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LENGTH GROUP AND SPECIES-SPECIFIC GROWTH RESPONSES OFCARNIVOROUS FISH UNDER CHRONIC EXPOSURE OF METALS MIXTURE

Z. Bashir[1], M. Javed[2] and S. Abbas[3]*

[1,2]Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad-38040, Pakistan
[3]Institute of Zoology, Faculty of Life Sciences, Gulab Devi Educational Complex, Lahore, Pakistan
*Corresponding author's e-mail address: sidraabbas39@gmail.com

ABSTRACT

The present research study was conducted to determine the effect of sub-lethal (1/3rd of LC_{50}) concentrations of heavy metals mixture (Cd+Cr+Co+Cu+Ni) on the growth performance of two carnivorous fish species viz. *Channa marulius* and *Mystus seenghala*, under laboratory conditions. The statistical differences among various growth parameters of the treated and control fish were analyzed by employing the Factorial experiments under RCBD and Tukey's/Student Newman-Keul tests. The three length groups (50, 100 and 150 mm) of each fish species were divided into two groups i.e., treated and control. For a period of 120 days, each group of fish species was exposed to Cd+Cr+Co+Cu+Ni mixture, separately and growth of the treated fish was compared with that of the control fish group. The fish growth trials were conducted, by selecting a group (n=10) of each length group and fish species. The experiments were conducted with three replications for each exposure concentration, separately. The growth performance of three length groups (50, 100 and 150 mm) of metals mixture treated and control fish groups was monitored in terms of increase/decrease in average wet weights (g) and total lengths (mm), feed intake (g), condition factor and feed conversion ratio. The data on all the growth variables of fish were collected, on weekly basis and analyzed statistically. The Results showed that all the three (50, 100 and 150 mm) length groups of Cd+Cr+Co+Cu+Ni mixture treated fish revealed significantly reduced growth, in terms of increase in average wet weights, total lengths and feed intake as compared to the three length groups of control fish. However, the treated fish showed significantly higher values of condition factor (K) as compared to the control fish. Regarding overall means, the 150 mm fish showed significantly better average wet weight and total length increments, and feed intake while lower FCR as compared to 100- and 50-mm length groups. The 50 mm fish exhibited significantly better K value than that of 100- and 150-mm length groups of fish. During chronic Cd+Cr+Co+Cu+Ni mixture exposure, *Channa marulius* exhibited significantly ($p<0.01$) higher average wet weight and total length increments, feed intake and condition factor while lower FCR as compared to *Mystus seenghala*.

Key words: *Channa marulius*, *Mystus seenghala*, length groups, metals mixture, chronic exposure, growth.
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INTRODUCTION

Aquatic ecosystems are being contaminated by heavy metals because of both natural and anthropogenic sources. The main sources of water pollution include municipal, industrial, and agricultural runoff, resulting into heavy metals release into aquatic ecosystems. Elevated concentrations of heavy metals are capable to produce hazardous effects on the aquatic life whenever come into contact with a natural ecosystem (Elbeshti *et al*., 2018). Despite wastewater management efforts in Pakistan, heavy metals are still posing huge health hazards to aquatic organisms due to rapid industrialization and use of agricultural chemicals containing metals (Ashraf *et al*., 2018).

Metals are serious pollutants of aquatic environments causing alterations in the metabolic, physiological and structural systems of both the animals and plants (Jabeen *et al*., 2012). Fish are known as the key indicators of metal's pollution of the freshwater eco-systems. As the fish grows under contaminated environment, their muscles, liver and intestine become profoundly contaminated with metals (Ahmad *et al*., 2011). This in turn can cause severe hazards to humans who consume this contaminated fish (Godwin *et al*., 2011). In the natural habitats, animals are exposed to a number of metals at the same time and the metals toxicity exhibits diverse effects on their life (Naz *et al*., 2013). The property of speciation and bioavailability of individual metal is affected when the number of metals coexists in water. This is due to competition among different metals for their uptake routes, target sites, transport and excretion mechanism within the fish (Abbas *et al*., 2018).

Among various metals, cadmium is highly toxic due to its non-biodegradibility. Disturbance in respiration and reduction in growth may occur when fish are chronically exposed to waterborne cadmium (Witeska *et al*., 2006). Chromium plays a crucial role in the fish metabolism as it is an essential trace element. It is documented that due to its environmental persistence and

ability to produce hazarduous effects, chromium can cause carcinogenesis that may eventually lead to mortality of the carnivorous fish (Sthanadar *et al*., 2013). Copper is also known for its toxic effects to fish species. Physiology, growth and reproduction of fish are critically affected by chronic exposure of Cu (Zhao *et al*., 2012). Nickel and cobalt can adversely affect the growth of fish by causing different biochemical and behavioral alterations in the fish (Azmat *et al*., 2012).

Heavy metal contamination in the freshwater ecosystems of Pakistan has become hazardous to the natural fish fauna, particularly to the carnivorous fish species like *Channa marulius* and *Mystus seenghala* that are about too extinct in the freshwaters. These carnivorous fish species are existing at the highest trophic level of aquatic food chain, therefore, have higher ability to bio-magnify the metals from the aquatic ecosystems (Javed *et al*., 2016). *Channa marulius* is also known as snakehead fish and belongs to the genus "channa" and family "channidae". Itis broadly distributed in Pakistan, China and India, and usually resides in the irrigation canals, rivers and freshwater ponds (Ashraf *et al*., 2011). It can flourish in weedy marshes, swamps and can tolerate brackish water to some extent (Deshmukh *et al*., 2011). *Mystus seenghala* is extensively distributed in Pakistan, India, Burma, Nepal, Sirilanka, Veitnam and Thailand. It is one of the most important primary food fish in Thailand and other parts of the Southeast Asia (Athikesavan *et al*., 2006). The fingerlings of *Mystus seenghala* feed on insects while adults feed on alive shrimps, fishes and molluscs. It also feeds on dead fish and other organisms present in the ecosystem. *Mystus seenghala* is euryhaline and estuarine catfish that survives in low salinity conditions and is a threatened fish species (Rafique and Khan, 2012).

Due to higher rate of heavy metal's toxicity in the natural freshwater ecosystems, these carnivorous fish species have faced considerable decline in population over the last few years (Javed *et al*., 2017). The existing literature on the impact of heavy metals on fish is mainly concerned with the individual metal's effects while the studies on metals mixture toxicity to the carnivorous fish species are scarce (Dondero *et al*., 2011). Therefore, the present study was conducted to determine the growth performance of three length groups (50, 100 and 150 mm) of *Channa marulius* and *Mystus seenghala* during chronic exposure of Cd+Cr+Co+Cu+Ni mixture under laboratory conditions.

MATERIALS AND METHODS

These experiments were performed at the Fisheries Research Farms, Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad. The length groups viz. 50, 100 and 150 mm of *Channa marulius* and *Mystus seenghala* were obtained from Head Qadirabad and kept in laboratory conditions for acclimatization for ten days in cemented tanks and were fed with 45% crude protein rich pelleted feed. After acclimatization, ten specimens of each length group of one fish species were stocked in the glass aquarium, separately. One group related to each length group of one carnivorous fish species was exposed to metals mixture (treated) while the other group was kept in clean metal free environment (control). Three replicates for each length group and fish species under treated and control media, separately, were used for the growth trials. Same was done for growth studies of second carnivorous fish species. In this way, a total of 180 fish specimens related to each carnivorous species were utilized for growth trials. The experiment was laid under the Randomized Complete Block Design (RCBD). Chemically pure cadmium chloride ($CdCl_2.H_2O$), chromium chloride ($CrCl_3.6H_2O$), cobalt chloride ($CoCl_2.6H_2O$), copper sulphate ($CuSO_4.5H_2O$) and nickel chloride ($NiCl_2.6H_2O$) compounds were mixed, separately, in deionized water for the preparation of stock solutions. The Cd+Cr+Co+Cu+Ni mixture stock solution was prepared by mixing the individual metals on ion equivalence basis (1:1). The treated fish were exposed to $1/3^{rd}$ of LC_{50} of metals mixture as determined by Javed (2015). The chronic exposure of Cd+Cr+Co+Cu+Ni mixture at sub-lethal levels was given to each length group viz. 50, 100 and 150 mm of two fish species, separately, in glass aquaria at constant water pH, temperature and total hardness of 8, 28°C and 250 mgL^{-1}, respectively. To avoid sudden stress on the fish, the metals mixture solution was given in three divisions of its total concentration. Constant aeration was supplied to all the test media with an air pump fixed with a capillary system. Three length groups of *Channa marulius* and *Mystus seenghala* were exposed, separately, to the following sub-lethal concentrations for 120 days:

Fish species	Length groups	$1/3^{rd}$ of LC_{50} (mgL^{-1})
Channa marulius	50 mm	40.99±0.07
	100 mm	41.81±0.08
	150 mm	46.46±0.10
Mystus seenghala	50 mm	6.50±0.01
	100 mm	8.28±0.02
	150 mm	11.77±0.06

Each test was performed with three replications for each exposure concentration. During growth trials, the fish were fed with feed having 45% crude protein and 3.50 $Kcalg^{-1}$ gross energy, to satiation, twice daily. The

growth performance of three length groups viz. 50, 100 and 150 mm of *C. marulius* and *M. seenghala* were examined, separately, in terms of increase or decrease in average wet weights (g) and total lengths (mm), feed intake (g), condition factor and feed conversion ratio, on weekly basis. During Cd+Cr+Co+Cu+Ni mixture exposure period, the physico-chemical parameters of the test media viz. pH, temperature (°C), dissolved oxygen (mgL^{-1}), carbon dioxide (mgL^{-1}), total ammonia (mgL^{-1}), total hardness (mgL^{-1}), calcium (mgL^{-1}), magnesium (mgL^{-1}), sodium (mgL^{-1}) and potassium (mgL^{-1}) were also monitored by following the methods as described in APHA (2012).

Statistical analyses: Three replications (blocks) were used for each length group, treatment and fish species. The differences among various growth parameters (increase/decrease in average wet weights (g) and total lengths (mm), feed intake (g), condition factor and feed conversion ratio) of 50-, 100- and 150-mm length groups of metals mixture treated and control fish (*Channa marulius* and *Mystus seenghala*) were analyzed statistically through Factorial experiments under Randomized Complete Block Design (RCBD) by using Statistix[8.1] computer software. The means were compared for statistical differences by employing Tukey's/Student Newman-Keul tests ($p<0.05$).

RESULTS AND DISCUSSION

During the period of this experiment, it was found that all the control fish species of 50-, 100- and 150-mm length groups exhibited significantly ($p<0.05$) higher growth in terms of their average wet weights and total length increments, and feed intake as compared to the Cd+Cr+Co+Cu+Ni mixture treated fish length groups (Tables 1, 2 and 3). However, the metals mixture treated fish showed significantly better K while lower FCR than the control fish (Tables 4 and 5). In metals mixture stressed media, the lower growth rate of fish may be due to interaction among metals in the mixture causing toxicity to the fish (Hussain *et al*., 2010). Ramesha *et al*. (2003) examined the chronic effects of individual metals (Hg, Cd) and Hg+Cd mixture on the growth performance of the common carp (*Cyprinus carpio*) and concluded that single metal did not affect different stages of fish development to larger extent, but metals mixture posed more harmful effects not only on the development but also growth rate of the fish. The results of present study are similar to the findings of Giguere *et al*. (2004) who recorded that control fish, *Perca flavescens* (yellow perch) gained significantly higher wet weights and total lengths than that of metals treated fish. Syvokiene *et al*. (2003) performed an experiment to investigate the effect of Cu, Cr, Ni, Zn, Pb, Fe, Cd and Mn mixture on the rainbow trout larvae and found that the growth was significantly decreased under the stress of heavy metals mixture.

There existed age/size specific variations in the growth responses of two carnivorous fish species. The 150 mm length group of fish showed higher average wet weight and total length increments and feed intake as compared to 100 and 50 mm length groups of fish (Tables 1, 2 and 3). Among the three length groups of fish, 50 mm fish exhibited significantly higher condition factor than other length groups (Table 4). Condition factor actually represents the condition related to health and wellbeing of a fish. Hence, the current findings suggested that younger fish was in a better condition than older fish, in both metals mixture treated and control media. The 50 mm length group of fish showed significantly better feed conversion ratio (Table 5) than 100 and 150 mm fish suggesting that the fish of smaller size/length is more efficient in utilizing feed than the larger size/length fish. Ameer *et al*. (2013) found that under chronic metals (Cd, Zn and Cu) exposure, 120-day fish age group showed significantly higher wet weight increment than 90- and 60-day fish age groups. The results of present study revealed that fish of greater size exhibited more tolerance to the stress of metal contaminated water hence showed higher weight gain than the smaller sized fish. Shafiq *et al*. (2012) reported that 90-day age group of *Cirrhina mrigala* exhibited significantly higher feed intake and better feed conversion ratio than 65- and 45-day fish age groups, under sub-lethal mixed (water-borne and dietary) nickel exposure for 12 weeks. The present results are in contradiction to Abbas and Javed (2016a) who reported significantly higher condition factor values for 120-day age group of cobalt treated fish as compared to 90- and 60-day age groups.

During sub-lethal Cd+Cr+Co+Cu+Ni mixture exposure, *Channa marulius* exhibited significantly higher average wet weight and total length increments, feed intake and condition factor while lower feed conversion ratio than *Mystus seenghala*, at $p<0.05$ (Tables 1, 2, 3, 4 and 5). Such species-specific growth responses are due to the fact that different species have variable metabolic rates and capabilities to tolerate the stressful conditions caused by the xenobiotics. Abbas and Javed (2016b) also reported significantly variable growth responses of five fish species under sub-lethal metals mixture (Zn+Pb+Mn) exposure. *Ctenopharyngodon idella* showed higher average increase in fork length, followed by *Labeo rohita*, *Cirrhina mrigala*, *Catla catla* and *Hypophthalmichthys molitrix*. Ahmed *et al*. (2016) found that different fish species have variable tendencies to accumulate metal in their bodies thus altering their growth patterns. Javed (2012) found that *Catla catla* exhibited significantly higher wet weight increment than *Cirrhina mrigala* and *Labeo rohita* during sub-lethal Cu+Cd+Zn+Ni+Co mixture exposure for a period of 12

weeks. However, higher condition factor value was recorded for *C. mrigala*, followed by *L. rohita* and *C. catla*. Naz *et al*. (2013) also found statistically significant and variable growth patterns among fish species (*Hypophthalmichthys molitrix*, *Ctenopharyngodon idella*, *Cirrhina mrigala*, *Labeo rohita* and *Catla catla*) when exposed to 1/3rd of LC_{50} concentrations of Pb+Zn+Mn mixture for 84 days. During growth trials, the feed intake by all the fish species followed the order: *H. molitrix>C. idella>C. mrigala>L. rohita>C. catla* while feed conversion efficiency followed the order: *L. rohita>C. mrigala>H. molitrix>C. idella>C. catla*.

Table 1. Increase in average wet weights (g) of carnivorous fish species..

Length groups x Treatments		
Length groups	Treatments	
	Metals mixture	Control
50 mm	0.12±0.03 b	0.18±0.07 a
100 mm	0.11±0.02 b	0.29±0.14 a
150 mm	0.18±0.07 b	0.36±0.18 a
Length groups x Species		
Length groups	Species	
	Channa marulius	*Mystus seenghala*
50 mm	0.19±0.08 c	0.11±0.02 c
100 mm	0.25±0.12 b	0.15±0.05 b
150 mm	0.30±0.15 a	0.24±0.11 a
Treatments x Species		
Treatments	Species	
	Channa marulius	*Mystus seenghala*
Metals mixture	0.15±0.05 a	0.12±0.03 b
Control	0.35±0.17 a	0.21±0.09 b

Means with similar letters within a single row and *column is statistically non-significant at p<0.05.

Table 2. Increase in average total lengths (mm) of carnivorous fish species.

Length groups x Treatments		
Length groups	Treatments	
	Metals mixture	Control
50 mm	0.61±0.25 b	1.19±0.42 a
100 mm	0.92±0.35 b	1.52±0.51 a
150 mm	1.19±0.42 b	2.36±0.60 a
Length groups x Species		
Length groups	Species	
	Channa marulius	*Mystus seenghala*
50 mm	1.13±0.40 c	0.67±0.27 c
100 mm	1.61±0.54 b	0.82±0.32 b
150 mm	2.08±0.56 a	1.47±0.49 a
Treatments x Species		
Treatments	Species	
	Channa marulius	*Mystus seenghala*
Metals mixture	1.11±0.39 a	0.70±0.29 b
Control	2.11±0.57 a	1.28±0.45 b

Means with similar letters within a single row and *column is statistically non-significant at p<0.05.

Table 3. Feed intake (g) of carnivorous fish species.

Length groups x Treatments		
Length groups	Treatments	
	Metals mixture	Control
50 mm	0.20±0.08 b	0.50±0.21 a
100 mm	0.37±0.15 b	0.92±0.37 a
150 mm	0.66±0.26 b	1.36±0.50 a
Length groups x Species		
Length groups	Species	
	Channa marulius	*Mystus seenghala*
50 mm	0.49±0.20 c	0.21±0.09 c
100 mm	0.75±0.31 b	0.54±0.23 b
150 mm	1.23±0.46 a	0.79±0.33 a
Treatments x Species		
Treatments	Species	
	Channa marulius	*Mystus seenghala*
Metals mixture	0.52±0.22 a	0.30±0.13 b
Control	1.12±0.42 a	0.72±0.29 b

Means with similar letter s within a single row and *column is statistically non-significant at p<0.05

Table 4. Condition factor of carnivorous fish species.

Length groups x Treatments		
Length groups	Treatments	
	Metals mixture	Control
50 mm	2.89±0.60 a	1.68±0.47 b
100 mm	0.75±0.29 a	0.59±0.26 b
150 mm	0.38±0.19 a	0.28±0.14 b
Length groups x Species		
Length groups	Species	
	Channa marulius	*Mystus seenghala*
50 mm	2.64±0.56 a	1.94±0.51 a
100 mm	0.80±0.32 b	0.55±0.24 b
150 mm	0.39±0.20 c	0.28±0.13 c
Treatments x Species		
Treatments	Species	
	Channa marulius	*Mystus seenghala*
Metals mixture	1.62±0.45 a	1.06±0.40 b
Control	0.93±0.36 a	0.77±0.30 b

Means with similar letters within a single row and *column is statistically non-significant at p<0.05.

Table 5. Feed conversion ratio of carnivorous fish species.

Length groups x Treatments		
Length groups	Treatments	
	Metals mixture	Control
50 mm	2.28±0.43 b	2.79±0.46 a
100 mm	4.30±0.67 a	3.42±0.57 b
150 mm	4.21±0.64 a	3.79±0.60 b
Length groups x Species		
Length groups	Species	
	Channa marulius	*Mystus seenghala*
50 mm	2.92±0.49 c	2.15±0.40 c
100 mm	3.48±0.49 b	4.24±0.65 a
150 mm	4.60±0.70 a	3.40±0.56 b

Treatments x Species

Treatments	Species	
	Channa marulius	*Mystus seenghala*
Metals mixture	4.14±0.63 a	3.05±0.52 b
Control	3.20±0.54 b	3.48±0.59 a

Means with similar letters within a single row and *column is statistically non-significant at $p<0.05$.

Conclusions: It was concluded that after 120-day growth trials with fish, all the three (50, 100 and 150 mm) length groups of Cd+Cr+Co+Cu+Ni mixture treated fish showed significantly lesser growth in terms of increase in average wet weights, total lengths and feed intake as compared to the three length groups of control fish. However, the metals mixture treated fish showed significantly higher condition factor and lower FCR than the control fish. Regarding overall means, the 150 mm fish showed significantly better average wet weight and total length increments, and feed intake while lower FCR as compared to 100- and 50-mm length groups. The 50 mm fish exhibited significantly better K value than that of 100- and 150-mm length groups of fish. Among two carnivorous fish species, *Channa marulius* exhibited significantly ($p<0.01$) higher average wet weight and total length increments, feed intake and condition factor while lower FCR as compared to *Mystus seenghala*, during chronic metals mixture exposure.

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