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Comparative morphometrical analyses of normal erythrocytes of two air-breathing fishes *Channa punctatus* and *Anabas testudineus*

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Abstract

The investigation was carried out to study the sex wise normal red blood cells (RBCs) morphometry of Channa punctatus and Anabas testudineus. In fish hematology for clinical diagnosis and cytomorphological study the present study is required to compare the red blood cell morphometry. To compare the sizes of normal RBCs in two different species an attempt was made. By the study of cells of blood in many different species, fishes provide an interesting comparison of size of cell in relation to their activities and habits a trial was made to study morphometric data of blood cells in Channa punctatus and Anabas testudineus. In Channa punctatus and Anabas testudineus morphometrical features of RBCs provide a baseline data which will be helpful for further comparison with other species and also diagnostic interpretation of normocytic, microcytic and macrocytic anemias can be known for these species by which their physiological conditions can be known and they can be treated accordingly with their environment.

Keywords: RBCs, male *C. punctatus*, female *C. punctatus*, male *A. testudineus*, female *A. testunidieus*

Introduction

Analyses of morphometry of blood cells play a significant role in fish[1]. Analyses of cells of blood in many different fish species provides an interesting comparison of cell size with respect to their activities and habits[2]. By the size of erythrocyte types of anemia's, stress and bio-physio-pathological aspects in fish can be ascertained[3].

In fish for veterinarians and fish health professionals there are few diagnostic tools are available to evaluate disease and abnormalities. Besides, there are limitations regarding data of blood cells and size of blood cells[2]. This investigation of the study was carried out to study the morphometrical features of normal red blood cells of two different fish species namely *Channa punctatus* and *Anabas testudineus* with respect to sex. As in these species of fish morphometry of red blood cells were not well defined and not well documented, the study is designed to determine the size of red blood cells.

One of the best indicators of body health are blood cells and are very important in immune system[2]. In fish blood cells there was a very little attention has been devoted for the structure of blood cells[2]. In internal physiological conditions and external stimuli blood cells are very sensitive[3]. In the blood of fish and human erythrocytes are the dominant cell type and after erythrocytes, thrombocytes are the most abundant cells[4]. In the vast majority of fish species and most other vertebrates' erythrocytes are the dominant cell type in blood[5,6]. In vertebrates on the life history strategies, key information was provided by RBCs[7]. Several diagnostic tools are there for fish health professionals and veterinarians to study the fish health and abnormalities in them. However, there is limitation relating to reference data of fish red blood cell size[8]. According to previous author the size of mature erythrocytes was not sex- dependent[9]. The investigation of this study was to gain a basic data regarding the morphological and morphometrical characteristics of the red blood cells by which their physiological conditions can also be known.

Materials and methods

Blood samples collection, smears preparation, staining and morphometric study: The fishes were collected from the local cultured pond and weight of each fish is approximately 100g each. For the morphological and morphometrical analyses of red blood cells caudal vein was the site of blood collection and immediately preparation of blood smears were done after collection of blood and Giemsa stain was used for staining. The length & breadth of RBCs & their respective nucleus of the both the sexes, i.e., male and female of *Channa punctatus* and *Anabas testudineus* were measured with the help of CC130-1.3 mega pixel microscopic camera (Mfg. by Catalyst Biotech, Maharashtra, India) connected to microscope (M. No. BD-08 B, S. No. 21320 Mfg. By B.D. Instrumentation, Ambala Cantt, India) under 40X objective.

Photomicrography: Photomicrography of red blood cells was done by Axio imager A2 microscope connected with camera and computer by using 40X objective.

Statistical analyses: For analyses of statistical parameters statistical software Microsoft Office Excel 2007 was used using students t-test and PAST software for Turkey's pair wise comparison test. Measurement of both length and breadth of RBCs were done in micron meter (µm). Differences were classified as $p<0.05$ means significant and $p<0.01$, $p<0.001$ means highly significant.

Results and discussion

Fish has nucleated, and are more metabolically active RBCs than mammalian RBCs and significant role was played in blood chemistry[10]. Morphology of erythrocyte is one of the most sensitive indicators of toxic impact of various environmental factors on fish[11]. Morphometry of RBCs and their nucleus were measured in micron meter (µm). To monitor the health status of fish; fish hematology is gaining increasing importance[12].

Some earlier workers have described in the evolutionary system of vertebrate circulatory system erythrocytes are considered as the centre piece[13]. By the measurement of erythrocytes, information about the genome size of a species can be known[14,15]. Some earlier workers have emphasized that erythrocytes may be used in ploidy determination[16,17]. For the assessment of air pollution in animals, as a use of diagnostic assay size of erythrocytes are used[18]. Main function of erythrocyte is gaseous exchange. In comparison to larger erythrocyte, smaller erythrocyte has higher rate of gaseous exchange[19,20].

In the current study the morphology of RBCs of both *Channa punctatus* (Figure-1) and *Anabas testudineus* (Figure-2) were observed. The study of morphometrical parameters of red blood cells of *C. punctatus* with respect to sex were recorded in which nine parameters were observed (Table-1). For mean length of RBCs highly significant difference at $p<0.001$ is observed between the two sexes. For area of RBCs highly significant at $p<0.01$ is observed between the both sexes. Observance of significant difference was there at $p<0.05$ for breadth of nucleus of RBCs, for area of nucleus and ratio between area of RBCs and area of nucleus (A/A') between the two sexes.

For all the nine parameters between the two sexes of *Anabas testudineus* there was no significant difference was noticed (Table-2).

The comparative study of morphometry of red blood cells of *C. punctatus* and *A. testudineus* was recorded (Table-3) and expressed in mean±S.E. For length of RBCs, the highest value (11.33±0.23) was observed in female *C. punctatus* and lowest value (10.04 ±0.21) was observed in male *C. punctatus* among the two species of two sexes (Figure-3). Highly significant difference is there between male *C. punctatus* and female *C. punctatus* (p=0.000165). Significant difference (p=0.01715) is there between male *C. punctatus* and male *A. testudineus*. Significant difference (p=0.024) is there between male *C. punctatus* and female *A. testudineus.*

For breadth of RBCs, the highest value (7.91±0.22) was observed in female *C. punctatus* and lowest value (7.02±0.19) was observed in female *A. testudineus* (Figure-4). Significant difference (p=0.01563) is there between female *C. punctatus* and female *A. testudineus.*

For length and breadth ratio (L/B) female *A. testudineus* has highest value (1.57±0.05) and lowest value (1.36±0.04) was observed in male *C. punctatus* (Figure-5). Highly significant difference (p=0.009158) is there between male *C. punctatus* and female *A. testudineus.*

For area of RBCs female *C. punctatus* has highest value (279.45±8.48) and lowest value (238.85±10.12) was observed in male *C. punctatus* (Figure-6). Highly significant difference (p=0.00506) is there between male *C. punctatus* and female *C. punctatus*. Highly significant difference (p=0.006852) is also there between female *C. punctatus* and female *A. testudineus.*

For the nucleus length male *A. testudineus* has highest value (5.40±0.18) and lowest value (4.25±0.19) was observed in female *C. punctatus* (Figure-7). Significant difference (p=03742) is there between male *C. punctatus* and male *A. testudineus*. Highly significant difference (p=001287) is there between female *C. punctatus* and male *A. testudineus*. Significant difference (p=0.01686) is there between female *C. punctatus* and female *A. testudineus*.

For the nucleus breadth female *A. testudineus* has highest value (2.93±0.13) and lowest value (2.20±0.11) was observed in female *C. punctatus* (Figure-8). Highly significant difference (p=0.001113) is there between female *C. punctatus* and male *A. testudineus*. Highly significant difference (p=0.000543) is also there between female *C. punctatus* and female *A. testudineus*.

For nucleus length and breadth ratio (L'/B') female *C. punctatus* has highest value (2.08±0.14) and lowest value (1.80±0.09) was observed in male *C. punctatus* (Figure-9). No significant difference is there between the different sexes and among the different sex of each species.

For nucleus area, male *A. testudineus* has highest value (49.11±2.67) and lowest value (30.20±2.25) was observed in female *C. punctatus* (Figure-10). Between female *C. punctatus* and female *A. testudineu* highly significant difference (p=000173) is there. Between female *C. punctatus* and female *A. testudineus* highly significant difference (p=0.000236) is there.

For the ratio between area of RBCs and area of nucleus (A/A') the highest value (11.15±0.98) was observed in female *C. punctatus* and lowest value (5.62±0.46) was observed in male *A. testudineus* (Figure-11). Highly significant difference (p=0.006298) is there between male *C. punctatus* and female *C. punctatus*. Highly significant difference (p=0.000138) is there between female *C. punctatus* and male *A. testudineus*. Highly significant difference (p=0.000138) is there between female *C. punctatus* and female *A. testudineus*.

Table-1: Sex wise size of red blood cells of *Channa punctatus* measured in micron meter (µm).

Parameters	Male		Female	
	Mean	SEM	Mean	SEM
Length of RBCs (L)	10.04***	0.21	11.33***	0.23
Breadth of RBCs (B)	7.52	0.22	7.91	0.22
L/B	1.36	0.04	1.48	0.05
Area of RBCs (A)	238.85**	10.12	279.45**	8.48
Length of nucleus of RBCs (L')	4.58	0.25	4.25	0.19
Breadth of nucleus of RBCs (B')	2.60*	0.12	2.20*	0.11
L'/B'	1.80	0.09	2.08	0.14
Area of nucleus (A')	38.98*	3.49	30.20*	2.25
A/A'	7.78*	0.81	11.15*	0.98

Figures in parentheses represent the number of observations in each case. SEM: Standard error mean. *Significant at $p<0.05$, **highly significant at $p<0.01$, ***highly significant at $p<0.001$.

Table-2: Sex wise size of red blood cells of *Anabas testudineus* measured in micron meter (µm).

Parameters	Male (n=30)		Female (n=30)	
	Mean	SEM	Mean	SEM
Length of RBCs (L)	10.87^{NS}	0.13	10.84^{NS}	0.19
Breadth of RBCs (B)	7.27^{NS}	0.12	7.02^{NS}	0.19
L/B	1.50^{NS}	0.03	1.57^{NS}	0.05
Area of RBCs (A)	248.74^{NS}	5.63	240.01^{NS}	8.82
Length of nucleus of RBCs (L')	5.40^{NS}	0.18	5.15^{NS}	0.20
Breadth of nucleus of RBCs (B')	2.89^{NS}	0.12	2.93^{NS}	0.13
L'/B'	1.97^{NS}	0.11	1.84^{NS}	0.10
Area of nucleus (A')	49.11^{NS}	2.67	48.14^{NS}	3.13
A/A'	5.67^{NS}	0.43	5.62^{NS}	0.46

Figures in parentheses represent the number of observations in each case SEM depicts standard error mean NS depicts not significant.

Table-3: Comparative analyses of size of red blood cells of *Channa punctatus* and *Anabas testudineus* measured in micron meter (µm)

Parameters	*Channa punctatus*		*Anabas testudineus*		F-value
	Male (Mean± S.E) (n=30)	Female (Mean± S.E) (n=30)	Male (Mean± S.E) (n=30)	Female (Mean±S.E) (n=30)	
Length of RBCs (L)	10.04 ±0.21 [a]	11.33±0.23 [a]	10.87±0.13 [a]	10.84±0.19 [a]	7.75**
Breadth of RBCs (B)	7.52±0.22	7.91±0.22 [b]	7.27±0.12	7.02±0.19 [b]	3.35*
L/B	1.36±0.04 [a]	1.48±0.05 [a]	1.50±0.03	1.57±0.05	3.57*
Area of RBCs (A)	238.85±10.12[a]	279.45±8.48 [a, b]	248.74±5.63	240.01±8.82 [b]	5.06**
Length of nucleus of RBCs (L')	4.58±0.25 [a]	4.25±0.19 [b]	5.40±0.18 [a, b]	5.15±0.20 [b]	6.10**
Breadth of nucleus of RBCs (B')	2.60±0.12	2.20±0.11 [b]	2.89±0.12 [b]	2.93±0.13 [b]	7.19**
L'/B'	1.80±0.09	2.08±0.14	1.97±0.11	1.84±0.10	1.22[NS]
Area of nucleus (A')	38.98±3.49	30.20±2.25 [b]	49.11±2.67 [b]	48.14±3.13 [b]	9.65**
A/A'	7.78±0.81 [a]	11.15±0.98 [a, b]	5.67±0.43 [b]	5.62±0.46 [b]	13.21**

[1]Mean±SE with similar superscripts in the same row indicates significant difference at p<0.05 and at p<0.01 [2]Significant at p<0.05 is indicated by *. ** means significant at p<0.01and NS means not significant. [3]Figures in parentheses represent the number of observations in each case SE: Standard error, F value: Fischer's value.

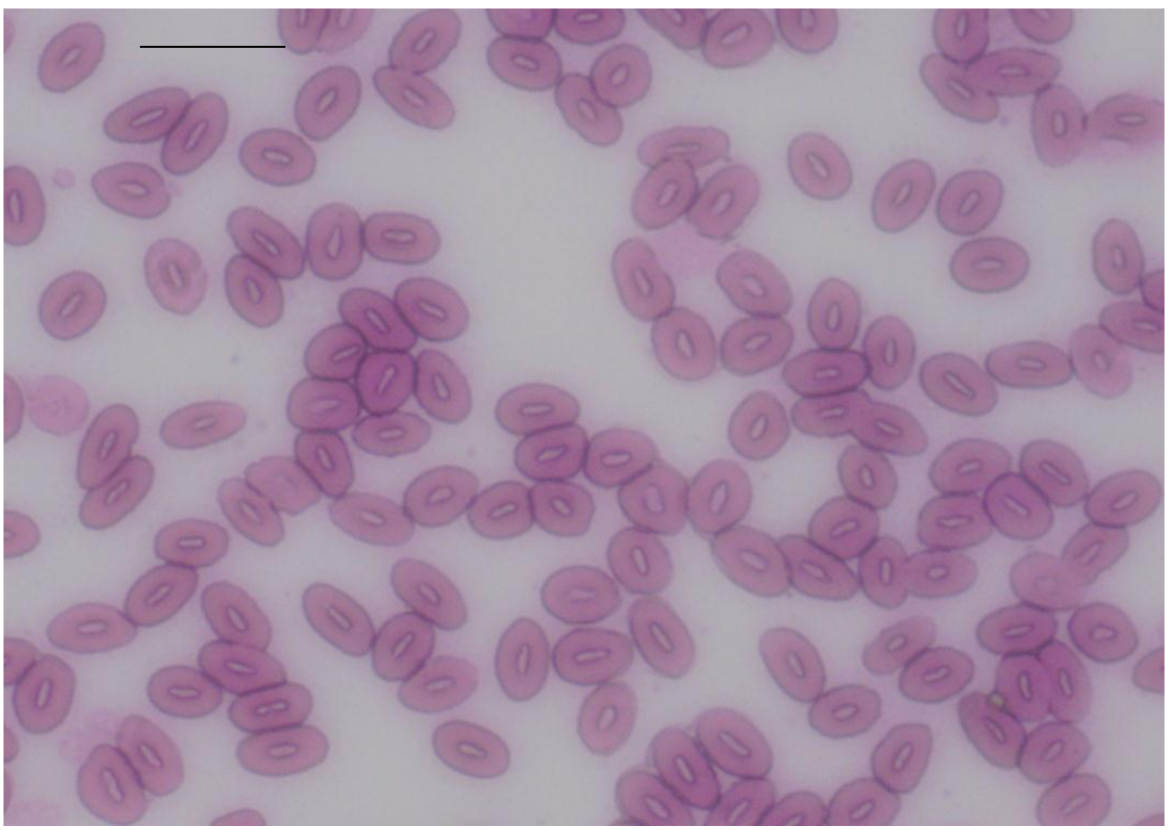

Figure-1: Red blood cells of *Channa punctatus*.

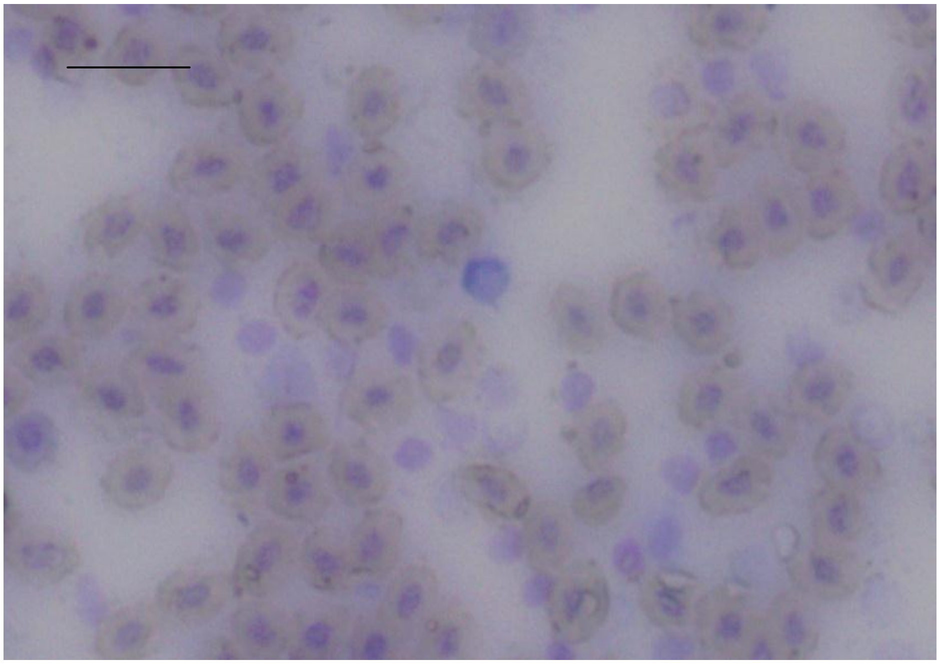

Figure-2: Red blood cells of *Anabas testudineus*.

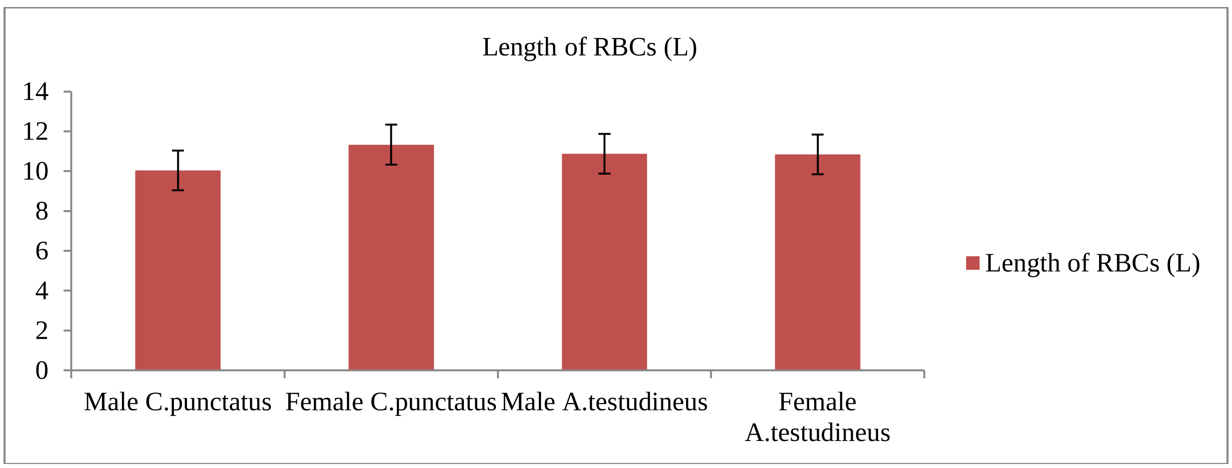

Figure-3: Sex-wise differences of length of RBCs in *Channa punctatus* and *Anabas testudineus*.

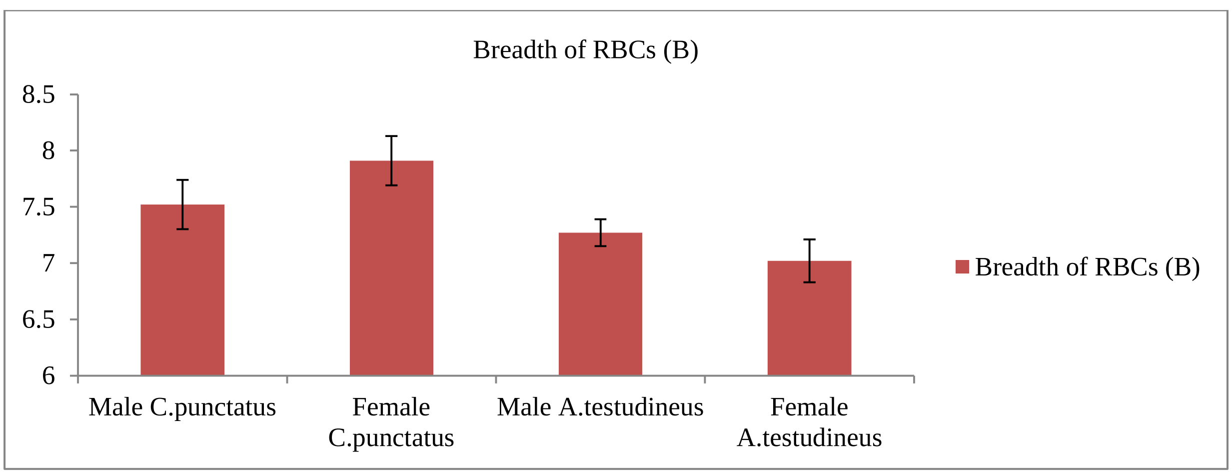

Figure-4: Sex-wise differences of breadth of RBCs in *Channa punctatus* and *Anabas testudineus*.

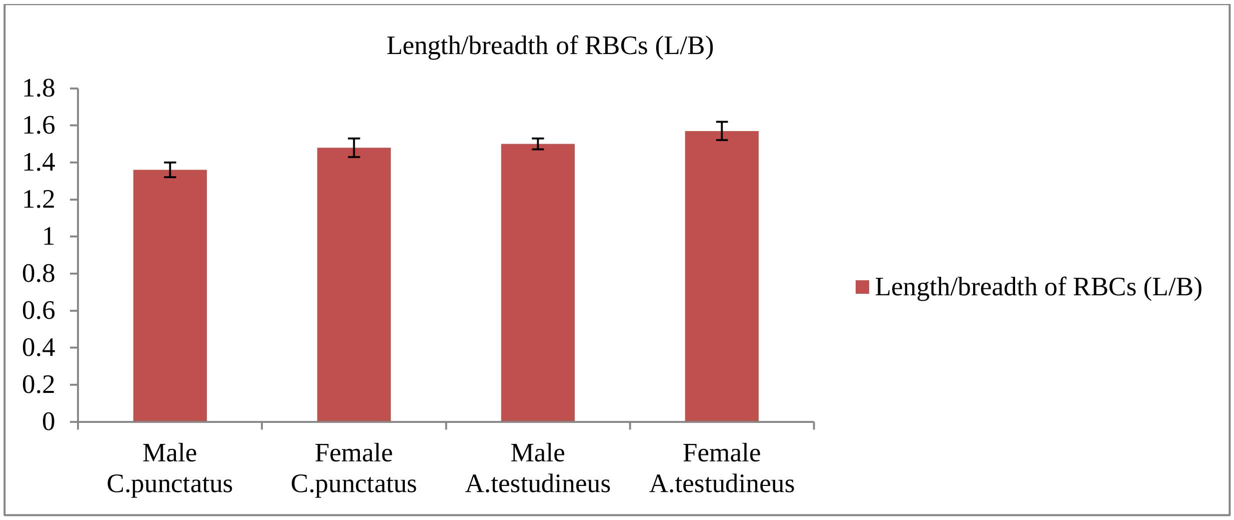

Figure-5: Sex-wise differences of ratio of length and breadth of nucleus of RBCs in *Channa punctatus* and *Anabas testudineus*.

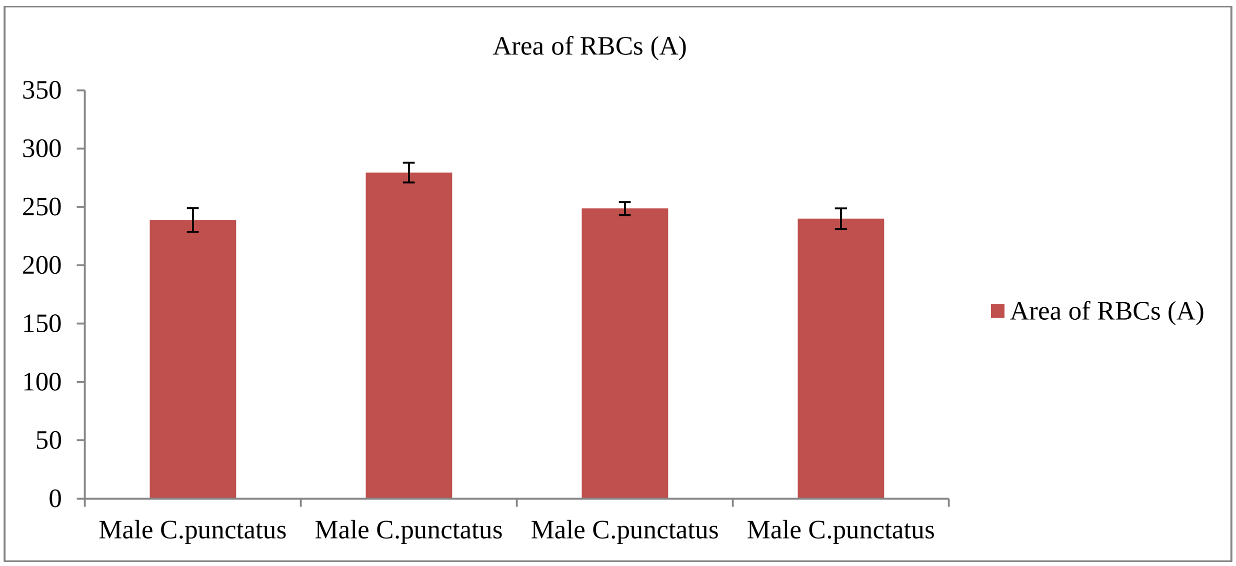

Figure-6: Sex-wise differences of area of RBCs in *Channa punctatus* and *Anabas testudineus*.

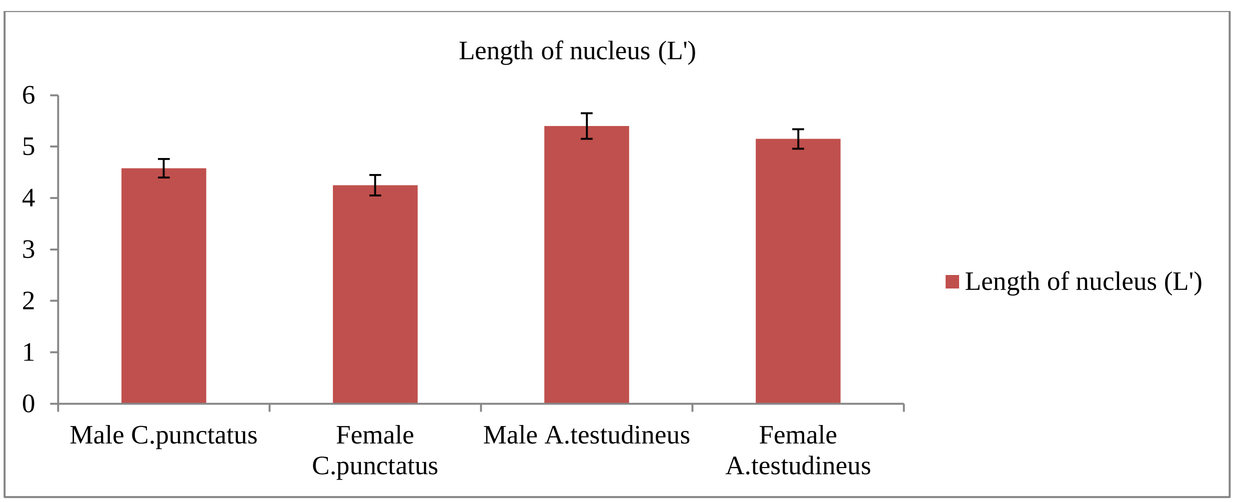

Figure-7: Sex-wise differences of length of nucleus in *Channa punctatus* and *Anabas testudineus*.

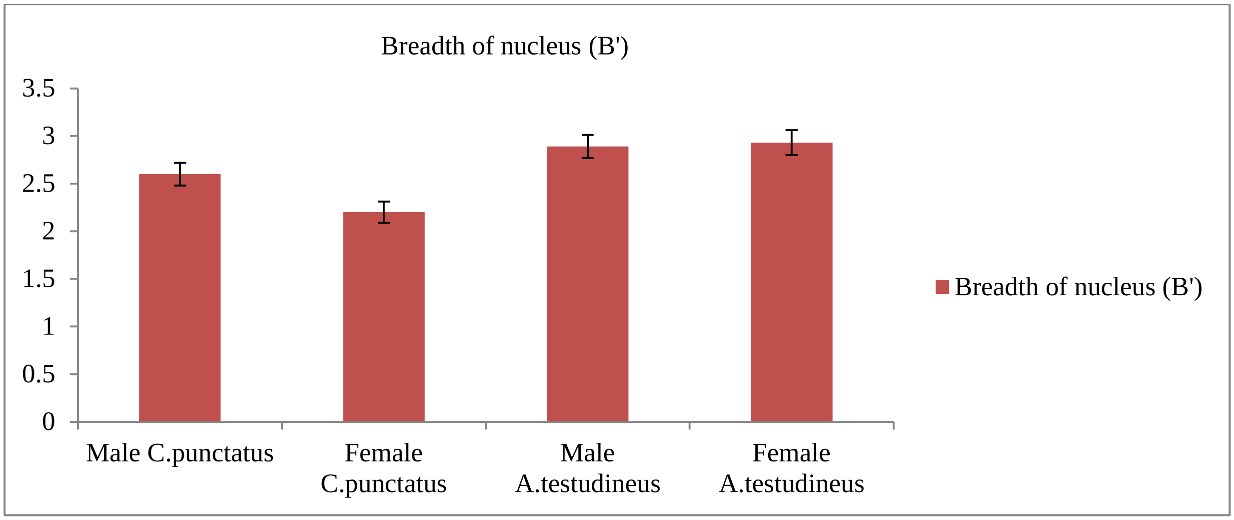

Figure-8: Sex-wise differences of breadth of nucleus in *Channa punctatus* and *Anabas testudineus*.

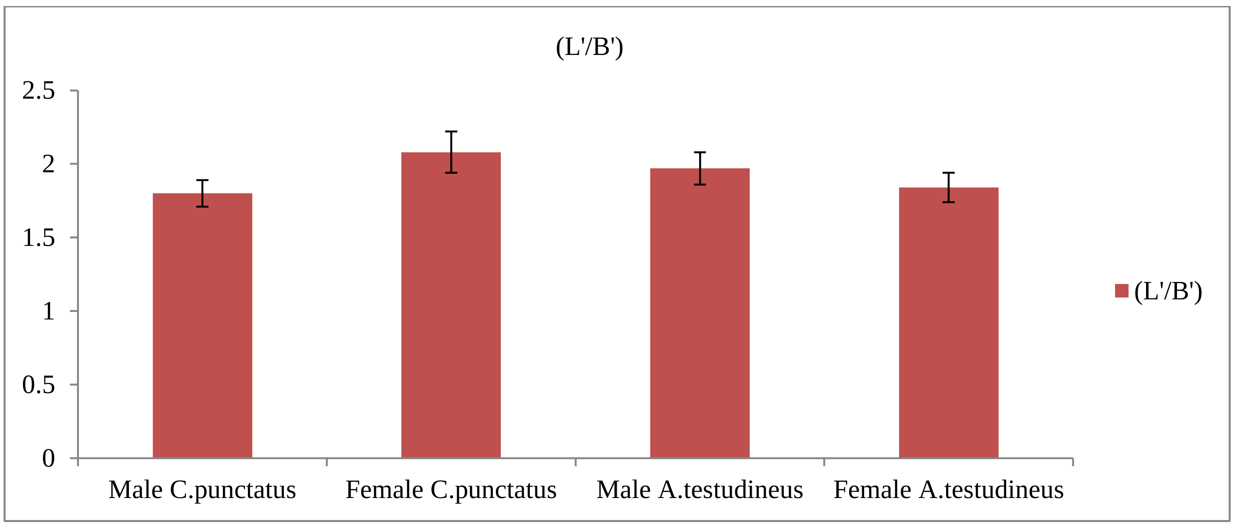

Figure-9: Sex-wise differences of ratio of length of nucleus of RBCs breadth of nucleus of RBCs in *Channa punctatus* and *Anabas testudineus*.

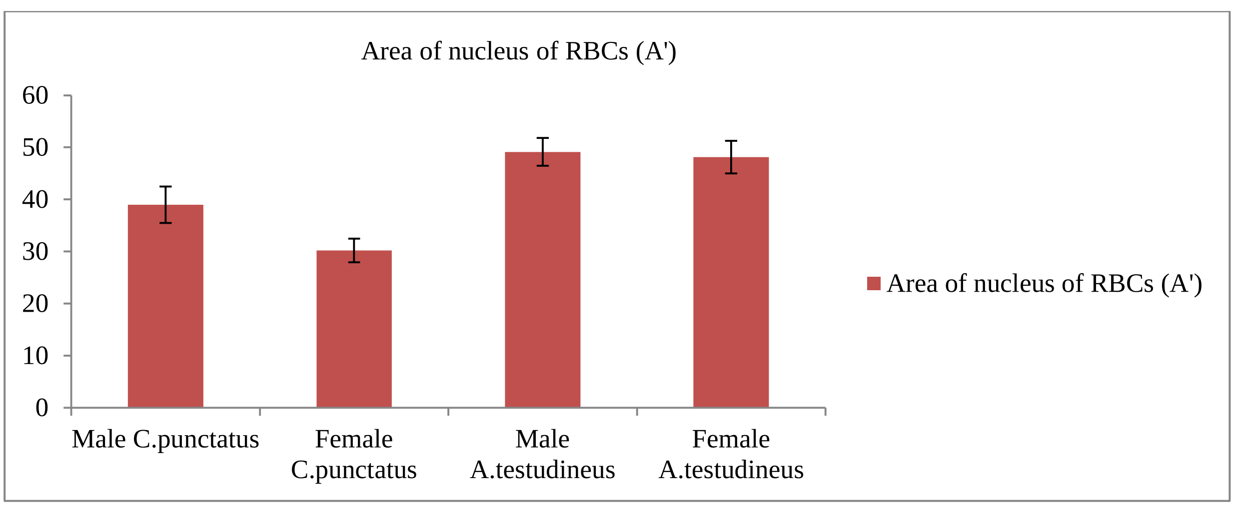

Figure-10: Sex-wise differences of area of nucleus of RBCs in *Channa punctatus* and *Anabas testudineus*.

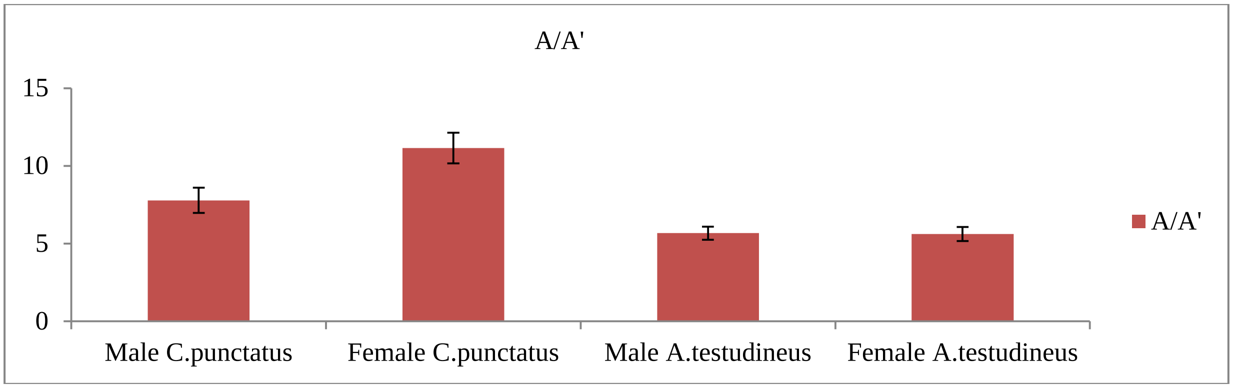

Figure-11: Sex-wise differences of area of RBCs and area of nucleus in *Channa punctatus* and *Anabas testudineus*.

Conclusion

The present study concludes that both sex and species have effect on the morphometric parameters of erythrocytes in both *C. punctatus* and *A. testudineus*. The current study indicates a basic data on the morphometry of RBCs of *Chana punctatus* and *Anabas teutudineus* which will be helpful for further comparison with other species and also diagnostic interpretation of normocytic, microcytic and macrocytic anemias can be known for these species by which their physiological conditions can be known and they can be treated accordingly with their environment.

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