INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
11–701/ZIP/2012/14–2–281–285
http://www.fspublishers.org

Full Length Article

Growth Responses of Fish under Chronic Exposure of Waterborne and Dietary Metals

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ABSTRACT

Three fish species viz. *Catla catla, Labeo rohita* and *Cirrhina mrigala* were exposed, separately to waterborne and dietary sub-lethal concentrations (1/3 of LC_{50}) of copper (Cu), cadmium (Cd) and zinc (Zn) for 12 weeks to monitor their growth responses. During initial hours of treatments, all the three fish species showed hyperactivity and reduced exploratory behavior, which was significantly more pronounced in the fish exposed to waterborne than dietary metals. All the three fish species showed significantly variable responses toward feed intakes, weight gains and FCE under both waterborne and dietary exposure of metals. However, the fish grown under metal free water (control) exhibited significantly better growth. Among treatments, dietary Zn caused significantly higher weight gains, while that of Cu attributed significantly lower weights in fish. Feed intakes in all the three fish species dropped significantly due to waterborne exposures with the lowest being observed due to Cu and Zn. Fish appetite increased significantly due to dietary exposure of metals. However, higher feed intakes did not product into better FCE or weight increments in comparison to the control fish. Among the three treated fish species, *C. catla* exhibited significantly better FCE, followed by that of *C. mrigala and L. rohita*. Fish growth showed strong relationships with condition factor and feed intakes under chronic exposure of metals. Among the three treated fish species, *C. catla* exhibited significantly better FCE, followed by that of *C. mrigala and L. rohita*. Therefore, toxicity of different metals did not only influence the fish appetite but acclimation and FCE also that ultimately affected the fish growth.

Key Words: Fish; Growth; Copper; Cadmium; Zinc

INTRODUCTION

Water is the primary exposure route of metallic toxicity to the aquatic organisms including fish. However, toxicity originating from contaminated diets appears to be another pathway in establishing toxicity of metals to the fish. The environmental criteria and other risk assessments associated with metallic ion toxicity have been focused on waterborne toxicity, while some research efforts have indicated that dietary exposure of various metals can be imperative for different fish species and exposure variables (Kim & Kang, 2004; Hoyle *et al*., 2007; Hashemi *et al*., 2008; Naeem *et al*., 2011). In an aquatic environment, fish are directly allied with their inherent physiological ability to cope-up with the contaminated water. Metals are unique in their characteristics from the other contaminants due to their non bio-degradability in the environment. Various metals can cause adverse effects on the immune system of fish leading to growth reduction, enhanced vulnerability to diseases, mortality (More *et al.,* 2003) and reduction in fish reproduction capability (Meyer *et al*., 2005). Heavy metals can be taken up by the aquatic organisms through several routes i.e., via body surface, gills, contaminated feed or may be by a combination of both water-borne and dietary exposures. However, which route is more important would depend upon the prevailing environmental conditions (Depledge *et al*., 1994). Dietary uptake of metals is considered a main cause of long-term contamination in a wild fish (Dallinger *et al*., 1987) and hence increased the interest of toxicologists in the nutritional aspects of toxicity in fish (Handy, 1996). Ascertaining the impacts of dietary metals on fish in the laboratory would also be similar to what would occur in natural water systems containing contaminated natural diets. Biological methods are practiced worldwide for appraisal of toxic effects of individual metals, their mixtures and sewage on various test-organisms to ascertain their physiological sensitivity, behavioral patterns and morphological indices. In nature, the organisms are mostly affected by long-term influence of low concentration of metals. In laboratory tests, chronic studies of sub-lethal toxicity are applied in these cases (Kazlauskiene *et al*., 2003) and evaluated practical changes in fish physiology and ultimately affecting the fish growth by long-term chronic exposures. This facilitates the evaluation of sub-lethal concentrations on the growth, behavior, physiology and biology of organisms to forecast their adaptive capabilities to cope-up with and the toxicity of metals (Galvez *et al*., 1998).

To cite this paper: Javed, M., 2012. Growth responses of fish under chronic exposures of waterborne and dietary metals. *Int. J. Agric. Biol.*, 14: 281–285

Fish requires Cu and Zn as micronutrients (Watanabe *et al*., 1997) and can obtain these metals from water and diet (Wood, 2001). However, their higher intakes could cause deleterious effects on fish growth (Hayat *et al*., 2007). Cu is beneficial at low levels, however, may also be potentially toxic at elevated concentrations (Moore & Ramamoorthy, 1984). Cadmium is highly toxic metal (Roesijadi & Unger, 1993) that can influence many physiological processes like secretion function of the pituitary gland in fish (Pundir & Saxena, 1992). The rivers in Pakistan have been affected badly due to dumping of industrial wastes containing high toxicity of Cu, Cd and Zn that is affecting the indigenous fish fauna. Therefore, in order to conserve these indigenous major carps viz. *C. catla L. rohita* and *C. mrigala* in the natural aquatic habitats, it is imperative to determine their growth potentials under chronic effects of water-borne and dietary metals to predict possible impacts of persistent metal's pollution on fish.

MATERIALS AND METHODS

Juvenile major carps viz. *C. catla, L. rohita* and *C. mrigala* were acclimatized to laboratory conditions in clean tap water for two weeks. After acclimatization, groups (n=10) of three fish species were grown, separately, under water-borne and dietary sub-lethal concentrations of Cu, Cd and Zn for 12 weeks with three replications for each growth trail. Pure chloride compounds (Aldrich, USA) of Cu ($CuCl_2$. $2H_2O$), Cd ($CdCl_2$. H_2O) and Zn ($ZnCl_2$) were used to prepare the stock solutions and further dilution in de-ionized water up to the desired sub-lethal concentrations for three fish species. The average hardness of aquarium water was maintained at 200±1.00 mg L^{-1} as $CaCO_3$ at pH 7±0.05 and temperature of 30±0.05°C. Ten fish of each species, with three replications, were grown separately under the following sub-lethal (1/3 of LC_{50}) waterborne and dietary concentrations of Cu, Cd and Zn, as determined by Javed *et al*. (2008), are given in Table I:

Table I: Heavy metal concentrations in water and diet of three fish species

Metal	Fish species	Water-borne Concentrations (mg L^{-1})	Dietary Concentrations (µg g^{-1})
Copper	*Catla catla*	19.44	57.06
	Labeo rohita	24.24	60.53
	Cirrhina mrigala	20.07	58.56
Cadmium	*Catla catla*	51.69	57.74
	Labeo rohita	51.08	60.69
	Cirrhina mrigala	51.47	56.25
Zinc	*Catla catla*	17.32	63.94
	Labeo rohita	28.48	74.41
	Cirrhina mrigala	25.84	65.92

Waterborne toxicity growth trials: Three fish species were grown, separately, in glass aquaria which were filled with 50 L de-chlorinated tap water of desired sub-lethal concentration of metals. However, control fish were grown under metal free environment for comparison. At the beginning and end of each test, water samples were taken and analyzed for the corresponding metal concentrations following SMEWW (1989) through Atomic Absorption Spectrophotometer (Analyst 400 Perkin Elmer, USA). The fish were fed on crumbled feed (35% DP and 2.90 Kcal g^{-1} DE), to satiation, once daily for 12 weeks.

Diet-borne toxicity growth trails: The solutions were prepared in acidified water containing desired sub-lethal concentration of each metal viz. Cu, Cd and Zn, separately, and mixed well with the ingredients and pellets of desired 35% DP and 2.90 Kcal g^{-1} DE prepared by using a 2 mm diameter module. After preparation, all the diets were packed in plastic bags and stored at -20°C. Fish were fed to satiation twice a day. Ten fish of each species were grown in glass aquaria, with three replications, containing clean ground water and fed the diets containing the required sub-lethal dietary metals viz Cu, Cd and Zn, separately, for 12 weeks. However, control fish were fed with metal free diet.

The growth performance of each species, exposed to waterborne and dietary metals, were monitored in terms of increase in wet weights, condition factor, feed intake and FCE on weekly basis for 12 weeks. The results of these experiments are expressed as means ± SD. The raw data were verified for normality of distribution and homogeneity of variances. Analysis of variance and comparison of mean values were performed to determine statistical differences among various parameters under study (Steel *et al*., 1996). Relationships among various parameters were also determined by computing Pearson correlation coefficients.

RESULTS AND DISCUSSION

All the three fish species, grown under sub-lethal toxicity of metals, showed hyperactivity and reduced exploratory behavior. However, these behavioral responses were significantly more pronounced in the fish exposed to waterborne than dietary metal treatments. No fish mortality occurred during the whole experimental period. Metals are known to excite the fish activity as physical irritant to a potentially wide assortment of external tissues, causing increase in metabolic rate (Scarfe *et al.,* 1982). All the three fish species responded immediately to the exposure of water-borne metals showing hypersensitivity and reduced exploratory behavior (Steele, 1983; Koltes, 1985). The exposure of fish to Cu, Cd and Zn exerted significant impact on the wet weight increments of all the three fish species (Meyer *et al*., 2005). However, the fish grown under metal free water (control) exhibited significantly better growth. Waterborne Cu, Cd and Zn treatments caused significantly ($p<0.05$) lesser weight gains of 6.53±0.03, 4.94±0.04 and 3.74±0.03 g in *C. catla, L. rohita* and *C. mrigala,* respectively (Table II). Overall performance of three fish species was significantly lower under waterborne treatments. However, *C. catla* attained significantly better

Table II: Growth performance of fish under chronic stress of metals

Fish Species	Waterborne Exposure			Dietary Exposure			
	Cu	Cd	Zn	Cu	Cd	Zn	Control
Gain in Weight(g)							
Catla catla	6.53±0.03 f	7.85±0.01 e	6.05±0.03 f	16.53±0.02 d	20.95±0.09 c	26.90±0.04 b	33.75±0.02 a
Labeo rohita	5.31±0.05 e	4.94±0.04 f	3.42±0.01 g	17.69±0.06 d	21.95±0.02 c	23.87±0.06 b	32.39±0.03 a
Cirrhina mrigala	7.02±0.04 f	8.08±0.03 e	3.74±0.03 g	22.37±0.03 b	18.57±0.03 d	20.43±0.07 c	43.06±0.03 a
Overall Means	**6.29±0.88 e**	**6.96±1.75 e**	**4.40±1.44 f**	**18.86±3.09 d**	**20.49±1.74 c**	**23.73±3.24 b**	**36.40±5.81 a**
Condition Factor (K)							
Catla catla	1.03±0.01 d	1.81±0.01 c	1.03±0.01 d	1.93±0.01 c	2.62±0.01 b	3.07±0.01 a	2.84±0.01 a
Labeo rohita	0.34±0.01 d	1.91±0.01 c	1.85±0.01 c	2.46±0.01 b	2.87±0.01 b	3.31±0.01 a	3.14±0.01 a
Cirrhina mrigala	0.92±0.01 c	2.02±0.01 b	1.01±0.01 c	1.78±0.01 b	2.87±0.01 a	2.67±0.01 a	2.98±0.01 a
Overall Means	**0.76±0.37 f**	**1.91±0.11 d**	**1.30±0.48 e**	**2.06±0.36 e**	**2.79±0.14 b**	**3.02±0.32 a**	**2.99±0.15 a**
Feed Intake (g)							
Catla catla	8.67±0.02 g	10.57±0.08 e	9.90±0.03 f	18.80±0.03 c	24.16±0.03 b	27.89±0.03 a	15.40±0.02 d
Labeo rohita	9.47±0.03 f	10.23±0.03 e	11.59±0.04 d	20.47±0.03 b	28.39±0.06 a	28.33±0.03 a	15.79±0.03 c
Cirrhina mrigala	11.54±0.04 d	12.08±0.09 d	7.00±0.04 e	25.90±0.02 b	27.56±0.03 a	26.63±0.02 b	15.57±0.02 c
Overall Means	**9.89±1.48 f**	**10.96±0.98 e**	**9.45±2.32 f**	**21.72±3.71 c**	**26.70±2.24 b**	**27.62±0.88 a**	**15.59±0.19 d**
FCE (%)							
Catla catla	75.31±0.02 d	74.27±0.09 d	61.11±0.06 e	87.92±0.05 c	86.71±0.06 c	96.45±0.03 b	219.15±0.03 a
Labeo rohita	56.07±0.03 e	48.29±0.03 f	29.51±0.07 g	86.42±0.07 b	77.31±0.03 d	84.25±0.07 c	205.51±0.07 a
Cirrhina mrigala	60.83±0.02 e	66.89±0.02 d	53.43±0.05 f	88.37±0.08 b	67.38±0.07 d	76.72±0.08 c	276.55±0.09 a
Overall Means	**64.07±9.02 d**	**63.15±8.39 d**	**48.02±9.48 e**	**87.57±1.02 b**	**77.13±9.67 c**	**85.81±9.96 b**	**233.74±37.70 a**

Condition factor (K) = W × 10^5 ÷ L^3 where W = Wet fish weight (g); L = Wet fork length (mm)

FCE= gain in weight (g) /feed intake (g) x 100; Mans with similar letters in a single row are statistically non-significant at $p<0.05$

Table III: Relationships among fish growth parameters

Metal		*Catla catla*			*Lebeo rohita*			*Cirrhina mrigala*		
		Increase in weight	Feed Intake	FCR	Increase in weight	Feed Intake	FCR	Increase in weight	Feed intake	FCR
Waterborne Exposure										
	Feed Intake	0.67666	-	-	0.81230	-	-	0.66311	-	-
	FCE	0.45091	0.32092	-	0.01592	0.43841	-	0.64491	0.37573	-
Cu	Condition Factor	-0.70001	-0.48068	0.24321	0.39917	-0.45045	0.13787	0.26806	0.24648	-0.02360
	Feed Intake	-0.33467	-	-	0.26859	-	-	-0.11715	-	-
	FCE	0.62506	-0.11605	-	0.46005	0.26309	-	0.69561	-0.10244	-
Cd	Condition Factor	0.73593	0.06563	0.74384	0.50649	0.48429	0.76216	0.60416	-0.44290	0.46249
	Feed Intake	0.54262	-	-	0.02303	-	-	0.70369	-	-
	FCE	0.40073	-0.13753	-	0.03724	0.37935	-	0.72259	0.46279	-
Zn	Condition Factor	0.88669	0.52868	0.14256	0.58640	-0.53116	-0.34509	0.85333	0.72774	0.70397
Dietary Exposure										
	Feed intake	0.57277	-	-	0.68085	-	-	0.73374	-	-
	FCE	0.59308	-0.04972	-	0.57855	0.07844	-	0.03748	0.67064	-
Cu	Condition Factor	0.80210	0.10127	-0.08122	0.43789	0.40742	0.36996	0.14920	-0.10780	0.35192
	Feed Intake	-0.11478	-	-	0.01569	-	-	0.21502	-	-
	FCE	0.83903	0.29828	-	0.85318	0.37920	-	0.85855	0.10512	-
Cd	Condition Factor	0.69354	-0.38203	-0.65137	0.86708	-0.06680	0.82164	0.55434	-0.08686	0.77936
	Feed intake	-0.07400	-	-	0.23748	-	-	0.17053	-	-
	FCE	0.77898	0.53678	-	0.91644	-0.11850	-	0.93328	0.01844	-
Zn	Condition Factor	0.68654	-0.46570	-0.87508	0.76505	0.02322	0.88659	0.24128	0.18247	0.21661

Critical value (2 tail, 0.05 = + or – 0.57400)

weight gains than the other two fish species. Dietary Zn treatment caused significantly higher weight gains while that of Cu attributed significantly lower weights in fish. Waterborne and dietary exposure of metals exerted significant impact on the condition factor of all the three fish species also. Ali *et al.* (2003) reported significant impacts of different sub-lethal concentrations of water-borne Cu on the behavioral response and growth performance of *Oreochromis niloticus*. Heinsbroek *et al.* (2007) reported significant influence of dietary cobalt on the growth and feed conversion efficiency of eels.

The effects of metal's toxicity on the fish are dependant upon concentrations and their types (Kazlauskiene & Burba, 1997; Vosyliene *et al*., 2003; Hussain *et al.*, 2011). Mixtures of Cu+Zn+Cr+Ni+Fe have also been reported to cause adverse effects on the growth of rainbow trout (Kazlauskiene & Stasiunaite, 1999). *L. rohita* showed significantly higher sensitivity towards metallic toxicity, followed by that of *C. catla* and *C. mrigala* with significant ($p<0.05$) differences among them for their weight escalations. Exposure of waterborne metals caused significantly lower weight gains in all the three fish species than dietary metals (Table II). Feed intakes in fish were significantly ($p<0.05$) higher due to dietary than waterborne treatments. However, this increased feed intake, due to dietary exposure of metals, did not product into significantly better FCE in comparison with control fish. All the dietary treatments caused significantly better FCE to the fish than that of waterborne treatments. Among the three treated fish species, *C. catla* exhibited significantly better FCE, followed by that of *C. mrigal and L. rohita* (Table II). Fish increments showed significantly positive relationship with feed intake and condition factor under both waterborne and dietary treatments (Table III). Heavy metals are known to change the feeding patterns of fish (James *et al*., 2003). The growth of fish is commonly used as a receptive and unswerving end point in chronic indication of toxic effects of a number of different biochemical and physiological processes, which are more revealing to assess the effects on specific processes associated with bioenergetics, such as feeding, assimilation, excretion and metabolism (Bhavan & Geraldine, 2000). Subathra and Karuppasamy (2007) reported reduction in feeding parameters that decreased with increasing concentration and period of Cu exposure to the fish, *Mystus vittatus*. Reduced feeding activity has also been reported in common carp (DeBoeck *et al*., 1997) and *Perca flaviatilis* (Collvin, 1985) due to metallic toxicity.

The relationship between weight increment and condition factor of *C. catla* appeared significantly positive under all the waterborne and dietary treatments. In *L. rohita,* weight increments were positively correlated with feed intake and FCE while these relationships were significant ($p<0.05$) under dietary treatments only. *C. mrigala* exhibited significantly positive correlation of their weight increments with feed intake, FCE and condition factor under both waterborne and dietary treatments except for feed intake under waterborne Cu. Feed intake of fish showed positively significant correlation with condition factor of fish under waterborne Zn only, while the same with FCR was positively significant under waterborne Zn and dietary Cd treatments (Table III). Vincent *et al*. (1996) reported decreased feed conversion ratio in *C. catla* with increasing waterborne Cr concentrations. High levels of feed ingestion were suggestive of reduction in assimilated energy that ultimately product in terms of reduced fish weight. Ali *et al.* (2003) reported significant effect of sub-lethal water-borne Cu concentrations on the feed consumption, weight escalations, specific growth rate, feed conversion ratios and condition factor of *O. niloticus*. Dietary metal exposures to the fish resulted into significantly better condition factor than that exhibited due to exposure of waterborne metals. Vosyliene *et al.* (2003) reported toxic effects of metals on the fish that exerted significant impact on their feed intakes (De Boeck *et al.,* 1997). Hansen *et al*. (2002) observed variable growth in *Oncorhynchus mykiss* at various concentrations of waterborne Cu. The response of *L. rohita* towards condition factor revealed better feed intake that did not result into significantly higher weight gains that were significantly lesser than that of both *C. catla* and *C. mrigala*. Similarly, Ali *et al*. (2003) observed significant variations in specific growth rate and condition factor of *O. niloticus* grown under different sub-lethal concentration of metals. Sherwood *et al*. (2000) observed adverse effects of Zn, Cd and Cu on the growth performance, feed intake and feed conversion efficiency of yellow perch.

Acknowledgement: This research work was financially supported by the Higher Education Commission, Islamabad under the project # 803.

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(Received 16 December 2011; Accepted 27 January 2012)