

E-ISSN: 2320-7078

P-ISSN: 2349-6800

[www.entomoljournal.com](http://www.entomoljournal.com)

JEZS 2020; 8(5): 1503-1507

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Received: 13-07-2020

Accepted: 15-08-2020

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## Revision of some morphometric characteristics and building the standard haematological reference value in *Rita rita* from Pakistan

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### Abstract

This study was aimed to measure some morphometric and haematological characters of *Rita rita* captured in the L.B.D canal from river Ravi. The morphometric measurement was carried through measuring tape and scale rod and weight was taken with the help of digital balance. The haematological analysis was carried out through the Neubauer haemocytometer. The weight of fish was  $86.55 \pm 52.23$  g, length with tail  $18.72 \pm 3.66$  cm, total length without tail  $15.40 \pm 2.79$  cm, length of tail/caudal fin  $3.34 \pm 1.05$  cm, and the girth/width  $10.88 \pm 2.23$  cm. The distance between eyes was  $2.08 \pm 0.41$  cm, between nostrils  $1.23 \pm 0.30$  cm. Length of mandibles was  $2.14 \pm 0.69$  cm, maxilla  $2.31 \pm 0.74$  cm, width of head  $4.25 \pm 1.65$  cm, diameter of eye  $0.25 \pm 0.10$  cm. Length of dorsal fin was  $4.57 \pm 1.51$  cm, width  $2.71 \pm 0.83$  cm, length of adipose fin  $2.17 \pm 0.50$  cm, width fin  $0.72 \pm 0.2$  cm, length of anal fin  $2.34 \pm 0.54$  cm, width  $1.84 \pm 0.57$  cm, length of pectoral fin  $3.63 \pm 0.90$  cm, width  $2.15 \pm 0.53$  cm, length of pelvic fin  $2.16 \pm 0.87$  cm and width  $1.40 \pm 0.42$  cm. The number of rays of the dorsal fin was  $6+1 \pm 0.30$ , anal fin  $9+1.36$ , pectoral fin  $6+1 \pm 0.98$ , pelvic fin  $7+1.02$ , and tail /caudal fin  $17+1.31$ . The relationship between length and weight showed that the relationship was non-linear and growth was allometric. Regarding the haematological analysis, RBCs were  $4.92 \pm 0.45 \times 10^6/\text{mm}^3$ , haemoglobin  $8.6 \pm 2.11$  g/dL, WBCs  $2.32 \pm 0.30$ , platelets  $53.4 \pm 11.07$ , neutrophils  $7.4 \pm 3.2\%$ , Lymphocytes  $89.2 \pm 4.26\%$ , Monocytes  $2 \pm 1.55\%$  and Eosinophils  $1.2 \pm 0.4\%$ . The alkaline phosphatase (ALP) and acid phosphatase (ACP) was above than reference value which showed that river pollution has started damaging the body organs of fish.

**Keywords:** Haematology, morphometry, Pakistan, reference value, Rita

### Introduction

*Rita rita* (Hamilton) is freshwater fish and found in ponds, lakes, rivers, canals, and streams [1]. Its body is elongated, broad from the front, and narrow towards the tail. Skin is slippery has no scales and contains dorsal, pectoral, pelvic, anal, and caudal fin/tail. Its caudal fin is forked like. All the fins have fin rays except the adipose fin [2]. The body color of young fish is greenish-brown from the upper side and silvery brown from the back of the body. The body of the *R. rita* is very plucky, hard, and without any scale. The abdomen is wide and smooth. The head is large, low, wide, and dorso-ventrally smooth. The mouth is lower and sloppy. The teeth are not of one fixed type. The upper jaws are just like villi projections, having a cone shape and slightly bent. The lower jaw is just like molar teeth in shape and connected to the top of the mouth cavity. Three types of burbles are present on the maxilla, mandibles, and near the nasal cavity. The burbles on the maxilla and mandibles are long. The nasal burbles are short. Two fins are present on its dorsal side, first is large and has a spine. The second fin is adipose which has no rays or spine in it. The pectoral fin also has spines on both sides but these spines are not large then the spine of the dorsal fin. The tail is branched with no spines.

*R. rita* feeds in the bottom but sometimes also from the column. Feeding consists of algae, higher plants, insects, crustaceans, and rotifers [3]. It is found in South Asian countries like India, Bangladesh, Pakistan, Nepal, Afghanistan, and Myanmar [4-7] however, its status is endangered in Bangladesh and threaten in India [8]. Its population is decreasing rapidly due to over-exploitation, pollution, loss of breeding grounds, high heavy metals concentration in water, and changes in the natural ecosystem [9].

It is a very important catfish from the market point of view [10, 11]. It has rich protein content and good taste [12]. In different countries, it is also used as ornamental fish [13]. *R. rita* is a riverine bottom dweller and prefers muddy or clear water [13, 14].

Further, it is freshwater but can bear low salinity conditions [4]. It can also live in a low dissolved oxygen amount, high turbidity, and an odd environment. This species has also been used as a pollution indicator because it can bear the high limits of fluctuations in its natural environment [15, 16]. Therefore, this study was designed to measure some morphometric and haematological parameters of *R. rita*.

## Materials and Methods

### Study site and sampling

*R. rita* was sampled from LBDC located in Okara, Punjab, Pakistan, and originated from river Ravi. A total of 51 fish samples were collected from ten different localities of LBDC in district Okara.

### Morphometry

All measurements were taken with the help of measuring tape and scale. The larger length was taken with measuring tape and the smaller length with the scale. All measurements were taken in cm except weight which was taken in grams. The weight of the fishes was measured with electric balance and expressed in grams. The total length of fish was measured from head to tail. This length was started from maxilla to tip of the caudal fin and expressed in cm. The total length of fish was measured without the tail. This length was started from the maxilla to the end of the body (start of caudal fin/tail). The total length of the tail/caudal fin was measured from the start of the tail/caudal fin to the tip of the tail/caudal fin. The width of the head was measured from one barbell to another. The total length of the dorsal fin was measured from the point of attachment with the body to the tip of the fin. The width of the dorsal fin was measured from the first hard ray to the last ray of the fin. The length of the adipose fin was measured from the point of attachment with the body to the tip of the fin. The width of the adipose fin was measured from the start of the lobe to till the end of the lobe. The total length of the anal fin was measured from the point of attachment with the body to the tip of the fin. The width of the anal fin was measured from the first ray to the last ray of the fin. The girth of the fish was taken with the help of measuring tape. This measurement was a complete round of measuring tape around the body. The total length of the pectoral fin was measured from the point of attachment with the body to the tip of the fin. The width of the pectoral fin was measured from the first hard ray to the last ray of the fin. The total length of the pelvic fin was measured from the point of attachment with the body to the tip of the fin. The total length of the anal fin was measured from the point of attachment with the body to the tip of the fin. The diameter of the eyes was measured from one side to another side with the help of a scale. Distance between eyes was measured from the inner side of one eye to the inner side of another eye. The distance between nostrils was measured from one nostril to another. The length of mandibles was measured from the point of attachment to the tip of the mandible. The length of the maxilla was measured from the point of attachment to the tip of the maxilla. The number of rays was counted with the help of applying a hand lens. The number of soft and hard rays were counted separately. To find the weight-length relationship, first, the values of *a* and *b* parameters were found out with slope and intercept in the regression analysis. Firstly took the data of weight and length and then the logarithmic values of each weight and length were found by using  $y = \log(\text{value})$  function.

The purpose of finding the values of *a* and *b* parameters was to find the weight-length relationship by using the following equation:

$$W = aL^b$$

In excel we insert a dotted graph by using values of weight and length. The length was on X-axis and weight on Y-axis. Then draw the regression line on the graph. Then we did a regression analysis of length and weight relationship for the estimation of parameters. For this purpose, Microsoft Excel was used to perform the regression analysis.

### Haematology

The blood for haematological analysis was collected from the heart through cardiac puncture. The needle of the syringe was inserted on the ventral side between the pectoral fins. The blood was collected in an EDTA tube.

The haemoglobin, RBCs, WBCs, and platelets were counted in haematological analysis after dilution. The Neubauer haemocytometer (Blaxhall, 1972) was used to counts WBCs, and RBCs and Sali's haemoglobinometer were used for the calculation of haemoglobin. The value of PCV (Packed Cell Volume) was estimated via Koestner, Nelson [17]. The MCV, MCH, and MCHC were measured with standard formulas of Borensztein, De Gregorio [18].

### Activity of acid phosphatase (ACP) and alkaline phosphatase (ALP)

The activity of both enzymes was measured according to Michell, Karnovsky [19] and Estiarte, Peuelas [20]. In general methodology, the reaction mixture consisted of 0.7 ml sodium acetate buffer with pH 5. Then it was mixed with 0.25 ml 5 mM p. nitro-phenyl phosphate. This mixture acted as a substrate which was again mixed with 0.5 ml enzyme. All the mixture contents formed a total volume of 1 ml. this reaction mixture was incubated at 37 °C for 30 minutes. To this reaction mixture, 4 ml of 0.1 N NaOH was added and once again incubated it in the incubator for 30 minutes at 37 °C.

In the case of ALP, the substrate solution was the mixture of 0.5 ml buffer of glycine having pH 7.8, 10 mM 0.2 ml MgCl<sub>2</sub>, and 5 mM p. nitro-phenyl phosphate having a volume of 0.25. Finally, about 0.05 ml of the enzyme was added and all the reaction mixture was equal to 1 ml. this mixture was then incubated at 37 °C for 30 minutes. The activities of both enzymes were measured by color (yellow color measurement) of p. nitro-phenol using a synergy (HT) Multi-Mode Microplate Reader, (Winooski, VT, USA).

All the readings and results were in mean and standard deviation of all samples was compared through ANOVA by using IBM SPSS (v 21) software.

### Results

Result revealed that this fish had large head region compared to other fishes. The width of the head ranged from 1.8-8cm with an average size of  $4.25 \pm 1.67$ cm. The diameter of eyes was 0.1-0.5 cm with average size  $0.25 \pm 0.12$ cm, distance between eyes 1.5-3cm with average size  $2.08 \pm 0.41$ cm, distance between 0.7-2cm with average size  $1.23 \pm 0.30$ cm. The length of mandibles was from 1.1-3.5cm with an average size of  $2.14 \pm 0.69$ cm. The length of the maxilla was 1-3.8cm with an average size of  $2.31 \pm 0.74$ cm (Table 1).

**Table 1:** Measurements of various mouth parts of *R. rita* recorded from LBDC in okara.

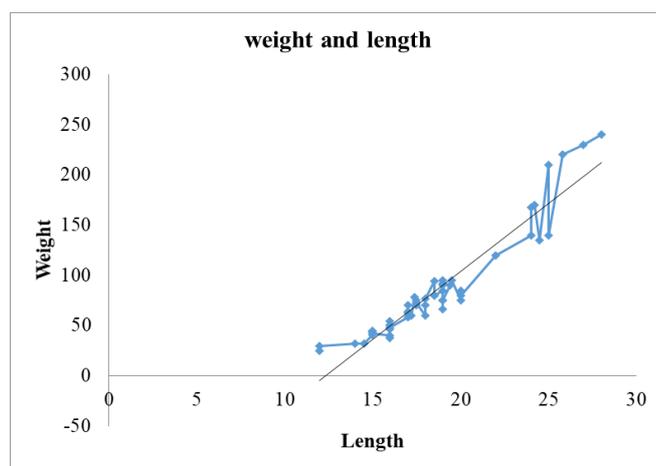
Measurements n=51	Mean± S.D	Range (Min-Max)
Distance between eyes (cm)	2.082 ± 0.41	1.5-3
Distance between nostrils (cm)	1.23 ± 0.30	0.7-2
Length of mandibles (cm)	2.14 ± 0.69	1.1-3.5
Length of maxilla (cm)	2.31 ± 0.74	1-3.8
Width of head (cm)	4.25 ± 1.67	1.4-8
Diameter of eyes (cm)	0.25 ± 0.12	0.1-0.5

The fishes also have several fins with variable shapes and length. The length of the dorsal fin was in the range of 2-7.9cm with the average size of  $4.57 \pm 1.51$ cm, width 1.2-4.2 cm average size  $2.71 \pm 0.83$  cm, adipose fin 1.2-4.2 cm average size  $2.17 \pm 0.50$  cm, width 0.4-1.2 cm with the average size of  $0.72 \pm 0.22$  cm (Table 2).

A nonlinear relationship was found between weight and length of captured *R. rita*. It was because *R. rita* is a small length fish which also does not get much weight. The non-linear growth is due to environmental factors such as temperature, unavailability of food, large heavy metal concentration, and maybe over-hunting. The graph is non-linear that's why growth is allometric (Fig. 1).

**Table 2:** Measurements of fins of *R. rita* recorded from LBDC in okara

Measurements N= 51	Mean± S.D	Range (Min-Max)
Length of dorsal fin (cm)	$4.57 \pm 1.51$	2-7.9
Width of dorsal fin (cm)	$2.71 \pm 0.83$	1.2-4.2
Length of adipose fin (cm)	$2.17 \pm 0.50$	1.2-3.3
Width of adipose fin (cm)	$0.72 \pm 0.22$	0.4-1.2
Length of anal fin (cm)	$2.34 \pm 0.54$	0.9-3.3
Width of anal fin (cm)	$1.84 \pm 0.57$	1-3.1
Length of pectoral fin (cm)	$3.63 \pm 0.90$	2-6
Width of pectoral fin (cm)	$2.15 \pm 0.53$	1.1-3.2
Length of pelvic fin (cm)	$2.16 \pm 0.87$	1.1-5.9
Width of pelvic fin (cm)	$1.40 \pm 0.42$	0.6-2.9
No. of rays of dorsal fin	$6 \pm 1 \pm 0.30$	6-7
No. of rays of anal fin	$9 \pm 1.36$	6-12
No. of rays of pectoral fin	$6 \pm 1 \pm 0.98$	5-8
No. of rays of pelvic fin	$7 \pm 1.02$	5-9
No. of rays of tail	$17 \pm 1.31$	14-20



**Fig 1:** Weight length relationship of *Rita rita* fish

The weight of fishes was  $86.55 \pm 52.23$  g. The total length with the tail was  $18.72 \pm 3.66$  cm. The total length without a tail was  $15.40 \pm 2.79$  cm. The length of tail was  $3.34 \pm 1.05$  cm. The girth/width of fish was  $10.88 \pm 2.23$  cm (Table 3).

In regression analysis value of R-square was 0.99, which showed that the relationship was fitted to be good<sup>21</sup>. The values of intercept and X-variables (Slope) of P-value were non-significant which showed that there was non-linear growth. There was not too much difference in the weight-length relationship due to various factors as mentioned earlier. In table 4, a and b values are calculated through regression analysis which is a useful parameter and can be used to calculate length or weight if one value is known using the following formula:

**Table 3:** Length and weight of different body parts of *R. rita* recorded from LBDC in okara.

Measurements N=51	Mean± S.D	Range (Min-Max)
Weight (g)	$86.55 \pm 52.23$	25-240
Total length with tail (cm)	$18.72 \pm 3.66$	12-28
Total length without tail (cm)	$15.40 \pm 2.79$	10-22
Length of tail (cm)	$3.34 \pm 1.05$	1.5-6
Girth (width of fish)(cm)	$10.88 \pm 2.23$	6.5-17

$$W = aL^b$$

Whereas w is weight, L is the length and "a" and "b" are coefficients calculated from regression analysis<sup>[22]</sup>.

**Table 4:** Regression analysis for the calculation values of parameters of a and b

Tl A.M±S.D	Tw A.M±S.D	log10tl A.M	log10tw A.M	a	B	Standard Error	R Square
$18.72 \pm 3.67$	$86.55 \pm 52.23$	1.26	0.10	0.72	1.41	0.001317679	0.997881645

**Table 5:** Estimation of coefficients for weight-length relationship

	Coefficients	Standard Error	t Stat	P-value	95% cl of a		95% cl of b	
					Lower limit	Upper limit	Lower limit	Upper limit
Intercept	-0.33	0.01	-116.25	1.67 <sup>NS</sup>	-0.34	-0.33	-0.34	-0.33
X Variable 1	0.34	0.01	151.93	3.48 <sup>NS</sup>	0.34	0.35	0.34	0.35

*Rita rita* blood contains average RBCs  $4.92 \pm 0.45 \times 10^6/\text{mm}^3$ , haemoglobin  $8.6 \pm 2.11$  (g/dL), WBCs  $2.32 \pm 0.30 \times 10^3/\text{mm}^3$ ,

platelets  $53.4 \pm 11.07 \times 10^3/\mu\text{l}$  neutrophils  $7.4 \pm 3.2$ , lymphocytes  $89.2 \pm 4.26\%$ , monocytes  $2 \pm 1.55\%$  and eosinophil  $1.2 \pm$

0.4%.

The average PCV percentage in the blood of *R. rita* fish was  $25.8 \pm 6.32\%$ , MCV  $51.792 \pm 8.34$  fl, MCH  $17.306 \pm 2.75$  pg, and MCHC  $33.33 \pm 0$  g/dL.

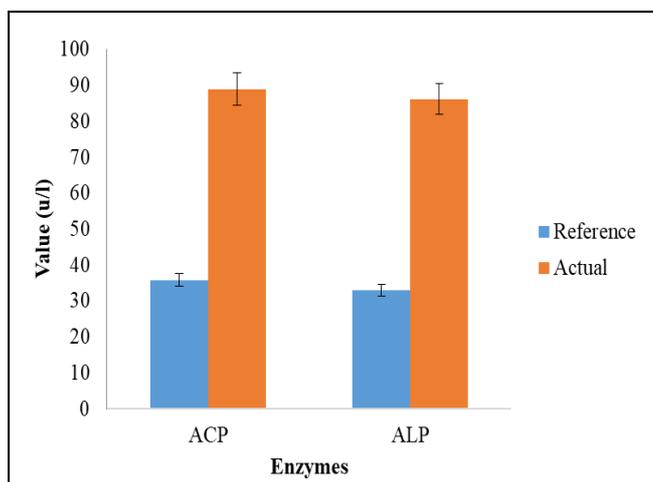
**Table 6:** Some hematological parameters of *R.rita* recorded from LBDC in okara

Samples N=5	Mean± S.D	Range (Min-Max)
RBCs ( $\times 10^6/\text{mm}^3$ )	4.92±0.45	4.3-5.6
Haemoglobin (g/dL)	8.6±2.11	6.8-11.4
WBC ( $\times 10^3/\text{mm}^3$ )	2.32±0.30	2-2.8
Platelets ( $10^3/\mu\text{l}$ )	53.4±11.07	37-69
Neutrophils (%)	7.4±3.2	3-12
Lymphocytes (%)	89.2±4.26	82-95
Monocytes (%)	2±1.55	1-5
Eosinophil (%)	1.2±0.4	1-2

**Table 7:** Value of some hematological parameters of *R.rita*

Samples (N= 5)	Mean± S. D
PCV (%)	25.8 ± 6.32
MCV (fl)	51.792 ± 8.34
MCH (pg)	17.306 ± 2.75
MCHC (g/dL)	33.33 ± 0

ALP and ACP enzymes which are not released in normal body functioning. These enzymes are released only when there is any damage in the body organs like kidney, liver, gills, muscles, heart, or any other organ of the body. The ACP release especially when tumour formation takes place in the body. The normal value of ACP and ALP is 35.9 u/L and 32.9 u/L and our calculated value of ACP and ALP in blood was 88.99 u/L and 86.17 u/L respectively which showed there was damage in the organs of fish or maybe the formation of the tumour. This damage may be due to toxic substances or may be due to high heavy metal in the water.



**Fig 2:** Change in the level ALP and ACP in *R. rita* captured from LBDC

## Discussion

*Rita rita* fish is a freshwater catfish. It is locally called as “Khagga” or “Desi-Khagga”. It is usually found in the ponds, streams, canal, and rivers of Pakistan, India, Bangladesh, and Sri Lanka [1].

In the present study, 51 fishes were captured from LBD canal Okara, Punjab Pakistan. All specimens were evaluated for the morphometric analysis. The diameter between the eyes was

from 0.1-0.5cm. The average diameters of all specimens were  $0.51 \pm 0.12$ cm. The distance between the eyes was from 1.5-3 cm. The average distance between the eyes of all specimens was  $2.08 \pm 0.41$ cm. The average diameter of the eye was also measured by Jalbani, Narejo [23] which was  $0.32 \pm 0.04$  cm, they captured the fishes from the Indus river near Jamshoro, Sindh.

The length of the dorsal fin was ranging from 2-7.9 cm, width from 1.2-4.2 cm. The length of the pectoral fin was  $3.63 \pm 0.90$ cm and width  $2.15 \pm 0.53$ cm. The fins were also measured by Jalbani, Narejo [23], the average pectoral fin length of captured fishes was  $0.78 \pm 0.15$ cm.

The weight of fish was  $86.55 \pm 52.23$  g. The total length with the tail was  $18.72 \pm 3.66$  cm. The total length without the tail was  $15.40 \pm 2.79$ cm. Some morphometric characters of the same fish are also studied by Khan, Narejo [24] and their weight ranged from 75 to 625 g and total length ranged from 10.1 to 40 cm of 60 specimens of *Rita rita* collected from Indus river near Jamshoro. The total weight and length of *Rita rita* fish were also measured by Mitra, Ganguly [25] and according to them, the weight of fish ranged from 200-400 grams and the length of fish ranged from 20-30 cm of specimens which were collected from Ganga river India. Similarly, in a study by Jalbani, Narejo [23], the average length of fish was  $18.11 \pm 8.32$  cm. The average tail length of the fish was  $20.35 \pm 5.18$ cm. In comparisons of some morphological parameters of *Rita rita*, there were slight variations in the weight, length of fish, length of fins, and the number of rays of fins in given studies. It may be due to different localities of water bodies, changes in aquatic environmental conditions like salinity, pH, temperature, heavy metal concentration, or due to other toxic materials. The major factor which causes variation were seasonal capturing of fishes from LBD canal and seasonally drying of LBD canal.

The graph between weight and length shows that there was a non-linear relationship. It was due to several; environmental factors such as temperature, unavailability of food, high heavy metal concentration, seasonal drying of the water body, seasonal capturing, and low DO. Then calculate the values of a and b parameters using regression analysis for  $W = aL^b$ .

Mean value of RBCs count was  $4.92 \times 10^6/\text{mm}^3$ , WBCs  $2.32 \times 10^3/\text{mm}^3$ , haemoglobin 8.6 g/dL, the platelets  $53.4 \times 10^3/\mu\text{l}$ . Percentage values of Neutrophils, Lymphocytes, Monocytes, and Eosinophil 7.4%, 89.2%, 2%, and 1% respectively. The mean PCV percentage was 25.8%, MCV 51.792 fl, MCH 17.306 pg and MCHC 33.33 g/dL. The haematological analysis on the same fish was also conducted by [26] mean RBCs count was  $2.16 \times 10^6/\text{mm}^3$ , mean WBCs count  $1.9-2.6 \times 10^3/\text{mm}^3$ , mean haemoglobin 14 g/dL. The RBCs count was calculated by us were high because there will be low dissolved oxygen in water, many pathogens, and more diseases which increase the number of RBCs, WBCs, and haemoglobin.

Alkaline phosphatase (ALP) and Acid Phosphatase (ACP) are widely distributed in the living world [27]. The activity of ALP is usually found in the kidney, liver, gills, and heart [27]. Alkaline phosphatase (ALP) is a protein enzyme [28]. Acid phosphatase stored in lysosomes Alkaline phosphatase (ALP) and Acid Phosphatase (ACP) are enzymes that are not released in normal conditions of internal organs of fish like kidney, liver, gills, and heart. ALP and ACP enzymes usually release in abnormal body conditions in a disease condition. ACP mostly release when tumour formation occurs. The reference value of ACP and ALP is 35.9 u/L and 32.9 u/L and

our calculated value of ACP and ALP in blood was 88.99 u/L and 86.17 u/L. The values of alkaline phosphatase (ALP) and Acid phosphatase (ACP) were higher than the reference value which showed that pollutant of various origins has started to damage the different organs and tumour formation might also be started in some part of the body.

It was concluded that the fish was small in size and weight does not considerably increase as the length increases. The length-weight relationship was non-linear and allometric. The blood parameters showed fluctuation might be due to water pollution, oxygen level, several pathogens, and certain diseases.

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