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Assessing Growth Responses of Fish Exposed to Heavy Metals Mixture by Using Regression Analyses

Saima Naz,* Muhammad Javed and Amara Tahir
Department of Zoology and Fisheries, University of Agriculture, Faisalabad-38040, Pakistan.

Abstract.- The aim of this study was to determine the growth responses of five fish species *viz.*, *Catla catla*, *Labeo rohita, Cirrhina mrigala, Ctenopharyngodon idella* and *Hypophthalmichthys molitrix*, separately, under chronic exposure of metals mixture (Zn+Pb+Mn) at sub-lethal concentrations (1/3rd of LC_{50}) for 12 weeks. Fish were kept under constant water temperature, pH and total hardness of 29ºC, 7.25 and 225mgL^{-1}, respectively. The growth parameters *viz.* increase in average weights (g), fork and total lengths (mm), feed intakes (g), feed conversion efficiency and condition factor were monitored on daily basis during test period. The relationships among growth parameters of metals mixture stressed as well as unstressed (control) fish were computed by using regression analyses. Results showed that, among treated fish species, the weights were negatively and significantly correlated with their fork and total lengths, but for control fish species, the relationships were positive and statistically non-significant. The condition factors and feed intakes of treated and control fish species were correlated positively and significantly with fish weight escalations, showing significant dependence of weight increments on condition factor and feed intake by the fish. Negative and statistically non-significant relationships between feed conversion efficiency and the feed intakes were exhibited by all the treated and control fish species.

Key words: Feed intake, condition factor, major and chinese carps, Zn + Pb + Mn mixture, heavy metals.

INTRODUCTION

Lethal and sub-lethal effects such as changes in behaviour, reproduction, development, biochemistry and pathology cause toxicity to living organisms (Olufayo and Alade, 2012). Natural and anthropogenic sources continuously discharge heavy metals into aquatic ecosystem (Oymak *et al.*, 2009). Heavy metals represent a fundamental group of aquatic pollutants due to their long persistence, toxicity, bio-accumulative as well as non-biodegradable properties in the food chain (Uysal *et al.*, 2008; Moorthikumar and Muthulingam, 2010). There was little concern about environmental contamination during early use of metals but, in the present era, the effect of heavy metals on aquatic organisms is attracting widespread interest, predominantly in water pollution related studies (Ololade and Oginni, 2010). Fish have been used in a number of scientific research works as a tool of bio-monitoring of aquatic contaminants because fish as aquatic inhabitant cannot get rid of the lethal effects of various pollutants in natural waters (Olaifa *et al.*, 2004). Increasing population and establishment of industries in the urban areas of the Punjab (Pakistan) have resulted in the discharge of heavy metals and their compounds into the rivers. Among them, Zn, Pb and Mn are representative heavy metals that are known to affect the growth performance of fish, when present beyond the permissible limits (Raymond and Okieimen, 2011). Numerous studies on injurious effects of metals on fish deal with individual metal (Palaniappan and Karthikeyan, 2009; Godwin *et al.*, 2011), however, metals mixtures induce greater toxic effects on aquatic organisms than that of single metal (Javed, 2012).

Global aquaculture production is largely influenced by the culture of major carps as well as other cyprinids. Growth rates, intensity of feeding and feed conversion efficiency (FCE) are the foremost variables for the commercial aquaculture enterprises (Bostock, 2010). Understanding the relationships among these variables is fundamental for proper feeding of fish as the amount of the daily feed ratio, frequency and timing of the feedings as well as presentation of the predetermined ratio are the key factors of feed management strategies which directly influence the growth and feed conversion rates (Bascinar *et al.*, 2007). FCE combines feed intake (FI) and growth rate and acts as a limiting

* Corresponding author: sam_ali_3@yahoo.com
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factor to evaluate growth responses of fish (Doupe and Lymbery, 2003). FI inevitably influences the growth rates of fish, as there exists intricate relationships among feeding, growth, and other key elements of fitness (Gregory and Wood, 1999).

The application of different statistical methods has increased tremendously in recent years for analyzing data related to aquatic toxicology (Hernandez *et al*., 2005). Regression analyses are used to identify the most important variables affected by the exposure of heavy metals and their mixture as well as to study the relationships between them (Vallvey *et al*., 2006). Regression analyses also depict usefulness of statistical tools for evaluation and interpretation of large complex data sets to get better information about harmful effects of heavy metals on aquatic animals (Smolders *et al.*, 2004).

The objective of this experiment was the use of regression model as a statistical tool to identify the toxic effects of metal mixture on the growth responses of major and Chinese carps and interrelationships among their growth parameters. Fish seed collected from polluted sites of the rivers has been found to deliver stunted and variable growth. This necessitated planning a research project to help the farmers in obtaining higher fish yields that would exercise positive effects on their socio-economic conditions.

MATERIALS AND METHODS

The trials were conducted at Fisheries Research Farms, University of Agriculture, Faisalabad, beginning from 01 June 2010 to 01 September 2010. Five fibreglass experimental aquaria, each with a full volume of 70 L, were used in the experiment.

Randomized complete block design (RCBD) was followed to conduct this experiment. 90 days old juveniles of *Catla catla, Labeo rohita, Cirrhina mrigala, Ctenopharyngodon idella* and *Hypophthalmichthys molitrix* having the average weights of 3.31±1.85, 5.69±2.07, 4.52±2.01, 4.13±1.65 and 3.79±11.70g, respectively were investigated for their growth responses during chronic exposure of Zn+Pb+Mn mixture. Juveniles of these fish species were obtained from Fish Seed Hatchery, Faisalabad and kept in cemented tanks for acclimatization at experimental conditions for 2 days before the trial started. After the fish species were allocated and acclimatized, each fish was weighed as well as fork and total lengths were also measured individually. After obtaining initial weights and lengths of fish species, they were divided into two groups. One group was kept in metal free water as control while other was exposed to sub-lethal levels of Zn+Pb+Mn mixture. The trail was conducted with three replicates for each fish species under constant laboratory conditions at constant pH (7.25), water temperature (29°C) and total hardness (225 mgL^{-1}). One third of metal mixture (Zn+Pb+Mn) LC_{50} concentrations *viz*., 18.59±1.09, 21.03±1.13, 18.21±1.34, 18.84±1.68 and 17.85±1.36, mgL^{-1} (Javed and Yaqub, 2010) were used as sub-lethal levels for *C. catla, L. rohita, C. mrigala, Ctenopharyngodon idella* and *H. molitrix,* respectively. Fish were fed with commercial feed having crude protein: 30%, digestible protein: 35% and digestible energy: 2900 kcal/kg, throughout the trial. The feeding was conducted twice a day to visible satiation. Leftover feed and fecal waste were removed from all the aquaria through vacuum pumps. Daily feed consumption (feed given to fish), FCE and condition factor (CF) were recorded. In addition, all the fish were weighed individually at the end of the experiment. Total lengths of the fish were also measured to calculate CFs. From the data obtained during the trials, FI= Feed consumed per day / mean body weight x 100, FCE= weight gain/feed intake x 100 and CF= $[(W/L^3) \times 10^5]$ were determined.

Statistical analysis

The data obtained from this experiment was statistically analyzed using MICROSTAT package of the computer by following Steel *et al.* (1996). One way analysis of variance (ANOVA) and Tuckey's Student Newman-Keul test at 95% confidence interval ($p<0.05$) were also employed to compare mean values of various growth parameters as well as to find-out significance of interactions. The data on all the growth parameters were subjected to regression analyses to determine relationships among them by using the equation y= a + bx.

RESULTS AND DISCUSSION

Growth responses of fish species

Exposure of Zn+Pb+Mn mixture caused minimum growth in all the five fish species than that of control. Chronic exposure of Zn+Pb+Mn mixture to *Catla catla, Labeo rohita, Cirrhina mrigala, Ctenopharyngodon idella* and *Hypophthalmichthys molitrix* gave significantly ($p<0.05$) lower average weights of 0.94, 0.93, 0.94, 0.94 and 1.03g, than that of unstressed fish with the average increments of 1.90, 2.25, 2.22, 1.89 and 2.16 g, respectively (Table I). Among metal mixture exposed fish, *Hypophthalmichthys molitrix* had significantly higher feed intake of 2.19 g, followed by that of *Ctenopharyngodon idella, Cirrhina mrigala, Labeo rohita* and *Catla catla* with the average FIs of 2.04, 1.98, 1.98 and 1.85 g respectively. Metal mixture treatments also exerted significant impacts on both CF and FCE with the mean values of 2.33 and 111.58 % observed for *Cirrhina mrigala* and *Labeo rohita,* respectively (Table II).

The toxicity exerted on fish due to effects of metal mixtures varied significantly (Vosyliene and Jankaite, 2006; Bu-Olayan and Thomas, 2008). Adverse impacts on fish growth and health were induced by sub-lethal concentrations of metal mixture as evident from fish CF (Table II). Significantly variable responses toward FIs, FCE, weight and length increments were exhibited by all the five fish species due to significant impacts on their growth induced by the exposure of metal mixture (Naeem *et al.,* 2011). Various studies of the effect of metals on fish deal with exposure to a single metal. However, contaminated water bodies usually contain escalated levels of metals in a mixture form (Jabeen *et al.,* 2012). Therefore, it is quite difficult to compare the results obtained for exposure to a single metal under laboratory conditions with those from natural conditions. It is necessary to compare the effects of metals in mixture forms, while explaining interactions among various metals that may induce additive, antagonistic or synergistic effects (Marr *et al.,* 1998). Significant effects on fish appetite caused by stress of metal mixture resulted in to significant change in FCE of fish species (Hayat *et al.,* 2007). Significant changes in the FI, body ion regulation and growth have also been reported in rainbow trout exposed to Zn, Cu and Cd (McGeer *et al.,* 2000).

Relationships among growth parameters

Knowledge of quantitative parameters such as condition factor, growth, weight-length relationship, recruitment and mortality of fishes is an imperative tool for the study of fishing biology, particularly when the species lies at the bottom of the higher food web (Lizama and Ambrosio, 2002). Relationships among growth parameters help fisheries scientists to convert growth in- length equations to growth-in-weight in models of stock assessment (Morato *et al.,* 2001; Stergiou and Moutopoulos, 2001), compare life history and morphological features of populations residing in different regions (Stergiou and Moutopoulos, 2001), biomass estimation from length frequency distributions (Petrakis and Stergiou, 1995; Dulcic and Kraljevic, 1996) as well as determining fish condition (Petrakis and Stergiou, 1995).

The high values of R^2 (coefficient of determination) for each regression equation predicted higher reliability of these regression models. In the present investigation, among the treated fish species, the weights were negatively and significantly correlated with their fork and total lengths, but for control fish species, the relationships were positive and statistically non-significant (Table III). These results are in accordance with the findings of Sasi (2008). His results showed significant correlations between fork and total lengths versus fecundity. The relationships for fork and total length with fecundity showed correlation coefficients of 0.358 and 0.630, respectively. Length-weight relationships of five fish species were estimated by Fafioye and Oluajo (2005) in Epe Lagoon, Lagos. They calculated 95% confidence interval of length for about 76.19% of the fish species while length-weight relationships of 35 fish species were estimated by Agboola and Anetekhai (2008) from Badagry Creek, Lagos.

The information about CF is necessary for understanding the life cycle of fish species as well as it also contributes to adequate management of fish species that assists in maintaining equilibrium in the ecosystem (Lizama and Ambrosio, 2002). In

Table I.- **Analysis of variance on wet weights (g), fork length and total length (mm) of five fish species.**

S. O. V	D.F	Mean squares		
		Average weight (g)	Average fork length (mm)	Average total length (mm)
Weeks	11	1.119 $^{p<0.05}$	0.614 $^{p<0.05}$	0.655 $^{p<0.05}$
Species	4	0.647 $^{p<0.05}$	1.870 $^{p<0.01}$	0.935 $^{p<0.01}$
Treatment	1	0.062 $^{p<0.01}$	3.881 $^{p<0.05}$	4.157 $^{p<0.05}$
Species x Treatment	4	0.538 $^{p<0.05}$	2.113 $^{p<0.05}$	2.493 $^{p<0.05}$
Error	238	0.014	0.093	0.121

Comparison of means

Week #	Average weight (g)	Average fork length (mm)	Average total length (mm)
1	1.14 ± 0.01 **g**	0.85 ± 0.03 **fg**	0.86 ± 0.08 **f**
2	1.30 ± 0.02 **f**	1.22 ± 0.04 **b**	1.33 ± 0.03 **a**
3	1.63 ± 0.07 **c**	1.18 ± 0.04 **c**	1.29 ± 0.06 **ab**
4	1.70 ± 0.07 **b**	1.06 ± 0.02 **ef**	1.02 ± 0.06 **e**
5	1.38 ± 0.05 **ef**	1.09 ± 0.07 **d**	1.31 ± 0.04 **a**
6	1.33 ± 0.01 **f**	1.23 ± 0.04 **b**	0.10 ± 0.06 **d**
7	1.62 ± 0.06 **c**	1.19 ± 0.05 **c**	1.32 ± 0.03 **a**
8	1.59 ± 0.02 **d**	0.98 ± 0.08 **f**	1.15 ± 0.08 **cd**
9	1.49 ± 0.01 **e**	1.32 ± 0.07 **a**	1.09 ± 0.07 **d**
10	1.59 ± 0.06 **d**	0.10 ± 0.09 **g**	1.17 ± 0.08 **c**
11	1.68 ± 0.06 **bc**	1.10 ± 0.09 **d**	1.16 ± 0.04 **c**
12	1.79 ± 0.03 **a**	0.89 ± 0.07 **fg**	1.22 ± 0.07 **b**

Species	Average weight (g)	Average fork length (mm)	Average total length (mm)
Catla catla	1.42 ± 0.08 **b**	1.15 ± 0.06 **b**	1.19 ± 0.04 **b**
Labeo rohita	1.59 ± 0.08 **a**	1.33 ± 0.05 **a**	1.33 ± 0.03 **a**
Cirrhina mrigala	1.58 ± 0.09 **a**	1.05 ± 0.08 **c**	1.16 ± 0.02 **c**
Ctenopharyngodon idella	1.41 ± 0.06 **b**	0.95 ± 0.09 **d**	1.11 ± 0.02 **d**
Hypophthalmichthys molitrix	1.59 ± 0.05 **a**	0.94 ± 0.03 **d**	1.02 ± 0.01 **e**
Treatment			
Treated	0.96 ± 0.06 **b**	0.98 ± 0.07 **b**	1.05 ± 0.03 **b**
Control	2.08 ± 0.07 **a**	1.19 ± 0.05 **a**	1.27 ± 0.05 **a**

Means with different letters in a column differ significantly (p<0.05) from each other

Species x treatment

Species	Treatments					
	Average weight (g)		Average fork length (mm)		Average total length (mm)	
	Treated	Control	Treated	Control	Treated	Control
Catla catla	0.94 **b**	1.90 **a**	0.93 **b**	1.38 **a**	0.99 **b**	1.40 **a**
Labeo rohita	0.93 **b**	2.25 **a**	1.01 **b**	1.66 **a**	0.98 **b**	1.67 **a**
Cirrhina mrigala	0.94 **b**	2.22 **a**	1.16 **b**	1.95 **a**	1.02 **b**	1.29 **a**
Ctenopharyngodon idella	0.94 **b**	1.89 **a**	0.91 **b**	0.99 **a**	1.23 **b**	1.98 **a**
Hypophthalmichthys molitrix	1.03 **b**	2.16 **a**	0.91 **b**	0.98 **a**	1.04 **b**	2.10 **a**

Means with different letters in a row under each growth parameters differ significantly (p<0.05) from each other

Table II.- **Analysis of variance on feed intake (g), condition factor and feed conversion efficiency (%) of five fish species.**

S. O. V	D.F	Mean squares		
		Feed intake (g)	Condition factor	Feed conversion efficiency (%)
Weeks	11	0.971 $^{p<0.05}$	2.721 $^{p<0.05}$	5132.773 $^{p<0.05}$
Species	4	1.123 $^{p<0.05}$	0.696 $^{p<0.05}$	15598.081 $^{p<0.05}$
Treatment	1	0.000 $^{p<0.05}$	130.129 $^{p<0.05}$	251538.968 $^{p<0.05}$
Species x Treatment	4	1.351 $^{p<0.05}$	0.720 $^{p<0.05}$	18807.152 $^{p<0.05}$
Error	238	0.609	0.732	71.498

Comparison of means

Weeks#	Feed intake (g)	Condition factor	Feed conversion efficiency (%)
1	1.87 ± 0.05 **g**	1.79 ±0.07 **j**	101.68 ± 0.09 **c**
2	1.75 ± 0.09 **h**	1.86 ± 0.06 **i**	104.95 ± 0.08 **c**
3	1.80 ± 0.08 **i**	1.81 ± 0.05 **j**	120.55 ± 0.06 **a**
4	1.76 ± 0.09 **h**	2.14 ± 0.09 **h**	117.11 ± 0.05 **b**
5	2.07 ± 0.06 **f**	2.24 ± 0.07 **e**	85.26 ± 0.04 **f**
6	1.91 ± 0.05 **g**	2.19 ± 0.06 **g**	83.05 ± 0.04 **fg**
7	2.20 ± 0.05 **b**	2.19 ± 0.05 **g**	94.87 ± 0.08 **de**
8	2.09 ± 0.03 **f**	2.40 ± 0.04 **f**	99.40 ± 0.04 **d**
9	2.13 ± 0.03 **d**	2.29 ± 0.08 **e**	77.67 ± 0.03 **g**
10	2.23 ± 0.02 **a**	2.50 ± 0.08 **c**	97.28 ± 0.08 **d**
11	2.12 ± 0.01 **e**	2.65 ± 0.09 **b**	87.03 ± 0.07 **f**
12	2.18 ± 0.05 **c**	2.68 ± 0.07 **a**	92.47 ± 0.06 **e**

Species	Feed intake (g)	Condition factor	Feed conversion efficiency (%)
Catla catla	1.85 ± 0.07 **d**	2.24 ±0.09 **c**	10.87 ± 0.07 **e**
Labeo rohita	1.98 ± 0.09 **c**	2.27 ± 0.07 **b**	111.58 ± 0.08 **a**
Cirrhina mrigala	1.98 ± 0.08 **c**	2.33 ± 0.05 **a**	105.10 ± 0.06 **b**
Ctenopharyngodon idella	2.04 ± 0.07 **b**	2.24 ± 0.04 **c**	73.58 ± 0.06 **d**
Hypophthalmichthys molitrix	2.19 ± 0.05 **a**	2.07 ± 0.03 **d**	91.93 ± 0.04 **c**
Treatment			
Treated	2.01 ± 0.05 **a**	1,63 ± 0.06 **b**	70.17 ± 0.06 **b**
Control	2.01 ± 0.06 **a**	2.83 ± 0.04 **a**	123.04 ± 0.07**a**

Means with different letters in a column differ significantly (p<0.05) from each other

Species x treatment

Species	Treatments					
	Feed intake (g)		Condition factor		Feed conversion efficiency (%)	
	Treated	Control	Treated	Control	Treated	Control
Catla catla	1.79 **b**	1.91 **a**	1.72 **b**	2.76 **a**	96.79 **b**	104.95 **a**
Labeo rohita	1.83 **b**	2.12 **a**	1.61 **b**	2.94 **a**	91.84 **b**	131.31 **a**
Cirrhina mrigala	1.19 **b**	1.76 **a**	1.67 **b**	2.98 **a**	59.31 **b**	150.89 **a**
Ctenopharyngodon idella	1.99 **b**	2.10 **a**	1.55 **b**	2.94 **a**	48.58 **b**	98.58 **a**
Hypophthalmichthys molitrix	2.23 **b**	2.15 **a**	1.60 **b**	2.53 **a**	54.36 **b**	129.49 **a**

Means with different letters in a row under each growth parameter differ significantly (p<0.05) from each other

Table III.- Regression equations for relationships between different growth parameters of treated and control fish.

	Regression Equation (y = a + bx)	r	R^2
Control			
	Increase in weight (g) = 2.58 + 0.008 ^{NS} fork length (mm) (0.97)	0.284	0.198
	Increase in weight (g) = 2.53 + 0.047 $^{p<0.05}$ total length (mm) (10.08)	0.956	0.986
	Increase in weight (g) = 2.01 + 0.288 $^{p<0.01}$ feed intake (g) (3.01)	0.991	0.990
	Feed conversion efficiency = 84.06 + 15.057 $^{p<0.01}$ increase in weight (g) (9.40)	0.954	0.999
	Feed conversion efficiency = 2.54 – 0.004 ^{NS} feed intake (g) (5.52)	0.301	0.097
	Condition factor = 1.68 + 0.575 $^{p<0.01}$ feed intake (g) (1.20)	0.983	0.999
	Condition factor =1.81 + 0.396 $^{p<0.01}$ increase in weight (g) (3.45)	0.997	0.974
Treated	Increase in weight (g) = 1.36 – 0.090 $^{p<0.05}$ fork length (mm) (5.04)	0.984	0.998
	Increase in weight (g) = 1.48 - 0.198 $^{p<0.05}$ total length (mm) (7.08)	0.976	0.986
	Increase in weight (g) = 1.71 + 0.280 $^{p<0.01}$ feed intake (g) (0.01)	0.992	0.990
	Feed conversion efficiency = 51.77 + 14.48 $^{p<0.01}$ increase in weight (g) (5.01)	0.954	0.999
	Feed conversion efficiency = 79.25 – 4.522 ^{NS} feed intake (g) (0.10)	0.246	0.167
	Condition factor = 1.07 + 0.278 $^{p<0.01}$ feed intake (g) (2.01)	0.943	0.989
	Condition factor = 1.18 + 0.335 $^{p<0.01}$ increase in weight (g) (0.81)	0.987	0.954

(Values within brackets show standard errors)

r = Correlation coefficient
R^2 = Coefficient of determination

the present experiment, the CFs and FIs of treated and control fish species were correlated positively and significantly with fish weight escalations, showing significant dependence of weight increments on CF and FI by the fish. Moreover, CFs of both treated and control fish species were directly and significantly dependant upon the trends of fish to gain weight and the amount taken up by the fish (FI) during the whole study period (Table III). These correlations show similarity with the findings of Jabeen *et al.* (2004). CFs of different tropical fish species were investigated and reported by Saliu (2001) and Lizama *et al.* (2002). In the present investigation, negative and statistically non-significant relationships between FCE and the FIs were exhibited by all the treated and control fish species (Table III). Jabeen *et al.* (2004) reported significantly positive correlation between FCE and average body weight of *Cirrhinus mrigala* in case of fish meal (0.672), cotton seed meal (0.598), while for barely (0.571) the relationship was non significant. The correlations between FI and FCE recorded in the present study are similar to the results given by Faturoti (1989), who investigated that the FI, FCE and protein intake were positively correlated with average gain in fish weight. Minimum feeding frequency increased coefficients of variation for weight in sunfish (Wang *et al.*, 1998) and rainbow trout (Bascinar *et al.*, 2001), but no significant differences were recorded between feeding frequency treatments in Arctic charr (Petursdottir, 2002) and halibut (Schnaittacher *et al.*, 2005).

CONCLUSIONS

The growth parameters *viz.*, increase in average weights (g), fork and total lengths (mm), (FCE), condition factor (CF) and feed intakes (FIs) (g) were significantly better in unstressed (control) fish species. The regression analyses showed that, among treated fish species, the weights were negatively and significantly correlated with their fork and total lengths, but for control fish species, the relationships were positive and statistically non-significant. The CFs and FIs of treated and control fish species were correlated positively and significantly with fish weight escalations, showing significant dependence of weight increments on CF and FI by the fish. Negative and statistically non-significant relationships between FCE and the FIs were exhibited by all the treated and control fish species.

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