



Effect of Body Size on Metal Concentrations in Wild *Puntius chola*

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Abstracts

In this study, relationships of body size (length and weight) with metals *i.e.*, zinc, iron, copper, manganese and calcium in freshwater wild *Puntius chola* (whole fish) have been derived. Metal concentration levels in dried fish powders have been determined by flame atomic absorption spectrometry following wet digestion method involving the use of HNO₃ and H₂O₂. Mean concentrations ($\mu\text{g g}^{-1}$ dry weight) of the metal in carcasses of wild *Puntius chola* have been found to be as 170.8 (Zn), 538 (Fe), 21.2 (Cu), 8.8 (Mn), and 69.10 (Ca, mg g^{-1} dry weight). Cadmium concentration was below the limit of detection of the technique. It was observed that all metals except copper showed significant positive correlation ($p < 0.001$) with total body weight or total body length. All metals, except cadmium which was 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). Calcium was 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). Manganese showed positive allometry *i.e.*, significant proportional increase in metal concentration with increase in body length which suggests that the metal is probably accumulated at higher rate compared to its rate of excretion as the fish grows. However, zinc, iron and copper showed negative allometry to an increase in total length, *i.e.*, significant proportional decrease in metal concentration with increase in body length which suggests that these metals are probably accumulated at lesser rate compared to its rate of excretion as the fish grows.

Keywords: Body size, metal concentrations; *Puntius chola*.

Introduction

Human activities have increased the levels of heavy metals in 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 [1]. 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 bioindicator of the wider conditions of water quality.

The use of fish as bio indicator 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 fishes throughout the world [2-13].

Puntius chola is a fresh water fish. It is locally called as "chiddu" in Pakistan. This freshwater fish inhabits in streams, rivers, canals, beds, hoars, ponds, in undated fields, occurs mainly in shallow waters. It is omnivorous fish which feeds on wide variety of food like worms, crustaceans, insects, and also feeds plant matter, so having variable feeding habits. It has second trophic level feeds offshore through 24 hours period. It can tolerate a pH range of 6.0 to 6.5, lives in tropical climate between temperature

ranges 20 to 25°C. It has prolific breeding, high growth rate but minimum population, with doubling time less than 15 months. The maximum length of swamp barb is 15.0 cm [14]. It is a harmless fish and has commercial importance in fisheries and aquarium. This fish is found in Pakistan, India, Nepal, Bangladesh, Sri Lanka and Burma [15]. A recent paper has presented the weight, length and condition factor relationships of wild *Puntius chola* from Islamabad, Pakistan [16]. The present work was aimed to examine dynamics in the proportion of metal accumulation in relation to growth of wild *Puntius chola*.

Materials and Methods

Sample Collection and Storage

Fifty two wild *Puntius chola* whole fish samples of body length ranging from 5.0-9.5 cm and body weight 1.95-13.02 g were obtained from Fish Hatchery Reservoir, Islamabad, Pakistan using a cast net and were transported live in plastic containers to the Laboratory. Fishes were removed from plastic containers and anaesthetized using MS222 (Sandoz). These were then blotted dry with filter paper and the weight of each fish was determined using an electrical balance (AW220, Shimadzu, Japan) to the nearest 0.01 g. Total body length were measured to nearest 0.01 cm using measuring tape. All measurements were made from tip of maxilla to the tip of caudal fin ray. These fish were placed in a pre-weighed aluminium 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

A wet digestion method reported by Ansari et al. [17] was used to prepare the sample solutions. To weighed amounts (0.3-1.0 g) of dried fish sample powder, 5 ml of freshly prepared aqua regia was added. The solution was refluxed for 30 minutes and then cooled down to room temperature (25 °C). 10 ml of deionised water added and solution was filtered (if necessary) using Whatman # 42 filter paper and then diluted up to 25 ml with deionised water. Sample solutions were then stored in clean polyethylene bottles for

metal analysis. Dogfish liver CRM (DOLT-2) from National Research Council, Canada was also digested and then 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 and Ca, 422.7. 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 *Puntius chola* (whole fish) on dry and wet weight basis are presented in Table 1. Cadmium levels were below the limit of detection of the technique used. Results have been validated by analyzing Dogfish liver CRM (DOLT-2) from National Research Council of Canada for which recoveries of Zn, Fe, Cu and Mn were 100 ± 5 %.

Table 1. Mean and standard deviation values of metal concentrations in carcasses (whole body) of farmed *Puntius chola* (n = 52)

Metal	Concentration* ($\mu\text{g/g}^{-1}$)	
	Dry weight	Wet weight
Zn	170.8 ± 34.2	47.6 ± 11.5
Fe	538 ± 238	133.6 ± 75.2
Cu	21.2 ± 18.4	5.9 ± 5.1
Mn	8.8 ± 5.4	2.5 ± 1.5
Ca**	69.10 ± 17.87	19.2 ± 5.7

* Mean \pm one standard deviation; ** (mg/g⁻¹)

As variations were found to be related to body weight or body length, regression analysis was performed to assess the size dependence of these metals. Parameters of the relationships are given in Tables 2 & 3. The allometric approach [18,19] 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 body weight or length.

Table 2. Regression parameters for determining the burden of a metal in *Puntius chola*. Log body burden Metal (g) = a + b (Log total wet body weight (g)) for *Puntius chola*. Underlined values are not significantly different from b =1.0 (P < 0.05) and n =52

Wet body Weight (g)	Metal	a	b	S.E (b)	r
1.95	Zn	-4.2588	<u>0.8920</u>	0.1157	0.7774 *
	Fe	-3.7123	<u>0.7132</u>	0.1815	0.5330 *
To	Cu	-5.0883	<u>0.6252</u>	0.3238	0.2957 ns
	Mn	-6.1471	<u>1.5593</u>	0.4598	0.4777 *
13.02	Ca	-1.8265	<u>1.1264</u>	0.1366	0.7976 *

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

* = P < 0.001; ns= non significant P>0.05

Table 3. Regression parameters for determining the burden of a metal in *Puntius chola*. Log body burden metal (g) = a + b (Log total length (cm)) for *Puntius chola*. Underlined values are not significantly different from b =3.0 (P < 0.05) and n =52

Body length (cm)	Metal	a	b	S.E (b)	r
5.0	Zn	-5.7052	2.5089	0.1184	0.7652 *
	Fe	-4.8921	2.0340	0.1817	0.5320 *
to	Cu	-6.1269	1.7882	0.3237	0.2961 ns
	Mn	-8.3736	<u>4.0232</u>	0.4722	0.4314 *
9.5	Ca	-3.6505	<u>3.1651</u>	0.1405	0.7844 *

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

* = P < 0.001; ns= non significant P>0.05

It was observed that all metals except copper showed significant positive correlation ($p < 0.001$) with total body weight or total body length. All metals, except cadmium which was 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). Calcium was 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). Manganese showed positive allometry *i.e.*, significant proportional increase in metal concentration with increase in body length which suggests that the metal is probably accumulated at higher rate compared to its rate of excretion as the fish grows. However, zinc, iron and copper showed negative allometry to an increase in total length, *i.e.*, significant proportional decrease in metal concentration with increase in body length which suggests that these metals are probably accumulated at lesser rate compared to its rate of excretion as the fish grows.

The values of metal concentrations obtained with *Puntius chola* were compared with the values already reported in the literature for other carps (Table4). It was observed that inter specific variations exist which could possibly be due the nature of their habitat, feeding habits, meat quality, gradual or quick accumulation of pollutants entering the aquatic ecosystem. Toxic metal such as cadmium appear to be either not or rarely accumulated in *Puntius chola* which may be due to pollution free environment or species specific.

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

Metal	Metal Concentration * ($\mu\text{g g}^{-1}$)				
	Catla catla ⁹		Oncorhynchus mykiss ²	Puntius chola ^a	
	Dry weight	Wet weight	Wet weight	Dry weight	Wet weight
Zn	106.5 \pm 9.2	23.0 \pm 3.2	25	170.8 \pm 34.2	47.6 \pm 11.5
Fe	669 \pm 196	145 \pm 44	12.00	538 \pm 238	133.6 \pm 75.2
Cu	45.6 \pm 46.9	9.5 \pm 9.6	1.2	21.2 \pm 18.4	5.9 \pm 5.1
Mn	40.3 \pm 12.2	8.5 \pm 2.5	1.81	8.8 \pm 5.4	2.5 \pm 1.5
Ca **	45.88 \pm 0.81	9.73 \pm 2.01	5.16	69.10 \pm 17.87	19.2 \pm 5.7

* Mean \pm one standard deviation; ** mg g⁻¹ dry weight; a Present work

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