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Role of Waterborne Copper on Survival, Growth and Feed Intake of Indian Major Carp, *Cirrhinus mrigala* Hamilton

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Abstract The effect of copper on survival, growth and feed intake of Indian major carp, *Cirrhinus mrigala* (Hamilton) fry (0.92 ± 0.28 g) was studied for 60 days. Survival rates of the fish exposed to control (0.02), 0.10, 0.15 and 0.23 mg L^{-1} copper were 100%, 83 ± 3%, 58 ± 6%, and 50 ± 4%, respectively. Average daily growth was significantly ($p < 0.05$) lower at 0.15 and 0.23 mg L^{-1} of copper. There was almost no growth at 0.23 mg L^{-1} of copper. Feed intake rates reduced significantly ($p < 0.05$) at all the copper treatments. The copper accumulation in the fish increased with increasing concentrations of the metal.

Keywords Copper · Growth · Feed intake · *Cirrhinus mrigala*

Copper has been used for many years as an important chemical in fresh water farm ponds and aquaculture operations. It is used as a prophylactic agent for a variety of fish diseases and parasites. It is a ubiquitous trace metal that is required by aquatic organisms (Ogino and Yang 1980; Satoh et al. 1983). It is a co-factor for several proteins that carry out fundamental functions in growth and development (Linder 1991; Fairweather-Tait 1997; Uauy et al. 1998). It plays an important role in the absorption and metabolism of iron and zinc, and is involved in electron transport. It has a function in hemoglobin formation and in several enzyme systems such as cytochrome *c* oxidase, tyrosinase, copper/zinc superoxide dismutase (CuZnSOD), catalase. But at high concentrations, copper is toxic, so homeostatic mechanisms are required to regulate internal levels of copper (Harris 1991; Pena et al. 1999). Young animals are apparently more prone to deficiency or toxicity because of increased demands for growth, and because they have a high efficiency of absorption coupled with immaturity of the excretion system (Kamunde et al. 2002). Copper can also be used to control algae in ponds, including filamentous and higher algae such as 'Chara' (*Chara* sp.). Copper usually, as copper sulfate, has a long history of use in aquaculture to control pests, as micronutrient in agriculture, or as molluciside, which is used during the preparation of ponds before flooding and stocking. A complex compound of copper with ammonia (copper ammoniate) $[Cu(NH_3)_4(H_2O)SO_4]$ is used for controlling ectoparasites of fish. However, the bioavailability of copper varies to a great extent depending on the water chemistry. Very few reports are available regarding the effect of copper in fish growth (Buckley et al. 1982; Ali et al. 2003; James et al. 2003; Boeck et al. 2004).

Cirrhinus mrigala (Hamilton), locally called mrigal, is one of the Indian major carps being cultured extensively in aquaculture practices in India. It is the natural inhabitant of the freshwater bodies of the rivers of Northern India, Bangladesh, Burma and Pakistan (Jhingran 1991). It has been transplanted into waters of peninsular India for aquaculture (Talwar and Jhingran 1991). Mrigal is next to Rohu (*Labeo rohita*) and Catla (*Catla catla*) in importance for culture and is a bottom feeder usually feeding on plankton (Jhingran and Khan 1979; Rath 1993). Jhingran and Khan (1979) also reported about the contribution of

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mrigal on total fish productions in composite fish culture among Indian major carps. In the present investigation, an attempt has been made to study the effect of different concentrations of waterborne copper on survival, growth and feeding of *C. mrigala*.

Materials and Methods

Two hundred fry of *C. mrigala* were collected from a private hatchery (Kalinga Hatchery, Guriapukuri) for the experiment of copper. The average weight of the fish was 0.92 ± 0.28 g of the same stock. The fish were brought to the laboratory in buckets of well-oxygenated water and stocked in four tanks of 400 L of well-oxygenated water. The fishes were acclimatized for 7 days under laboratory conditions (dissolved oxygen 7.07 mg L^{-1}, pH 7.6, alkalinity as $CaCO_3$ 130 mg L^{-1} and water hardness as $CaCO_3$ 145 mg L^{-1}). During acclimatization, water was changed on alternate days, and the fishes were fed with powdered feed of 3% body weight twice daily at 0800 and 1800 hours.

Stock solution of 10,000 mg L^{-1} copper was prepared using analytical grade (Fisher, Chennai, India) of cupric chloride [copper (II) chloride] in 1 L double distilled water and then diluted with freshwater to obtain the desired concentrations. In the present study, three concentrations, i.e., 0.1, 0.2 and 0.3 mg L^{-1} Cu were used as treatments.

The experiments were conducted in 12 rectangular glass tanks (30 × 30 × 38 cm) of 34.2 L capacity. The water used for the experiment was dechlorinated tap water. Each tank contained well-oxygenated 20 L water. Three concentrations of copper (0.1, 0.2 and 0.3 mg L^{-1}) were used to assess the effect on the growth and feed intake of *C. mrigala*. One control was maintained. Three replications were kept for each concentration of copper and also for the control. Treatments were randomly assigned among tanks for the experiment. No addition of copper was made to the controls. The medium was thoroughly mixed after the addition of chemical in the tanks, and 12 fishes (fry) were introduced in each tank for the experiment. All the tanks were aerated for 18 h day^{-1}. Aeration was stopped for 6 h to avoid super saturation. Fishes were given 60 days exposures for all the treatments including controls.

The fishes were fed with powdered feed (proximate composition of feed was crude protein 40–43%, crude fat 5–8%, moisture 8–10%, ash 12–15% and energy 4–5 kcal g^{-1}) at 3% body weight twice daily at 0800 and 1800 hours for the duration of the experiment. The feed was provided to the fish in feeding trays.

Observations were made every morning at 0800 hours and the dead fishes were removed from the tank whenever they appeared. The experiment was conducted using the static renewal method with half of the test solutions renewed on alternate days. In all the tanks including controls, copper concentration was confirmed at 24 h intervals throughout the experiment. The measured quantity of water samples was digested with concentrated nitric acid on hot plate at 70°C and the concentrate was transferred to a volumetric flask and diluted to the mark with double distilled water. The concentration of copper was determined with an atomic absorption spectrophotometer (AAS, Perkin–Elmer model 3110) in flame mode at the wavelength of 324.7 nm. The AAS was calibrated using standard solutions (Merck, Darmstadt, Germany) of copper. For this experiment, the control and the three (0.1, 0.2 and 0.3 mg L^{-1}) different treatments correspond to the values (mean ± SD, n = 60) of 0.02 ± 0.005, 0.10 ± 0.01, 0.15 ± 0.02 and 0.23 ± 0.02 mg L^{-1} of total copper. Therefore, the results were made on the basis of the measured concentrations (the actual levels) of copper in waters.

All fecal matter was siphoned off before the next feeding. Feeding rates were adjusted for fish mortality by subtracting the average initial fish weight from the total initial fish weight. After 2 h of feeding, unconsumed feed was removed by pipetting, placed on filter paper and dried to constant weight. The percentage of feed intake was expressed as $(A - B) \times 100/A$, where A is the amount of feed supplied (g) and B is the amount of feed (g) remaining after 2 h of feeding.

Various water quality parameters were measured weekly following standard methods (APHA 1989). The methods used for measurement of different parameters were as follows: water temperature using a thermometer, pH using an electronic pH meter, conductivity using an electronic conductivity meter, dissolved oxygen using Winkler's method, total alkalinity using methyl red indicator and sulfuric acid titration method, and total hardness using Eriochrome Black T indicator and ethylene diamine tetracetic acid titration method.

After 60 days, the experiment was terminated and the weight of each fish in wet condition for all the treatments were measured individually. The weight gain percentage was calculated as $[(C - D)/D] \times 100$, where C is the final weight (g) and D is the initial weight (g). The survival rate was also calculated for all the treatments including controls. Average daily growth (g day^{-1}) was calculated at the end of the experiment as [(final weight − initial weight)/number of days].

After taking the weight of the fish exposed to copper including controls at the end of the experiment, they were used for the estimation of copper accumulation in the whole fish. Fish were killed by decapitation, and the whole fish at wet condition were taken in conical flasks and were digested with 10 mL concentrated nitric acid on a hot plate at 120°C until the vapor and the acid fluids inside the flask

turned clear. After completion of the digestion process, the samples were cooled and filtered using the Whatman filter paper 42 and the final volume made up to 25 mL with the double distilled water. The filtrate was subjected to copper analysis in AAS (Perkin–Elmer, model 3110) adopting suitable measuring condition. The accumulation of copper was measured at the wavelength of 324.7 nm. The ASS was initially calibrated using standard solutions of copper.

All the results were subjected to statistical evaluation. One-way analysis of variance (ANOVA) with Duncan multiple range tests was applied to find out the significant differences among treatment means using the SAS computer software for growth, survival rate, feed intake, and accumulation of copper of the fish.

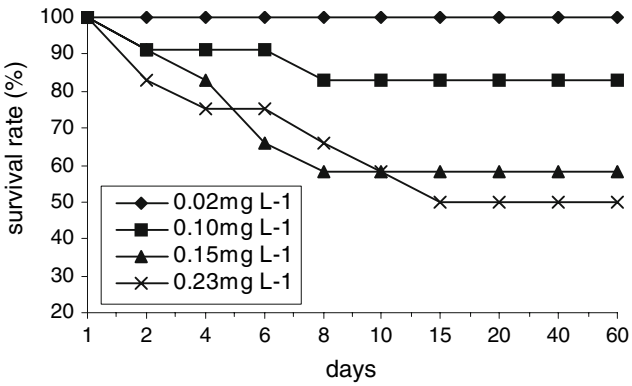

Fig. 1 Effect of copper on survival (%) of *Cirrhinus mrigala* with time (day)

Results and Discussion

Survival rate, growth, feed intake and accumulation of copper of *C. mrigala* exposed to different concentrations of Cu are shown in Table 1. Survival rate of the fish with time is also shown in Fig. 1. Significant difference ($p < 0.05$) in fish survival was observed among control (0.02 mg L^{-1}), 0.10, and 0.15 mg L^{-1} of Cu levels. However, no significant difference in fish survival was recorded between 0.15 and 0.23 mg L^{-1} of Cu levels. From the table it is evident that Cu had a significant ($p < 0.05$) effect on fish growth as the fish grew faster at control compared to 0.15 mg L^{-1} Cu. There was almost no growth of the fish at 0.23 mg L^{-1} of Cu. No significant difference ($p > 0.05$) in fish growth could be observed between control and 0.10 mg L^{-1} Cu level. Copper had a significant effect on the feed intake of fish. Feed intake decreased in the 0.23 mg L^{-1} Cu level compared to 0.10 mg L^{-1} Cu level. Significant differences ($p < 0.05$) in feed intake were observed among control (0.02 mg L^{-1} Cu) and different concentrations of Cu treatments. Copper accumulation in fish increased with increasing concentration of the metal. The highest Cu accumulation (4.59 ± 0.21 mg kg^{-1}) occurred at 0.23 mg L^{-1} levels while the lowest accumulation (0.22 ± 0.08 mg kg^{-1}) occurred at control (0.02 mg L^{-1} Cu).

Some important water quality parameters from *C. mrigala* fry exposed to waters of different Cu levels are presented in Table 2. The water quality parameters were optimum for the growth of the fish.

Trace metals have varied effects on cells, some of them are absolutely required for the functioning of both prokaryotic and eukaryotic cells, but others are toxic to cells and organs through different pathways and in different degrees. Cells have developed mechanism to keep toxic metal species away from critical targets. Contamination of natural waters due to trace metals has become inevitable because of rapid industrialization globally. Information on the lethal and sub lethal effects of metals on aquatic organism is voluminous (Eisler et al. 1979). In the present study, when the fishes were treated with higher doses of $CuSO_4$, some morphological changes in the body of the fishes were observed. The fishes were less active; eyes became brownish in color; color of the fins and scales was changed to faint blue and also reddish swellings were observed on the paired fins. The inactivity of the copper treated fish could be due to less oxygen uptake in the presence of the metal. Another reason of inactivity may be the effect of trace metals on brain as observed by Weinsberg et al. (1995).

The present study indicates that *C. mrigala* fry exposed to 0.10 mg L^{-1} copper showed a reduction in growth

Table 1 Effect of different concentrations of copper in water on mean (± SD) survival, growth, feed intake and accumulation of *Cirrhinus mrigala* after 60 days of exposure

Copper exposure (mg L^{-1})	Initial weight (g)	Final weight (g)	Growth (g day^{-1})	Survival rate (%)	Feed intake (%/body weight)	Accumulation (mg kg^{-1})
Control (0.02)	0.85 ± 0.15	2.02 ± 0.13	0.019 ± 0.006^{a}	100^{a}	5.80 ± 0.15^{a}	0.22 ± 0.08^{a}
0.10	0.87 ± 0.22.	1.92 ± 0.30	0.017 ± 0.005^{a}	83 ± 3^{b}	5.13 ± 0.17^{b}	2.55 ± 0.10^{b}
0.15	0.92 ± 0.13	1.48 ± 0.08	0.009 ± 0.002^{b}	58 ± 6^{c}	3.81 ± 0.19^{c}	3.56 ± 0.16^{b}
0.23	1.06 ± 0.17	1.12 ± 0.14	0.001 ± 0.001^{c}	50 ± 4^{c}	3.18 ± 0.22^{d}	4.59 ± 0.21^{c}

Values followed by the same superscripts in a column are not significantly different at the 0.05 levels

Table 2 Physiochemical parameters of water for copper experiment

Treatments (mg L^{-1})	Temperature (°C)	pH	Dissolved oxygen (mg L^{-1})	Alkalinity (mg L^{-1} as $CaCO_3$)	Hardness (mg L^{-1} as $CaCO_3$)	Conductivity (m mhos cm^{-1})
Control (0.02)	26 ± 2	7.6 ± 0.2	7.6 ± 0.42	134 ± 14	140 ± 12	0.287 ± 0.018
0.10	26 ± 3	7.6 ± 0.2	7.5 ± 0.50	134 ± 14	130 ± 10	0.287 ± 0.013
0.15	26 ± 2	7.7 ± 0.3	7.6 ± 0.40	128 ± 17	140 ± 14	0.292 ± 0.015
0.23	26 ± 2	7.6 ± 0.2	7.4 ± 0.60	124 ± 15	140 ± 18	0.297 ± 0.015

(weight) of 10.3% to that of the control (0.02 mg L^{-1}) for 60 days of exposure. Those exposed to 0.15 and 0.23 mg L^{-1} copper showed a reduction to 52.1 and 94.9%, respectively. The present study also indicates that feed intake of *C. mrigala* exposed to 0.10 mg L^{-1} of copper was significantly ($p < 0.05$) lowered to 11.6% compared to that of the control. Those exposed to 0.15 and 0.23 mg L^{-1} of copper showed a reduction in feed intake of 34.3 and 45.2%, respectively. The lesser-feed utilization may be because of the toxic effect of copper on fish by impairing normal physiological functions. Copper acts as a cofactor for a number of key proteins (i.e., superoxide dismutase, ceruplasmin). As with iron, copper's flexible redox state plays a vital role in cellular respiration, with cytochrome c oxidase being an important copper protein. Copper is thus an essential element, and daily dietary requirements for fish are in the region of 15–60 μ mol (1–4 mg) Cu kg^{-1} dry mass (Lanno et al. 1985; Watanabe et al. 1997). However, in excess, copper is toxic. High concentrations of waterborne copper affect branchial function, the main toxic action being impairment of sodium homeostasis (Lauren and McDonald 1985). Thus, if the concentration of copper differs from the actual physiological requirements, it may lead to either a toxic effect or an inhibition of growth. The toxic effect of Cu also is related to its capacity for catalyzing oxidative reactions, leading to the production of reactive oxygen species (Lopes et al. 2001). These highly reactive compounds may also induce tissue alterations and physiological derangement in fish (Varanka et al. 2001). Fish exhibits intense excitation and difficulty in breathing in toxic solutions of copper. $CuSO_4$ in water medium dissociates into Cu^{++} and SO_4^{--} ions. The free copper ion is toxic to fish. A layer of mucus generally covers the skin and gills, which prevent an exchange of gases, and damage to respiratory epithelium of fish causes suffocation (Metelev et al. 1983). On exposure for longer periods, increased accumulation of toxic copper in the body might be the cause of decreased rate of feeding, growth and survivability.

Buckley et al. (1982) reported that Coho salmon, exposed to sublethal levels of aqueous copper (1/4 and 1/2 LC_{50}) lost appetite and ceased growing or showed decreased rates of growth. Ali et al. (2003) reported that weight gain and specific growth rate of *Oreochromis niloticus* exposed to 0.15, 0.30 and 0.50 mg L^{-1} of copper in water decreased significantly ($p < 0.05$) as compared to control and this decrease was linearly correlated with the increase of copper concentration in water. They also reported that the exposure of the fish to different copper levels in water significantly reduced ($p < 0.05$) their food consumption as compared with the control. James et al. (2003) reported that exposure for 140 days of an ornamental fish; *Xiphophorus helleri* to sublethal concentrations (0, 0.04, 0.08 and 0.012 mg L^{-1}) of copper reduced the rates of food intake and growth in relation to copper concentrations. Boeck et al. (2004) reported that copper exposure for 28 days to 0.807 M affected both growth and feeding behavior in common carp and also at 0.557 M, growth was affected despite normal food consumption.

The accumulation of trace metals in the tissues of aquatic organisms may cause various physiological defects and mortality (Karakoc 1999). In this study, copper accumulation in fish increased with increasing concentration of the metal. Copper levels were also elevated in various organs (liver, gills and kidneys) of the fish (Buckley et al. 1982; Boeck et al. 2004). The copper level in the whole body and liver increased significantly ($p < 0.05$) with the copper concentration increase in water (Ali et al. 2003). Copper accumulation in the fish linearly increased with increase in exposure period up to 140 days and also with increase in tested copper concentration (James et al. 2003). Wu et al. (2003) reported that copper contents in the whole body of newly hatched tilapia (*Oreochromis mossambicus*) larvae significantly increased following copper exposure (0, 30, 50 and 100 μg L^{-1}) for 96 h.

From our results, it is evident that the maximum acceptable level of copper (Cu) in water could be <0.10 mg L^{-1} for the rearing of *C. mrigala* fry based on survival, growth and feed intake by the fish.

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