DSFH water.

Materials and Methods

This study was conducted indoors using 12 2,271-L circular fiberglass tanks. Each tank was filled with 1,279 L water from Lake Diversion, the hatchery water source, which was passed through both a rapid sand filter and an ultraviolet (UV) filter. The treated water was examined for the presence of *P. parvum* using a hemacytometer, then tested for ichthyotoxicity using established protocols (Appendices A and B). Calcium carbonate alkalinity was determined with a HACH Model AL-DT Alkalinity Test Kit. Aeration in each tank was provided by compressed air and tanks were covered with netting to prevent fish escapement. On 15 January 2002, the day following filling, tanks were randomly assigned to treatments. Treatments were 0.5 mg Cu/L, 1.0 mg Cu/L and 2.0 mg Cu/L as copper sulfate pentahydrate (100 % active ingredient) or untreated (controls). Five rainbow trout were placed into each tank. Temperature, dissolved oxygen, and pH were measured daily in each tank. Copper analysis from water samples taken from the tanks was performed using the bathocuproine method (Appendix D) at 4 and 24 hours post-treatment. The 4-hour samples were preserved with HCl (APHA 1995) and analyzed at the same time the unpreserved 24-hour samples were analyzed. Dead fish were removed from each tank and the numbers recorded daily. The study lasted 7 days. The water quality and fish mortality (or survival) data were analyzed by analysis of variance with differences among treatment and control groups considered significant at $P \leq 0.05$.

Results and Discussion

The water used for the study was determined to be free of *P. parvum* and ichthyotoxin. No cells were found when the water was examined microscopically, and toxin was not detected with the bioassay. Furthermore, water quality did not differ significantly among treatment and control groups (Table 1).

The concentrations of free copper in the 24-h water samples were slightly higher than those in the 4-h samples for all treatments (Table 2), although one would expect to find less free copper in the 24-hour samples. Apparently, it is important to analyze for copper in water samples as soon as possible after collection since copper can be adsorbed onto sample containers (APHA 1995) or form complexes (Boyd 1990). The preservation of the 4-hour water samples with hydrochloric acid was intended to prevent copper adsorption; however, some adsorption appears to have occurred even with the addition of the acid. Whether the added acid was insufficient to stabilize all of the free copper was unclear. The concentrations of copper in the control ponds were 0.043-0.053 mg Cu/L, reflecting the ambient concentrations of copper in the lake water used for the study. This observation suggests that the ambient copper concentration of water should be considered in the calculation of copper treatments to better determine actual concentrations of copper exposed to fish.

The concentrations of copper added to the water declined very rapidly to mean cumulative percent declines of 69.5-74.3 after 4 h and 64.9-71.2 after 24 h. Essentially, the lowest concentrations were achieved after the first 4 h. This agrees with other reports (e.g., McIntosh 1975; Button et al. 1977) that most of the soluble copper applied to pond water disappears quickly.

The survival of rainbow trout ranged from 74 to 100% and did not significantly differ among treatments and the control, perhaps due to small sample size. Whereas none of the fish died in the control tanks, there was progressively higher mortality among the treatment groups as copper concentration increased. Additionally, the onset of mortality was sooner at higher copper concentrations. Although the differences in survival were not statistically significant, these differences could be biologically or economically important. A 74% survival over a 7-day period could be unacceptable, whereas a 94% survival might be acceptable. Further research should be done with higher sample sizes before conclusions can be drawn about the effect of copper on the survival of trout in the DSFH water.

TABLE 1.—Mean (±SD) values of dissolved oxygen, temperature, pH, and copper concentrations measured at 4- and 24-hr post-treatment (percent declines in parentheses) in study tanks and survival of rainbow trout exposed to copper sulfate treatments for seven days.

Variable	Copper sulfate treatment (mg Cu/L)			
	0	0.5	1.0	2.0
Dissolved oxygen (mg/L)	10.14 ± 0.29	10.19 ± 0.35	10.20 ± 0.33	10.14 ± 0.32
Temperature (ºC)	11.82 ± 0.45	11.60 ± 0.44	11.68 ± 0.45	11.77 ± 0.47
pH	7.99 ± 0.04	7.99 ± 0.05	8.00 ± 0.04	8.01 ± 0.04
24-h copper (mg/L)	0.043 ± 0.014	0.152 ± 0.006 (69.5 ± 1.2)	0.274 ± 0.033 (72.6 ± 3.3)	0.514 ± 0.029 (74.3 ± 1.4)
24-h copper (mg/L)	0.053 ± 0.016	0.175 ± 0.021 (64.9 ± 4.1)	0.327 ± 0.037 (67.3 ± 3.7)	0.576 ± 0.046 (71.2 ± 2.3)
Survival	100	93.3 ± 11.5	86.7 ± 11.5	73.3 ± 11.5