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# The Morphometrical Characterisation of Normal Blood Cells of Two air-breathing fishes, *Clarias batrachus and Anabas testudineus*

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## Abstract

*Morphometrical analysis of blood cells plays a major role in fish pathology. Study of blood cells in many different species of fishes provides an interesting comparison of cell size in relation to their activities and habits. For morphometrical analysis of blood cells blood was drawn from the caudal vein and blood smears were prepared immediately after blood was obtained and stained with Giemsa stain. Blood cells and its nucleus length and breadth were measured in micron meter. In this investigation five types of blood cells were found those are erythrocyte, lymphocyte, monocyte, eosinophil and neutrophil but no basophil was observed in Anabas testudineus as well s in Clarias batrachus. Nucleus length and breadth could not measured in granulocytes because of lobed nucleus. Erythrocyte cell length and breadth shows highly significant difference ($p<0.001$) with respect to sex in C .batrachus and A.testudineus and also between these two different fishes. Erythrocyte nucleus length and breadth shows significant sexual difference ($p<0.001$) between different fishes taken for this study. C.batrachus only shows significant sexual difference in nucleus length and breadth which is absent in A.testudineus. Lymphocyte cell length do not differ significantly but cell breadth shows significant sexual dimorphism at $p<0.05$ only in A.testudineus. Lymphocyte nucleus length and breadth shows highly significant difference ($p<0.001$) between these two different air breathing fishes taken for this study but does not show sex wise difference. Among the granulocytes eosinophil and neutrophil do not differ significantly sex wise as well as between these two species. Monocyte cell length ($p<0.05$) and breadth ($p<0.01$) differ significantly between these two species of fish but does not show sex wise difference.*

**Keywords:** *Anabas testudineus*, *Clarias batrachus*, Blood cells, Morphometry.

## Introduction

Morphometrical characterization of blood cells plays an important role in fish pathology. In most mammals, erythrocytes are enucleated and biconcave. Fishes, reptiles and birds has elliptical erythrocytes with nucleus. Erythrocytes size of fish varies among different species. Extending over periods of year, the red blood cells size in vertebrate was studied [1]. Oval and round cells of blood were studied in the skate and dogfish[2]. Morphology of peripheral blood cells of desert horned viper was observed[3]. Results of relative dimensions of red blood cells of vertebrates, especially birds, Emu were reported[4] and the results of red blood cell size of many researchers were tabulated[5]. Few reports on blood cell measurements are there because many species are not studied till now. Study of blood cells size in different species can give us information about cell size in order to their habit and habitat.

The erythrocytes morphology was reported for 7 freshwater fish species[6]. They found that shape and size of nucleus differ significantly among the species. However nuclear shape and size varies less within species as compared to among the species. On the basis of morphological difference in nuclear shape, leukocytes in mammals are easily identified. Neutrophils are phagocytic cells that help the cell to kill and digest microorganisms. Eosinophils are specialized cells of the immune system actively involved in the defence against allergic reaction. Due to variation in morphology of these white blood cells in different animals it is difficult to identify these cells on the basis of morphology. Veterinary hematology uses Leishman, Wright and Giemsa stain to identify blood cells. Basophils are present in few animals, and this cell type is reported in a few species. Circulating blood has also immature leukocytes. Thus, cytochemical staining of piscine leukocytes may be helpful in identification of cell types and also useful in identifying immunological cell types associated with developmental and pathological processes.

Morphometry is a quantitative description of geometrical structures in all dimensions[7-8]. It provides a numerical objectification of the most suitable modifications unavailable to visual estimation, and as such has clinical and research applications that are becoming more numerous, especially in cytology and histopathology[9-11]. This study analyzes the morphometry of normal blood cells of Clarias batrachus and *Anabas testudineus* as the morphometrical characterization of blood cells of these two species is not well defined in literature. So, this investigation is planned to determine the size of blood cells and its nucleus. This information will be helpful in

determining the health status of the *C.batrachus* and *A. testudineus.*

## Material and Methods

In the present investigation, 15 males and 15 females of each species were collected from a freshwater pond at Khurda District, Bhubaneswar, Odisha and were transported to the laboratory. Then blood was collected from live fish putting it on a bed in a tray. Fish head was covered with a damp cloth then a small sample of whole blood was drawn from the caudal vein. Immediately blood smears were prepared after blood was collected in EDTA (Ethylenediaminetetraacetic Acid) vials and stained with Giemsa stain (prepared from Giemsa powder (Qualigens CAS NO. 51811-82-6 Product No. 39382, Thermo Fisher Scientific India Pvt. Ltd., Mumbai, Maharashtra, India). From each slide length and breadth of blood cells and nucleus were measured with the help of 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 Morphometry of cells and nuclei were expressed as mean±SE in male and female fish of each species. The cellular and nuclear dimensions were compared using the method One-Way Analysis of Variance (ANOVA) followed by Tukey's pair wise comparison tests. All these statistical analysis were performed using the statistical software Microsoft Office Excel 2007 and Paleontological Statistics (PAST) Version 2.17

## Results and Discussion

Morphometry of blood cells and nucleus are measured in micron meter (µm) (table -1). Fish erythrocytes resemble their avian/reptilian counterpart; they are oval in shape with abundant smooth eosinophilic cytoplasm and a central, oval-shaped condensed nucleus. Cell size varies greatly within species. Erythrocyte cell length and breadth shows highly significant difference ($p<0.001$) between male and female of *C. batrachus* and *A. testudineus* as well as there is significant difference between these two species. Erythrocyte nucleus length and breadth shows highly significant difference ($p<0.001$) with sexual dimorphism in *C. batrachus* but *A. testudineus* does not show sexual difference and it shows significant difference ($p<0.001$) between the mentioned species. Gaseous exchange is the main function of erythrocytes. The rate of gaseous exchange is higher in smaller erythrocytes than larger one [12-13]. The size of erythrocyte determine the position of a species in the evolutionary scale. The sizes of erythrocytes are larger and possess nuclei in lower vertebrates like cyclostomes, elasmobranches and urodeles but in higher vertebrates (mammals) the erythrocytes are smaller and nuclei is absent[14].

Fish lymphocytes are small round cells and a rim of smooth light blue cytoplasm around the large oval-round condensed nucleus. The lymphocytes of some fish, especially elasmobranch species shows "blebs" or out pocketings of the cell membrane[15-16]. Cell length of lymphocyte does not show significant difference between the sexes as well between the two different fishes taken for present study. Lymphocyte cell breadth shows significant difference ($p<0.05$) between *A.testudineus* male and female but there is no sex wise significant difference in *C.batrachus* as well as between the two species taken for this study. The lymphocytes nucleus length and breadth differ significantly ($p<0.001$) between two different air breathing fishes but does not show sex wise difference.

**Table-1**
**Blood cell cytomorphometry of *Clarias batrachus and Anabas testudineus***

| Cell types | Cell /nucleus | Parameters | Clarias batrachus | | Anabas testudineus | | F value |
|---|---|---|---|---|---|---|---|
| | | | Male(30) | Female(30) | Male(30) | Female(30) | |
| Erythrocyte | Cell | Length | $8.72\pm0.14^{a,x}$ | $9.57\pm0.15^{b,c}$ | $9.83\pm0.15^{y,e}$ | $8.30\pm0.10^{d,f}$ | 25.58*** |
| | | Breadth | $7.09\pm0.19^{a,x}$ | $8.19\pm0.14^{b,c}$ | $8.034\pm0.20^{y,e}$ | $6.82\pm0.24^{d,f}$ | 11.47*** |
| | Nucleus | Length | $3.70\pm0.11^{a,x,g}$ | $5.06\pm0.13^{b}$ | $5.014\pm0.18^{y}$ | $4.92\pm0.16^{h}$ | 18.51*** |
| | | Breadth | $2.45\pm0.12^{a,x,g}$ | $4.09\pm0.12^{b,i}$ | $3.39\pm0.18^{y,j}$ | $3.94\pm0.18^{h}$ | 22.42*** |
| Lympho cyte | Cell | Length | 10.63±0.26 | 10.04±0.18 | 10.02±0.26 | 10.34±0.12 | $1.709^{NS}$ |
| | | Breadth | 8.41±0.20 | 8.66±0.19 | $8.34\pm0.30^{a}$ | $9.12\pm0.18^{b}$ | 2.932* |
| | nucleus | Length | $7.25\pm0.24^{x,g}$ | $6.94\pm0.19^{c}$ | $6.20\pm0.25^{y}$ | $5.44\pm0.25^{h,d}$ | 11.7*** |
| | | Breath | $5.78\pm0.22^{g}$ | $5.36\pm0.23^{c}$ | 5.06±0.25 | $4.38\pm0.22^{h,d}$ | 6.161*** |
| Monocyte | Cell | Length | $9.60\pm0.15^{a}$ | $9.73\pm0.27^{i,c}$ | $8.70\pm0.20^{b,j}$ | $8.79\pm0.30^{d}$ | 4.916** |
| | | Breadth | 8.01±0.14 | $8.20\pm0.20^{a}$ | $7.16\pm0.29^{b}$ | 7.35±0.39 | 3.375* |
| Eosinophil | cell | Length | 9.12±0.24 | 8.79±0.24 | 9.80±0.30 | 9.03±0.35 | $2.223^{NS}$ |
| | | Breadth | 7.64±0.24 | 7.26±0.28 | 7.80±0.34 | 7.58±0.37 | $0.5326^{NS}$ |
| Neutrophil | cell | Length | 9.85±0.25 | 9.99±0.19 | 9.81±0.20 | 9.33±0.24 | $1.586^{NS}$ |
| | | Breadth | 8.32±0.19 | 8.48±0.23 | 7.75±0.21 | 7.87±0.28 | $2.238^{NS}$ |

Figures in parentheses indicate number of observations *Significant at $p<0.05$, **significant at $p<0.01$ and *** significant at $p<0.001$. Consecutive Letters in the superscript represents significant differences among the rows.

Fish monocytes are large cells with a large nucleus, occupying one third of the cell. The cytoplasm contains small scattered bluish granules. Monocytes are phagocytic in function. Morphometry of monocyte shows significant difference in length ($p<0.01$) and breadth ($p<0.05$) of blood cells between the *A.testudineus* and *C.batrachus* but does not reflect sexual dimorphism. In fish, granulocytes Viz. neutrophils and eosinophils are the most common but does not show significant difference between these two fishes while basophils are much rarer. Basophils are not found in both the species.

Among white blood cells, lymphocytes of *C.batrachus* male show highest cell length and monocyte of *A.testudineus* male shows lowest cell length (figure-1). Lymphocyte of *A.testudineus* female has highest cell breadth and *A.testudineus* male's monocyte has lowest cell breadth (figure-2). Lymphocyte's Nucleus length and breadth has heighest value in *C.batrachus* male and lowest in *A.testudineus* female (figure-3 and figure-4). Erythrocytes expressed lower values in cytomorphometry compare to leukocytes.

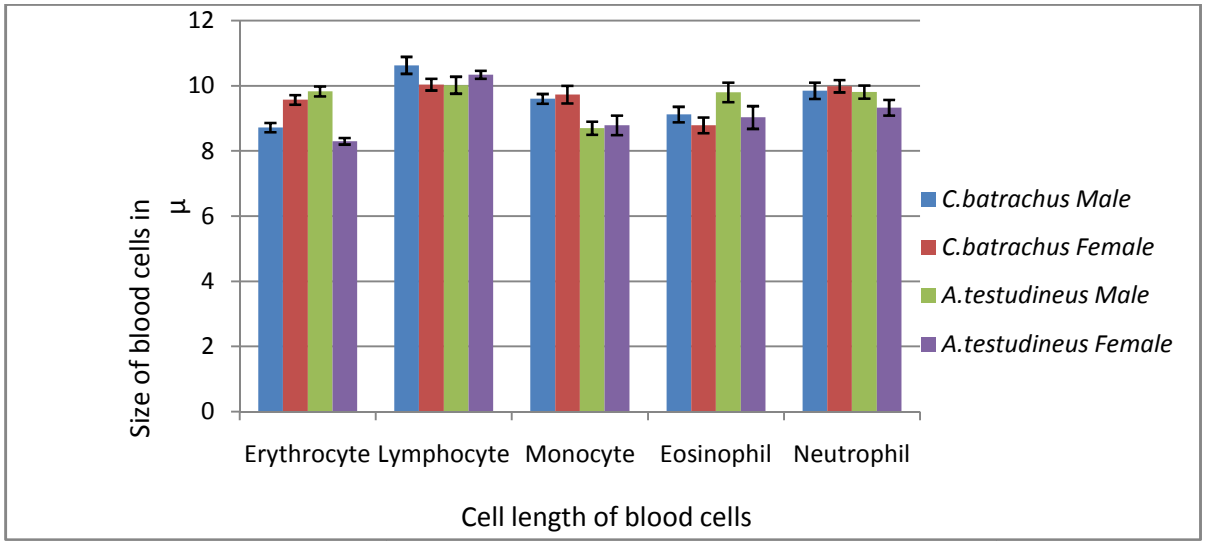


**Figure-1**
**Comparison of length of blood cells of male and female *C.batrachus and A.testudineus***

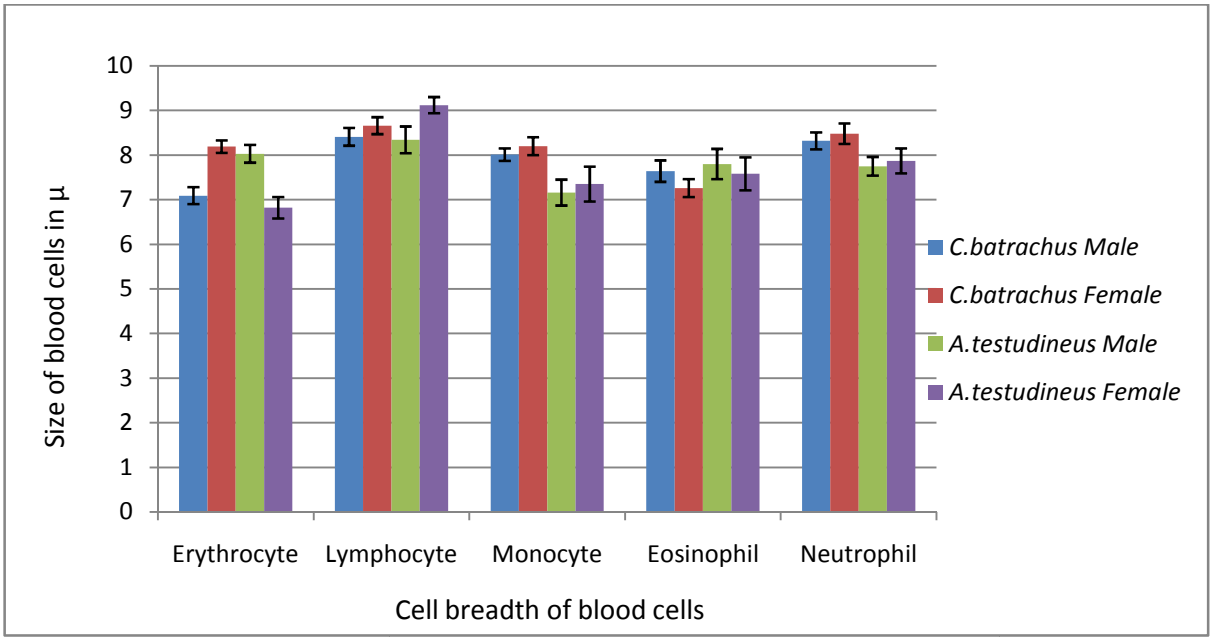


**Figure-2**
**Comparison of breadth of blood cells of male and female *C. batrachus* and *A. testudineus***

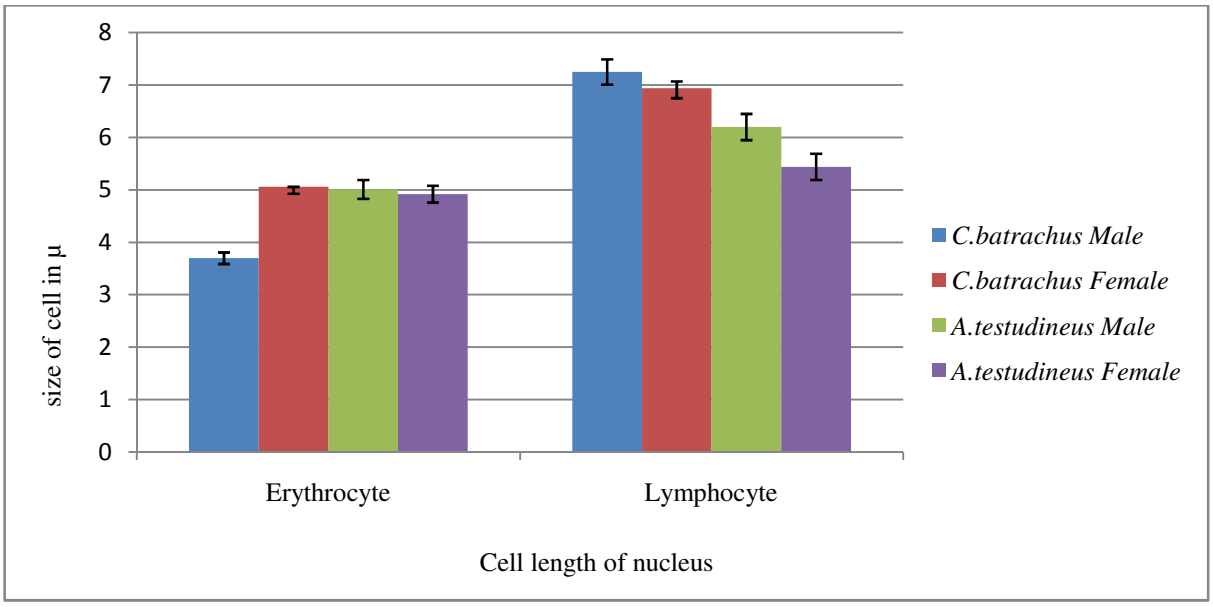


**Figure-3**
**Comparison of blood cells nucleus length of male and female *C.batrachus and A.testudineus***

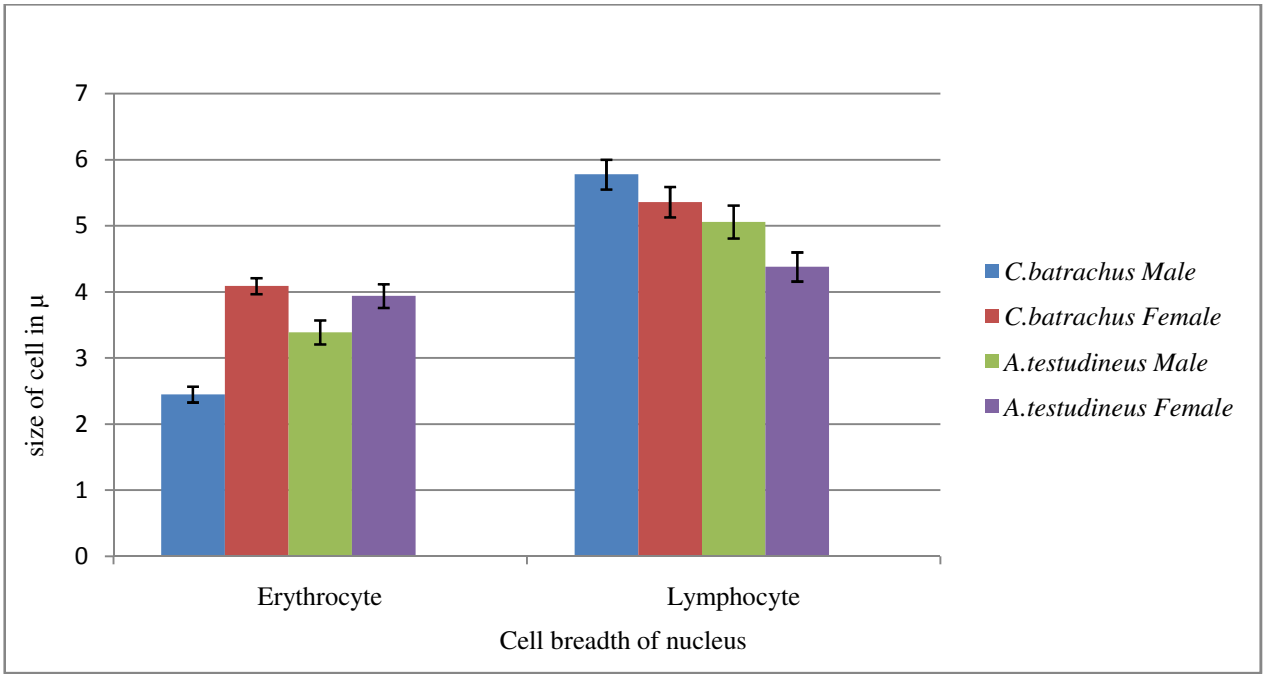


**Figure-4**
**Comparison of blood cells nucleus breadth of male and female *C.batrachus and A.testudineus***

The present investigation reveals significant difference in morphometry of blood cell and nuclei between the *A.testudineus* and *C.batrachus*. Only one type of blood cell i.e., erythrocyte shows sex wise difference in each fish. This may be due to change in environment, habitat or due to sexual dimorphism.

## Conclusion

This investigation may be helpful in determining the health status of these fishes. The evaluation of morphological parameters will detect early signs of clinical pathology as well as presence of disturbance in the aquatic environment. The abnormal cells would indicate the problems or pollution in the water quality of the pond where the species inhabits. Therefore, the blood cell analyses of fresh, marine and brackish water fishes and comparative study of blood cells of those fishes would throw light on health status of fishes.

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