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Copper Toxicity on Growth and Reproductive Potential in an Ornamental Fish, *Xiphophorus helleri*

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Abstract

The effect of copper toxicity on growth, gonad development and reproductive performance in an ornamental fish, *Xiphophorus helleri* was studied for 140 days. The 96 h LC50 value of copper for *X. helleri* was 0.361 $mg\cdot l^{-1}$. Exposure of animals to sublethal concentrations of copper reduced the rates of food intake and conversion and conversion efficiency; also reproductive indices like gonad weight, gonadosomatic index and fertility showed significant reduction in relation to copper concentration. Metabolic parameters like oxygen consumption and opercular beat, on the other hand, exhibited opposite trend. Reduction in the rate and efficiency of conversion negatively reflected on the reproductive performance of experimental fish. Test fish reared in tap water (control) and lowest concentration of copper (0.04 ppm) bred two times and released more number of young ones than other exposures. Control individual released 228 young ones whereas those exposed to 0.04, 0.08 and 0.12 ppm copper released only 72, 29 and 17 young ones during the experimental period of 140 days. Sublethal concentrations of copper, eventhough, reduced the number of young ones production, did not cause mortality. Vertebral deformities (scoliosis) particularly tail curvature was observed in male *X. helleri* exposed to the highest concentration of copper.

Introduction

Among the ornamental fishes, red swordtail, *Xiphophorus helleri* is a delight in the aquarium with their dazzling good look and posturing behaviour. They are not only an economically important ornamental fish but also biologically interesting. They are able to exploit a wide range of habitats, as they can prosper in brackish lagoons as well as in freshwater ponds, lakes and streams.

Swordtails are marketed all over the world and yield a good amount of foreign exchange (James and Sampath 1998). Ornamental fishes are exposed to increasing problem of aquatic pollution and many species can only be saved through breeding programs. The breeding of ornamental fish, therefore, not only provides an income to many people but also contributes to the conservation of fish species (Bassleer 1994). Previous studies have documented heavy metal toxicity on growth (Lett et al. 1976), uptake and elimination of metals (Cuvin and Furness 1988; Giles 1988), reproduction (Kaviraj 1983; Nicoletto and Hendricks 1988) and metal removal by bio-chemical agents (James et al. 1992, 2000) in cultivable fishes. However, toxic effects of heavy metal on growth and reproduction in ornamental fishes were not studied by many workers. Ornamental fishes are not sturdy like edible fishes and they are easily susceptible to diseases and pollution. The findings of copper toxicity on aquarium fish could be applicable to cultivable fishes too. Hence, the present study was undertaken to study the toxic impact of copper on growth and reproductive performance in an economically important ornamental fish, *Xiphophorus helleri*.

Materials and Methods

Experimental animal

Broods of red swordtail fish, *X. helleri* were procured from the aqua farms and brought to the laboratory in polythene bags containing aerated water. Male and female *X. helleri* in the ratio of 1:2 were maintained in cement tank (1.75'x1.5'; 110 l capacity) containing 90 l of well water. They were fed *ad libitum* with minced pieces of beef liver twice a day and aquarium water was completely changed twice in a week. The cement aquaria were profusely provided with *Hydrilla* plant for breeding of *X. helleri*. Floating plants offer best protection for the fry, as parents spend most of their time near the surface, sometimes feeding on their young ones. Young ones after their release were immediately transferred to another cement tank. The fry were fed on *Artemia* nauplii for first seven days and later on with chopped beef liver twice a day.

Copper toxicity

Two series of experiments were conducted in the present study. In the first series of experiment, 96 h LC50 value of copper for *X. helleri* was determined following the method of Finney (1971). A stock solution of copper was prepared by dissolving 3.93 g of analar grade (Merck) copper sulphate ($CuSO_4.7H_2O$) in 1 l of distilled water and then diluted with freshwater to obtain the desired concentration. Forty five days old healthy juveniles of *X. helleri* (17.2 ± 0.47 mm) were exposed to different concentrations of copper (0.18, 0.24, 0.30, 0.36, 0.42, 0.48 and 0.54 ppm or $mg{\bullet}l^{-1}$) in plastic troughs containing 10 l of test media and mortality was recorded for 96 h. A static renewable bioassay method was adopted to determine the 96 h LC50 (Sprague

1973) and juveniles were not fed during the bioassay test. The test media were changed daily at 0800 h to maintain the constant toxic concentrations (Sprague 1971) during the bioassay test. The 96 h LC50 value of copper for *X. helleri* was 0.361 ppm and its 95% confidence limits were –0.62 (lower) and 3.74 ppm (upper). One third of the LC50 value was taken as the maximum sublethal concentration in the present study.

In the second series of experiment, sublethal effects of copper on feed intake, growth and reproductive performance in *X. helleri* were studied. Forty five days old active juveniles of *X. helleri* were selected from the stock and divided into four groups of 25 each and exposed to four sublethal levels of copper (0, 0.04, 0.08 and 0.12 ppm). Triplicates were maintained for each group. The experiment was conducted in epoxy-coated cement tank containing 80 l of test medium for 140 days till one breeding; the epoxy coating prevents the metal adsorption (James et al. 2000). The experimental media were changed once in two days before feeding to maintain the constancy of metal concentration in the experimental medium. The hydrobiological parameters like dissolved oxygen, temperature, hardness, pH were monitored biweekly and these parameters averaged to 4.12 ml O_2^{-1}, 29.1 ± 0.7°C, 304 ± 13 mg $CaCO_3$ l^{-1} and 7.2 ± 0.2 respectively.

Feed consumption

The experiment was conducted over a period of 140 days in two phases. In the first phase of the experiment, feeding parameters, gonad weight and gonadosomatic index (GSI)and metal accumulation of test animals were measured at an interval of 20 days. The experimental fish was fed, weighed quantity of beef liver *ad libitum* in a feeding tray for twice in a day at 0600 and 1800 h. Unconsumed feed was removed after 1 hr of feeding and dried in hot air oven at 80°C for two days. The feed consumption (mg) was estimated by subtracting the amount of unconsumed dry feed from the total dry weight of feed offered.

$$\frac{\text{Feeding rate}}{\left(\text{mg} \cdot \text{g}^{-1} \text{ live fish} \cdot \text{day}^{-1}\right)} = \frac{\text{Feed consumption (mg)}}{\text{Initial wet weight of fish (g) x Duration (days)}}$$

Growth

Before commencing the experiment, total wet weight of test fish in each exposure was weighed and simultaneously a sample of five fish from the stock was sacrificed to estimate the water content (Maynard and Loosli 1962). Initial dry weight of test animals in all exposures was arrived based on this estimation. At an interval of 20 days until the completion of experiment, all animals in each exposure were collected and wet weight was taken. Wet weight was converted to dry weight using the percentage water content of control animals sacrificed at the beginning. Growth (conversion) was estimated taking the difference in dry weight between the beginning and at the end of every 20 days. Conversion rate and efficiency were calculated from the following formulae.

$$\text{Conversion rate } \left(\text{mg} \cdot \text{g}^{-1} \text{ live fish} \cdot \text{day}^{-1}\right) = \frac{\text{Conversion (mg)}}{\text{Initial wet weight of fish (g) x Duration (days)}}$$

$$\text{Gross conversion efficiency (\%)} = \frac{\text{Feed conversion (mg)}}{\text{Feed consumption (mg)}} \text{x} 100$$

Respiration and gonad estimations

For oxygen estimation, a sample of three fish in triplicate tanks were taken from each sublethal concentrations and separately maintained in 1 l glass container containing respective concentrations of copper and aquatic oxygen consumption and opercular beat were estimated for a period of 1 h duration; estimation was done once in 20 days interval during the experiment. Before commencement of breeding on day 80, three females from each replicate tank were sacrificed and ovaries were dissected out and weighed. From these weight (mg), the gonadosomatic index (GSI) was computed following Dahlgren (1979).

$$\text{Gonadosomatic index (\%)} = \frac{\text{Wet weight of gonad (mg)}}{\text{Wet weight of fish (mg)}} \text{x} 100$$

Fish, feed samples, unconsumed feed and ovary were weighed in an electrical monopan balance to 0.1 mg accuracy.

Fertility

After sexual maturity on day 80, fertility was estimated in addition to feeding and growth estimations in brooders in the second phase of the experiment. Two female broods were randomly chosen from each replicate exposures and reared with a male in the same tank till they bred in all the experimental groups. The remaining test animals in the experimental tanks were removed and maintained separately to monitor the vertebral deformities during the experimental period of 140 days. Before parturition, the mature female can be recognised by the increased size of its belly and a conspicuous dark pregnancy spot on its ventral side. To minimize the metal absorption by the plant *Hydrilla* sp., it was placed in the aquarium only at the time of partiurition to give protection to the fry. Female broods of swordtail released young ones at 27-32 days (James 1998) interval and they were isolated from their parents and counted. The number of intrafollicular embryos produced by a female fish is known as fertility (Dahlgren 1979). Mortality and developmental abnormalities were also observed in the fry. Number of days taken for breeding of each group was recorded. Student 't' test was applied to determine the significance of mean values between control and different experimental groups. ANOVA was applied to detect the significant effects of copper concentrations and rearing period against the chosen physiological parameters in *X. helleri*. Tukey's multiple comparison test was applied to compare all the mean values of chosen feeding parameters and copper accumulation in fish body (Zar 1974).

Cu level in water and fish

Copper content in the 3 fish body (dry matter) and water were estimated at 20 days interval from 0 to 80th day and at the end of the experiment on 140th day. Since the breeding experiment commenced after 80th day, the broods were not sacrificed for metal analysis during the period between 80 and 140 days and were sacrificed at the end of the experiment on day 140. Three replicates of fish samples were digested with a mixture of concentrated nitric acid (HNO3) and perchloric acid (HClO4) in the ratio of 1:2 until the formation of a white residue at 1000C in a water bath. The cooled residue was dissolved completely by adding in HCl and made up to 25 ml with distilled water (FAO 1975). The copper concentration in the water was estimated following the method of APHA (1993). The solution was filtered through cotton wool and the filterate was subjected to metal analysis in atomic absorption spectrophotometry (GBC 906 AA model). The instrument was calibrated using standards prepared from copper sulphate.

Results and Discussion

Feeding and metabolism

The present study revealed that sublethal levels of copper significantly ($P < 0.05$) reduced the chosen feeding parameters and reproductive performance in *X. helleri*. The feeding parameters were greater in control fish and decreased with increasing concentrations of copper in experimental fish. The conversion rate of control fish was 20.8 $mg \cdot g^{-1}$ live fish$\cdot day^{-1}$ and it declined to 11.6, 8.5 and 4.8 $mg \cdot g^{-1}$ live fish$\cdot day^{-1}$ in fish exposed to 0.04, 0.08 and 0.12 ppm of copper on day 20 respectively. Similar trend was also obtained for feeding rate and conversion efficiency. ANOVA showed that copper concentrations and rearing period significantly ($P < 0.01$) affected the rates of feeding and conversion and conversion efficiency (not rearing period) in *X. helleri*. Tukey's test revealed that feeding rate was significantly ($P < 0.05$) affected in fish exposed to copper at 0.08 and 0.12 ppm, conversion rate in all tested copper concentrations and conversion efficiency in the highest concentration only over control treatment (see Table 1). Control fish elicited 2-3 times more feeding rate and 4 times more conversion rate as compared to fish exposed to highest concentration of copper (Table 1). The average number of opercular beats and rate of oxygen consumption were significantly (One-way ANOVA: $P < 0.01$) increased with increasing concentrations of copper during the experiment (Fig.1). The fish exposed to tap water, 0.04, 0.08 and 0.12 ppm copper showed 0.60, 0.78, 0.85 and 1.23 mg O_2 $g^{-1} \cdot h^{-1}$ of oxygen consumption and 12.53, 14.65, 16.66 and 22.70 times opercular beats per minute respectively during the experimental period of 140 days. The increased oxygen consumption and opercular activity may be due to the copper distress (Brafield and Mathiessan 1976; James 1990). The reduction in conversion rate (growth) and conversion efficiency observed in *X. helleri* exposed to sublethal levels of copper might have been evidently due to the inhibition of feed intake and increased cost of metabolism. Increased number of opercular beats and rate of oxygen consumption were observed in copper exposed fish (Fig.1) which might have reflected on the growth reduction (Table 1) and reproduction (Table 2; Fig.2). *Heteropneustes fossilis* exposed to mercury exhibited a slow growth rate due to

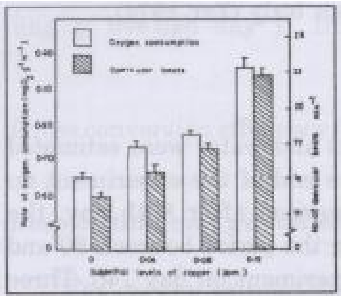
Fig. 1. Sublethal effects of copper on rate of oxygen consumption and number of opercular beats in *Xiphophorus helleri*.

Table 1. Sublethal effects of copper on the rates of feeding and conversion (mg•g-1 live fish•day-1) and conversion efficiency (%) in *Xiphophorus helleri*. Each value is the mean ($\overline{X}$ ± SD) of three observations.

Rearing period (days)	Sublethal levels of copper (ppm)			
	0	0.04	0.08	0.12
Feeding rate				
20	66.9 ± 4.3	45.0 ± 2.7	38.7 ± 3.6	21.8 ± 1.8
40	57.8 ± 5.2	36.9 ± 3.1	28.6 ± 2.1	20.9 ± 2.1
60	54.0 ± 3.8	30.6 ± 2.1	26.0 ± 2.4	19.8 ± 1.9
80*	42.1 ± 2.8	26.6 ± 2.6	22.5 ± 1.9	17.6 ± 1.4
100	18.1 ± 1.1	13.1 ± 0.8	12.8 ± 0.9	12.7 ± 0.8
120	21.6 ± 1.5	15.0 ± 0.5	14.3 ± 1.0	13.1 ± 1.1
140	27.4 ± 2.1	17.0 ± 1.1	14.6 ± 1.1	13.3 ± 0.9
Conversion rate				
20	20.8 ± 1.1	11.6 ± 0.5	8.5 ± 0.4	4.8 ± 0.2
40	17.1 ± 1.0	7.9 ± 0.4	6.1 ± 0.3	3.7 ± 0.3
60	15.5 ± 1.3	7.1 ± 0.7	5.3 ± 0.4	3.5 ± 0.3
80*	14.4 ± 0.7	6.1 ± 0.5	4.1 ± 0.3	2.8 ± 0.2
100	5.6 ± 0	3.4 ± 0.1	2.9 ± 0.1	2.5 ± 0.2
120	6.5 ± 0.2	3.9 ± 0.2	3.4 ± 0.2	2.8 ± 0.2
140	3.6 ± 0.4	1.9 ± 0.1	1.7 ± 0.2	0.4 ± 0
Conversion efficiency				
20	31.1 ± 2.1	25.7 ± 2.6	22.0 ± 2.0	21.8 ± 2.5
40	29.6 ± 1.2	21.5 ± 1.9	21.2 ± 2.4	17.9 ± 1.1
60	28.8 ± 2.9	23.3 ± 2.1	20.5 ± 1.8	17.5 ± 1.3
80*	34.2 ± 3.4	22.8 ± 1.2	18.2 ± 1.9	15.9 ± 1.7
100	31.2 ± 2.7	26.3 ± 2.1	23.0 ± 2.2	19.6 ± 2.2
120	30.1 ± 2.9	26.0 ± 2.8	24.1 ± 2.6	21.2 ± 1.9
140	13.3 ± 0.9	11.4 ± 1.0	11.5 ± 1.1	3.2 ± 0

*Breeding commenced.
Tukey's Test
Cu concentrations (ppm) : 0 0.04 0.08 0.12
Feeding rate : $\mu_1 = \mu_2 \neq \mu_3 \neq \mu_4$
Conversion rate : $\mu_1 = \mu_2 \neq \mu_3 \neq \mu_4$
Conversion efficiency : $\mu_1 = \mu_2 = \mu_3 \neq \mu_4$
≠ Cu treatments significant over other exposures at $P < 0.05$.

poor feed intake and the utilization of the greater proportion of the feed energy for metabolism (James et al. 1992). Lett et al. (1976) attributed the growth reduction in copper exposed *Salmo gairdneri* partly to increased metabolic cost and reduced food consumption. Mathers et al. (1985) reported that as high fraction of food energy was utilized for increased metabolic activities, less energy was left for growth when exposed to PCP in *Micropterus salmoides*.

Growth reduction during breeding

The rate and efficiency of conversion were reduced in *X. helleri* just prior to and during the breeding periods. For instance, the conversion rate of control fish prior to its second breeding was 6.5 $mg \cdot g^{-1}$ live $fish \cdot day^{-1}$ on day 120 and it significantly ($t = 9.18$; $P < 0.01$) declined to 3.6 $mg \cdot g^{-1}$ live $fish \cdot day^{-1}$ after breeding on day 140. The reproductive cycle of swordtail fish is short. As more assimilated feed energy is required for gonad development and production of fry, the fish is left with minimum energy for growth (Table 1). *X. helleri* being an ovo-viviparous fish (young ones are developed from the eggs which have been retained in the mother's body, but without any close tissue connections between these two for the supply of extra nourishment to the embryo) nourishes embryo from the feed stored in the eggs. During development, the absorption of organic substances and water molecules from the maternal body (Balinsky 1970), could result in considerable amount of energy loss in female *X. helleri*. Rathinam (1993) observed loss of body weight during parturition in the live-bearers *M. latipinna* and *X. helleri*. The present study also showed that copper exposures highly reduced the rate

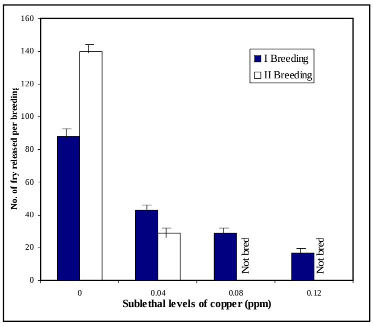

Fig. 2. Sublethal effects of copper on fertility in *Xiphophorus helleri* exposed for a period of 140 days

Table 2. Effect of sublethal levels of copper on gonad weight and fertility in *Xiphophorus helleri*. Each value is the mean ($\overline{X}$ ± SD) of six observations.*P<0.01; ** P<0.05

Parameters	Sublethal levels of copper (ppm)				ANOVA F-Value
	0	0.04	0.08	0.12	
Gonad weight (mg wet wt.)	43.0 ± 2.2	20.0 ± 1.1	16 ± 0.9	13 ± 0.5	11.3*
Gonadosomatic index (%)	2.2 ± 0.1	1.1 ± 0	1.0 ± 0	0.7 ± 0	13.5*
No.of days taken for first breeding	105 ± 5	109 ± 6	129 ± 4	132 ± 2	-
No.of fry produced in first breeding	88 ± 6	43 ± 5	29 ± 4	17 ± 2	8.1*
No.of days taken for second breeding	133 ± 6	139 ± 4	-	-	-
No.of fry produced in second breeding	140 ± 9	29 ± 2	Not bred	Not bred	-
Total number of fry produced	228 ± 8	72 ± 3	29 ± 4	17 ± 2	3.7**

and efficiency of conversion in *X. helleri* and it negatively reflected on the reproductive performance.

Reproduction and Cu accumulation

Like feeding parameters, gonad weight, GSI and fertility of *X. helleri* were significantly (One-way ANOVA: $P < 0.05$) decreased with increasing concentrations of copper (Table 2). Control fish registered 4 times more gonad weight and 13 times more number of fry production than those exposed to the highest concentration of copper. Fish reared in control water and lowest concentration of copper (0.04 ppm) bred two times and released more fry than other groups. For instance, control fish released 228 fry whereas fish exposed to 0.04, 0.08 and 0.12 ppm copper released only 72, 29 and 17 fry during the experimental period of 140 days (Table 2; Fig.2). Sublethal concentrations of copper even though it reduced the number of fry production, did not cause mortality. Table 3 reveals that, copper could not be detected in the control water throughout the experimental period. However, negligible variation was observed in the experimental exposures. Copper accumulation in the test fish *X. helleri* has linearly increased with increase in exposure period from day 0 to 80 and 140 and also with increasing tested copper concentration. Tukey's test showed significant ($P < 0.05$) accumulation of copper in fish exposed to 0.12 ppm as compared to other exposures. Accumulation of copper in fish body of *X. helleri* during exposures resulted in drastic reduction in growth, gonad weight, fry production and breeding frequency. Nicoletto and Hendricks (1988) found high levels of mercury accumulation in females of four different species of centrarchid fishes at the onset of reproduction which affected the egg production. Selenium exposure adversely affected the development of ovaries and caused the reproductive problems in red ear sunfish *Lepomis microlophus* (Sorensen 1988) and razorback sucker fish *Xyrauchen texanus* (Hamilton and

Table 3. Sublethal effect of copper on the distribution of copper in water and fish *Xiphophorus helleri* exposed for 140 days as a function of exposure period. Each value is the mean ($\overline{X}$ ± SD) of three estimations.

Exposure period (days)	Water ($mgCu \cdot l^{-1}$)				Fish body ($mgCu \cdot g^{-1}$ dry weight)			
	Sublethal levels of copper (ppm)							
	0	0.04	0.08	0.12	0	0.04	0.08	0.12
0	nd	0.040	0.080	0.120	nd	nd	nd	nd
20	nd	0.037	0.072	0.115	nd	0.004	0.006	0.009
40	nd	0.036	0.074	0.112	nd	0.007	0.010	0.015
60	nd	0.035	0.075	0.114	nd	0.009	0.016	0.023
80	nd	0.037	0.071	0.113	nd	0.013	0.023	0.031
140	nd	0.034	0.072	0.111	nd	0.019	0.036	0.043

nd : Not detectable
Tukeys test : Cu accumulation in fish body = $\mu_1 = \mu_2 = \mu_3 \neq \mu_4$; $P<0.05$

Waddell 1994). Choudhary et al. (1981) reported that an increasing concentrations of malathion reduced the feeding parameters and gonadosomatic index in *Heteropneustes fossilis*.

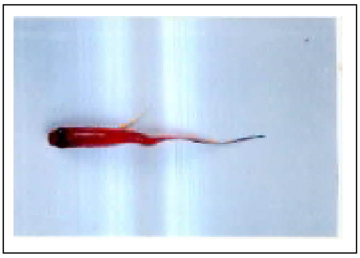

Fig. 3. Vertebral deformity (scoliosis) in *Xiphophorus helleri* exposed to the highest level of copper (0.12 ppm)

Vertebral deformity

Vertebral deformity (scoliosis) particularly tail curvature was observed in five male *X. helleri* exposed to the highest concentration of copper on 92nd day of exposure (Fig.3). Vijayram et al. (1990) reported vertebral deformities in *Anabas testudineus* exposed to 0.25 and 1.00 ppm of cadmium on 109 and 86th day of exposure period respectively. The prolonged exposure of *X. helleri* to highest concentration of copper produced scoliosis and it may be due to the vertebral demineralization (Ca, P and Mg) resulting in weakening and shortening of the bone. An imbalance of mineral metabolism, Ca, P and Mg was observed in *A. testudineus* exposed to cadmium (Vijayram et al. 1990). The incidence of scoliosis has been reported in *Channa striatus* (Bhaskaran 1980) and *Rana tigrina* tadpoles exposed to methyl parathion (Kennedy and Sampath 2001).

The following observations were made in the present investigation: i) The feeding parameters were higher in control fish and decreased with increasing concentrations of copper in test fish, ii) copper exposures increased the metabolic indicators like oxygen consumption and opercular beats and reduced the feed consumption which negatively reflected on growth and reproductive performance, iii) copper accumulation was significantly high in fish exposed to the highest level of copper and it caused the vertebral deformity in male *X. helleri*.

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