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## RESEARCH ARTICLE

# NORMAL HAEMOCYTOLOGICAL AND SERUM BIOCHEMICAL PROFILE OF *Catla catla* (HAMILTON, 1822) AS A DIAGNOSTIC TOOL IN AQUACULTURE

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## ABSTRACT

The aim of the present study was to obtain a baseline data on the blood cell size, morphology, differential leucocyte count, haematological parameters of and biochemical parameters in *Catla catla* since there is a limited information in the published literature. Blood samples were collected from the caudal vein of apparently healthy fish (male: n=15 and female: n=15) were collected. Haematological and biochemical values of the blood samples were determined using standard techniques. The morphological features of blood cells were described according to observations made by light microscopy. The various types of blood cells measurement were carried out with the help of Microscope Eyepiece Digital Camera and computer. From microscopic observation blood cells such as erythrocytes, five types of leucocytes: lymphocytes, monocytes, heterophils, neutrophil and eosinophils, were distinguished and characterised. Statistical analysis revealed haemoglobin, PCV ($p<0.001$), lymphocytes , heterophils ($p<0.05$) and cell morphometry except heterophil in *Catla catla* were significantly influenced by sex.



## INTRODUCTION

The Indian major carp *Catla calta* is a demanded commercial fish in India which is widely accepted as food by the consumers because of their taste and flesh. *Catla catla* contributes a major portion to the freshwater fish production. Haematological evaluation of fish is not routinely used in establishing the diagnosis of fish diseases, but it can be useful in the detection of diseases affecting the cellular components of blood. Certain diseases of fish result in anemia, leukopenia, leukocytosis, thrombocytopenia, and other abnormal changes of the blood cells. Haematological characteristics are an important tool that can be used to understand as an effective and sensitive index to monitor physiological and pathological changes in fishes. Changes in haematological parameters depend upon the aquatic biotope, fish species, age, and sexual maturity and health status (T. Patriche *et al*, 2011; D. Radu *et al*, 2009). Haematological and biochemical studies help in understanding the relationship of blood characteristics to the habitat and adaptability of the species to the environment. The fish haematological parameters such as RBC, WBC, Hb and PCV values etc., are thus shown to be influenced by many factors including environmental factors (Pandey, 1977).

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So it is more beneficial to aqua culturists to study the varying aspects of haematological and biochemical changes in different conditions.

## MATERIALS AND METHODS

Apparently healthy adult live specimens of *Catla catla* were collected from a freshwater pond of Kausalyaganga, Bhubaneswar Odisha. Fifteen adult specimens of almost equal size (length and weight) from each sex of the species were utilized in the present investigation. After collection, the live adult fishes were maintained in the aquarium for acclimatization prior to initiation of experiment. Blood samples were taken from the caudal vein. Blood was collected in the morning hours to avoid diurnal variation. Collected blood was transferred from the syringe to an eppendorf tube and kept undisturbed for clotting. After following retraction of clot, the supernatant serum was pipetted into another eppendorf tube. The serum was then used for all biochemical investigations. For other haematological investigations the collected blood was transferred from the syringe to an ethylenediaminetetraacetic acid (EDTA) tube then blood was mixed well without frothing. Haematological investigations included morphology and morphometry of blood cells, packed cell volume (PCV), hemoglobin content (Hb), total erythrocyte or red blood cell count (TEC/RBC), total leucocyte or white

blood cell count(TLC/WBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), differential leukocyte count (DLC). Serum biochemical parameters included estimation of total protein, albumin, globulin, cholesterol and glucose. Haemoglobin concentration was determined by indirect acid haematin method (Sahli, 1990). The microhematocrit tube was utilized for manual packed cell volume (PCV) determination, was centrifuged for 15 min at 3,500 rpm and interpreted by visual inspection (Mcinroy, 1953). Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) were determined as per following formula.

MCV (fl) = PCV/ RBC x 10.
MCH (pg) = Hb / RBC x 10
MCHC (%) = Hb / PCV x 100

For Morphometry and differential leucocyte count (DLC) blood smears were prepared on clean grease free slides [BLUE STAR PIC-2, POLAR INDUSTRIAL CORPORATION, MUMBAI, MAHARASHTRA, INDIA]. The air dried blood smears were fixed using methanol [QUALIGENS PRODUCT NO.34457, THERMO FISHER SCIENTIFIC INDIA PVT. LTD., MUMBAI, MAHARASHTRA, INDIA] and stained with Leishman's stain [QUALIGENS PRODUCT NO.38854, THERMO FISHER SCIENTIFIC INDIA PVT. LTD., POWAI, MUMBAI, MAHARASHTRA, INDIA]. Blood smear from individual fish was examined using a compound microscope (MICROSCOPE H 600 WILOZYT PLAN, HELMUT HUND GMBH, WETZLAR-NAUBORN, GERMANY). Different types of blood cells present in the smear were identified (T.W. Campbell, 2006) Slides were viewed in zigzag pattern, covering all parts of the blood smear and leukocytes were counted in each field of view until 100 cells were counted. Photograph of blood cells as well as cytomorphometry of cells and nuclei (30 observations per type of cell) was undertaken 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 [MICROSCOPE H 600 WILOZYT PLAN, SERIAL NO. 1024980, HELMUT HUND GMBH, WETZLAR-NAUBORN, GERMANY] and computer. Serum biochemical parameters such as total protein, albumin, globulin, cholesterol and glucose were estimated using standard kits (CREST BIOSYSTEMS, ALTO SANTACRUZ BAMBOLIM COMPLEX, GOA-403 202, INDIA). The results are presented as means ± SE. Difference in parameters in male and female fishes were analyzed using t-test and statistical significance was tested at $p< 0.05$, $p<0.01$and $p<0.001$ level. Statistical assessment of result was carried out using the Microsoft Office Excel 2007.

## RESULTS AND DISCUSSION

The aim of this investigation is to find out the baseline value of haematological parameters of *Catla catla* (Table 1).The present finding shows the average of RBC in male and female fish was 1.89±0.08 x $10^6$ and 1.69±0.07x $10^6$/mm$^3$ blood, respectively, this finding is similar with the findings of S.C. Pradhan *et al.* (2012a) in male (1.644 x $10^6$/mm$^3$) and female (1.44 x $10^6$/mm$^3$) *Catla catla* .However this value was lower than other freshwater fishes like *Clarias batrachus* 2.1 x $10^6$/mm$^3$ and *Labeo rohita* 2.0 x $10^6$ /mm$^3$ (S. Sudha and V. Santosh, 2012).The mean value of haemoglobin content of *Catla catla* in male and female fish was 7.53±0.27 and 6.56±0.36 of blood, respectively. There are significant differences between sexes. The annual mean value of WBC in male and female fish was 3.754 x 103 and 4.124 x 103/mm$^3$ of blood, respectively. Female fish had higher values than that of male fish this may be due to egg carriage stage, infection or adverse condition in female (L.S. Smith, 1986).PCV is higher in males than that of females and is significantly different. The result of PCV of this study for male and female are 24±1.15% and 20.93±0.90% respectively .Males have higher value of PCV than females which is perhaps because of relatively higher RBC counts in males. The MCV value of male and female fish was 132.19±11.07 and 126.11±6.8 fl. There was no significant difference in male and female fish. The higher haematological values in favour of male fish may be attributed to physiologically activeness than the female fish. However, MCHC and MCH values did not show any marked difference with respect to sex.

**Table 1. Haematological parameters of Male and Female *Catla catla***

| Parameters | Male(15) | Female(15) |
|---|---|---|
| RBC(106mm$^{-3}$) | 1.89±0.08 | 1.69±0.07 |
| HB((g/dl) | 7.53±0.27* | 6.56±0.36* |
| WBC(10$^3$ mm$^{-3}$) | 4.37±0.36 | 5.06±0.32 |
| PCV (%) | 24±1.15* | 20.93±0.90* |
| MCV (fl) | 132.19±11.07 | 126.11±6.81 |
| MCH (pg) | 41.48±3.08 | 39.57±2.52 |
| MCHC (%) | 31.64±0.51 | 31.13±0.66 |

Figures in parentheses indicate number of observations
*Significant at p<0.05.

Differential leucocytes count (DLC) revealed among all the leucocytes, the % of lymphocytes was highest in the *Catla catla* followed by heterophil, neutrophils, monocytes and eosinophils(Table -2). Basophils could not be observed .The percent of neutrophil, monocytes and eosinophils are found to be high in relation to the percent of lymphocytes in male specimens. Statistically analysis (*t*-test, p<0.05) reveals that lymphocyte and neutrophil differ significantly between male and female specimens. An increase in the percentage of polymorphonucleocyte (neutrophil, monocyte and eosinophil) indicates infection in fishes A. Sahan *et al.* (2007).

**Table 2. Differential leucocyte count of Male and Female *Catla catla***

| Parameters | Male(15) | Female(15) |
|---|---|---|
| Lymphocyte (%) | 66.93±0.67*** | 70.93±0.65*** |
| Monocyte (%) | 7.33±0.38 | 6.93±0.41 |
| Eosinophil (%) | 3.66±0.37 | 3.13±0.35 |
| Heterophil (%) | 18.53±0.55*** | 15.73±0.84*** |
| Neutrophil (%) | 3.53±0.36 | 3.26±0.26 |

Figures in parentheses indicate number of observations
**significant at p<0.01 and *** significant at P <0.001.

A clear understanding of the blood cell morphology is essential for each species of fish in order to establish complete blood cell count reference ranges. Fish erythrocytes resemble their avian/reptilian counterpart. Cell size varies greatly with species. As well as between sex. Blood cell sizes and erythrocyte dimensions of male and female *Catla catla* are

shown in Table 3. The erythrocytes are the predominant cell type found in blood and their size varies significantly between male and female.

Erythrocyte in Catla Catla were oval in shape with abundant smooth eosinophilic cytoplasm and a central, oval-shaped condensed nucleus (Fig.1 a).

**Table 3. Cytomorphomety of blood cells of *Catla catla***

| SL NO | Cell type | Cytoplasm/Nucleus | | Sex | |
|---|---|---|---|---|---|
| | | | | Male(n=30) | Female(n=30) |
| 1 | Erythrocyte | Cytoplasm | Mean length in µm | 10.32±0.18* | 9.83±0.24* |
| | | | Mean breadth in µm | 7.77±0.17** | 8.48±0.25** |
| | | Nucleus | Mean length in µm | 6.47±0.19 | 6.23±0.25 |
| | | | Mean breadth in µm | 4.12±0.15** | 4.98±0.22** |
| | | | Mean area in $\mu m^2$ | 9.83±0.29* | 10.54±0.21* |
| 2 | Lymphocyte | Cytoplasm | Mean length in µm | 8.03±0.28* | 8.75±0.25* |
| | | | Mean breadth in µm | 6.61± 0.25 | 6.98±0.20 |
| | | Nucleus | Mean length in µm | 5.15± 0.24 | 5.60±0.25 |
| | | | Mean breadth in µm | 10.75±0.41 | 10.12±0.23 |
| 3 | Eosinophil | Cytoplasm | Mean length in µm | 11.88±0.32** | 10.62±0.26** |
| | | | Mean breadth in µm | 9.83±0.30** | 8.70±0.26** |
| 4 | Monocyte | Cytoplasm | Mean length in µm | 10.75±0.41 | 10.12±0.23 |
| | | | Mean breadth in µm | 9.07±0.34 | 8.45±0.26 |
| 5 | Neutrophil | Cytoplasm | Mean length in µm | 11.88±0.32** | 10.62±0.26** |
| | | | Mean breadth in µm | 9.83±0.30** | 8.70±0.26** |
| 6 | Heterophil | Cytoplasm | Mean length in µm | 10.92±0.30 | 10.72±0.29 |
| | | | Mean breadth in µm | 8.62±0.32 | 8.96±0.31 |

Figures in parentheses indicate number of observations **significant at p<0.01 and *** significant at P <0.001.

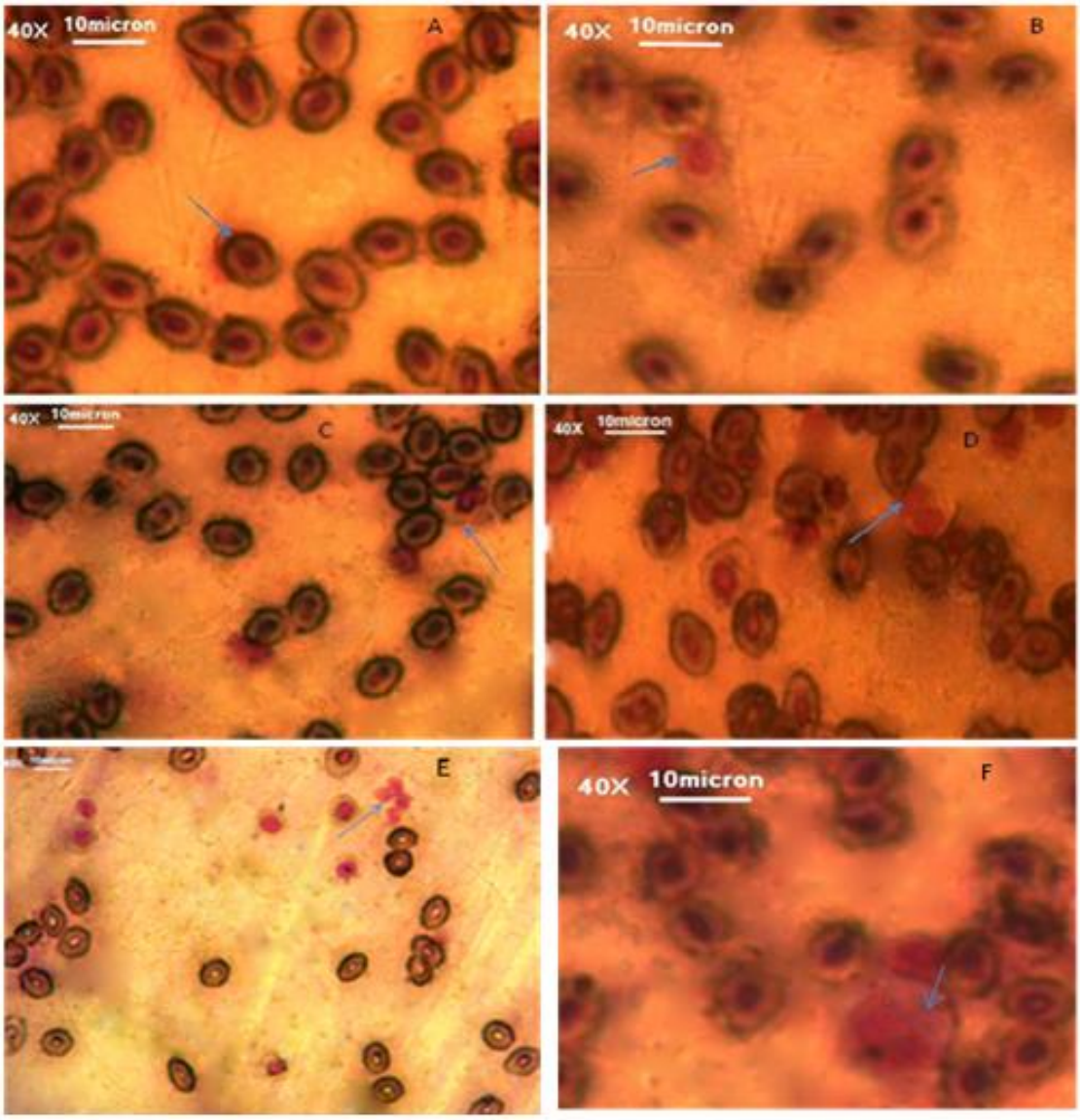


**Figure 1. Blood cells pictures of *Catla catla***
**(A)Erythrocyte (B) Lymphocyte (C) Monocyte (D) Eosinophil (E) Neutrophil (F) Heterophil**

According to M.M. Wintrobe (1933) the erythrocyte size reflects the position of a species on the evolutionary scale: in lower vertebrates and those with a not so successful evolutionary past, i.e., in cyclostomes, elasmobranches and urodeles, the erythrocytes are large, but in higher vertebrates (mammals) the same cells are smaller and do not contain nuclei. Erythrocytes are the dominant cell type in the vast majority of fish species blood. One of the most important functions of erythrocytes 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). Five types of leucocytes were identified: lymphocyte, heterophil, neutrophil eosinophil, and monocyte (Fig.1). Descriptive statistics for the leucocytes are presented in Table 3. Similar to those found in birds and reptiles, fish lymphocytes are small round cells with a high N:C ratio and a rim of smooth light blue cytoplasm around the large oval-round condensed nucleus (Fig.1b). Lymphocytes are one of the most important cells to impact on a fish's immune response.

These cells produce antibodies by specific immunity and increasing macrophages. An increase of such immune cells can promote a fish's defence to an adverse condition (Jalali *et al.,* 2009). In the present study, lymphocytes in female were significantly ($p<0.05$) higher than male. The monocytes mostly had a kidney shaped nucleus, which was less intensely chromatin stained than in lymphocytes. The monocyte cytoplasm was blue grey and covered a larger area (Fig.1c). Monocytes were larger than lymphocytes; they were round cells with a rough membrane. In the present study monocyte cell length and breadth does not vary significantly. Eosinophils are large, round, frequently irregularly outlined cells. They have an eccentric, round or sometimes bilobed nucleus. The cytoplasm is full of acidophilic granules (Fig.1d). The nucleus varied clinally from spherical to deeply segment. The neutrophil (Fig.1 e) was the least common cell type; these were large, distinctly round cells with a compact nucleus and abundant neutral to slightly eosinophilic cytoplasm that was largely devoid of granular material; those granules that were present appeared neutral to slightly basophilic. Heterophils were of similar overall size to erythrocytes, but round rather than elliptical. The cytoplasm varied from neutral to distinctly basophilic and was replete with indistinct, irregular, slightly eosinophilic granules (Fig.1 f).

Biochemical parameters have higher value in female than male except albumin and cholesterol but not significantly different (Table 4).

**Table 4. Biochemical parameters of Male and Female *Catla catla***

| Parameters | Male(15) | Female(15) |
|---|---|---|
| Protein(g/dl) | 5.97±0.29 | 6.34±0.44 |
| Albumin(g/dl) | 3.35±0.17 | 3.79±0.23 |
| Globulin(g/dl) | 2.62±0.34 | 2.55±0.43 |
| Glucose(mg/dl) | 95.73±2.64 | 101.74±3.35 |
| Cholesterol(mg/dl) | 272.06±14.33 | 252.36±8.65 |

Figures in parentheses indicate number of observations.

Although rich protein content was seen among fishes of all the living organisms and fish protein was readily and easily digestible of all animal proteins and also fishes are very cheap. The present study reports, *Catla catla* was having the protein content of 5.97±0.29 and 6.34±0.44 g/dl in male and female fish respectively .It is identified that, unlike red meats, eggs and dairy products, fish provides very high quality protein (Sabry, 1990) Serum protein and globulin are associated with stronger innate response in fish. In this investigation globulin content was found in male and female fish was 2.62±0.34 and 2.55±0.43 g/dl respectively. Albumin help in lipid transporation in fish blood (Andreeva, 1999) The mean value of albumin is higher in female fish than male .*Catla catla* is a Carnivorous fish species show an impaired ability to clear excesses in blood glucose levels and therefore have been traditionally considered as relatively glucose passionate species (Moon, 2001).However, glucose is also essential for brain function (Soengas and Aldegunde , 2002) suggesting the existence of a glucose sensing system in fish. The level of glucose found in present study is 106.91±4.3 and 113.13±5.68 mg/dl in male and female fish respectively. Cholesterol content was found highest in male than female in this experiment but difference was insignificant.

**Conclusion**

The results of our research provide a contribution to the knowledge of the characteristics of blood cells, haematological and biochemical parameters of the *Catla catla* under the normal conditions employed in this study. This investigation may be helpful as a tool to monitor the health status of this and other related fish species. The evaluation of haematological parameters will help in early detection of clinical pathology as well as the presence of disturbance in the environment.

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