

Alteration in Hematological and Biochemical Parameters in Fresh water Catfish *Clarias batrachus* as compared to *Cyprinus Carpio*

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Abstract

Upper Lake of Bhopal is large manmade perennial Lake of Madhya Pradesh, which supports a wide variety of Flora and Fauna. The natural and human caused stresses, overexploitation of resources, habitat variation, lack of natural habitat area, formation of dams, turning of rivers for urbanization reduce the biodiversity of Fish. Many physiological variations occur in response to environmental disturbances. Among which *Clarias batrachus* is one of the commonly reared fish in Asian countries is popular for its tasty flesh, rapid growth and high market price has been declared 'Endangered' by IUCN 2017 due to limited availability in the wild. The objective of this study to evaluate the Hematological parameters of threatened catfish *C. batrachus* and compared with standard hematology of found fish species. Hematological parameters are valuable for fish health status and as stress indicators in fishes. In the present study, the hematological parameters such as red blood cell (RBC), white blood cell (WBC), hematocrit (Hct), hemoglobin (HB), mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCVC) and biochemical such as blood glucose, total serum protein and urea were determined. Total 20 samples of experimental fish were collected from the upper lake of Bhopal during the month of October-December 2020 for hematological analysis. Among the hematological parameters, the significance decreases in RBC, Hematocrit, Hemoglobin,

MCV, MCH, MCHC, Total serum protein and Blood Urea was recorded when compared to control group ($P < 0.05$) and significant increase in WBC and Blood glucose was noticed. The results showed that the low values of RBC, Hb, Hct, Red blood corpuscles determination indicated the presence of anemia in the fishes of Upper Lake Bhopal. The variation in environmental and physiological condition may cause stress to the fish which may turn disturbance in the blood parameters affecting the survival of the fish.

Keywords: Hematological and biochemical parameters, *Clarias batrachus*, *Cyprinus carpio*, Upper Lake Bhopal.

INTRODUCTION

Over the years several species of freshwater have become threatened especially due to natural and human caused stresses, overexploitation of resources, habitat variation, lack of natural habitat area, formation of dams, turning of rivers for urbanization which reduced water flows in river, unsupportable fishing, introduction non-native fish species and climate change. Madhya Pradesh bestowed with 180 % fish species which contributes 29 % of fish fauna. Upper Lake of Bhopal is most important Lake of Madhya Pradesh supports a wide variety of flora and fauna. The upper of Bhopal along with its tributaries is considered the lifeline of this capital city of Madhya Pradesh. The Lake basin is projected to undergo compound impounding, with Weir, Channels, and dams on the lakes. These dams and their reservoirs are bringing drastic changes in the lentic ecosystem. In the process of changes the biodiversity of lake is shifting from the predominant lentic community to a lotic assemblage for categorizing the status of fish fauna abundance of yesteryears as compared to present, may serve as yardstick Napit 2013. All over the world diversity and ecosystem both are experiencing major threat for aquatic environment. *Clarias* species (Walking catfish) is a widely distributed fish which constitutes one of the major fisheries in Asia and Africa. Among which *Clarias batrachus* is one of the commonly reared fish in Asian countries and is an edible fish found in ponds, river and stream. *Clarias batrachus* is popular for its tasty flesh, rapid growth and high market price. Magur (*Clarias magur*) has been declared 'Endangered' by IUCN in 2017 due to limited availability in the wild. Argungu *et al.*, (2013) noted that the walking catfish is threatened and is becoming critically endangered in its native range in Asia. The periods of drought and human disruption of the walking catfish natural habitat has contributed to its decline in Malaysia and India. Railo *et al.*, (1985) reported that the blood parameters of diagnostic importance are erythrocyte and leukocytes count, hemoglobin, hematocrit, leukocyte differential counts would readily respond to incidental factor such as physical stress and environmental stress due to water contaminants. Hematological

parameters of fish are closely related to the responses of fish to environmental and biological factors can seasonally affects on blood parameter changes in fish such as the reproduction cycle, diet, temperature, pH and photo period. The measurement of blood parameter is commonly used as diagnostic tool in bio monitoring by which acute and chronic patho physiological changes attributable to nutrition, water quality and disease are detected (Adams *et al.* 1996). Hematological and biochemical indices provide considerable information about fish oxygen transport capacity, immune potential, disease, stress, nutritional status, intoxication etc. stress may fluctuate the values of red blood parameters (Ht, Hb, RBC, MCV) and various biochemical parameters (glucose, Total serum protein, Blood urea). The aim of the present paper was to study different Hematological and Bio-chemical Parameters of Threatened Fish species *C.batrachus* and to compare the Hematological parameters with Standard Hematology of found fish species.



Image.1 Collection of Fish Sample from Upper Lake of Bhopal.

MATERIAL AND METHODS

Collection of samples

Total 20 Live specimens of the fishes were collected from the Upper Lake of Bhopal **Image.1** with the help of fisherman in the month of in the month of October-December 2020. The fishes were brought to laboratory in a big bucket and transported in 1000 Liter of aerated container. Fishes were acclimatized for the week time feeding with earth worm, boiled eggs and small guppy fishes to avoid possible effect of

starvation on any of the hematological parameters before they used for experiment. Fishes were measured and weighted for total length (TL) by using centimeter scale to the nearest 1.0cm level and total weight with the help of digital balance to the nearest 1.0 gram. Fish were ranging a length of 10-17.5 cm and weighting 15-80 gram were selected.

Size of control group depends upon the size of total population and in study we should take around size of control group is 10-20 %. Thus, 3 fishes were used as control group (*Cyprinus carpio*) Fishes were in length (16-20cm) and weight of (25-90g) used as ready stock for experimental work.

Blood Sampling

The blood was collected from the caudal peduncle **Image.2** with the aid of 2 ml disposable plastic syringe and 21 gauge disposable hypodermic needle. Blood samples were collected in heparinized tubes (Wintrobe 1934, Yokoyama, 1947). Heparin was used as 2 drops as per 5 milliliter of blood (Hessr, 1960). The sample was then mixed gently and thoroughly.



Image.2 Collection of Blood Sample from caudal region of *C. batrachus*

Hematological and Biochemical Analysis-

Total count of RBC and WBC

Total count of RBC, Total red blood cells and Total White blood cells were counted using an improved Neubaur's hemocytometer (Shah and Altindag 2004; Shah and Altindag 2005).

Estimation of Hemoglobin and Hematocrit

Hemoglobin was determined with Hemoglobin test kit (DIAGNOVA, Ranbaxy, India) using the cyanmethemoglobin method. Hematocrit was determined by the methods of Blaxhall and Daisley (1973).

Blood Glucose

Blood glucose was estimated by Nelson-Somogyi method as described by Hawks physiological chemistry (Oser1965).

Total Serum Protein

Protein was determined by Folin-Ciocalteu method as modified by Zak and Cohen (1961).Bovine serum albumin was used as a reference standard.

Blood Urea

Blood urea was measured by diacetyl monoxide method. Urea present in serum reacts directly with diacetyl monoxide in the presence of Fe^{3+} ions and thiosemicarbazide at $100^{\circ}C$ to form red colored salt complex in acidic medium which is measured at blue green wave length(520 to 540nm).

Corpuscular values

The MCV, MCH and MCHC were calculated by standard formula.

$$\mathbf{MCV (fl)} = \frac{\mathbf{Hematocrit}}{\mathbf{Red Blood Cell}} \times 10$$

$$\mathbf{MCH (pg)} = \frac{\mathbf{Hemoglobin(g/dl)}}{\mathbf{Red Blood Cell}} \times 10$$

$$\mathbf{MCHC (g/dl)} = \frac{\mathbf{Hemoglobin}}{\mathbf{Hematocrit}} \times 100$$

DATA ANALYSIS

All data obtained were subjected to one-way analysis of variance (ANOVA) using GraphPad Prism software. Significant differences were determined at 95 % level of probability ($\alpha =0.05$) where ANOVA indicated the presence of a significant difference. Values of all measured variables are articulated as means \pm SED.

Table 1: Hematological and biochemical analysis of *Clarias batrachus* when compared to *Cyprinus carpio*.

Parameters	<i>C.carpio</i>	<i>C.batrachus</i>
Total RBC ($10^6/\mu\text{l}$)	1.44 \pm 0.22	0.49 \pm 0.05
Total WBC ($10^3/\mu\text{l}$)	14.04 \pm 1.49	20.04 \pm 0.26
Hemoglobin(g/dl)	16 \pm 0.98	5.07 \pm 0.30
Hematocrit (%)	30.17 \pm 0.51	9.38 \pm 0.53
MCV (fl)	220.55 \pm 35.67	233.18 \pm 57.90
MCH (pg)	114.29 \pm 10.28	146.62 \pm 25.59
MCHC (g/dl)	97 \pm 0.64	57.57 \pm 4.60
Blood Urea (mg/dL)	2.51 \pm 0.21	10.02 \pm 0.35
Blood Glucose(mg/dL)	84.88 \pm 3.44	110.19 \pm 4.50
Total Serum Protein (g/dL)	10.33 \pm 0.41	2.28 \pm 0.12

Each value represent mean \pm SED; n=20

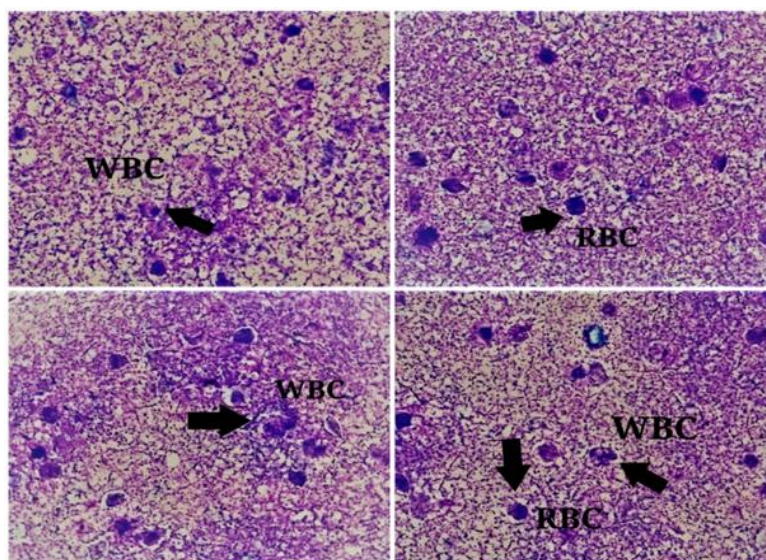


Image 3: Blood smear of *C. batrachus* showing anemia.

RESULT AND DISCUSSION

Table 1 shows the mean of each blood parameter together with the standard error of each mean. specific variations between the species were detected in all hematological

and biochemical values. fishes differ significantly in their activity patterns in response to various pollutants, the blood parameters like red blood Corpuscles (RBC), White blood corpuscles (WBC), Hemoglobin (Hb), Packed cell volume (PCV), Mean corpuscular volume (MCV), Mean corpuscular Hemoglobin (MCH), Mean corpuscular Hemoglobin concentration (MCHC) and biochemical parameters Total serum protein, Blood Glucose, Blood urea are commonly studied in fishes to estimate the impact of pesticides in aquatic life.

The erythrocyte count (RBC) measures number of red blood cells present in blood. The erythrocyte count of healthy controls group showed a mean value of 1.44 ± 0.22 million/ mm^3 and Threatened fish *C. batrachus* showed a mean value of 0.49 ± 0.05 million/ mm^3 . According to Fazio *et al.*, (2019) RBC determined automatically in different fish species was from 0.81 to $3.73 \times 10^6/\mu\text{L}$. in healthy catfish, the standard number of erythrocytes is 3.18×10^6 cells/ mm^3 (Alamanda *et al.*, 2007)). Based on the results of erythrocytes calculation in catfish samples in Upper Lake of Bhopal, it showed that they were below the normal range of erythrocytes levels in catfish. The variation may be because of differences in species or due to environmental factors such as habitat reduction, inflow of sewage due to agricultural activities. Hematological studies done by Kim *et al.*, (1976) reported that the mercury accumulates in the fish blood. The values mentioned above showed a significant decrease when compared to the control ($P < 0.05$). Decline in RBC values and anemia were reported in fishes such as *Salvalinus fontinalis* (Holcombe *et al.*, 1976). The low erythrocytes will cause fish to be unable to take in large amount of oxygen although the availability of oxygen in the waters is sufficient. As a result, fish will experience anoxia (lack of oxygen) (Ikun *et al.*, 2013). Fish suffering from with anemia can exhibit decreased values of all red blood parameters (Witeska, 2015).

White blood cells play an important role in the defense mechanism of the fish and consist of granulocytes, monocytes, lymphocytes and thrombocytes. The result of the total count of White blood cells showed that the blood of control fish showed a mean value of $14.04 \pm 1.49 \text{ mm}^3 \times 10^3$. The White blood cell of Threatened fish species *C. batrachus* showed a mean value of $20.04 \pm 0.26 \text{ mm}^3 \times 10^3$. The values mentioned above showed a significant increase when compared to control group ($P < 0.05$). Based on our result obtained from Upper Lake, indicating that the catfish are in an unhealthy state or in stressful condition. In comparison to control group blood of experimental group *C. batrachus* showed higher concentration of leukocytes. Fish that experience stress caused by changes in environment conditions and because of foreign bodies show a response to an increase in the number of leukocytes cells (Hastuti and Subandiyono, 2014). According to Svobodovan *et al.*, (2012) WBC in *C. carpio* was $10-80 \times 10^3/\mu\text{L}$. Fazio *et al.*, (2019) reported that WBC determined automatically in

different fish species ranged from 9.41 to $829.33 \times 10^3/\mu\text{L}$. The increased production of white blood cells counts in African catfish shows the body resistance response to foreign substances that cause disease

The Hemoglobin test is a measure of how much hemoglobin protein is present in blood and it found to be in the control fish showed mean value of 16 ± 0.98 g/dL and threatened fish *C. batrachus* showed the mean value of hemoglobin 5.07 ± 0.30 . According to Fazio *et al.*, (2019) Hb determined automatically in different fish species was from 4.70 to 16.6 g/dL. Chandra lekha and Dutta (2012) observed hematological changes in *Heteropneustes fossilis* viz. total erythrocytes count and Hemoglobin content. The Threatened fish species showed a significant decrease when compared to control group ($P < 0.05$). Panigrahi and Mishra (1978) observed reductions in Hemoglobin percentage and red blood cell count of the fishes *Anabas scandens* treated with mercury. The decrease in Hemoglobin and MCH indicates In hypochromia, this type of anemia found in chronic infection or chronic disease, it is called normochromic microcytic anemia (Gautam and Gupta 1989). Witeska *et al.*, (2016) reported that Hb in *C. carpio* is in the range 34.1 to 114.3 g/dL. Normal catfish (*Clarias sp*) hemoglobin concentration ranges from 10-14 g/dl. (Oluah *et al.*, 2020).

The Based on our result it can be noted that the hematocrit value of threatened catfish from the Upper Lake is low. If the fish is affected by disease, the blood hematocrit content will be abnormal, and if hematocrit is low, the erythrocyte count is also low. Hematocrit of control group in the present work was within the range of $30.17 \pm 0.51\%$ and for threatened fish species *C. batrachus* showed a mean value of 9.38 ± 0.53 . The Hematocrit value of *C. batrachus* showed a significant decrease when compared to control group ($P < 0.05$). The lower values of hematocrit in the fishes are probably due to anemia or hemodilution (Wedemeyer *et al.*, 1976). Lipika patnaik *et al.*, (2006) observed significant reduction in Erythrocytes, Hemoglobin and Hematocrit, in fish *C. batrachus* when exposed to pesticide Carbaryl for 96 hrs. Hematocrit appears to be positively correlated with RBC count, so a decrease in Hematocrit is observed (Fazio *et al.*, 2013). Blaxhall and Daisley (1973) have reported the possibility of using hematocrit as a tool in the aquaculture and fishery management for checking the anemic condition. In the case of *C. carpio* the range obtained by different authors was 14.0-44.0 % (Witeska *et al.*, 2016). Hematocrit value of healthy catfish is 30.8-45.5 % (Alamanda *et al.*, 2007).

MCV, MCH and MCHC refer to calculated secondary red cell indices. The investigation of these parameters may be useful in diagnostics of some fish diseases, e.g., anemia. The increase of MCV response to anemic state in some fish species. Increased values of MCV and MCH were observed in fish suffering from ichthyophthiriasis (Witeska *et al.*, 2010a). a Low MCHC values indicates hypochromia

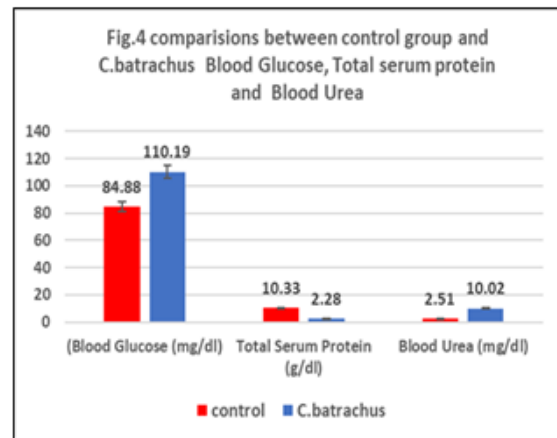
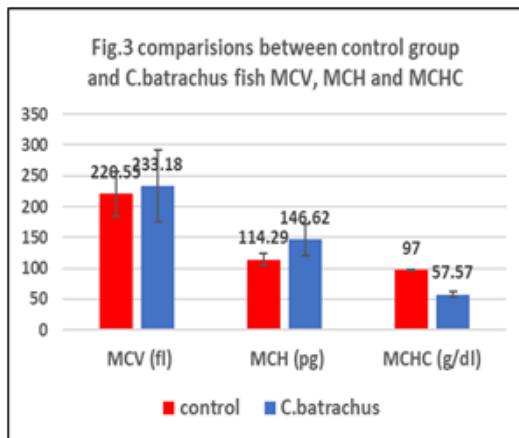
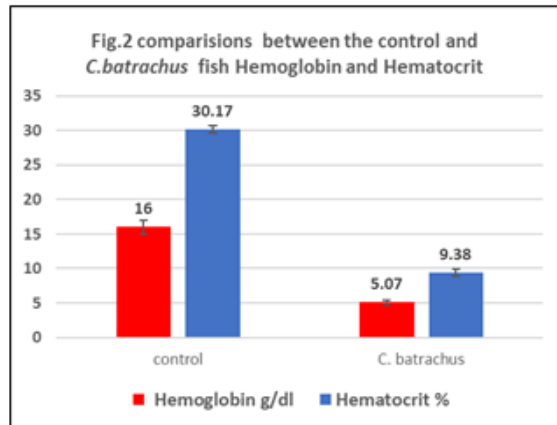
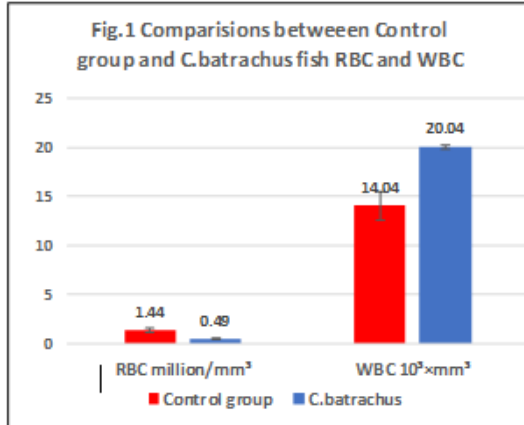
and have less color than normal is a sign of anemia due to iron deficiency. A low MCH means that blood cells contain less hemoglobin than normal. Chronic exposure to cypermethrin, a synthetic pyrethroid insecticide, may cause enlargement of erythrocytes and abnormal erythrocyte morphology yielding a macrocytic anemia. (Saxena and Seth 2002). Witeska *et al.*, (2016) indicate that MCV for *C. carpio* was in the range of 130.9-367.3 fL, MCH 31-8-139.0 pg and MCHC 150-446 g/L.

Blood Glucose were significantly high in this study. The distinct content of blood sugar fluctuates among species. Blood Glucose level of *C. batrachus* 110.19 ± 4.50 mg/dl are higher than that of control group 84.88 ± 3.44 mg/dl. According to (Patriche 2009) normal blood glucose level of fish is 40-90 mg/dl. The increased level of glucose is a manifestation for the higher needs of tissues to fuel the metabolic needs of osmoregulation and an important source of energy for maintaining homeostasis in fish during chronic stress (Pickering *et al.*, 1982). Increased in level blood glucose in fish specifies that where were fish inhibit, were contaminated by pollutants. The increase in blood Glucose is mainly instigated by cortisol-mediated gluconeogenesis that also inhibits cellular uptake of circulating glucose consequently increasing the levels of blood circulation. Nemosok and Bores (1982) reported that blood glucose appeared to be a sensitive indicator of environmental stress in fish.

Urea levels were significantly raised in this study. A high blood urea concentration was recorded in *C. batrachus* 10.02 ± 0.35 mg/dl when compared to control group 2.51 ± 0.21 mg/dl. The Urea level observed in *C. batrachus* was about two times higher than those reported by Agbede *et al.*, (1999) and Oyelese *et al.*, (1999) for adult catfish species. A high blood urea concentration recorded in *M. cephalus* is likely to be a sign of stress associated with the increase in the cholesterol level (Borges *et al.*, 2007). Urea level greater than 7.1 mg/dL or lower than 1.8 mg/dl is abnormal. Abnormal urea level reveals congestive heart failure, gastrointestinal bleeding and kidney disease. The elevated level of blood urea nitrogen (BUN) is probably not indicative of renal disease in the fish (Mensinger *et al.*, 2005) and it is more likely associated with gill or liver disease.

Serum proteins play an important role in transport of different substances, defense of the organism against pathological agents, osmotic regulation, and some other functions (Rudneva *et al.*, 2011). The present study reports, control group was having the highest Total Serum protein content 10.33 ± 0.41 g/dl when compared to *C. batrachus* 2.28 ± 0.12 g/dl correspondingly. The decrease in the concentration of total serum protein in many disease statuses is due to decrease capacity of synthesis and reduces absorption or protein loss through hemodilution (Patriche *et al.*, 2011; Stosik *et al.*, 2001). Decrease in serum protein level may be attributed to stress to fulfill an increase element for energy by the fish to cope with environmental condition

exposed to toxicant (Jenkins and Smith 2003). Neff (1985) has opined that decline in protein content may be related to impaired food intake, increased energy cost of homeostasis, tissue repair and detoxification mechanism during stress.



CONCLUSION

According to the result of our research provide a contribution to the consciousness of the characteristic of Hematological and biochemical parameters of Threatened catfish *C. batrachus*. Hematological and biochemical parameters recognized as important tool for monitoring fish health and also help in monitoring any variation in the quality of water. The present investigation indicating that the fish was suffering from anemia which suggest that the pesticides may be weaken the immune system and may result in severe physiological problems, eventually leading to the death of the fish. The significant threatened effect of environmental risk factors on health profile of fish *Clarias batrachus* reveals that the varieties of pesticides, chemicals that are used in crop cultivation may affect hematology of aquatic animals. The effect of heavy metals is likely to be used as bio indicator to monitor the quality of water in Upper Lake

Bhopal and is very harmful when combined with other pollutants. All the hematological parameter studied under this investigation proved the effect of pesticides and decline of natural habitat altering blood environment of *Clarias batrachus*. Based on our result, it shows that human consumption has a physiological effect on the fish and hematological parameters are useful tools for detecting this.

ETHICS APPROVAL

Not applicable. An approval of the ethics committee is not required.

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