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Influence of water quality on some haematological parameters during breeding phase in the freshwater fish, *Notopterus notopterus*

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Abstract
The influence of water quality on blood haematological parameters was investigated during breeding period (spawning) in the fresh water fish, *Notopterus notopterus.* This fish is available in large numbers in all the three aquatic bodies, two rivers (Bheema and Kagina rivers) and one small streem (Saradagi Nala). Temperature, oxygen and pH are important environmental factors considered for the present study and these are found to ensure the fish to survive, its distribution and normal reproductive functioning of the fish in an aquatic body. The reproductive activity was assessed based on gradual changes in the gonadosamatic index (GSI) which increases on approaching breeding period during June- July and spawning occurs in August by the onset of rains. The change in the blood parameters such as haematocrite (Hct), haemoglobin (Hb) concentration and RBC count was observed. The relationship between haemoglobin and oxygen differs between loading and unloading sites. The haematological parameters such as RBC count, haematocrite (Hct) and haemoglobin were compared and found to be differed with reference to water dissolved oxygen, hydrogen ion concentration and temperature, there is lower RBC count, subsequently lower haemoglobin and haematocrite in the fish collected from Kagina river than the fish from other two aquatic bodies, this may be due to difference in the O_2 or CO_2 transport as a result of increased metabolic activity. The less number of erythrocyte count are likely due in part to an increase in oxygen consumption and metabolic rates corresponding to the rise in water temperature. High level of dissolved oxygen, moderate hydrogen ion concentration and temperature of water creates a suitable ground for its normal reproductive physiology of the fish.

Keywords: Influence, quality, haematological, parameters, *Notopterus notopterus*

Introduction
Red blood cell indices are measurements that describe the size and oxygen-carrying protein (hemoglobin) content of red blood cells. The indices are used to help in the differential diagnosis of anemia. They are also called red cell absolute values or erythrocyte indices. These tests are the haematocrite, haemoglobin, and red blood cell count. The haematocrite is a measure of red blood cell mass, or how much space in the blood is occupied by red blood cells. The hemoglobin test is a measure of how much hemoglobin protein is in the blood. The red blood cell count (RBC) measures the number of red blood cells present in the blood. Red blood cell indices are additional measurements of red blood cells based on the relationship of these three test results. It has been observed that blood parameters such as haematocrite (Hct), haemoglobin (Hb) concentration and RBC count are related to environmental factors such as water temperature and salinity (Graham, 1997) [5]. Additionally, the relationship between haemoglobin and oxygen differs between loading and unloading sites and shows adaptations not only to environmental conditions but also to metabolic requirements, both of which govern oxygen availability and transport to tissues (Weber and Wells, 1989) [9]. Such adaptations may involve quantitative changes in total Hb content, or qualitative changes in Hb–oxygen–binding properties, and may appear both at the inter– and intra–specific level (Weber and Wells, 1989) [9]. Thus, the remarkable diversity of oxygen transport properties results from evolutionary processes through subtle sequence differences in haemoglobin that appear to match the varied metabolic demands of animals with the environmental oxygen supply (Wells, 1999) [10]. The relationships between the haematocrite, the hemoglobin level and the RBC are converted to red blood cell indices through mathematical formulas. In the present study, 10 specimens of fresh water fish, *Notopterus notopterus* were collected from each three aquatic

bodies separately and haematological parameters were analysed to find out if any influence of water quality on some blood parameters. The three aquatic bodies were located around 45 km away from Kalaburagi city. The fish, *N. notopterus* is available in large numbers and thrives well in the aquatic bodies of Kalaburagi.

Material and Methods

Live specimens of the Indian fresh water fish *N. notopterus*, 10 fishes each obtained from the aquatic bodies situated at, Bheema River, Kagina River and Saradgi nala (Small stream) separately near Kalaburagi, Karnataka State, India and transported in aerated containers to the laboratory. They were given a minimum period of a weeks to acclimatize to laboratory conditions during which time they were fed with earth worms, boiled eggs and guppy fishes obtained locally to avoid the possible effect of starvation on any of the hematological parameters. All sexes were used without discrimination. The fish were caught individually in a small hand net from the containers. The gonado somatic index (GSI) was determined by deviding gonad weight by body weight.

The dissolved oxygen (DO) of the water samples collected from different three aquatic bodies was determined by using modified Winkler's method APHA, AWWA, WCPF, 1985). The water temparature: was recorded at the site of collection using an ordinary mercury thermometer to the nearest of 0.1^0C (Water teperature at depth of 8 cm) and pH of the water at the aquatic body was measured by pH meter. The fish were then placed belly upwards and blood samples obtained from the caudal circulation with the aid of a heparinized 2 cm disposable plastic syringe and a 21 gauge disposable hypodermic needle. The samples were then mixed gently but thoroughly. Some blood samples were used for the measurement of haematocrite, haemoglobin concentration and red blood cell count. Hemoglobin was measured using the standard cyanmethemoglobin method described by Baker and Silverton, (1976) [2]. RBC count was determined using Neubaur's haemocytometer according to Dacie and Lewis, (1984) [3]. Haematocrite (Hct) value was determined by standard Wintrobe method, and expressed in percentage. Blood sample were loaded in Wintrobe tubes and spun in a centrifuge at 3000 rpm for 5 min and measured. The blood specimen is diluted 1:200 with RBC diluting fluid and cells are counted under high power objective by using Neubaeur slide. The numbers of cells in undiluted blood are calculated and reported as the number of cells per cu mm (μl) of whole blood. The total leukocytes count is enumerated by using an improved Neubauer haemocytometer, as described by the method of Dacie and lewis, (1984) [3]. Blood drawn up to 0.5 mark of a WBC pipette and wiped out the excess blood outside the pipette by using cotton then drawn diluting fluid up to 11 mark the content in the pipette is mixed and after five minutes by discarding few drops filled the counting chamber and allowed the cells to settle for two to three minutes focused on one of the area (each having 16 small squares) by turning objective to low power objective. Cells were counted in all four chambers. Calculated cells are reported as the number of cells per liter cu mm (ml) $\times 10^6$

Results

The present study is to find out the influence of water quality considering dissolved oxygen (DO), hydrogen ion concentration (pH) and water temperature on blood haematological levels during breeding period of the fish, Notopterus notopterus, where in the GSI is maximum. River Bheema (Aquatic No.1) situated at 40 kms away from Kalaburagi city is continuously flowing water without much submerged vegetation. River Kagina situated (Aquatic No.2) at 40 kms is continuously flowing water. Saradgi Nala – Small stream (Aquatic No.3) situated around 15 kms away from Kalaburagi. In all the three aquatic bodies studied, the dissolved (DO) oxygen level is high in aquatic body 3 Saradagi nala followed by Bheema River and Kagina River. The dissolved oxygen values of aquatic bodies 1, 2 and 3 are, 10.9, 9.6 and 12.1 mg/l respectively. Hydrogen ion concentration (pH) values of aquatic bodies Bheema, Kagina River and Saradgi nala studied are in the following order 8.4, 8.9, 8.5 respectively. The water temperatures of the aquatic body Kagina River to be higher, the readings of the thermometer for the three aquatic bodies are 30^0, 32^0, and 29^0 respectively.

In the present study the erythrocyte count found to be less in the fish from Kagina river, the erythrocyte count in the fish collected from Bheema river and Saradgi nala were found to be same, the data recorded are 1.44 ± 0.18 million/mm^3 (Bheema river), 1.16 ± 0.03 million/mm^3 (Kagina river), and 1.45 ± 0.03 million/mm^3 (Saradgi nala) significance found to be F^{value} = 5.86, P = 0.01, the means are significantly different, in the fish, *N. notopterus.* The hemoglobin test is a measure of how much hemoglobin protein is present in the blood. In the present study the haemoglobin content was higher in the fish from Saradgi nala followed by Bheema and Kagina Rivers. The data are 6.55 ± 0.26, (Bheema river), 6.26 ± 0.32 (Kagina river), and 6.76 ± 0.32 (Saradagi nala), significance found for F^{value} = 2.18, P > 0.05. The means are not significant (Table 1. and Fig -1, 2). Haematocrite is a blood test that measures the percentage of red blood cells found in whole blood. This measurement depends on the number of red blood cells and the size of red blood cells. In the present study haematocrite percentage was found to be almost same in the fish collected from all the aquatic bodies. The data are 20.41 ± 1.17 (Bheema), 20.00 ± 1.41 (Kagina) and 20.16 ± 1.16 (Saradgi) significance found as F^{value} = 0.0.16, P > 0.05, increase in the RBC count reflects the higher percentage of haematocrite in the fish *N. notopterus* collected from Bheema river

Discussion

The breeding period of the fish, *Notopterus notopterus* happens to be during June- July during which time the analysis of blood parameters was undertaken when gonado-somatic index (GSI) increases with maximum weight of the gonad was found. The analysis of the peripheral blood of fish serves for diagnostic purposes, to evaluate the condition of the fish and to assess the suitability of environmental condition. The fish lives in a very intimate contact with their environment and are therefore very susceptible to physical and chemical changes of the aquatic body, which may have effect on their blood components (Fazio *et al;* 2013) [4]. The blood cells represent a free connective tissue type, which neither maintain intimate connections with other cells, nor possess intercellular substances, constituting the homeostatic force of the organism (Kalashnikova, 1976) [6]. Blood parameters of fish *N.notopterus* collected from three different aquatic bodies and compared with each other, indicate that there is lower in the RBC count of the fish collected from Kagina river than the other aquatic bodies may be due to

stimulation of erythroprotein by elevated demands of O_2 or CO_2 transport as a result of increased metabolic activity. The less in erythrocyte count are likely due, in part, to a decrease in oxygen consumption and metabolic rates corresponding to rise in water temperatures (Zaunuy and Carrillo 1985; Martinez *et al*., 1994) [11, 8]. Haematocrite provides measurements of red blood cells (erythrocytes) in the whole blood, while the haemoglobin within those erythrocytes is the main transport mechanism for oxygen and carbon dioxide in the blood. The decrease in the haemoglobin and haematocrite may be because of less in the erythrocyte numbers. In the present study the fish collected from aquatic body Kagina were found to be less in the haemoglobin and haematocrite level and this may be due to low dissolved oxygen and high temperature. Haematocrite and haemoglobin in striped bass (*Morne saxtalis*) were highest during fall and winter and lowest during summer (Lochmiller *et al.*, 1989) [7]. Changes in the haemoglobin content of the blood in response to the environment might come about either by a change in the number of erythrocytes or by a change in the haemoglobin concentration of the individual cells (Anthony, 1961) [1]. For the manual procedures, the aforesaid numerical values are calculated from total number of red cells, the haemoglobin concentration (per unit volume of whole blood) and the haematocrite, these are called indices.

Table 1: Showing dissolved oxygen (DO) hydrogen ion concentration (pH) and water temperature of the three aquatic bodies of Kalaburagi and haematological parameters of the fresh water fish, *Notopterus notopterus* during breeding phase (spawning) of the reproductive cycle

Aquatic Body/Parameters	DO	pH	Temperaturere In degrees	RBC Count	Haemoglobin (Hb)	Haematocrite (Hct)
1) Bheema River	10.9	8.4	30º C	1.44 ± 0.18	6.55 ± 0.26	20.41 ± 1.17
2) Kagina River	9.6	8.9	30º C	1.16 ± 0.03	6.26 ± 0.59	20.00 ± 1.41
3) Saradgi Nala	10.8	8.5	29º C	1.45 ± 0.03	6.76 ± 0.32	20.16 ± 1.16

Values are expressed as mean ±SD, N=06

Statistical significance (One way ANOVA) Erythrocytes (RBC) countin millions/mm3, P-0.01, Haemoglobin (Hb) in g/dl, Fvalue =2.18, Haematocrite (Hct) in %, F value =0.016.

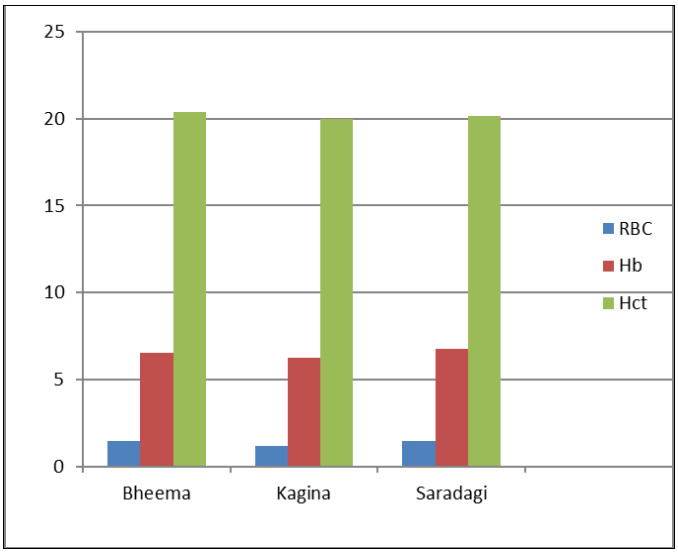

Fig 1: Showing percentage of Red blood cells (RBC), haemoglobin (Hb), haematocrite (Hct) in Bheema, Kagina River and Saradgi Nala

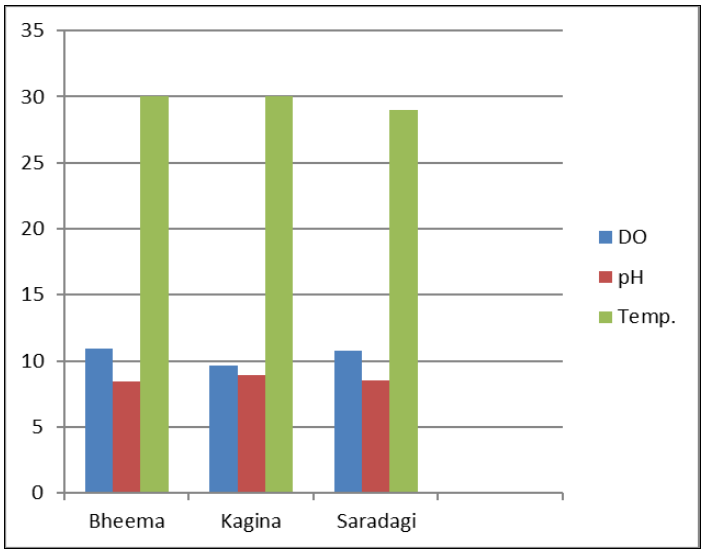

Fig 2: Showing percentage of dissolved oxygen (DO) hydrogen ion concentration (pH) and water temperature in Bheema, Kagina River and Saradgi Nala

Conclusions

In conclusion, based on the results obtained for haematological contents in response to oxygen availability, difference in the hydrogen ion concentration and temperature of the aquatic body indicate that there is influence of these parameters on the blood levels of hematology in the fresh water fish *N. notopterus*. The water body Bheema River provides better environmental conditions compared to other two aquatic bodies Saradgi nala and Kagina River.

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