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Effects of growth in *Mugil cephalus* juveniles sub-chronically exposed to copper, lead and zinc

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ABSTRACT

In this study, *Mugil cephalus* was exposed to copper, lead and zinc under sub-chronic toxicity test to investigate the effects of growth in terms of gain in length, weight and condition factor of the test organism. The chronic values for copper, lead and zinc was 28, 140, and 93 μg/l respectively. From the data obtained, it is clear that the concentrations of copper, lead and zinc significantly reduced the growth of Juvenile *Mugil cephalus* when compared with control. Growth effects was observed with the lower concentrations of copper and zinc when compared with lead, at higher concentrations of copper, lead and zinc the relationship with growth was highly significant at $P< 0.001$. Finally the relationship between concentrations of heavy metals and the growth of the juvenile *Mugil cephalus* were significant at $P<0.05$, 0.01 and 0.001.

KEYWORDS

Condition factor copper;
Lead;
Mugil cephalus;
Zinc.

INTRODUCTION

Estuarine are important areas for the reproduction and growth of many fish and crustacean species. Many fish species utilize estuaries as nurseries for feeding and growth during their planktonic phase[15]. There is a growing concern for chemicals and metals that are suspected of disrupting reproduction in aquatic organisms. These are tied to observations in humans and wildlife over the last 40 years of worrying trends of adverse effects[3]. Cadmium and lead are not required even in small amounts by living organisms. However, other heavy metals are potentially harmful to most organisms at some level of exposure and absorption[17]. Most commonly used stressor end points are variables related to growth performance. The body size correlates with many ecological as well as life history traits and thus influence the abundance of species as well as population structure and dynamics[11]. Growth rate has been frequently used as a measure of performance of the individual and is believed to be a more appropriate measure of toxicological effects. Essential and non-essential metals can produce toxic effects in fish by disturbing their growth, physiology, biochemistry, reproduction and mortality. Hence, fishes are considered as one of the best indicators of heavy metal contamination in coastal environment[28].

Behaviour studies are useful for studying effects of environmental pollutants because it can provide a bioassay to determine an ecological death that may occur after much lower exposures to the toxicant[2]. Altered behaviours caused by exposure to pollutants may hence cause serious risks to the success of animal populations and disrupt aquatic communities[12]. Condition Factor (CF), which assumes that heavier fish of a given length is in better condition, is able to indicate fish fitness under stress of pollution as metabolic trade-off is required to deal with detoxification and the energy available for growth may thus be reduced. CF in fish was reported to decline upon exposure to heavy metals[1, 4, 16].

In environmental studies, biomarkers have the advantage of detecting early adverse effects resulting from stress exposure, well before they become visible at the population level. In addition, a suite of biomarkers, as measurable endpoints at molecular, cellular and physiological levels, may be necessary for a successful perception on the health status of populations, and once selected for a particular case, they can be adapted to different ecological scenarios[10]. Hence, in the present study the sub-chronic effects of copper, lead and zinc was studied in the juveniles of *Mugil cephalus*.

MATERIALS AND METHODS

Juvenile specimens of *Mugil cephalus* (1.5 ±0.3cm in length and 0.22 ±0.02g) were collected from Ennore creek (13°13’54.48" N, 80°19' 26.60" E, Tamilnadu, India). Juveniles of *M.cephalus* were immediately transported to the laboratory in air-filled plastic bags. Test organisms were acclimatized in glass aquaria with aerated natural filtered seawater for a period of 8 days with 29 PSU salinity, temperature of 29 ±2 °C, dissolved oxygen of 5.9 mg/l and pH of 8. Captured wild organisms were quarantined immediately with Oxytetracycline. After a day of acclimatization, *M.cephalus* was then fed with pellets of rice bran and oil cake. The dead animals were removed immediately. The remaining detritus were removed by siphoning[23].

Prior to toxicity tests and stock solution preparations, all the glassware’s were washed in 10 per cent nitric acid and rinsed with deionized water. Stock solutions of copper, lead and zinc were freshly prepared by dissolving the proper metal salts ($CuCl_2$ for Cu, $Pb(NO_3)_2$ for Pb and $ZnSO_4.7H_2O$ for Zn in deionized (double distilled) water with glass standard flasks. Stock solutions were acidified by the addition of 0.1 ml of concentrated nitric acid per litre of stock solution[6]. Fresh stock solutions were prepared daily. These solutions were serially diluted to get the experimental concentration for the toxicity test.

The experimental method includes static renewal (24-hour renewal) test by following the method of[24]. Five concentrations in a geometric series including control were prepared for the test for 30 days for short-term chronic toxicity test[25]. Toxicant and seawater were replaced on daily basis. Each series of test chambers consisted of duplicates with 10 animals in a 5 L glass trough. Test chambers were loosely covered to reduce evaporation, to minimize the entry of dust into solutions, and to prevent loss of test animals. All the experiments were conducted at salinity of 28 PSU, temperature of 28 ±2 °C, dissolved oxygen of 5.6 mg/l and pH of 8.01 with gentle aeration. Test animals were fed regularly three times a day.

Commencement of the introduction of test organisms to the chronic toxicity test all the test organisms were subjected to physical measurements in terms of length and weight. Correspondingly, at the end of the test the survived organisms underwent physical measurements. The calculated values were compared with control values. Condition factor (K) of the experimental animal was calculated by[27],

$$K = \frac{100W}{L^3}$$

The Total Length (TL) of the test organism was measured from the tip of the anterior or part of the mouth to the caudal fin (fish) using ruler calibrated in centimeters. Test organisms were measured to the nearest centimeter. Weight was measured after blot drying with a piece of clean hand towel. Weighing was done with a tabletop digital weighing balance (Metller), to the nearest gram. One-way ANOVA (Dunnett’s multiple comparison test) was carried out using Graphpad Prism Software.

RESULTS AND DISCUSSION

The well-being of the fish was degraded notably (P<0.001) when exposed to 10, 20, 40, 80 and 160 µg/l Cu. Significant (P<0.01) reduced weight was observed in 20 and 40 µg/l copper concentrations. There was significant (P<0.001) difference in length exposed to 10, 20, 40, 80 and 160 µg/l concentrations of copper. The condition of fish was significantly (P<0.001) reduced in 40 and 160 µg/l. Fish exposed to 20 and 80 µg/l for 30 days also showed significant (P<0.01) drop in condition factor. *M.cephalus* juveniles exposed to lead concentrations observed significant (P<0.001) reduced weight in 76, 114, 171 and 256 µg/l also significant (P<0.01) change was brought by 51 µg/l. The condition factor of the fish was reduced significantly (P<0.01) in 76 and 114 µg/l, severe changes was observed in 171 and 256 µg/l copper concentrations which were significant (P<0.001) with control. Significant (P<0.001) reduced weight was brought about in the 118 and 188 µg/l zinc concentrations, significant reduced weight was also observed in 46 (P<0.05) and 74 µg/l (P<0.01) zinc concentrations. The health of the fish was altered significantly (P<0.05) in 29 µg/l. Significant (P<0.01) reduction in condition factor was also observed in 46, 74 and 118 µg/l zinc concentrations. The change was extreme in 188 µg/l, and significant at P<0.001.

The fishes in the control were in good health when compared to treated fishes. Chronic exposures to copper affect the growth of juvenile fish[8]. Waiwood and Beamish[26] observed a 20 per cent reduction in growth rate in rainbow trout exposed to 23 µg/l Cu over a 30-day test. Similarly, Seim et al.[21] observed that fish exposed to 31 µg/l Cu were approximately 20 per cent smaller than controls. The present study indicates the results closer to the cited observations, in the short-term chronic toxicity test, 20 µg/l produced a significant (P<0.001 and P<0.01) effect on the growth of the *M.cephalus* and significant (P<0.01) effect upon condition factor. Waiwood and Beamish[26] showed that 4, 23, and 168 µg/l of copper could produce 20 per cent reduction in growth rate.

Studies on non-salmonid species showed a similar relationship where a reduction in growth was observed at concentrations that were lethal, but no effect on growth was observed at

TABLE 1 : Gain in weight (%), length (%) and condition factor (%) of *M.cephalus* exposed to copper, lead and zinc in sub-chronic toxicity test

Metal	Concentration (µg/l)	Gain in mean weight (%)	Gain in mean length (%)	Condition factor (%)
Cu	0	1.78 ±0.27	0.36 ±0.93	0.85 ±0.02
	10	1.47 ±0.28	0.63 ±1.74***	0.87 ±0.03
	20	1.15 ±0.50**	0.54 ±1.19***	0.72 ±0.04**
	40	0.08 ±0.68**	0.15 ±0.63***	0.61 ±0.01***
	80	-0.17 ±0.01	0.21 ±0.72***	0.67 ±0.02**
	160	0.16 ±0.02***	0.30 ±1.27***	0.60 ±0.03***
Pb	0	1.54 ±0.01	2.26 ±0.43	0.71 ±0.02
	51	0.85 ±0.17*	0.88 ±2.09**	0.63 ±0.03
	76	0.33 ±0.12***	1.29 ±0.13**	0.58 ±0.01**
	114	0.16 ±0.35***	0.97 ±1.12**	0.55 ±0.02**
	171	0.03 ±0.42***	0.06 ±0.08***	0.52 ±0.01***
	256	0.08 ±0.47***	0.00 ±0.01***	0.41 ±0.03***
Zn	0	2.23 ±0.50	3.00 ±0.85	0.84 ±0.01
	29	2.24 ±0.83	3.77 ±0.95	0.69 ±0.05*
	46	1.15 ±0.50*	1.68 ±1.20*	0.69 ±0.05**
	74	0.72 ±0.23**	0.99 ±0.67**	0.66 ±0.02**
	118	-1.35± 0.06***	-0.06 ±0.09**	0.67 ±0.03**
	188	-5.14 ±0.40***	-0.66 ±0.93**	0.53 ±0.02***

*****values are significant at P<0.001, ** values are significant at P<0.01, * values are significant at P<0.05. One-way ANOVA (Dunnett's multiple comparison test (α=0.05)); Values are the mean and standard deviation; The concentration used column (mg/l) contains '0' indicating control, in triplicate.**

sublethal exposures[9]. Reduced growth was observed in Atlantic salmon alevins exposed to 0.47 µg/l Cd[20]. In the present study, a significant decrease of growth rate as well as the decrease of the locomotion after exposure to even lower concentrations was observed. Similar results have been described earlier[19]. Szczerbik[22] reported the mean growth rate of fish in the group fed with a dose of 10,000 µg/g was significantly lower than in the other groups. The negative influence on growth is a well-known effect of heavy metal action in fish and other aquatic organisms[13].

The growth inhibition in the group receiving the highest heavy metal concentration observed in our experiment could be due to the influence of heavy metals on food intake and assimilation[5]. Waiwood and Beamish[26] observed reduced growth rates in response to copper in rainbow trout juveniles exhibiting depressed appetite and decreased food consumption. In our study, qualitative observations of fish feeding behavior indicated that fish in all groups fed actively, although a slight reduction in feeding activity level was observed in the highest copper exposure. The extent to which reduced growth caused by copper exposure is associated with suppressed feeding, which may be related to exposure levels. Fish growth becomes significantly reduced in copper treatments compared with controls over the exposures[29].

Increased metabolic demands divert resources from normal growth processes[14]. Waiwood and Beamish[26] presented evidence to suggest that exposure to copper influences the basal metabolic rate of salmonids, which could limit growth through decreased efficiency of energy utilization coupled with increased metabolic maintenance costs. Mathers et al.[18] reported that, high fraction of food energy was utilized for increased metabolic activities; less energy was left for growth in *Micropterus salmoides*. Dang and Wang[7] reported that the cadmium exposed and control fish, had no significant difference in wet weight, Standard Length (SD), condition factor (K). Present study reveals that the weight, length and the condition factor of test organisms varied significantly (P<0.001 and P<0.01) with exposed concentrations.

CONCLUSION

Mugil cephalus exposed to essential heavy metals showed significant gain in weight and gain in length with control, there was reduced growth induced by non-essential heavy metals and the condition factor also reduced significantly from control (P<0.001 and P<0.01). The sensitivity of a species to growth effects caused by copper, lead and zinc exposure may be influenced by the relative growth rate of the species. Environmental conditions can significantly influence the physiology of fishes, and therefore, modify the growth potential. Although a number of physiological effects of copper, lead and zinc have been well characterized in various fish species, relative to other metals, little is known about the toxic mechanism in fish. Heavy metals may have different behavioral effects at concentrations much less, than at which they have lethal effects, suggesting that regulatory pollution limits based upon standard toxicological studies may be too high to prevent damage to aquatic communities through the sublethal behavioural effects.

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