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Comparative cytomorphometry of red blood cells of some fishes

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Abstract

This study aims to compare the size of the red blood cells of different freshwater fishes to gain knowledge of their activity. In this study, 11 different freshwater fishes of four different order like Siluriformes, Cypriniformes, Perciformes, and Osteoglossiformes were selected. For analyses of cytomorphometry of blood cells, blood was collected from caudal vein, and blood smear was prepared at the site of collection. In order to obtain size of different blood cell types, 30 cells of each cell type for each fish were photographed and dimensions of cell-like length and breadth were measured using Microscope Eyepiece Digital Camera (CatCam130 – 1.3 Mega Pixel (MP), Code No. CC130, Catalyst Biotech, Maharashtra, India, attached to Hund Wetzlar Microscope GmbH, Wetzlar-Nauborn, Germany) and computer. This study confirms the cytomorphometry of red blood cells differ significantly at $p < 0.001$ concerning sex and species. The study will help in diagnosis which in turn will accelerate production of fishes.

Keywords: *Freshwater, Cytomorphometry, Red blood cells, Fish*

1. Introduction

Comparative hematological study, especially regarding the morphology of blood cells, is another segment of research in the field of aquaculture. Morphometrical analysis of blood cells plays a significant role in fish pathology. Study of blood cells in many different species gives knowledge on how the size of blood cells varies in relation to their activities. RBCs are the most abundant cell type in the bloodstream and are known for their roles in gas exchange and respiration. In mammals, mature RBCs are flexible, oval, biconcave disks that lack cell nuclei, organelles, and ribosomes. In nonmammalian vertebrates, RBCs are oval, flattened, biconvex disks with a cytoskeleton composed of a marginal band of microtubules and a cell nucleus and organelles in their cytoplasm, which allow them to de novo synthesize proteins and molecules in response to stress and stimuli (Nombela and Ortega-Villaizan, 2018). Study of vertebrate blood cells particularly fishes, attracted scientific attention when a detailed study of the red blood cells in scorpion fishes were carried out (Gulliver, 1875). Cytomorphometry of blood cells included the measurements of length, breadth, and area of both cell and nucleus as well as the estimation of N/C ratio and expressed in micrometer (μm). Due to the presence of a lobed nucleus, it is impossible to measure the nuclear length, breadth, and area of monocytes, neutrophils, and eosinophils. The data on cytomorphometrical analysis of fish blood cells are inadequate though it is

required not only to know the activity of fishes but also to classify different types of anemia. Therefore, in this investigation, comparative morphometry of different types of blood cells of some freshwater water fishes is taken for consideration in respect to sex, species, and habitat.

2. Materials and Methods

All procedures used for this investigation followed approved guidelines for the ethical treatment of animals. The fishes collected for this investigation from freshwater aquatic bodies are *Clarias batrachus* (Linnaeus, 1758), *Heteropneustes fossilis* (Bloch, 1794) , *Anabas testudineus* (Bloch, 1792), *Channa punctatus* (Bloch, 1793) , Channa striatus (Bloch, 1793) , *Oreochromis niloticus* (Linnaeus, 1758), *Labeo rohita* (Hamilton, 1822), Catla catla (Hamilton, 1822), *Cirrhinus mrigala* (Hamilton, 1822) , *Cirrhinus reba* (Hamilton, 1822) , *Notopterus notopterus* (Pallas, 1769) .

For analyses of cytomorphometrical of blood cells, blood was collected from caudal vein and blood smear was prepared at the site of collection. For smearing of blood, one drop of blood was taken on a clean grease free slide at one end immediately after collection. Another slide having a uniform edge was placed just on the drop of blood at an angle of 45° to the first and moved to touch the blood. The slides used for this study were of Blue Star Company, micro slides, Pic-2 manufactured by Polar Industrial Corporation, Mumbai India, ground edges and lint free packing, measuring 75 mm long, 25 mm wide and 1.45 mm thickness.

After this, the spreader slide was moved gently over the first slide without interruption until the end to have a uniform and smooth blood smear on the slide. Then the slide was air dried for 10 minutes. The dried blood smeared slide was kept over the staining rack over a well-leveled plane. The slide was then stained by Leishman's stain. From each specimen, ten slides were prepared and kept ready for observation of blood cells.

In order to obtain the size of different blood cell types, 30 cells of each cell type for each fish were photographed and dimensions of cell-like length and breadth were measured using Microscope Eyepiece Digital Camera (CatCam130 – 1.3 Mega Pixel (MP), Code No. CC130, Catalyst Biotech, Maharashtra, India, attached to Hund Wetzlar Microscope GmbH, Wetzlar-Nauborn, Germany) and computer. For this investigation, 30 cells, randomly selected from different smears from different fishes were measured except nuclear dimension of granuloytes because of indented of the nucleus.

From the measured data, the following parameters were calculated as stated below.

Area of elliptical blood cells (Erythrocyte) = π x LD /2 x SD/2

Where LD = Longer diameter of the cell, SD = Shorter diameter of the cell

Area of rounded blood cells (Leucocytes) = πr^2

Where r is the radius of the circular cell, r = Diameter/2

N/ C ratio =Nucleus area/Cell area

3. Statistical Analysis

Cytomorphometrical parameters were expressed as mean ± SE. The entire data obtained were subjected to Paleontological Statistics (PAST) Version 2.17 [Natural History Museum, University of Oslo] for One-Way Analysis of Variance (ANOVA) followed by Tukey's pair wise comparison tests. Differences were classified as significant at $p < 0.001$.

4. Results

This study records the morphometry (Table 1 and Figure 1) and morphology (Figure 2 to 12) of erythrocytes of different freshwater fishes. The morphometry of erythrocytes like cell length is found to be higher in males of *Cirrhinus mrigala* (11.31±0.21) of the order Cypriniformes whereas lower in females of *Anabas testudineus* (8.30 ±0.10) of Perciformes. The cell length varies significantly between the species of different orders and also between the different species of same order at the level $P<0.001$. Significant difference with respect to sex is observed in cell length of *Clarias batrachus* (males = 8.72±0.14 and females = 9.57±0.15), *Cirrhinus mrigala* (males = 11.31±0.21 and females = 9.75±0.31) and *Anabas testudineus* (males = 9.83±1.5 and females = 8.30±0.10).

Table 1: Comparative Cytomorphometry of Erythrocytes Among Freshwater Fishes

Morphometry of Erythrocyte			Cell Length	Cell Breadth	Cell Area	Nucleus Length	Nucleus Breadth	Nucleus Area	N/C Ratio
Siluriformes	*H. fossilis*	♂	9.97± 0.3a	8.39± 0.34a	67.96± 4.77a	6.24± 0.26a	4.72± 0.26a	24.69± 2.62a	0.35± 0.19a
		♀	9.78± 0.28,b	8.83± 0.28b	69.64± 4.11b	5.25± 0.22b	3.99± 0.22b	17.49± 1.70a,b	0.26± 0.02b
	C.batrachus	♂	8.72± 0.14a,c	7.09± 0.19a,b,	49.27± 2.07a,b,c	3.70± 0.11a,b,c	2.45± 0.12a,b,c	17.31± 0.5a,b,c	0.15± 0.01c
		♀	9.57± 0.15C,d	8.19± 0.14a,b,c	62.04± 1.72 c,d	5.06± 0.13a,c,d	4.09± 0.12,c,d	16.57± 0.87,c,d	0.27± 0.01
Perciformes	*L.rohita*	♂	10.29± 0.21c,e	8.68± 0.18a,c,d	70.91± 2.42c,e	6.70± 0.23,b,c,d,e	4.60± 0.26,c,	25.11± 2.03,c,e	0.35± 0.02
		♀	10.11± 0.19c,f	8.44± 0.22a,b,c,e	68.05± 2.76c,f	6.00± 0.23c	4.34± 0.20,c,e	21.25± 1.63,c	0.31± 0.019
	C.catla	♂	10.32± 0.18c,g	7.77± 0.17a,b	63.64± 2.24g	6.47± 0.19b, c,d,g	4.12± 0.15,c,f	21.37± 1.33,c,f	0.33± 0.17
		♀	9.83± 0.24c,h	8.48± 0.25a,b,c,f	67.03± 3.55 c h	6.23± 0.25, c,d,h	4.98± 0.22,c,g	25.63± 2.11,c,g	0.37± 0.01
	C.mrigala	♂	11.31± 0.21a,h,i	8.22± 0.23a,b	73.47± 2.83c,i	6.91± 0.24b, c,d,i	4.75± 0.26,c,,h	27.03± 2.57,b,c,d,f,h	0.35± 0.02
		♀	9.75± 0.31 l,j	8.09± 0.25a,b,g	67.72± 4.12 i,j	5.80± 0.26, c	4.52± 0.22,c,i	21.93± 2.39,c,h	0.34± 0.02
	C.reba	♂	10.85± 0.24c,d,k	8.41± 0.26a,b,c,h	73.17± 3.75c,k	6.30± 0.23, c,d,j	4.73± 0.26,c,,j	24.81± 2.28,c,i	0.33± 0.02
		♀	10.21± 0.24c,l	8.35± 0.22a,b,c,i	68.25± 3.14c,l	5.91± 0.23, c	4.47± 0.23,c,,k	21.94± 2.06,c	0.32± 0.02
	C.striatus	♂	10.22± 0.25c,m	8.21± 0.29a,b,j	67.64± 4.03c,m	5.87± 0.30, c,k	4.22± 0.22,c	20.77± 2.08,c,j	0.30± 0.02
		♀	10.21± 0.24c,n	8.41± 0.29a,b,c,k	69.07± 3.90c,n	6.30± 0.24, c,d	4.69± 0.22,c,l	24.25± 1.93,c	0.34± 0.01
	C.punctatus	♂	9.53± 0.17b,d, l,k,o	7.41± 0.25a,b,d l	55.96± 2.4i,k	5.45± 0.18, c,e,l,l	3.45± 0.12a,c,g, h,j,l,m	14.96± 0.81a,c,e, g,h,l,j	0.27± 0.01
		♀	9.63± 0.20,l,,k,p	8.23± 0.22a,b,l	63.37± 2.97,o	6.36± 0.22, c,d,m	4.84± 0.24,c,m,,n	25.11± 1.99,c,j,k	0.39± 0.02 b
	O. niloticus	♂	9.32± 0.19f,l,k	7.69± 0.14a,b	57.04± 2.05l,k	5.31± 0.17, c,e,g,l,n	4.24± 0.18,c,o	18.33± 1.33,c,l	0.32± 0.02
		♀	9.06± 0.18e,g,l,j, k,l,m,n	7.90± 0.19a,b	57.22± 2.48i,k	6.58± 0.20b,c, d,l,n,o	5.44± 0.22b,c,d, e,f,k,m,o,p	29.17± 1.94,c,d, f,l,m	0.50± 0.19,b,c,d
	A. testudineus	♂	9.83 ±1.5c,l,q	8.03 ±0.20a,b,m	62.75± 2.38,p	5.01± 0.18a,c,e,g,h, l,j,k,m,o,p	3.39± 0.18a,c,g, h,l,j,l,n,p	13.96± 1.18a,b,c,e, g,h,l,j,k,m,n	0.23± 0.02,d,e
		♀	8.30± 0.10a,b,d,e, f,g,h,l,j,k,l, m,n,o,p,r,q	6.82± 0.24a,b, d,e,f,g, h,l,j,k,l,m	44.88± 1.85a,b,d,e, f,g,h,j,k,l, m,n,o,p,q	4.92± 0.16a,c,f,g, h,l,j,k,m,o,q	3.94± 0.18,c,p	15.91± 1.24,c,g, h,m,n	0.45± 0.11,b
Osteoglossi-formes	*N. notopterus*	♂	9.73± 0.12,l,k,r	7.73± 0.19a,b	59.61± 1.47	6.48±0.14 b,c,d,n,p,q	4.27± 0.16,c,p	21.86± 0.97,c	0.37± 0.01
		♀	9.74± 0.22, l,r	7.80± 0.27 a,b	61.31± 3.27q	6.81±0.23 b,c,d,l, n,p,q	4.23± 0.26, c,p	23.89± 2.46,c	0.52± 0.14a, b,e
F-Value			8.969***	9.756***	5.677***	12.72***	8.468***	8.027***	3.367***

Note: Figures in parentheses represent number of observations; Mean ± SE bearing similar letters differ significantly in rows at *p* < 0.001; *** Significant at p < 0.001.
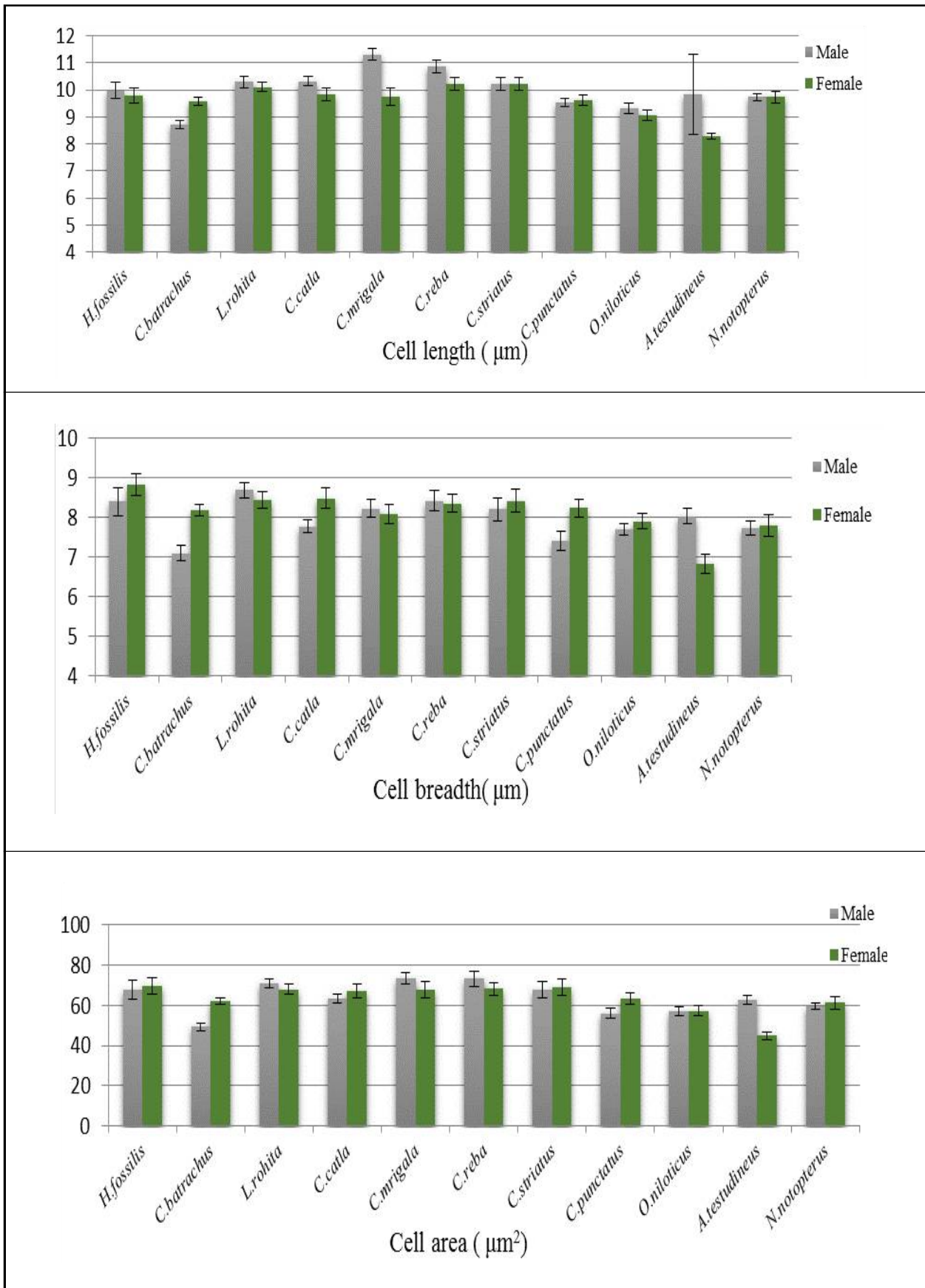
Figure 1: Comparative Morphometrical Analyses of Erythrocytes Among Freshwater Fishes
a. Cell Length, b. Cell Breadth, c. Cell Area, d. Nucleus Length, e. Nucleus Breadth, f. Nucleus Area, g. N/C Ratio
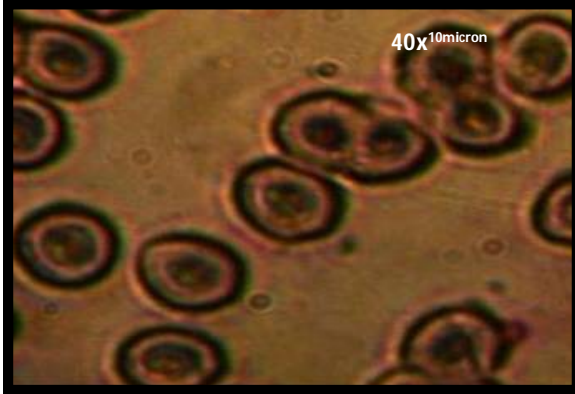
Nucleus length(μm)

Nucleus breadth(μm)

Nucleus area(μm²)

N/C ratio

Figure 1 (Cont.)
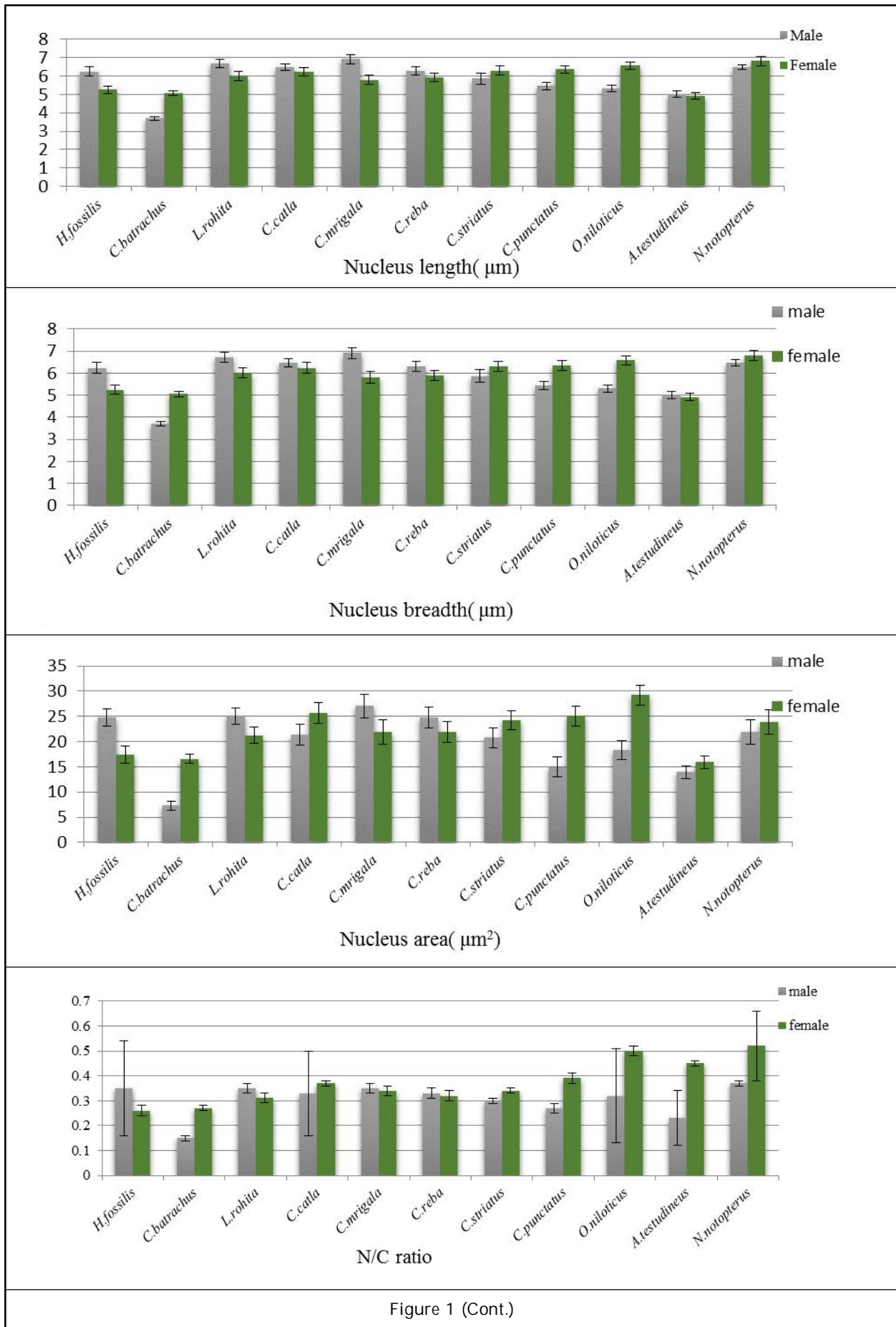
Figure 2: Erythrocyte of freshwater *H. fossilis*

Figure 3: Erythrocyte of freshwater *C. batrachus*
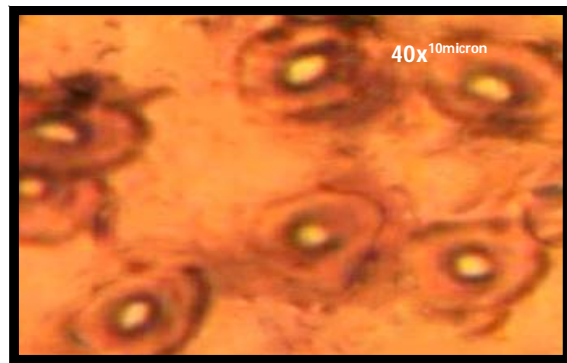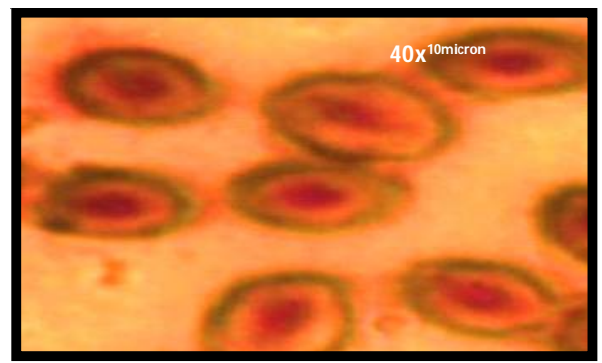

Figure 4: Erythrocyte of freshwater *L.rohita*

Figure 5: Erythrocyte of freshwater *C. catla*

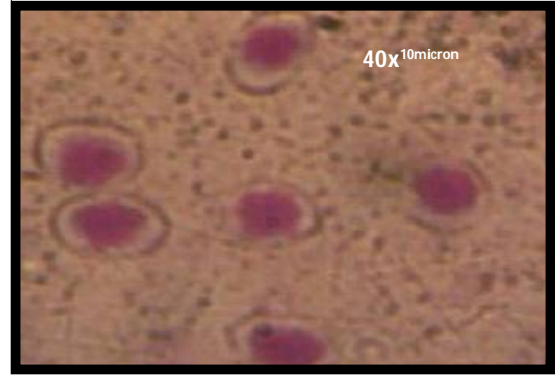**Figure 6: Erythrocyte of freshwater *C. mrigala***

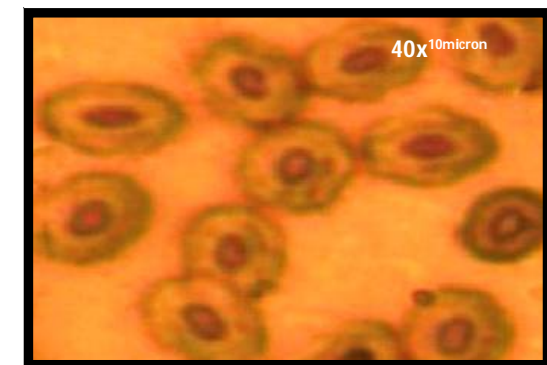**Figure 7: Erythrocyte of freshwater *C.reba***

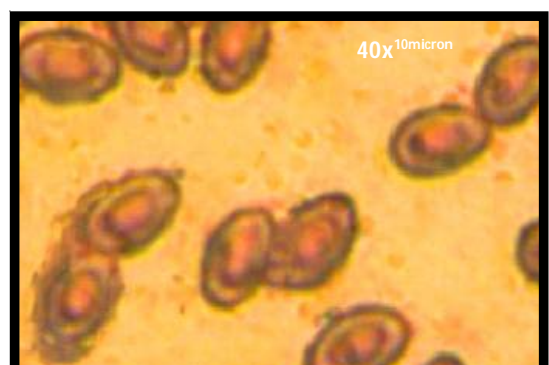**Figure 8: Erythrocyte of freshwater *C. striatus***

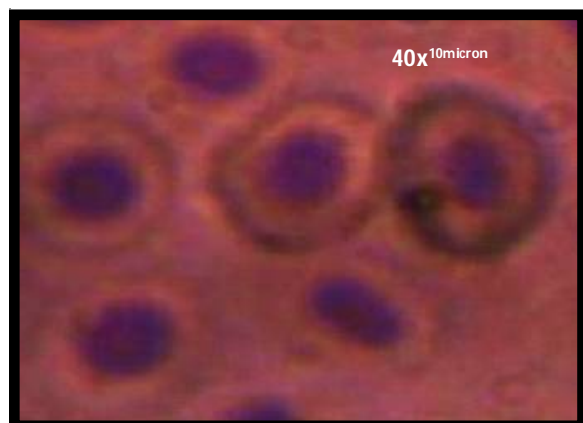**Figure 9: Erythrocyte of freshwater *C. punctatus***

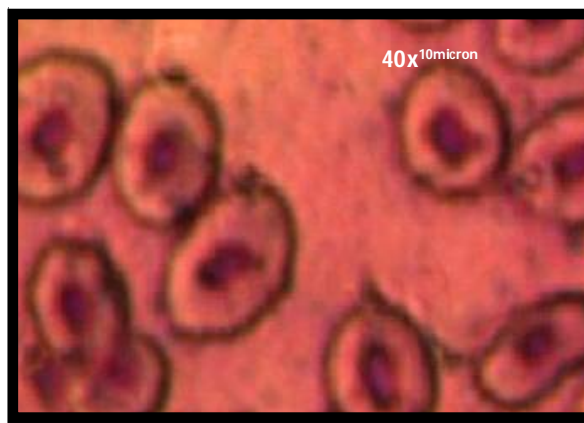**Figure 10: Erythrocyte of freshwater *O. niloticus***

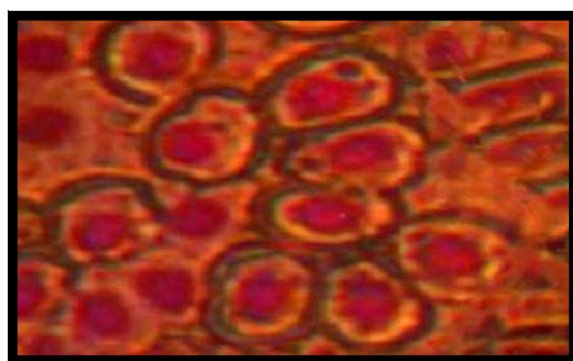

Figure 11: Erythrocyte of freshwater *A.testudineus*

Figure 12: Erythrocyte of freshwater *N. notopterus*

The cell breadth of erythrocyte shows higher value in *Labeo rohita* (8.68±0.18) and lower in *Anabas testudineus* (6.82±0.24). Sex wise significant difference in cell breadth is recorded in *Clarias batrachus* (males = 7.09±0.19 and females = 8.19±0.14), *Channa punctatus* (males = 7.09±0.19 and females = 8.19±0.14) and *Anabas testudineus* (males = 8.03±0.20 and females = 6.82±0.24) at $P < 0.001$. It is noted that area of erythrocytes reflects higher value in *Cirrhinus mrigala* (8.30 ±0.10) and lower in A*nabas testudineus* (8.30 ±0.10), but sex wise significant difference of erythrocyte area is found only in *Clarias batrachus*

(males = 49.27±2. 07 and females = 62.04±1.72), *Cirrhinus mrigala* (males = 8.03±0.20 and females = 6.82±0.24), *Channa punctatus* (males = 8.03±0.20 and females = 6.82±0.24) and *Anabas testudineus* (males = 62.75±2.38 and females = 44.88±1.85).

Morphometrical parameters of nucleus deviate significantly species-wise at $p < 0.001$. The length of the nucleus showed higher value in *Cirrhinus mrigala* (6.91 ±0.24) and decreased in *Anabas testudineus* (4.92 ±0.16). Sex-wise significant difference in nuclear length is noted in *Clarias batrachus* (males 3.70±0. 11 and females = 5.06±0.13) and *Cirrhinus mrigala* (males 6.91±0. 24 and females =5.80±0.26). The nuclear breadth (5.44±0.22) and area (29.17±1.94) have more value in *Oreochromis niloticus* of Perciformes while a lower value of nuclear breadth is noted in *Clarias batrachus* (2.45±0.12) of Siluriformes and the nuclear area is recorded in *Channa punctatus* (14.96±0.81) of Perciformes.

Sex wise significant variations in nuclear breadth is measured in *Clarias batrachus* (males = 2.45±0.12 and females 4.09±0.12), *Channa punctatus* (males = 3.45±0.12 and females = 4.84±0.24) and *Oreochromis niloticus* (males = 4.24±0.18 and females = 5.44±0.22) whereas, nuclear area shows sex wise significant variation in *Heteropneustes fossilis* (males = 24.69±2.62 and females =17.49±1.70), *Cirrhinus mrigala* (males = 27.03±2.57 and females = 21.93±2.39), *Channa punctatus* (males = 14.96±0.81 and females = 25.11±1.99) and *Oreochromis niloticus* (males = 18.33±1.33 and females = 29.17±1.94).

The N/C ratio shows more value in *Notopterus notopterus* (0.52±0.14) and less value in *Clarias batrachus* (0.15±0.01). The N/C ratio deviates significantly only with respect to species but not sex wise.

5. Discussion

The mean values for the size of the erythrocytes deviate significantly between species of fishes selected for this work, this result getting supported by the findings reported by Barron et al. (1956) and McKnight (1966). It is observed that erythrocytes of fishes in this investigation are usually elliptical in their shape and nucleated. This corroborates with the findings of Srivastava (1968) and Pandey et al. (1976) who observe similar shape in *Heteropneustes fossilis.* The comparative study of morphometry of erythrocytes reflects the highest length in slow moving fishes as compared to the active fishes.

In freshwater fishes, the highest length of erythrocytes is observed in the order Cypriniformes as compared to fishes of the order Siluriformes. Similarly, in brackish water, fishes coming under the order Perciformes have higher value in comparison to the Siluriformes. According to Witeska (2013), the activity of the animals and the size of the blood cells are closely related, i.e., the more active species have smaller erythrocytes and sluggish ones have larger corpuscles. According to Wintrobe (1933) the erythrocyte size reflects the position of a species on the evolutionary scale, i.e., in lower vertebrates and those with not successful evolutionary past, such as cyclostomes, elasmobranches and urodeles, the size of erythrocytes is large, but in higher vertebrates (mammals) the same cells are smaller and do not contain nuclei. Erythrocytes are the dominant blood cell type in the vast majority of fish species (Lisicic et al., 2013). Morphometric studies on the blood cells in *Cyprinus carpio, Ctenopharyngodan idella* and *Hypophthalmichthys molitrix* by Kumar (2016) reports that the number of erythrocytes is the most abundant blood cells followed by thrombocytes. One of the most important function of erythrocyte is carrying oxygen and carbon dioxide and the ratio of size to surface area is also a determining factor in the tissues. Thus, a small erythrocyte offers the possibility of a higher rate of exchange than a larger one (Hartman and Lessler, 1964; Sevinch et al., 2000).

Smith (1925) states that RBC size varies inversely with the metabolic activity and the red blood size has an adaptive value therefore, any adaptive reduction in metabolic rate leads to increase in cell size. There is a correlation between the dimension of erythrocytes and its oxygen carrying capacity. The concentration of haemoglobin (Hb) in the blood depends on the number of erythrocytes per unit volume of blood or by increasing the volume of red blood cells, or both. As a rule, the concentration of red blood cell (RBC) in the blood of homoeothermic vertebrates is always higher than poikilotherms and on the contrary, the mean diameter of RBC of homoeothermic animals are lower than that of poikilotherms. The size of erythrocytes of fishes found in this study is larger than mammal (Radhakrishnan et al., 1976). Moreover, erythrocytes of fishes have low N/C ratio. In this study, the normal N/C ratio ranges from 0.15 to 0.52 in freshwater fishes whereas 0.28 to 0.68 in brackish water fishes. This morphological peculiarity of the cell enables the erythrocytes to concentrate maximum amount of haemoglobin.

Totter (1956) observes cellular haematology of Salamander and reports banding pattern of erythrocytes which may be considered as shape change corresponding to disc sphere transformation in mammalian red blood cells. Fish RBCs, are integral in several biologic processes relevant to immunity, such as pathogen recognition, pathogen binding and clearance, and production of effector molecules and cytokines (Marin et al., 2018). The present observation on the morphometry of erythrocytes with eccentric nucleus of fishes is an evidence to support the phylogenetic transformation, of mammalian erythrocytes assuming a new shape and loses its nucleus. Therefore, erythrocyte of fishes may be a transitional stage towards evolution of erythrocytes of higher vertebrates.

6. Conclusion

This study records the activity of the fishes and the size of the blood cells are closely related, i.e., the more active species have smaller erythrocytes and sluggish ones have larger erythrocytes. The data obtained for cytomorphometry of blood cells of some freshwater fishes in this study may enhance the current understanding of the cytological values, which is expected to help ichthyologists and fish farmers for diagnosis, prognosis and treatment of different anaemia in fishes. This will accelerate in the production of healthy and disease-free fishes.

Compliance with Ethical Starndards

This investigation followed all the guidelines for the care and use of animals.

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Conflicts of Interest

First author declares that she has no conflict of interest.
Second author declare that he has also no conflict of interest.

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