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ORIGINAL ARTICLE

Hematological Parameter of *Heteropneustes fossilis* (Shing fish)

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Abstract

Background: The physiological conditions of fish are essential for the successful fish culture. **Objective:** The purpose of the present study was to see the haematological parameter of air breathing cat fish (*Heteropneustes fossilis*) of Bangladesh. **Methodology:** This animal study was conducted in the "Bargen lab" in the Department of Zoology at University of Dhaka as well as in "The Peoples Pathological lab" which was a private diagnostic laboratory at Dhaka city from July' 2008 to April' 2009 for a period of 9(nine) months. The fish was *Heteropneustes fossilis*. Haemocytometer including two graduated pipettes was used for counting leucocytes. **Result:** The result indicated eight types of blood cells in peripheral condition of *Heteropneustes fossilis*. The average cellular counts of *Heteropneustes fossilis* were erythrocytes 8.45×10^{6} m^{3}, leukocytes $15.44\times10^{3}m^{-3}$, Thrombocytes 34.72%, large lymphocytes 1.02%, small lymphocytes 26.7%, monocyte 3.9%, neutrophil 16.9%, eosinophils 6.97%, basophiles 8.6%, haemoglobin 11.7g $100ml^{1}$, erythrocyte sedimentation rate (ESR) 6.49g $100ml^{-1}$. The most of the hematological parameter showed intra specific variation except eosinophils and ESR of *Heteropneustes fossilis* (Shing fish). **Conclusion:** Hematological studies shows that the physiology of fish change with the change in the environment, time, season, maturity, nutritional state, activity level physical and chemical change in water.

Keywords: Hematology, E.S.R., *Heteropneustes fossilis*, EDTA, DC

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Introduction

Fish is a very important food in daily life. There is no other alternative to increase fish production by fish culture. However, inland production is decreasing gradually as a result of lack of proper scientific management, physiochemical changes, industrial pollution, and insecticide pollution (Siddiqui and Nassem 1961). Any changes in the environment would

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affect the physiology of the fishes. Therefore, normal haematological parameters can have significant meaning in the field of disease diagnosis (Siddiqui and Nassem 1979).

Haematological parameter is one of the most reliable tools for diagnosis of disease of the fishes (Beneriee 1964). The haemochemical analysis including blood parameters may be used to separate the fish groups. Fish have a close circulatory system with a clear cut single circulation from body to gills and return. This type of fish is very nutritional and delicious fish. It contain high amount of protein, calcium, iron, phosphorus, and vitamin (Mahajan and Dheer 1979). That's why they are always good for patient to recovering illness. They can live on land for a long time in few water so they are called "Geol fish".

It was therefore considered desirable to evaluate the hematological changes in the blood parameters of predatory fish. *Heteropneustes fossilis (*Bloch, 1797) resulting from the seasonal variation. The present work deals with the differential cell counts, total count of the WBC to see the change in hematological parameters due to seasonal variation, nature condition.

Methodology

This animal experimental study was conducted in the "Bargen lab" in the Department of Zoology at University of Dhaka as well as in "The Peoples Pathological lab" which was a private diagnostic laboratory at Dhaka city from July' 2008 to April' 2009 for a period of 9(nine) months. The fish was *Heteropneustes fossilis* (Bloch, 1797) which belongs to Heteropneustidae family was examined. Ethylene Diamine Tetra Acetic acid (EDTA) was used as anticoagulant. Haemocytometer, injection needle, electronic microscope, slides, reagent bottles, beakers, micropipettes, petri dish, glass road stinger and cloth were used. Haemocytometer including two graduated pipettes was used for counting leucocytes. At first blood was drawn exactly up to the marks 0.5 in a clean dry WBC pipette; then blood was wiped off from the outside of the pipette. After that, white cell was sucked and was diluted third exactly up to 11 mark. Air bubble was prevented to enter into the bulb; then the blood was mixed thoroughly followed by vigorous rotation was done to dilute the fluid in the bulb. Then the special cover slip was placed on the platform of the counting chamber followed by firm pressure till a service of concentric rings appears. Then the diluting fluid of the long stem was discarded which was about 1/3 of the bulb. The tip of the pipette was touched on the platform at the edge of special cover slip at about 45 angles. The chamber was filled by surface tension. Then it was kept in the counting chamber for 2 to 3 minutes for the cells to settle. The chamber was placed on the microscope and was focused the rulings under the microscope. The total numbers of leucocytes were estimated using the method given by Rahman (1989).

Results

A total number of 30 *Heteropneustes fossilis* were examined in this study. During examination of haematological parameters of *Heteropneustes fossilis* Erythrocytes or RBC are found ellipsoid from; nucleus was clearly visible and takes auricular purple stain with Giemsa stain cytoplasm is lightly stained of violet color. Leucocytes or WBC are two types- agranulocytes and granulocytes. Morphological identification of the blood parameters of *Heteropneustes fossilis* are given below: Erythrocytes are ellipsoid form; nucleus is clear by visible and takes auricular purple stain with Giemsa cytoplasm is lightly stained of violet color. Erythrocytes *Heteropneustes fossilis* are count (10.5×10^6 mm^{-3}) during July and

lowest count (06.5 X 10^6 mm^{-3}) during December'2008. The observed fluctuation in Erythrocytes counts of *Heteropneustes fossilis* to length of the fish are graphically presented in graph. Length group 10-12 cm showed highest count of erythrocytes 7.8X106 mm^{-3} and lowest count is 6.9 X 106 mm^{-3} in 12-14 cm group. Leucocytes are two types-Agranulocytes and Granulocytes Thrombocytes, Lymphocytes (large and small) Monocyte are in Agranulocytes. Neutrophil, eosinophils, basophiles are in Granulocytes. Thrombocytes constitute 52% to 58% of total leucocytes. They are oval with ellipsoid nucleus. Lymphocytes are immunocompetent of cells large lymphocytes 3% to 6% of total leucocytes. It has varied shapes from circular to amoeboid form. Small lymphocytes constitute only 2%-5% of total leucocytes. It is oval with an ellipsoid nucleus Monocyte is represent 1.6%-3.0% of total leucocytes and globular shape its nucleus has variable forms. Neutrophil are 20%-30% of total leucocytes.

Table 1: Monthly fluctuation of Haemotological parameters of *Heteropneustes fossilis*

Parameter	April'08	May'08	June'08	July'08	Aug'08	Sept'08	Oct'08	Nov'08	Dec'08	Mean ± SD
Erythrocytes (10^6mm^{-3})	8.0	9.5	10.0	10.5	8.5	8.0	8.2	7.0	6.5	8.45± 1.33
Leucocytes (10^6mm^{-3})	155	150	152	149	148	145	150	154	160	151.44± 4.42
Thrombocytes*	32.5	37.5	35	35.5	34	32.5	31.5	34	40	34.92± 2.58
L-lymphocytes*	0.4	0.4	.05	1.0	1.1	1.2	2.0	1.6	1.0	1.02± .54
S-Lymphocytes*	24.5	27.6	27.0	26.0	27.0	28.0	28.0	26.5	20.5	26.73± 252
Monocyte*	3.0	1.8	1.9	3.2	4.0	5.3	5.4	4.5	6.2	3.9± 1.56
Neutrophil*	21.2	18.0	16.0	16.5	16.0	17.2	16.8	15.4	15.2	16.93± 1.83
Eosinophils*	8.6	8.6	8.5	6.0	5.0	4.8	6.0	6.2	9	6.97± 1.69
Basophiles*	5.2	6.0	5.5	10.5	10.0	11.2	4.6	12.0	7.5	8.6± 2.6
Haemoglobin*	12.0	11.5	11.2	11.6	10.0	12.5	12.6	12.8	11	11.7± .40
ESR**	6.0	6.1	6.0	5.4	8	8.5	6.5	6.0	5.5	6.4± 1.08

*Figures show in percentage. **Figures show in mm in 1st hour.

They are generally round in shape and large its nucleus has a variable form. Eosinophils are in only 3%-6% of total leukocytes. They are typically amoeboid with irregular shape nucleus. Basophiles are rounded with founded nucleus. Platelets are absent in fish blood. Erythrocytes containing haemoglobin plays a vital role in vertebrate respiration. The studies of erythrocytes in *Heteropneustes fossilis* reveal a remarkable difference in their size and counts in different month in response to the physiological and environmental conditions in which they live Monthly fluctuation of erythrocytes showed decreasing trend in dry season and after breeding period. According to length fluctuation rate was decreasing in the lower length and increasing trend was observed in higher length and increasing trend was observed in higher length group and smaller weight group showed lower erythrocytes counts. Total leucocytes counts ranged from $145\times10^{-3}mm^{-3}$ to $160X10^{-3}mm^{-3}$ ± 4.42. Leucocytes counts of *Heteropneustes fossilis* increased with the increase of length and reach at peak(146 X 10^3mm^{-3}) in 16-18cm length group and lowest count (142 X 10^3mm^{-3}) was observed in 10-12 cm length group.

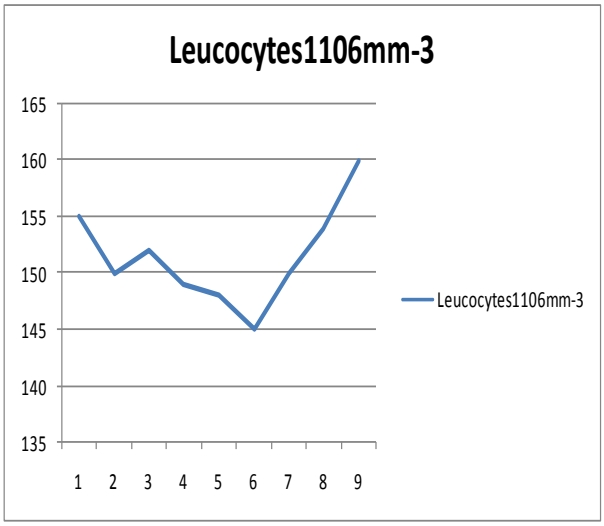

Figure 1: Showing of total leucocytes of *Heteropneustes fossilis*

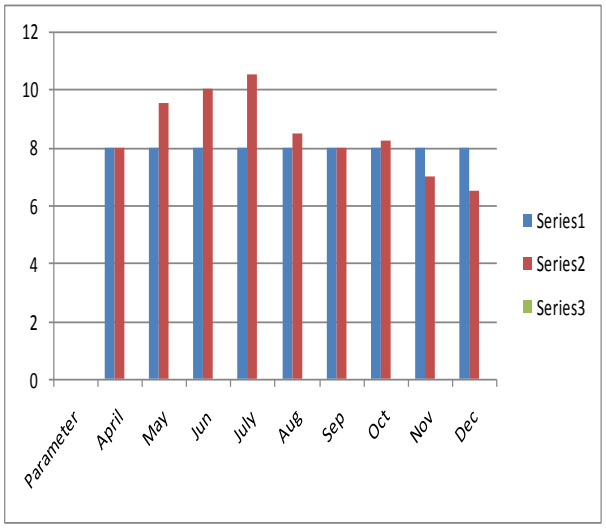

Figure 2: Showing of Erythrocytes of *Heteropneustes fossilis*

Thrombocytes counts ranged between31.5% to 40.0%of total leucocytes in *Heteropneustes fossilis*. Mean percent for *Heteropneustes fossilis* was 34.72 X $10^3 mm^{-3}$ Highest thrombocytes 35% were observed in (16-18 cm) length group lowest (31% ± 1.00) percent was in 10-12 cm length group. So the fluctuation rate of thrombocytes showed an increases trend of count in higher length and heavier group A range of 0.4% to 02.00% of large lymphocytes was observed in case of *Heteropneustes fossilis* with 1.02% ± 0.54 mean concentrations.

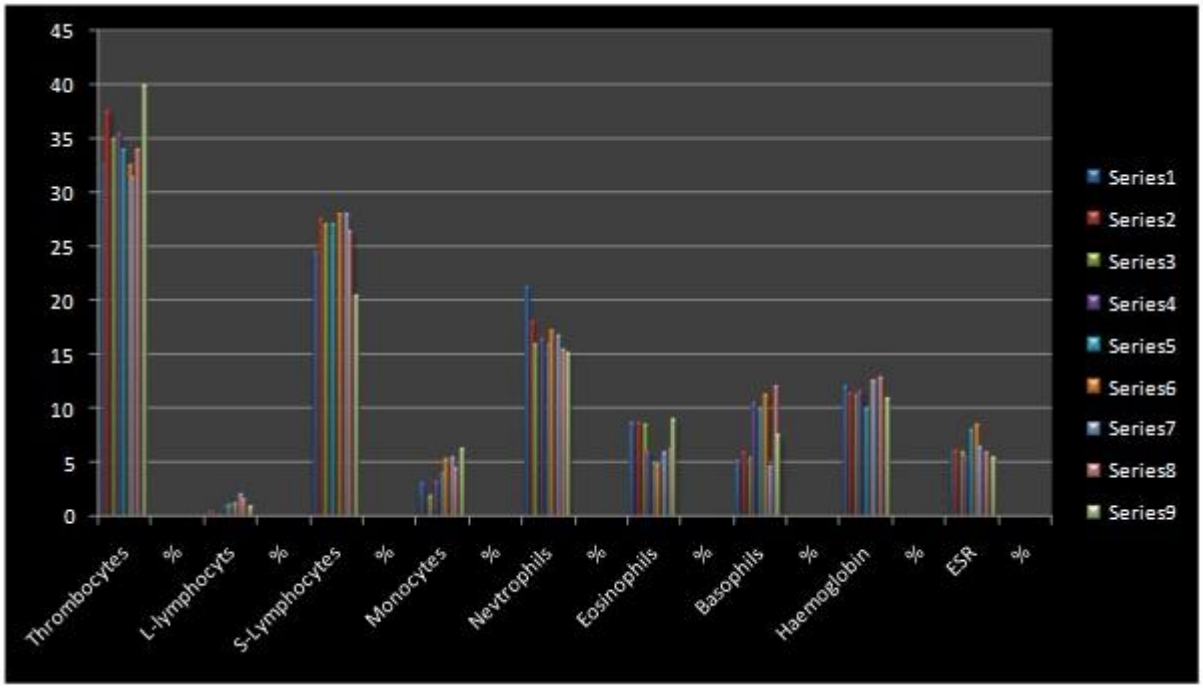

Figure 3: Showing the monthly fluctuation of Erythrocytes, Leucocytes, Thrombocytes, L-Lymphocytes, S-Lymphocytes, Monocyte, Neutrophil of Heteropneustes *fossilis*

The monthly fluctuation of *Heteropneustes fossilis* showed highest percent (2.00%) was observed during October'08 and lowest percent (0.4%) was during May'08'. Large lymphocytes showed an increasing trend of count in highest percent in (03%) in 16-18 cm length group and lowest percent (2.0%) in 10-12 cm length group. A range of 20.05% to 29.05% of small lymphocytes was found to constitute the total leucocytes with 26.73% ± 2.52 mean percent Highest percent (29.5%) observed in April' 08 and highest percent observed in 10-12cm group and lowest percent (26%)in 16-18 cm group. Percent of monocyte constituting the total leucocytes in *Heteropneustes fossilis* ranged between 1.8 to 6.2 with 3.9% ± 1.56 mean percent.

Monthly fluctuation should highest percent (6.2%) in December' 08 lowest percent of monocyte 1.8% was observed during May' 08, Highest rate (5.5%)) in 12-14cmandlowest percent (4.0%) in 14-16 cm length group and monthly fluctuation of *Heteropneustes fossilis* showed a decreasing trend fluctuation in the post breeding season and the values slowly upward during breeding season. Percent of neutrophil range between 15.2% and 28.0% with 21.2% with 16.93 ± 1.83 mean percents highest (21.2%) percent neutrophil found in *Heteropneustes fossilis* during April.08 and lowest percent (15.2%)was observed during December .08 Showed irregular fluctuation pattern of *Heteropneustes fossilis* with peak in percent of (17.5%) in14-16 cm length and in heavier group.

Table 2: Showing the length wish fluctuation of Erythrocytes leucocytes, Thrombocytes, L-lymphocytes, S-Lymphocytes, Mnocytes, Neutrophils of *Heteropneuestes fossilis*

Size	**Erythrocytes ($10^6 mm^{-3}$)**	**Leucocytes ($10^6 mm^{-3}$)**	**Thrombocytes**	**L-lymphocytes**	**S-Lymphocytes**	**Monocyte**	**Neutrophil**	**Eosinophils**	**Basophiles**	**Haemoglobin**	**ESR**
10-12 cm	7.8	142.0	31	2.0	30	5.0	19.0	4	10	11.0	6.0
12-14 cm	6.9	144.0	33	1.5	29	5.5	17.0	5	9	11.5	5.0
14-16 cm	8.5	145	34	2.5	27	4.0	17.5	6	11	12.0	6.5
16-18 cm	8.9	146	35	3	26	5.0	14.0	5	12	12.5	5.8

Percent of eosinophils range between 4.8% and 9.0% with 6.97% ± 1.69 mean monthly fluctuation of eosinophils found in peak percent (9.0%) during December' 08 and lowest percent (4.8%) was September' 08. Peak percent also found in higher length group and heavier group. Basophiles range in case of *Heteropneustes fossilis* between 5.20 % and 11.2% with 8.6% ± 2.6 mean. Highest percents of basophiles are (11.2%) during September' 08 lowest (5.2%) during April' 08. lowest percent found in smaller and lighter (12-14cm.) group. The above observation showed no definite pattern of variation in relation to weight of the fishes. The erythrocytic characteristics are represented by haemoglobin, haematocrit and Erythrocyte sedimentation Rate (ESR). They play a vital role in disease diagnosis and progress of diseases. In *Heteropneustes fossilis* the haemoglobin concentration ranged between 12.8g $100ml^{-1}$ was observed in May 08 lowest (11.70g 100 ml^{-1}) in August' 08. Increased level of haemoglobin with increase in length was recorded 12.5g $100m^{-1}$ ± 7.68 at 16-18 cm.

Table 3: Showing the weight wize fluctuation of Erythrocytes leucocytes, Thrombocytes, L-lymphocytes, S-Lymphocytes, Mnocytes, Neutrophils of *Heteropneuestes fossilis*

Size	Erythrocyles ($10^6 mm^{-3}$)	Leucocytes ($10^6 mm^{-3}$)	Thrombocytes	L-lymphocyts	S-Lymphocytes	Monocytes	Nevtrophils	Eosinophils	Basophils	Haemoglobin	ESR
50-60gm	7.0	155	32	1.0	27	3.0	22.0	8	4	11.0	6
60-70gm	6.5	152	34	1.5	27	4.0	20.0	8	6	12.0	5
70-80gm	6.2	154	33	1.2	27	5.0	21.0	4	6	11.5	5.5
80-90gm	8.0	156	31	1.5	34	4.0	22.0	10	5.5	12.5	5.8

Highest percent (12.50%) of haemoglobin was in 80-90 gm group. Erythrocyte sedimentation rate of *Heteropneustes fossilis* ranged between 8.5g $100ml^1$ with 5.4g g $100ml^{-1} \pm 1.08$ mean concentration..The observation reveals that E.S. R. increased in the dry season and the E .S. R. decreased during breeding season and then slowly upward ESR increased with the. Decrease of the length group. An increased trends showing the lower group ESR with increase in heavy fishes was recorded.

Discussion

Fisheries sector plays an important role in the economy of Bangladesh. As fish live in a very intimate contact with their environment, they are extremely dependent upon it (Mahajan and Dheer 1979). Any changes in the environment would affect the physiology of the fishes. The blood said to be a mirror in which all the vital process taking place in the organisms are reflected. The blood properties are indispensable for understanding the biological process, taking place in fish species (Hawkns and Thomasi 1971). The haematological assessment is the rising motive to prove any disturbances of environmental influences on the fishes. Fishes show abnormal effects due to food, habitat, temperature, pH, and many other elements of environment (Fange 1994). Fish blood is being studied in toxicological research and environmental monitoring as a possible indicator of physiological and pathological change in fishery management (Gupta and Gupta 1981).

It was therefore considered desirable to evaluate the haematological changes in the blood parameter of the predatory fish like *Heteropneustes fossilis* resulting from the seasonal variation. The present work thus deals with the differential cell counts, total count of W B C to see the change in haematological parameters due to seasonal variation nature condition. Gupta and Guota observed variation in blood parameters of different individuals of the same species regarding their length, weight ,diameter and count of different types of blood cells which might be due to the influence of pathological and physiological factor (Beneriee 1964). The present investigation puts together nine months observation on haematological parameters of *Heteropneustes fossilis* for their possible change in natural conditions. Erythrocytes of fish are nucleated and play a vital role in respiration .The monthly fluctuation is increases during breeding period , due to available of food nutrient , oxygen etc.A decreasing trend was observed during the post breeding period of shing fish In

case of small lymphocytes and it is a very lower stages but it was gradually increasing with the increasing of length. In fishes the significance of leukocytes cells and their biological function are not clearly understood. The different types of leucocytes have the different in number and morphology between species. In case of thrombocytes of *Heteropneustes fossilis* was shows slowly increasing trend in monthly fluctuation. A heigher number of large lymphocytes during the breeding period is found (Khaleoue 1978). The smaller fishes have lower percent of large lymphocytes which increased with increases of length, but small lymphocytes was higher in smaller length group of sizes (Vanerjee 1986). The lower percent of monocytes was observed in havier group of fishes but higher percent of neutrophil which increased with increases oflength.In case of eosiophils during post breeding period was increased slowly and the highest count was in December. The basophil showed a slowly decreasing pattern under investigation was observed and lowest count was in April. During the investigation it was reveals that the haemoglobin showed higher count during the breeding period and ESR has an decreasing trend in post breeding period.

Conclusion

The physiological changes are better understood by the changes in the blood composition. For diagnosis of physiological disorders and effects of environmental stresses and diseases of the various kinds, study and understanding of the blood physiology of the fish requires an instristic knowledge of the composition and function of the varies blood component in nature and under specific environmental condition to enable the establishment of a basic haematological profile for future use. However to establish the blood value a thorough understanding of the normal range ,mean with standard deviation and their various with length group, weight group, physiological and physical condition, of cultured procedures of fresh water is needed. It may be concluded that this types observation is needed for future use in order to development of fisheries sector. So Haematological profile of *Heteropneustes fossilis* is important in evaluating the health of fish.

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