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Morphometric studies of blood cells in *Cyprinus carpio, Ctenopharyngodan idella* and *Hypophthalmichthys molitrix* cultured fish in west Godavari region of Andhra Pradesh

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Abstract
The main objective of this study is to measure the blood cell dimension of the cultured fish *Cyprinus carpio, Ctenopharyngodan idella* and *Hypophthalmichthys molitrix*. Basic knowledge from this study is important for hematological research, cytological studies and clinical diagnosis. Since study of blood cells in many different species of fishes provides an interesting comparison of cell size in relation to their activities and habits an attempt was made to study morphometric data of blood cells in *Cyprinus carpio, Ctenopharyngodan idella and Hypophthalmichthys molitrix*. The fish were collected from the cultured pond in west Godavari region of Andhra Pradesh. For the 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. The cell size of Erythrocytes, Erythroblasts, Thrombocytes, Monocytes, Large lymphocytes, Small lymphocytes, Neutrophils, Eosinophils and Basophils were measured irrespective of the sex. Thrombocytes are the most abundant blood cells after erythrocytes and are recognized easily from lymphocytes by morphological features and size The size of blood cells (Range & Mean of long axis and short axis) was measured in micron meter (µm) for the three exotic carps. These types of studies on different blood cells size in different species can give us information about cell size in order to their habit and habitat and provides a numerical objectification of the most suitable modifications that can help in research applications.

Keywords: Morphometric, morphology, cell size, *Cyprinus carpio, Ctenopharyngodan idella* and *Hypophthalmichthys molitrix*

1. Introduction
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. The cytomorphological classification, staining characteristics and cytometric studies on fish blood corpuscles in Indian fish have not received considerable attention by earlier workers. This is because the cytometry of blood cells did not find much use in routine clinical studies in fish. However, the erythrocyte size finds multiple uses in ascertaining the types of anemia's, stress and bio-physio-pathological aspects in fish, Joshi (2000) [21].

There are few diagnostic tools available to veterinarians and fish health professionals to evaluate disease and abnormalities in fish. Besides, data regarding blood cell characteristics and blood cell size are limited. This study analyzes the morphometry of normal blood cells of the selected fish species *Cyprinus carpio, Ctenopharyngodan idella* and *Hypophthalmichthys molitrix* since morphometrical characterization of blood cells of these fish species was not well defined and literature in this area is isolated, old and often incomplete. So, this investigation is planned to determine the size of blood cells. Basic knowledge from this study is important for haematological research, conservation and clinical diagnosis so this type of research might provide some useful information for other researchers that could be used as a biomarker or as an available tool to diagnose and monitor disease in these species. Morphometry is a quantitative description of geometrical structures in all dimensions, Baak (1985) [3] and Vandiest and Baak (1991) [44]. 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, Oberholzer *et al.*, (1991) [31], Nafe (1991) [30] and Russack (1994) [37].

Among haematological indices, blood cells are one of the best indicators of body health. They are vitally important in the immune system. Despite of recent interest in the immune systems of fish species regarded as important in aquaculture, very little attention has been devoted to the structural features of fish blood cells. The purpose of this study was to obtain a basic knowledge about the morphological features of the blood cells. In the present study the three exotic carps *Cyprinus carpio, Ctenopharyngodan idella and Hypophthalmichthys molitrix* which are mostly cultured in West Godavari district region are selected for assessment of the morphological features of the blood cells.

2. Materials and Methods

Data of the different types of fish cultured in the West Godavari district was collected and of the different freshwater fish, the most widely cultured fish such as exotic carps *Cyprinus carpio, Ctenopharyngodan idella* and *Hypophthalmichthys molitrix* were selected irrespective of the sex. They are collected from the different areas of the selected mandals. Samples from each fish weighing between 460-590 gms and their total length between 21-33 cm were collected for the present study.

The 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. Blood smears, five for each fish, were prepared as described by Humason (1979) [20]. Air-dried smears were fixed for 1 minute in absolute methanol, stained with Wright-Giemsa (WG) fluid (Ellsaesser *et al*., 1984) [12] for 8 minute and washed twice in distilled water for 1 min, then they are air-dried. The stained smears were studied under a Micro Imaging System – image capture Computer system. The classification of fish blood corpuscles used in the present work is after, Mahajan and Dheer (1979) [25]. The size of cells (range & mean of long and short axis) were measured in the three exotic carps *Cyprinus carpio, Ctenopharyngodan idella and Hypophthalmichthys molitrix* with the help of Ocular micrometer The measurements were made for 50 erythrocytes and 25 numbers each of erythroblasts, thrombocytes, monocytes, large lymphocytes, small lymphocytes, neutrophils, eosinophils and basophils. All the statistical analysis were performed using the statistical software Microsoft Office Excel 2007.

3. Results and Discussions

Morphometry of blood cells were measured in micron meter (µm). The data collected from the species are presented in table-1. Erythrocytes, erythroblasts, thrombocytes and different types of leucocytes such as large lymphocytes, small lymphocytes, monocytes, neutrophils, eosinophils and basophils were distinguished and characterized. Thrombocytes are the most abundant blood cells after erythrocytes and are recognized easily from lymphocytes by morphological features and size. Fish haematology is gaining increasing importance in fish culture because of its value in monitoring the health status of fish (Hrubec *et al*., 2000) [19]. Knowledge of the haematological characteristics is an important tool that can be used as an effective and sensitive index to monitor physiological and pathological changes in fish (Kori-Siakpere *et al*., 2005) [22]. In India, Bagchi and Ibrahim (1974) [4], Pandey *et al.,* (1976) [32] and Goel *et al.,* (1984) [15] have measured some haematological parameters in Indian major carps and cat fishes, reared under different rearing conditions. Suryavanshi and Chakraborty (1972) [40], Tandon and Joshi (1975) [41] have attempted to establish the normal blood values of fish. Vazquez and Guerrero, (2007) [45] described the hematological indices on *Cichlasoma dimerus* and the morphological features of blood cells were described according to the observations made by light and electron microscopy.

In the present study Erythrocytes were the most abundant cells in smears of the peripheral blood of all the fish species. Two kinds of erythrocyte were found in all the fish species blood. Under the light microscope the predominant shape of the erythrocytes was elliptical with an oval, central or nearly central, dark purple-stained nucleus and blue–gray-stained cytoplasm. The surface of the cells was smooth. The other kind of erythrocyte was the immature erythrocyte, which could be larger or smaller than the mature variety. They were oval to round in shape and fewer in number, and are called erythroblasts. They had a magenta stained chromatin of reticular appearance and a blue-stained cytoplasm, darker than that of the mature erythrocyte. The division of the erythrocyte could both be observed occasionally. In fish blood the presence of erythroblasts indicates the erythrocytes of the fish are primitive and can proliferate in peripheral blood (Fijan, 2002a) [14]. All the fish species blood contained a larger number of mature erythrocytes than immature erythrocytes, in direct proportion to their stage of maturity; these proportions can therefore be used as erythropoietic activity change indicators (Fijan, 2002b) [13]. Because erythropoiesis in fishes can be affected by anaemia, temperature, seasonality (Lecklin and Nikinmaa, 1998) [23], and bleeding (Fijan, 2002b) [13], this could affect the number of circulating erythroblasts. The erythroblasts count may be an indicator of a regenerative response that can be modulated by anaemic processes and environmental factors such as temperature and oxygen availability.

Cell size varies greatly within species. Erythrocyte cell shows highly significant difference between the species, Acharya Gayatri and Mohanty Prafulla (2014) [1]. Gaseous exchange is the main function of erythrocytes. The rate of gaseous exchange is higher in smaller erythrocytes than larger one Hartman and Lessler (1964) [17], Sevine *et al*., (2000) [39] 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 are absent as reported by Wintrobe (1961) [48]. MCV is wet volume, whereas the diameters of erythrocytes were measured from dry smears and were used to calculate dry cell area as erythrocyte size. These different measurements between erythrocyte size and MCV should not be compared to each other. The erythrocyte indexes MCV and MCH have a wide range of physiological variation.

Erythrocytes expressed lower values in cytomorphometry compare to leukocytes. The present investigation reveals significant difference in morphometry of blood cell between the species. Only one type of blood cell, erythrocyte shows difference in each fish. This may be due to change in environment, habitat or due to sexual dimorphism. The cells

under light microscope appeared as seen in typical blood differential leukocytes count slide except mild variation in their sizes. Under these observations, the leukocytes recognized are neutrophils, basophiles and eosinophils in granulocytes while large and small lymphocytes and monocytes. Smaller numbers of leucocytes were distributed diffusely among the dense erythrocytes, either singly or as clumps. Rao and Behera (1973) [34] reported mean values of the RBC size (long axis X short axis) as 9.00 x 7.00 µm in *Catla calta*, whereas Qayyum and Naseem (1967) [33] reported the mean value of the RBC size (long axis x short axis) as 12.60 x 6.90 µm in *Cirrhinus mrigala* which are slightly lower in long axis and higher value for the short axis (11.12 x 9.51 µm).

Thrombocytes appeared as round, oval, spikeshaped, or fusiform cells. The oval and spike shaped thrombocytes were frequently observed. They occurred as single cells or in clusters and had a large central dark purple- stained nucleus with chromatin clusters which were more abundant in the spike-shaped and fusiform thrombocytes. There was little blue gray stained cytoplasm surrounding the nucleus. The thrombocytes of fish are not the same as those of mammals. In fish, they often have intact cell structures and, in addition to a role in blood coagulation, phagocytosis has been reported. This phagocytosis is doubted by Ellis (1976) [11]. Although fish blood thrombocytes have been described as the most abundant blood cells after erythrocytes, very little is known about their morphological features and functional properties (Rowley *et al*., 1988) [36], the data available on the percentage of thrombocytes in fish peripheral blood being very confused. Different reports have suggested they account for 70% and 3–13% of total blood leukocyte count, whereas Catton (1951) [8] observed no thrombocytes in *Perca fluviatilis* blood. In different fish species round, oval, spindle, and spike forms have also been described (Doggett and Harris, 1989; Lo´pez-Ruiz *et al*., 1992) [10, 24]. When smearing is carried out too slowly, or the blood is allowed to partially clot before smearing, then the thrombocytes are often seen in aggregates displaying a ragged appearance. When the blood is collected in a suitable anticoagulant such as sodium citrate or heparin, however, the thrombocytes appear oval or spindle shaped, and this is presumably their normal *in vivo* appearance (Rowley *et al*., 1988) [36].

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. In monocytes the nucleus stained dark blue and usually ovoid, kidney-shaped, or horseshoe-shape. The term monocyte/macrophage is widely used to refer to a fish blood cell type (Savage, 1983) [38] with phagocytic activity (Morrow and Pulsford 1980) [29] or morphophysiological features comparable with those of the monocytes of higher vertebrates (Rowley *et al*., 1988) [36]. Monocytes have been reported to be present in many fish species. It has, however, been reported that monocytes are absent from the blood of brown trout *Salmo trutta* (Blaxhall and Daisley, 1973) [5] and goldfish *Carassius auratus* (Weinreb and Weinreb, 1969) [47].

In some work the lymphocytes have been classified into large and small groups, Zhou *et al*., (2002) [50]. Barber *et al*., (1981) however, remarked that lymphocyte volumes changed continuously and there was no evidence that the function of a lymphocyte was related to its volume. Roubal (1986) [35] and Yuan *et al*., (1998) [49] described lymphocytes as one group. In the present study two different types of lymphocytes were identified as large and small lymphocytes based on the size which was evident. Lymphocytes are usually the most common leucocyte type present in the blood of some fish, accounting for as much as 85% of the total leucocyte population, excluding thrombocytes (Groff and Zinkl, 1999) [16]. Fish lymphocytes are small round cells and a rim of smooth light blue cytoplasm around the large oval-round condensed nucleus or slightly irregular nucleus was heavily stained purple. The size of the cells varied. They are generally classified as small or large Campbell and Murru, (1990) [6]. In the smaller lymphocytes the nucleus occupied most of the space in the cells whereas the large lymphocytes had more cytoplasm which, sometimes, is seen extended into pseudopodia or apophysis on its surface. Lymphocytes were oval and had a round purple-stained nucleus and a very dark blue-stained cytoplasm. The lymphocytes observed in our study took the general form reported by other workers and were morphologically similar their mammalian counterparts. According to Campbell (2004) [7], it is probable that the low density conditions and optimal water quality resulted in a healthy situation that inhibited the lymphocytosis produced during wound healing, inflammatory disease, parasitic infections and viral diseases. The lymphocytes of some fish, especially elasmobranch species shows “blebs” or out pocketing of the cell membrane Arnold (2005) [2], Clauss *et al*., (2008) [9]. Several authors have demonstrated a special interest in the leucocytes of teleost fishes with regard to their morphology and absolute values. Their investigations revealed a great diversity of morphological aspects in some types of leucocytes (Blaxhall and Daisley, 1973; Ellis, 1976; Ueda *et al*., 1997; Veiga, 1999) [5, 11, 43, 46]. In addition, the same type of leucocyte has been described with different names by different authors. Therefore the nomenclature concerning teleost leucocytes is confusing (Ueda *et al*., 2001) [42].

Available information about fish blood granulocytes is very confusing (Rowley *et al*., 1988) [36]. It is assumed that the three types of granulocyte described in higher vertebrates (heterophilic/neutrophilic, acidophilic, and basophilic) are also present in fish (Doggett and Harris, 1989; Meseguer *et al*., 1990) [10], although sometimes more than three (Mainwaring and Rowley, 1985a, b) [27, 26], or only one or two (Savage, 1983) [38] circulating types of granulocyte have been described for some fish species. In our study we found three kinds of granulocyte—neutrophils, eosinophils and basophils. The neutrophils were of several sizes, but usually larger than erythrocytes in volume, and of several shapes, for example spherical, pear-shaped, or irregular ellipse. The nucleus stained purple, usually ovoid and eccentric, with pale blue cytoplasm that may be extended into a blunt ended pseudopodium. Occasionally the nucleus may be observed as a ribbon-like structure across the diameter of the cell—horseshoe-shaped, band-shaped, two-segmented in shape, or several-segmented in shape and centrally or eccentrically located. The nucleus-to-cytoplasm ratio of granulocytes was smaller than that of monocytes and lymphocytes. The cytoplasm was stained light blue and the nucleus was also stained lighter than that of the monocyte and lymphocyte. The eosinophil was observed only rarely in the peripheral blood, being round or nearly round. There were large bilobed, trilobed, and tetralobed, eccentric nuclei stained dark purple in the cell. The cytoplasm was light red and full of large, spherical, bright red stained granules. The difference may be specific to the species or the biogeography of the fish

leucocytes. It is difficult to classify the leukocytes of fish. In addition to the numerous species of fish, the basis or standards of classification are not the same in different studies. Leukocytes at different stages of development occur in fish blood and they are difficult to identify (Hibiya, 1983) [18].

Table 1: The size of cells (Range & Mean of long axis and short axis) measured in micron meter (µm) for the three exotic carps

Cell type	*Cyprinus carpio*	*Ctenopharyngodon idella*	*Hypophthalmichthys molitrix*
Erythrocytes*	8.75-12.25 x 7-10.50 (10.21 x 9.22)	7.70-11.90 x 3.50-9.80 (8.60 x 6.20)	10.50-12.95 x 7.00-11.20 (11.93 x 8.32)
Erythroblasts*	9.10-12.75 x 8.75-11.25 (12.01 x 10;05)	9.10-11.90 x 7.00-9.41 (10.08 x 9.20)	13.30-16.80 x 10.85-12.60 (14.15 x 10.92
Thrombocytes#	3.50-5.60 x 3.50-4.90 (5.08 x 4.15)	4.20-6.30 x 3.10-3.90 (5.60 x 3.50)	7.00-9.80 x 5.00-5.95 (8.61 x 5.62)
Monocytes#	11.90-12.60 x10.85-12.25 (12.20 x 11.19)	8.75-10.85 x 7.00-9.80 (10.02 x 9.61	13.30-15.05 x 12.25-14.70 (14.12 x 13.62)
Large lymphocytes#	11.50-12.96 x 11.00-12.25 (12.60 x 11.85)	5.60-10.60 x 5.15-8.75 (9.10 x 8.01)	14.70-16.81 x 13.70-16.80 (16.03 x 15.13)
Small lymphocytes#	5.60-5.95 x 3.50-5.60 (5.91 x 4.12)	3.50-5.95 x 3.00-5.60 (5.13 x 4.02)	7.00-10.85 x 7.00-9.10 (9.03 x 8.11)
Neutrophils#	11.2-12.6 x 9-12.25 (11.55 x 10. 20)	7.00-10.15 x 7.00-9.80 (9.2 x 8.69)	11.20-13.30 x 10.25-13.30 (12.9 x 10.85)
Eosinophils#	9.80-11.95 x 9.45-11.20 (11.62 x 10.15)	5.95-9.45 x 5.60-7.70 (8.10 x 7.20)	10.00-11.90 x 8.75-10.50 (9.51 x 8.81)
Basophils#	9.10-10.50 x 8.75-10.15 (10.15 x 9.80)	10.20-11.95 x 9.10-11.20 (10.6 x 9.20)	6.30-9.10 x 5.25-6.65 (8.13 x 6.18)

* n=50 and #n=25 measurements for each type of cell.
All the statistical analysis was performed using the statistical software Microsoft Office Excel 2007.

4. Conclusions

The different cell morphology studied in the selected freshwater fish species in the present study exhibited different sizes in cell structure among the species. These types of studies on different blood cells size in different species can give us information about cell size in order to their habit and habitat and provides a numerical objectification of the most suitable modifications that can help in research applications in cytological and histopathological studies.

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6. References

1. Acharya Gayatri, Mohanty Prafulla K. The morphometrical characterisation of normal blood cells of two airbreathing fishes, *Clarias batrachus* and *Anabas testudineus* International Research Journal of Biological Sciences. 2014; 3(11):37-41.
2. Arnold JE. Hematology of the sandbar shark, *Carcharhinus plumbeus*: standardization of complete blood count techniques for elasmobranches. Veterinary Clinical Pathology. 2005; 34(2):115-123.
3. Baak JPA. The principles and advances of quantitative pathology, Anal. Quant. Cytol. Histol. 1985; 9:89-95.
4. Bagchi MM, Ibrahim KH. A note on the haematological study on rohu, *Labeo rohita* (Ham.). J. Inland Fish. Soc. India. 1974; 6:93-94.
5. Blaxhall PC, Daisley KW. Routine Haematological Methods for use Fish with Blood, J. Fish Biol. 1973; 5:771-781.
6. Campbell TW, Murru BS. An introduction to fish hematology. Camp. Cont. Ed. Vet. Sci. 1990; 12:525-533.
7. Campbell TW. Hematology of lower vertebrates. In: Proceedings of the 55th Annual Meeting of the American College of Veterinary Pathologists (ACVPC) & 39th Annual Meeting of the American Society of Clinical Pathology (ASVCP). ACVP and ASVCP, USA, 2004.
8. Catton WT Blood cell formation in certain teleost fishes. Blood. 1951; 6:39-60.
9. Clauss TM, Dove ADM, Arnold JE. Hematologic disorders of fish. Vet Clin North Am Exotic Anim Pract. 2008; 11:445-462.
10. Doggett TA, Harris JE. Ultrastructure of the peripheral blood leukocytes of *Oreochromis mossambicus.* J Fish Biol. 1989; 33:747-756.
11. Ellis AE. Leucocytes and related cells in the plaice *Pleuronectes platessa*. J Fish Biol. 1976; 8:147-153.
12. Ellsaesser C, Miller NW, Lobb CJ, Clem LW. A new method for the cytochemical staining of cells immobilized in agarose. Histochemistry. 1984; 80:559-562.
13. Fijan N. Composition of main haematopoietic compartments in normal and bled channel catfish. J Fish Biol. 2002b; 60:1142-1154.
14. Fijan N. Morphogenesis of blood cells lineages in channel catfish. J Fish Biol. 2002a; 60:999-1014.
15. Goel KA, Mishra BP, Kalpana Gupta, Sandhya Wadhwa A. comparative Haematological study on a few freshwater teleost. Indian J Fisheries. 1984; 31(1):108-112.
16. Groff JM, Zinkl JG. Hematology and clinical chemistry of Cyprinid fish. Common carp and goldfish. Vet. Clin. N. Am. Exot. Anim. Pract. 1999; 2(3):741-746.
17. Hartman FA, Lessler MA. Erythrocyte measurements in fishes, amphibia and reptiles, Biol Bull.1964; 126:83-88.
18. Hibiya T. Illustration of fish histology-normal and pathological organization. Kodansha, Tokyo. 1983, 65-71.
19. Hrubec TC, Cardinale JL, Smith SA, Haematology and plasma chemistry reference intervals for cultured tilapia

(Oreochromis hybrid). Vet Clin Pathol. 2000; 29(1):7-12.
20. Humason GL. Animal tissue technique. 4th Edn. WH Freeman and company, San Francisco, USA, 1979.
21. Joshi BD Some aspects of haematology of cold water fish: Cold water aquaculture and fisheries (Eds-H. R. Singh and W. S. Lakra). Narendra publishing house Delhi, 2000, 59-100.
22. Kori-Siakpere O, Ake JEG, Idoge E. Hematological characteristicof the African Snakehead, *Parachanna obscura*. AFJ Biot. 2005; 4(6):527-530.
23. Lecklin T, Nikinmaa M. Erythropoiesis in Arctic charr is not stimulated by anaemia. J Fish Biol. 1998; 53:1169-1177.
24. López Ruiz A, Esteban MA, Meseguer J. Blood cells of the gilthead seabream (*Sparus aurata* L.): light and electron microscopic studies. Anat Rec. 1992; 234:261-171
25. Mahajan CL, Dheer JMS. Autoradiography and differential haemoglobin staining as aids to the study of fish haematology. Experientia. 1979; 35:834-835.
26. Mainwaring G, Rowley AF. Studies on granulocyte heterogeneity in elasmobranchs. In: Manning MJ, Tatner MF (eds) Fish immunology. Academic Press, New York. 1985b, 57-69.
27. Mainwaring G, Rowley AF. Separation of leukocytes in the dogfish (*Scyliorhinus canicula*) using density gradient centrifugation and differential adhesion to glass coverslip. Cell Tissue Res. 1985a,; 241:283-290.
28. Meseguer J, Esteban MA, Garcı`a-Ayala A, Lo´ pez Ruı`z A, Agulleiro B. Granulopoiesis in the head-kidney of the sea bass (*Dicentrarchus labrax* L.): an ultrastructural study. Arch Histol Cytol. 1990; 53:287-296.
29. Morrow WJW, Pulsford A. Identification of peripheral blood leucocytes of the dogfish (*Scyliorhinus canicula* L.) by electron microscopy, J Fish Biol. 1980; 17:461-475.
30. Nafe R. Planimety in pathology: A method in its own right besides stereology in automatic image analysis, Exp. Pathol. 1991; 43:239-246.
31. Oberholzer M, Christen H, Ettlin R, Buser M, Oesereicher M Gschwind R. Some fundamental aspects of morphometry in clinical pathology, demonstrated on a simple, multipurpose analysis system, Analyt. Quant. Cytol. Histol. 1991; 13:316-320.
32. Pandey BN, Pajjdey PK, Choubey BJ, Dattamunshl JS. Studies on blood components of an air breathing siluroid fish *Heteropneustes fossilis* in relation to body size. Folia Haematol. 1976; 103:101-116.
33. Qayyum A, Nassem SM. A haematological study on mrigal *Cirrhiria mrigala* (Hamilton), Curr Sci. 1967; 36(16):435-6.
34. Rao MVS, Behera MK. Hematological study on freshwater teleost, *Catla catla* Current Science. 1973; 42(17):612-613.
35. Roubal FR. Blood and other possible inflammatory cells in the sparid *Acanthopagrus australis* (Gunther). J Fish Biol.1986; 28:573-594.
36. Rowley AF, Hunt TC, Page M, Mainwaring G. Fish. In: Rowley, A.F., Ratcliffe, N.A. (Eds.), Vertebrate Blood Cells. Cambridge University Press, Cambridge. 1988, 19-127.
37. Russack V. Image cytometry: Current applications and future trends, Crit. Rev. Clin. Lab. Sci. 1994, 31:1-34.
38. Savage AG. The ultrastructure of the blood cells of the pike *Esox lucius* L. J Morphol. 1983; 178:187-206.
39. Sevine M, Ugurtas IH, Yildirimhan HS. Erythrocyte measurements in *Lacerta rudis* (Reptilia Lacertidae), Turk J Zool. 2000; 24:207-209.
40. Suryavanshi S, Chakraborty JS. Studies on the blood glucose and pancreatic islets of the teleost, *Channa striatus* (Bloch). Proc. Indian Sci. Cong.1972; 59:487.
41. Tandon RS, Joshi BD. Studies on the physiology of blood of some freshwater fishes of India. 1. Normal blood glucose and lactic acid levels. J Inland Fish. Soc. India. 1975; 7:1-6.
42. Ueda IK, Egami MI, Sasso WS, Matushima ER. Cytochemical aspects of the peripheral blood cells of *Oreochromis (Tilapia) niloticus.* (Linnaeus, 1758) (Cichlidae, Teleostei)-Part II. Braz. J Vet. Res. Anim Sci. 2001; 38(6):273-277.
43. Ueda IK, Egami MI, Sasso WS, Matushima ER. Estudos hematol´ogicos em *Oreochromis* (*Tilapia*) *niloticus*. (Linnaeus, 1758) (Cichlidae, Teleostei). Brazilian Journal of Veterinary Research and Animal Science. 1997; 34(5):270-275.
44. Vandiest PJ, Baak JPA. Morphometry, In: Comprehensive cytology, Bibbo M., Ed. W.B. Saunders Company, Philadelphia. 1991, 946-964.
45. Vazquez GR, Guerrero GA. Characterization of blood cells and haematological parameters in *Cichlasoma dimerus* (Teleostei, Perciformes). Tissue Cell. 2007; 39:151-160.
46. Veiga ML. Aspectos morfologicos das celulas sanguineas, citoquımicos e ultraestruturais de trombocitos, neutrofilos e eosinofilos de Dourado *Salminus maxillosus* (Valenciennes, 1840) (Pisces, Characidae). Dissertac¸ao (Mestrado) Universidade Federal de S˜ao Paulo, Escola Paulista de Medicina, S˜ao Paulo. 1999, 95.
47. Weinreb EL, Weinreb S. A study of experimentally induced endocytosis in a teleost. I. Light microscopy of peripheral blood responses. Zoologica N Y. 1969; 54:25-34.
48. Wintrobe M M. Variations in the size and haemoglobin content of erythrocytes in the blood of various vertebrates, Folia Haematol. 1961; 51:32-49.
49. Yuan SQ, Zhang YA, Yao WJ. Micro and ultrastructure of peripheral blood cells of the Mandarin fish, *Siniperca chuatsi* (Basilewsky). Acta Hyrobiologica Sinica. 1998; 22:39-47.
50. Zhou Y, Guo WG, Yang ZG, Zhou XH, Zhang K, Wen XH, *et al*. Microstructure and Ultrastructure of the peripheral blood cells of European eel (*Anguilla anguilla*). Acta Zoologica Sinica. 2002; 48:393-401.