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# Dynamics of Leucocytic and Erythrocytic Variables in Relation to Seasonal Cycle in Adult *Channa Punctatus* (Bloch)

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## Abstract

The blood profile especially the dynamics of leucocytes and concurrent erythrocytic parameters of adult *Channa punctatus* (Bloch), a wetland air breathing fish was investigated in relation to seasonal variation. A yearlong study was based on monthly observation of the haematology of adult *C. punctatus* of both sexes. The values of Total Leucocytic Count (TLC), Leucocrit (Lct %) and relative population of neutrophil were increased in gravid fishes during the breeding season as well as in the recovery phase. The erythrocytic parameters also increased in the pre-breeding phase and attained the maximum values during the peak breeding season. The mean values for both leucocytic and erythrocytic parameters in *C. punctatus* obtained in this study are the baseline information to evaluate the gross health conditions.

**Keywords:** *Channa punctatus*, leucocytes, erythrocytes, Seasonal variation

## 1. Introduction

Blood is a convenient and easily accessible liquid which can be used as a sensitive index in understanding various physiological processes in fish throughout the year (Satheeshkumar *et al.*, 2012). The alterations in the haematological parameters of fish blood in relation to seasonal cycle have been observed by many researchers (Joshi and Tandon, 1977; Van Vuren and Hattingh, 1978; Lane, 1979; Mahajan and Dheer, 1979; Folmer *et al.*, 1992; Siddall *et al.*, 1995; Guijarro *et al.*, 2003; De Pedro *et al.*, 2005; Kohanestani *et al.*, 2013). The population of the tropical air breathing fish *Channa punctatus* (Bloch), previously highly abundant in the shallow wetlands of West Bengal, India is now dwindling in number and possibly faces threat of local extinction. The aim of the present study was to investigate blood profiles especially the leucocytic dynamics of adult *C. punctatus* in relation to seasonal variations. This information could aid in evaluating the response of different types of blood cells in a variety of conditions of physiological stress such as pollution, parasitism and bacterial infection (Roche and Bogé, 1996). A yearlong study was based on monthly observation of the haematological parameters of adult *C. punctatus*, with respect to Total Leucocytic Count (TLC), Leucocrit (Lct %) population, Differential count of various leucocytes and simultaneously certain erythrocytic parameters viz. Total Erythrocytic Count (TEC), Haemoglobin (Hb), Haematocrit (Ht %), and red cell indices viz. Mean Cell volume (MCV), Mean Cell Haemoglobin (MCH) and Mean Cell Haemoglobin Concentration (MCHC) were recorded to generate baseline data for evaluating gross health conditions of fish.

## 2. Materials and Methods

### *2.1 Fish stock collection, acclimatization and maintenance*

Live and healthy specimens (adults of both sexes) of *C. punctatus* were collected from local fish farm (Latitude: 22°31.115' N; Longitude: 88° 24.086' E) located at Kolkata, West Bengal, India throughout the year. The fish specimen were transported and acclimatized to the laboratory for 7 days in glass aquaria (size: 0.6m × 0.3m × 0.3m) under controlled laboratory conditions with continuous aeration. Fishes were fed *ad libitum* with *Tubifex* sp. and larvae of *Culex* sp. during acclimatization period only. The water was renewed every day to avoid accumulation of unutilized food and metabolic waste products.

### *2.2 Water monitoring*

Different water parameters like temperature, pH, hardness, dissolved oxygen and total alkalinity were measured following the standard methods (APHA, 2005). The respective values obtained are as follows: water temperature: 24ºC-26ºC; pH: 7.2-7.4; Hardness: 225-250 mg/l, Dissolved oxygen: 3.5-4.5 mg/l and Total alkalinity: 125-140 mg/l. The water parameters were checked before the experimental set up.

### *2.3 Experimental set up*

Studies on the seasonal changes of blood parameters were made from June, 2014 to May, 2015. The different months of the year were classified into five breeding phases based on reproductive maturity of the fish. The period from June to August was the peak breeding phase. The post-breeding months were September and October with no gonadal activity and the recovery phase was obtained during November and December. January and February months comprised the resting phase and finally, the period from March to May were the pre-breeding phase. At least 12 healthy adult fishes of both sexes were selected for a set of observations at each month. The specimens of adult *C. punctatus* selected for study was 81.25 ± 1.49 g in weight and 18.5 ± 0.65 cm in length.

*2.4 Blood sampling*

Free flowing blood was collected by severing the caudal peduncle without using anaesthesia. Minimum possible stress was allowed while collecting so as to minimize errors or fluctuations in blood parameters. For measurement of Total Erythrocytic Count (TEC), Total Leucocytic Count (TLC) and Haemoglobin (Hb) content, blood was taken directly from caudal blood vessel. Blood smears were also prepared from blood free from any anticoagulant. However, blood was collected in glass vial rinsed with 3.8 % sodium citrate (anticoagulant) for determination of Haematocrit (Ht) and Leucocrit (Lct) values. The measurements were made within 30 min of sampling. The weak and diseased fishes were rejected outright and the data of the fishes later found to be infected during the acclimatization period were not considered. Different blood parameters were measured following the methods earlier described (Hesser, 1960). Some modifications were made following the methods of Blaxhall and Daisley (1973), Dacie and Lewis (1984).

*2.5 Preparation of blood smears, staining and determination of Differential Count of WBC*

Blood smear was prepared from a small drop of blood taken directly in the centerline of a chemically cleaned slide 2-3 cm from one end. The film was immediately drawn by a rapid, smooth and forward movement of a spreader slide placed at an angle of 45° to the slide. For staining, the film was air-dried and flooded with Leishman stain and kept for 5 min. Double amount of freshly prepared distilled water (pH: 6.8) was added on the slide and stained for 30 min in the diluted stain. The slide was washed in a stream of distilled water until it acquired a pinkish tinge. The film was allowed to dry and placed under microscope for observation of leucocytic populations. Blood cells were observed under oil immersion magnification in a research microscope. The differential count of leucocytes was done by counting 150 cells of uniform blood smears avoiding extreme edges of the slides. The percentage of leucocytes was calculated from such observations.

*2. 6 Determination of Total Erythrocytic Count (TEC) and Total Leucocytic Count (TLC)*

The total count was done by Neubauer's improved double haemocytometer (Fein-OPTIK, Blankenburg, G.D.R.) using Hayem's solution as diluting fluid. Free flowing blood was drawn directly into the RBC and WBC pipettes respectively up to 0.5 mark. The tip of the pipettes was wiped with an absorbent tissue and the perfect volume was adjusted. The tip of the RBC pipette was brought immediately into the RBC diluting fluid placed in a watch glass and sucked up to 101 mark, therefore giving a dilution of 200 time. Similarly, blood in the WBC pipette was diluted with WBC diluting fluid up to 11 mark. In this case, 0.5 mark blood was diluted only 20 times. Both the pipettes were rotated for two minutes so as to avoid clumping of blood cells. A few drops of the diluted blood were discarded and the next drop was carefully taken free of any air bubble under the cover slip and spread to the both sides of the haemocytometer chambers. The blood was allowed to settle for a few minutes and counting was made under the microscope. For total count of erythrocytes, cells in five squares (80 smallest squares) of which four from the margin and fifth at the center were counted. The total number of RBC per cubic mm of undiluted blood was calculated by multiplying the total number in the five squares by 10,000 (the volume of each of the eighty smallest squares is 1/4,000 cubic mm). For computation of the total leucocytes, the number of cells in the four large squares was counted. The total number of leucocytes per cubic mm of undiluted blood was calculated by multiplying the total number of cells in the four large squares by 50.

*2.7 Haemoglobinometry*

The haemoglobin value was measured colorimetrically by Cyanmethaemoglobin method. In this method, blood is diluted in a solution containing potassium cyanide and potassium ferricyanide. All haemoglobin forms (viz. haemoglobin, methaemoglobin, carboxyhaemoglobin) except sulphaemoglobin are converted to Cyanmethaemoglobin (HiCN). The composition of haemoglobin reaction reagent was as follows: 200 mg potassium ferricyanide, $K_3 Fe (CN)_6$; 50 mg potassium cyanide, KCN; 140 mg of potassium dihydrogen phosphate, $KH_2PO_4$ dissolved in 1,000 ml distilled water with addition of 0.5 ml of Sterox SE. For this experiment, 20 µl of blood was added to 5 ml of haemoglobin reaction reagent in one test tube and the other test tube for blank contained 5 ml of the haemoglobin reaction reagent. The solutions were allowed to stand for 5 min to ensure completion of reaction to produce HiCN. The blank test tube was placed in the instrument and set in at 100 % T. The absorbance of the test solution was measured at a wavelength of 540 nm. The results were compared from haemoglobin standard curve to obtain the haemoglobin value.

*2.8 Determination of Haematocrit (Ht %) and Leucocrit (Lct %)*

The haematocrit and leucocrit values were estimated by microhaematocrit method (Wintrobe, 1967) using Wintrobe's tube, 110 mm long with a bore of 3 mm and marked in mm from 0 to 10 calibrated at 1 mm intervals. The graduated cell volume tube was filled up by anticoagulated blood from the bottom upwards exactly up to 10 mark using a capillary pipette. The tube was then centrifuged at 3,000 rpm for 25 min. The level of packed red cell volume and packed white cell volume respectively were noted and multiplied by 10 to obtain the percentage of haematocrit and leucocrit values.

*2.9 Measurement of absolute corpuscular values*

The red cell indices viz. Mean Cell Volume (MCV), Mean Cell Haemoglobin (MCH) and Mean Cell Haemoglobin Concentration (MCHC) are referred to as 'absolute' values. These values calculated from the red cell count (TEC), haemoglobin and haematocrit values using standard formulae (Hepler, 1966) are as follows: MCV (fl) = Haematocrit (%) × 10 / TEC, MCH (pg) = Haemoglobin (g %) × 10 / TEC and MCHC (g /l) = Haemoglobin (g %) × 100 / Haematocrit (%) value.

## 3. Results and Discussion

An overall increase in the Total Leucocytic Count (TLC) was observed during post-breeding season and recovery phase (September – December) months with its peak in December (Fig. 1a). In the breeding season (June-August), TLC

values also showed an increasing trend. The leucocrit values (Lct %) was almost showing similar changing pattern (Fig. 1b) like that of leucocytic population. The highest count of both TLC and Lct were found in December compared with the rest of the seasons.

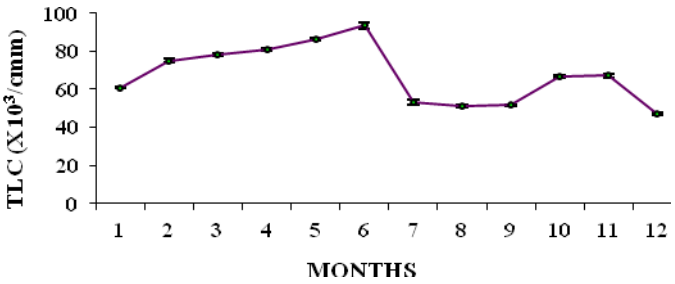


Fig. 1a

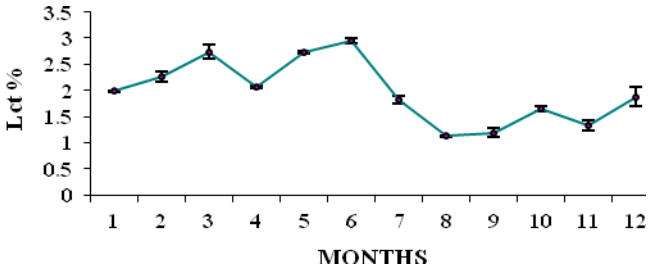


Fig. 1b

**Fig. 1:** Graphs representing monthly observations of TLC (Fig. 1a) and Leucocrit % values (Fig. 1b) of the peripheral blood of adult *C. punctatus*. Numbers 1-12 on the X - axis represents calendar months starting with June (1) and ending with May (12). TLC- Total Leucocytic Count and Lct- Leucocrit.

Within the leucocytes, the neutrophil population was decreased in pre-breeding season but increased during breeding and post-breeding seasons (Fig. 2a). Comparative cell numbers in eosinophil, basophil and monocyte were also counted during this study but there was no appreciable difference in the percentage of these cells. A high percentage of the leucocytes in the *C. punctatus* were small lymphocytes (25-54%) and large lymphocytes (19-36%) respectively, with variations among seasons (Fig. 2b). The relative percentage of large lymphocyte population (LL %) reached its highest value in March and that of small lymphocytes (SL %) in January respectively (Fig. 2b).

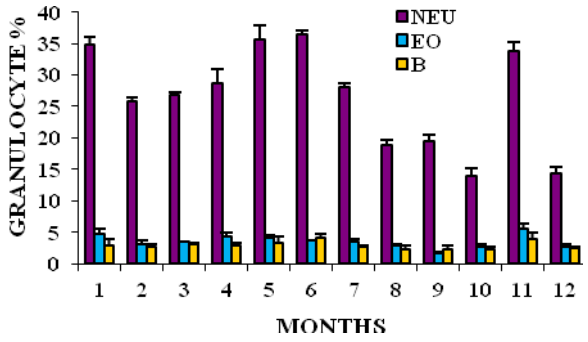


Fig. 2a

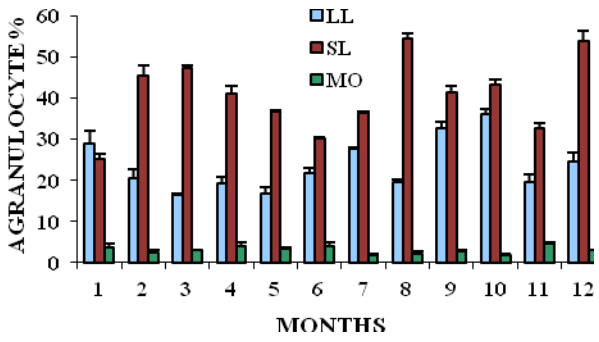


Fig.2b

**Fig. 2:** Graphs representing monthly observations of relative population of granulocytes (Fig. 2a) and agranulocytes % values (Fig. 2b) of the peripheral blood of *C. punctatus*. Numbers 1-12 on the X - axis represents calendar months starting with June (1) and ending with May (12). NEU- Neutrophil, EO- Eosinophil, B-Basophil, LL- Large lymphocyte, SL- Small lymphocyte and MO- Monocyte.

Fig.3 showed that values of TEC, Hb and Ht were found to be moderately high during breeding season (June-August) with their peak values in July (for both TEC and Hb) and in June respectively for Ht %. From August onwards, these values showed a decreasing trend during the post-breeding season. Equilibrium in the blood values were observed in the recovery phase (November and December) with little fluctuations during the resting phase. The TEC, Hb and Ht values were slightly elevated in the pre-breeding months. The above observations were true for both male and female fish.

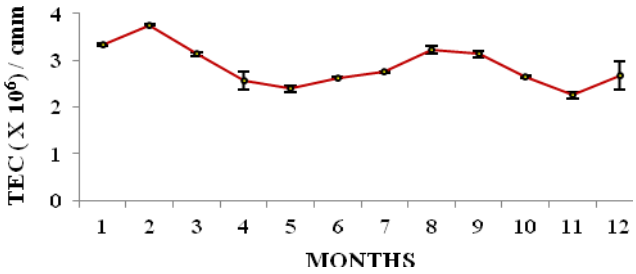


Fig. 3a

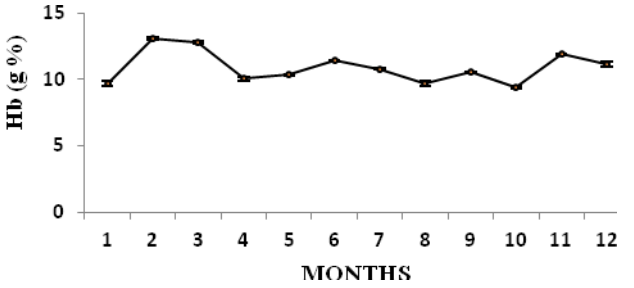


Fig. 3b

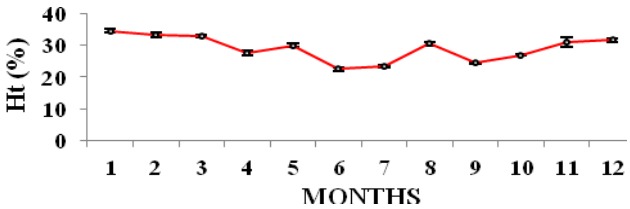


Fig. 3c

**Fig. 3:** Graphs representing monthly observations of TEC (Fig. 3a), Hb concentration (Fig. 3b) and Ht % values (Fig. 3c) of the peripheral blood of *C. punctatus.* Numbers 1-12 on the X - axis represent calendar months starting with June (1) and ending with May (12). TEC- Total Erythrocyte Count, Hb- haemoglobin and Ht- haematocrit.

Mean Cell Volume (MCV) of all fishes was noted to be highest in June and lowest in March (Fig. 4a). Both Mean Cell Haemoglobin (MCH) and Mean Cell Haemoglobin Concentration (MCHC) values were recorded highest in July. (Fig. 4b and 4c).

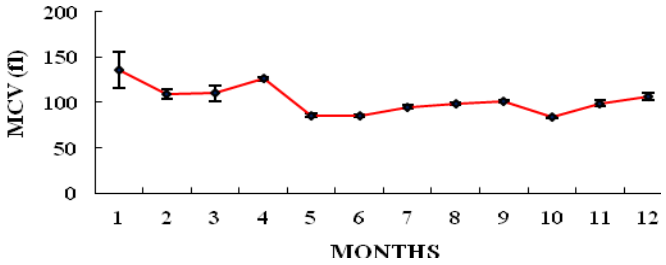


Fig. 4a

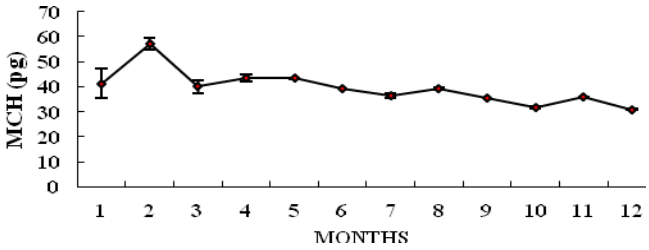


Fig. 4b

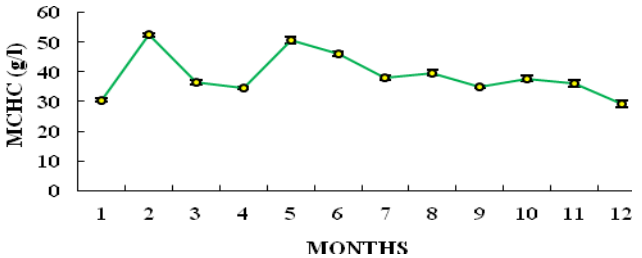


Fig. 4c

**Fig. 4:** Graphs representing monthly observations of MCV (Fig. 4a), MCH (Fig. 4b) and MCHC (Fig. 4c) values of the peripheral blood of *C. punctatus.* Numbers 1-12 on the X- axis represents calendar months starting with June (1) and ending with May (12). MCV- Mean Cell Volume, MCH- Mean Cell Haemoglobin and MCHC- Mean Cell Haemoglobin Concentration.

Seasonal water temperature fluctuations and day length can modulate the haematology of fish. Indeed, seasonal changes in body composition, haematology and blood biochemistry have been described in several fish species (Denton and Yousef, 1975; Banerjee, 1982; Jonsson and Jonsson, 1998; Swain *et al.*, 2007; Jerônimo *et al.*, 2011; Langer *et al.*, 2013). In this study, seasonal variations in haematological characteristics have been observed in adult *C. punctatus*. The leucocytic parameters viz. TLC, Lct and neutrophil population were elevated in gravid fishes during the breeding season (June- August) as well as in the recovery phase (November- December). This observation is in conformity with earlier report of Mahajan and Dheer (1979) who also observed the small decrease of TLC at the onset of breeding season but an appreciable increase of leucocytic population during the period of sexual activity (August and September) in female *C. punctatus*. Neutrophil population, however, decreased during the breeding season according to their observations. An increase in the lymphocytic population (LL and SL %) was observed during the pre-breeding season in the present study. Guijarro *et al*. (2003) found significant variations in the number of white and red blood cells, haematocrit and haemoglobin throughout the year in sexually mature male and female tench, *Tinca tinca*. They also found lowest values during autumn- winter months and the highest during summer with males exhibiting higher values than females. In the present study, the erythrocytic parameters viz. TEC, Hb and Ht values increased in the pre-breeding phase (March - May) and attained the maximum values during the peak breeding season (June - August). The fish sampled at this time of the year are mostly in maturing and gravid condition. The increased metabolic rate associated with gonadal maturation in the ripening fish necessitates gradual increase in the erythrocyte number and haemoglobin values as an adjustment to increased oxygen demand. The above erythrocytic parameters were significantly reduced in the fishes during post-breeding season.

## 4. Conclusions

There appears to be very little agreement with regard to variations of blood parameters in relation to breeding season in fish. This may be due to the fact that each species of fish requires different ranges of environmental cues and reproduces accordingly. The cumulative metabolic demand of individual fish may also direct the alterations of blood profile. Therefore, the average values for both leucocytes and erythrocytic parameters in *C. punctatus* obtained in this experiment are the baseline information to evaluate the gross health conditions.

## 5. Acknowledgement

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