



VARIABILITY IN THE HAEMOGLOBIN CONCENTRATION IN THE FRESH WATER FISH, NOTOPTERUS NOTOPTERUS COLLECTED FROM THREE AQUATIC BODIES

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ABSTRACT

Fish live in very intimate contact with their environment, and are therefore very susceptible to physical and chemical changes which may be reflected in their blood components. In the present study the haemoglobin, haematocrite and RBC count were measured in the fresh water fish collected from three aquatic bodies. The hematological levels in the fish along with physicochemical parameters from selected different water bodies are compared to understand the influence of physicochemical properties of water on blood haematological (haemoglobin, haematocrite and RBC count) levels. This study provides information on survival, health and physiological condition of the fish. Three types of aquatic bodies were selected are: Bheema river, Kagina river and Saradgi nala (Small stream) near Gulbarga, Karnataka State, The results indicated that the haemoglobin content was higher in the fish from aquatic body Saradgi nala followed by Bheema and Kagina River. 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. The haematocrite percentage was found to be almost same in the fish collected from all the aquatic bodies. The erythrocyte (RBC) count found to be decreased in the fish collected from aquatic body Kagina river, the erythrocyte count in the fish collected from Bheema and Saradgi nala were found to be same. Thus the variation of haematological parameters could be used as biomarker of environmental stress. The comparative studies on physicochemical characteristics of aquatic bodies were made and also compared with blood parameters of the fish, *N.notopterus*. The age related changes in the haemoglobin concentration (Hb) indicated that as the age increases Hb also increases.

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INTRODUCTION

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). 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 and 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). 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).

Before blood constituent data can be applied as a diagnostic tool, however, general patterns related to season, sex, and collection location need to be well documented and understood. The purpose of this study was to determine baseline levels of blood parameters for the fish, *N.notopterus*. Haematological measurements such as RBC, haemoglobin, haematocrite, were determined for fish collected from three different locations around Gulbarga region. Blood measurements were compared with regard to collection location.

MATERIAL AND METHODS

Live specimens of the Indian fresh water fish *N.notopterus* (about 30 fishes) were obtained from the aquatic bodies situated at, Bheema river, Kagina river and Saradgi nala (Small stream) near Gulbarga, 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.

The water sample was collected from different spots of the three aquatic bodies. The dissolved oxygen was determined by using modified Winkler's method APHA, AWWA, WCPF,

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1985) Hydrogen ion concentration (pH): The pH was determined by small hand pH meter dipping in to the water at the site of collection spots directly using LT-70-PH Hand-held digital pH meter. Water temperature was recorded at the site of collection spots using an ordinarymercury thermometer to the nearest of 0.1⁰C (Water teperature at depth of 8 cm).

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 use of plastic syringe is a necessary precaution with fish blood because contact with glass results in decreased coagulation time. The site chosen for puncture (about 3 to 4cm from the genital opening) was wiped dry with tissue paper to avoid contamination with mucus. The needle was inserted at right angle to the vertebral column of the fish and was gently aspirated during penetration. It was then pushed gently down until blood started to enter as the needle punctured a caudal blood vessel. Blood was taken under gentle aspiration until about 2ml has been obtained. Thereafter the needle was withdrawn and the blood gently transferred into heparinized plastic containers. 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. One portion of blood sample was used for the determination of haematological parameters. RBC count were determined using Neubaur's haemocytometer. 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.

Statistical treatment of the data: Difference in haematological parameters was studied by adopting varied statistical methods. Standard deviation and probability test i.e., 't' test were calculated. The student's t' test was carried out to know the levels of significance using the standard formula. All the values of p below 5% level are designated as significant, and the values above 5% level are designated as non-significant. One way ANOVA of the tests were carried out by using statistical software SPSS 7.5

Observations

The present study is an attempt to determine the favorable conditions in the aquatic bodies to find out the difference in the blood haematological levels of the fish collected from different aquatic bodies. The hematological levels in the fish along with physicochemical parameters from selected different water bodies are compared to understand the influence of physicochemical properties of water on blood haematological levels. This study provides information on survival and physiological condition of the fish. Three types of aquatic bodies were selected are: A).River Bheema situated at 40 kms away from Gulbarga city station called Yangunti is continuously flowing water without much submerged vegetation. B).River Kagina situated at 40 kms away from Gulbarga station called Dandoti is continuously flowing water. C).Saradgi Nala – Small stream situated at Saradgi around 15 kms away from Gulbarga.

In all the three aquatic bodies studied, the dissolved (DO) oxygen level is high in aquatic body Saradgi 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. The pH values of aquatic bodies Bheema, Kagina River and Saradgi nala studied are in the following order 8.4, 8.9, 8.5 respectively. In all the three aquatic bodies studied the water temperature of the aquatic body Kagina River to be higher. The readings of the thermometer for the three aquatic bodies are 30⁰, 32⁰, and 30⁰ respectively.

Haematological parameters: Analysis of the peripheral blood of fish serves for diagnostic purposes, to evaluate the condition of the fish, to evaluate the resistance of fish and to assess the suitability of environmental condition. 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). The erythrocytes count (RBC) measures the number of red blood cells presents in the blood. In the present study the erythrocyte count found to be decreased in the fish collected from aquatic body Kagina river, the erythrocyte count in the fish collected from Bheema and Saradgi nala were found to be same, the data recorded are 1.44 ± 0.18 million/mm³ (Bheema river), 1.16 ± 0.03 million/mm³ (Kagina river), and 1.45 ± 0.03 million/mm³ (Saradgi nala) significance found to be $F^{\text{value}} = 5.86$, $P = 0.01$, 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 aquatic body Saradgi nala followed by Bheema and Kagina River. The data are 6.55 ± 0.26 , in (Bheema river), 6.26 ± 0.32 (Kagina river), and 6.76 ± 0.32 (Saradagi nala), significance found for $F^{\text{value}} = 2.18$, $P > 0.05$. The means are not significant. Haematocrit 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 ± 1.12 (Bheema), 20.00 ± 1.41 (Kagina) and 20.16 ± 1.16 (Saradgi) significance found as $F^{\text{value}} = 0.016$, $P > 0.05$ increase in the RBC count reflects as higher in the haematocrite percent in the fish *N.notopterus*

The age related changes in the haemoglobin concentration indicated that as the age increases Hb also increases.

DISCUSSION

Any environmental disturbance can be considered a potential source of stress as it prompts a number of responses in the animal to deal with the physiological changes triggered by exterior changes, these responses can be detected in fish and in other vertebrates in the form of changes in the haematological and biochemical or alteration in the enzyme activity (Donaldson, 1981). Since measurement of haematological parameters reflect the poor or good condition of fish, more quietly then other commonly measured parameters and they respond quickly to changes in the environmental condition (Atkinson and Judd, 1978). Fish are useful bioindicators to evidence environmental degradation (Fausch *et al.*, 1990). Their haematology provides an important tool in the evaluation of its physiological status reflecting the relative health of the aquatic ecosystem. Jimena *et al.*, (2005) Studied on the normal rages of haematological indices in neo tropical fish *Corydoras paleatus* from an unpolluted area and reported that

discriminants analysis showed that haemoglobin is a key parameter to point out differences between population and different environmental conditions and it could be used as biomarker in future works evaluating the incidence of environmental stress on fish as well as pointing out changes in the water quality. Fish blood characteristics change in response to environmental condition, thus the variation of haematological parameters could be used as biomarker of environmental stress (Bridges *et al.*, 1976). The comparative studies on physicochemical characteristics of aquatic bodies were made and also compared with Hb. Htc and RBC count of the fish, *N.notopterus*. Haematological parameters also affected by external factors like seasonal dynamics, water temperature, environmental quality, food, stress etc (Rios *et al.*, 2002).

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 erythropoietin by elevated demands of O₂ or CO₂ transport as a result of increased metabolic activity. The less in erythrocyte count are likely due, in part, to an decrease in oxygen consumption and metabolic rates corresponding to rise in water temperatures (Martinez *et al.*, 1994). Haematocrite provides measurements of red blood cells (erythrocytes) in 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 lower in the haemoglobin and haematocrite level and this may be due to low dissolved oxygen and high temperature. Haematocrite and haemoglobin in striped bass (*Morone saxatilis*) were highest during fall and winter and lowest during summer (Lochmiller *et al.*, 1989). 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. 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.

CONCLUSIONS

The study of haematological and blood biochemical parameters are good indicators of identifying the health of fish. In conclusion, based on the results obtained for haematological contents show that the fish *N.notopterus* collected from different aquatic bodies are healthy and have favorable environmental conditions to thrive well. The water body Bheema River provides better environmental conditions compared to other two followed by Saradgi nala and Kagina river.

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