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Effect of Body Size on Metal Concentrations in Farmed *Cirrhinus mrigala*

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Abstract

In this study, metals such as zinc, iron, copper, manganese, sodium, potassium and magnesium in freshwater *Cirrhinus mrigala* (whole fish) have been analyzed and their relationships with fish length and weight have been derived. Mean concentrations ($mg \cdot g^{-1}$ dry weight) of the metals in carcasses of farmed *C. mrigala* have been found to be 65.7 (Zn), 50.3 (Mn), 538 (Fe), 24.1 (Cu), 1182 (Na), 9850 (K) and 2155 (Mg). Lead and nickel concentrations were below the limit of detection of the technique. All the metals show isometric relationships with increasing body weight of *C. mrigala*. Sodium indicates positive allometry whereas zinc shows negative allometry with increasing body length. However, copper, iron, potassium, magnesium and manganese show isometric relationships with increasing body length.

Introduction

Metals are natural constituents of the freshwater environment. Some metals are essential for life, some are merely beneficial and many are highly toxic. The concentrations at which metals may be considered important vary as some are essential at low concentration levels yet toxic at others. Human activity has increased the levels of metals in many of the natural water systems, which has raised concerns regarding metal bioaccumulation and human health hazards. With increasing public concern regarding environmental contamination, there is a growing need to monitor, evaluate, manage and remediate ecological damage (Kushlan 1993). In assessing the changing conditions within an ecosystem, measuring all components, functions, properties or values is impossible. Instead, selected biological components such as fish can serve as bioindicators of the wider conditions of water quality.

The use of fish as bioindicator of metals to study the pollution of the aquatic ecosystem due to toxic heavy metals is becoming popular. There is a growing interest in carrying out studies on metal levels of wild and cultured food fishes throughout the world (Shearer 1984; Jaffar et al. 1988; Rottiers 1993; Shackley et al. 1994;Salam et al. 1993, 1994, 1996, 1998). Jaffar et al. (1988) has suggested the need to correlate metal contents with growth of fish.

C. mrigala, locally called *mori* or *mrigal,* is one of the major carps being reared extensively for fish farming in Pakistan. It is the natural inhabitant of the freshwater sections of the rivers of Northern India, Bangladesh, Burma and Pakistan. It has been transplanted into waters of peninsular India for aquaculture (Talwar and Jhingran 1991). In natural waters, the fish shows a very rapid growth rate in the first four years of its life, followed by a period of slow growth in the next three years. The growth rate thereafter becomes even slower. The spawning season of *mrigal* depends on the onset and duration of the monsoon. It coincides with Southwest monsoon in India, Bangladesh and Pakistan (Jhingran ans Pullin 1985). *Mrigal* is next in importance for culture to *Rohu* and *Catla* and is a bottom feeder usually feeding on plankton (Jhingran and Khan 1979; Rath 1993). The contribution of *mrigal* on total fish productions in composite fish culture among major carps is reported by Jhingran and Khan (1979).

This study is part of the series to examine dynamics in the proportion of metal accumulation in relation to growth of an important edible fish, *C. mrigala,* cultured in Pakistan.

Materials and Methods

Sample collection and storage

Forty four immature *C. mrigala* whole fish samples of body length ranging from 10.1 to 34.0 cm and body weight 8.99 to 357.81 g were obtained from a commercial fish farm near Multan city (Haq Nawaz Fish Farm, Multan, Pakistan) using a cast net and were transported live in plastic containers to the Fisheries Research Laboratory of Bahauddin Zakariya University, Multan, Pakistan. Fishes were removed from plastic containers and anaesthetisized using MS222 (Sandoz). These were then blotted dry with filter paper and the weight of each fish was determined using an electrical balance (Chyo, Japan) to the nearest 0.01 g. Total body length were measured to nearest 0.01 cm using a measuring tape. All measurements were made from the tip of maxilla to the tip of caudal fin ray. These fish were placed in a preweighed aluminum foil tray in an electric oven (Gallenkamp, England) at 100°C until a constant weight was obtained. The dry carcasses were then crushed and powdered in an agate pestle and mortar.

Sample preparation and analysis

Sample solutions were prepared as follows: 1 g of dried fish sample powder was ashed in a muffle furnace at 500°C for 5 hours. The ash contents were dissolved in aqua regia (3 mL) and the solution was heated until dry. Then 3 mL of 1M HNO_3 was added, the solution was filtered (if necessary) and diluted up to 50 ml with deionized water. Sample solutions were then stored in clean polyethylene bottles for metal analysis. Dogfish liver CRM (DOLT-2) from the National Reseach Council, Canada was also ashed and analyzed following the same procedure.

Flame atomic absorption measurements were made with A-1800 atomic absorption spectrophotometer (Hitachi, Japan) following wavelengths (nm): Zn, 213.8; Fe, 248.3; Cu, 324.8; Mn, 279.6; Pb, 283.3; Ni, 232.0; Na, 589.0; K, 766.5 and Mg, 285.2. Analysis of each sample was made in duplicate. Calibration of the instrument was repeated after every ten samples during operation.

Results and Discussion

The mean and standard deviation values of different metals quantified in carcasses of farmed *C. mrigala* (whole fish) on dry weight basis are presented in table 1. Lead and nickel levels were below the limit of detection of the technique used. Results have been validated by analyzing dogfish liver CRM (DOLT-2) from NRC, Canada for which recoveries of Fe, Cu and Mn were 100 $\pm$ 5% except the Zn for which the recovery was 91 $\pm$ 3%. Strategies to improve Zn recoveries in freshwater fish samples is a subject of another paper which is under preparation.

As variations were found to be related to body weight or body length, regression analysis was performed to assess the size dependence of these meals. Parameters of the relationships are given in tables 2 and 3.

The allometric approach (Weatherley and Gill 1987; Salam and Davies 1994) was applied in which slope b of log-log regression of the relationship between total metal body burden and total body weight or length, when compared with b=1 or b=3 (an isometric slope) is a good predictor for isometric or allometric increase of these metals with increasing weight or length.

It was observed that all these metals showed significant positive correlation ($p<0.001$) with total body weight or total body length. All metals, except lead and nickel which were not quantifiable, were found to increase in direct proportion to an increase in body weight showing isometry (when the value of slope b is either equal to 1.0 or not significantly different from 1.0). Metals such as iron, copper, manganese, potassium and magnesium were found to increase in direct proportion to an increase in total length indicating isometric relationships (when the value of slope b is either equal to 3.0 or not significantly different from 3.0). Zinc shows negative allometry with increasing body length, i.e., significant proportional decrease in metal concentration with increase in body length which suggests that zinc is probably accumulated at a lesser rate compared to its rate of excretion as the fish grows. It seems an interesting characteristic of this fish species with regards to environmental pollution. Sodium shows positive allometry i.e., significant proportional increase in metal concentration with increase in body length which suggests that the metal is probably accumulated at a higher rate compared to its rate of excretion as the fish grows.

Table 1. Mean and standard deviation values of metal concentrations in carcasses (whole body) of farmed *C. mrigala* (n = 44)

Metal	Concentration* ($\mu g \cdot g^{-1}$ dry weight)
Zn	65.7 ± 7.4
Fe	538 ± 238
Cu	24.1 ± 8.5
Mn	50.3 ± 26.3
Na	1182 ± 272
K	9,850 ± 1,488
Mg	2,155 ± 613

*Mean ± one standard deviation.

The values of metal concentrations obtained with *C. mrigala* were compared with the values already reported in the literature for other carps (Table 4). It was observed that inter specific variations exist which could possibly be due to the nature of their habitat, feeding habits, meat quality, gradual or quick accumulation of pollutants entering the aquatic ecosystem. Toxic metals such as Pb and Ni appear to be either not or rarely a accumulated in *C. mrigala* which may be due to pollution free environment or species specific. Further work is needed to assess the degree of ac-

Table 2. Regression parameters for determining the burden of a metal in *C. mrigala*. Log body burden Metal (g) = a + b * (Log total wet body weight (g)) for *C. mrigala*. Underlined values are not significantly different from b =1.0 (P<0.05) and n =44

Wet body weight (g)	Metal	a	b	S.E (b)	r
8.99	Zn	-4.6951	<u>0.9295</u>	0.0572	0.9924***
	Fe	-3.7769	<u>0.8845</u>	0.2941	0.8302***
	Cu	-5.2251	<u>0.9715</u>	0.1532	0.9528***
to	Mn	-4.9529	<u>0.9769</u>	0.2589	0.8816***
	Na	-3.6954	<u>1.0786</u>	0.1099	0.9795***
	K	-2.680	<u>1.0259</u>	0.0801	0.9878***
357.81	Mg	-3.3829	<u>1.0441</u>	0.1304	0.9696***

a = Intercept; b = Slope; S.E (b) = Standard Error of slope; r = Correlation coefficient; *** = P < 0.001.

Table 3. Regression parameters for determining the burden of a metal in *C. mrigala*. Log body burden metal (g) = a + b * (Log total length (cm)) for *C. mrigala*. Underlined values are not significantly different from b =3.0 (P < 0.05) and n =44

Body length (cm)	Metal	a	b	S.E (b)	r
10.1	Zn	-6.5958	2.7961	0.0529	0.9935***
	Fe	-5.5742	<u>2.6515</u>	0.2956	0.8282 ***
	Cu	-7.194	<u>2.9082</u>	0.1589	0.9492 ***
to	Mn	-6.9455	<u>2.9346</u>	0.2592	0.8813 ***
	Na	-5.8916	3.2370	0.1131	0.9782 ***
	K	-4.7725	<u>3.0819</u>	0.0810	0.9875***
34.0	Mg	-5.5187	<u>3.1415</u>	0.1278	0.9709 ***

a = Intercept; b = Slope; S.E (b) = Standard Error of slope; r = Correlation coefficient; *** = P < 0.001.

Table 4. Comparative data of metal concentrations of various freshwater fish species (whole fish).

Metal	Metal Concentration* ($\mu g \cdot g^{-1}$ dry weight)			
	Catla catla[a]	*Labeo rohita*[b]	*Hypophthalmichthys molitrix*[c]	*Cirrhinus mrigala*[d]
Zn	106.5 ± 9.2	68.2 ± 12.4	58.2 ± 8.5	65.7 ± 7.4
Fe	669 ± 196	229 ± 162	510 ± 209	538 ± 238
Cu	45.6 ± 46.9	15.17 ± 6.3	BDL	24.1 ± 8.5
Mn	40.3 ± 12.2	18.2 ± 5.0	26.4 ± 15.2	50.3 ± 26.3
Na	2530 ± 421	5353 ± 1300	2659 ± 443	1182 ± 272
K	9636 ± 1914	4941 ± 214	7365 ± 752	9850 ± 1488
Mg	2236 ± 237	1536 ± 283	2292 ± 474	2155 ± 613

*Mean ± one standard deviation; a (Salam et al. 1998); b (Salam et al. 1993); c (Ansari et al. 2000); d (Present Work); BDL (Below Detection Limit)

cumulation of such toxic metals by *C. mrigala* from relatively polluted waters to arrive at definitive conclusions.

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