

Haematological and blood biochemical changes in the fresh water fish, *Notopterus notopterus* (Pallas) exposed to acidic medium

Raghavendra Kulkarni, Vinayak Barad

Department of Studies in Zoology
Gulbarga University, Gulbarga-585106, India

E-mail address: rskgug@gmail.com
Author for correspondence: rskgug@gmail.com

Keywords: Haematology, *Notopterus notopterus*.

Abstract. The present study was carried out to find out the changes induced by one of the stress conditions such as acidic medium exposure on some haematological and blood biochemical, parameters in the fresh water fish, *Notopterus notopterus*. Increase in the haemoglobin and haematocrite indicate haemoconcentration in the fish blood. Glucose, alkaline phosphatase, sodium and calcium, protein, BUN, creatinine, triglycerides, enzymes such as SGOT and SGPT were decreased in the fish *N. notopterus* exposed to acidic condition. The changes in the environmental factors such as acidic condition cause stress to the fish which may bring disturbance in the blood parameters effecting the survival of fish.

1. INTRODUCTION

Activities such as construction, clearing of vegetation, dumping of solid wastes, industrial and municipal effluents acidify the water body. These chemicals and particles in presence of rainwater and water vapour, readily form acids (and other corrosive chemical compounds), which build up in the atmosphere and are eventually washed out as acid rain, altering the pH of the recipient medium. The effluent arising from the industrial activities is discharged into surrounding water bodies thus contributing significantly to the alteration of the pH of the aqueous medium (Spiff and Horsefall, 1998). Physical (movement of body, fins, and opercular bones) and physiological (hematological parameters) attributes of fishes have been used as indicator of fish responses to its externalities (Casillas and Smith, 1977). It is consequently crucial to use these attributes in monitoring fish responses to increasingly acidic pH levels. (McWilliams and Potts, 1978), it was demonstrated that short term exposure of brown trout, *Salmo trutta*, to acid media produced significant changes in sodium fluxes and gill potentials. In acid media, positive changes in gill potentials could largely account for the increased loss of sodium from fish observed in these conditions.

In the present investigation effects of acidic condition on haematological and blood biochemical changes was observed in the locally available fresh water fish, *Notopterus notopterus*.

2. MATERIALS AND METHODS

Fresh water fish *Notopterus notopterus* (70-80 g body weight) were brought from Bheema River around 40 km away from Gulbarga. Fish were acclimatized for laboratory conditions for 7 days before the beginning of the experiment. Stable temperature of 27 ± 3 is an optimum temperature during the period of study. The pH was detected twice during experiment before and after the completion of experiment by hand pH-meter. Fish were fed with earth worm and boiled egg pieces once daily from the day of arrival until the end of the experiment. Daily change of water was around 10% that in the aquaria.

Experimental studies:

The fish; *N. notopterus* (twenty-20 number) were exposed to acidic medium at a pH-06 for three (3) days and the above exposure is considered as stress to the fish. The control group was maintained

simultaneously and was kept under optimal environmental conditions. The response of haematological and blood biochemical parameters were studied after the termination of exposure to acidic condition and also in the control group.

The experimental data was analyzed statistically by adopting varied statistical methods. The student 't' test was carried out to know the levels of significance using the standard formula. The experimental data was analyzed statistically by adopting varied statistical methods using statistical software S.P.S.S 7.5

Blood parameters:

Blood samples collected from caudal blood vessels, blood was separated in two portions, one portion was mixed with anticoagulant another portion of sample was centrifuged without anticoagulant for serum separation. Hemoglobin was measured using the standard cyanmethemoglobin method described by Baker and Silvertown (1976). Haematocrite 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. **Total Serum proteins (TP)** was measured by using the modified Biuret method, end point assay as described by Lawrence, (1986), **serum glucose** determined by (GOD-POD) Glucose oxidase – peroxidase, end point and assay method, **Blood urea nitrogen (BUN)** was determined by modified Berthelot method, **cholesterol** was determined by (CHOD-PAP) cholesterol oxidase - phenol aminophenazone method, **HDL** was determined by (CHOD-PAP) cholesterol oxidase - phenol aminophenazone method, **LDL** was determined by Friedewald's equation

$$\text{LDL} = \frac{\text{Total cholesterol} - \text{Triglycerides}}{5} - \text{HDL cholesterol}$$

Triglycerides (TG) were determined by (GPO-PAP) glycerol-3-phosphate oxidase - phenol aminophenazone end point assay method. **Creatinine** was determined by modified Jaffe's method Kinetic test without deproteinisation according to the Jaffe's method. **Serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT)** activity was assayed following modified International Federation for Clinical Chemistry (IFCC) method using commercial kit. Serum Alkaline phosphatase activity was determined by kinetic assay (IFCC) method using commercial kit. **Sodium and Potassium:** are determined by colorimetric method. **Calcium** was determined by Modified Arsenazo method.

Table 1: Showing haematological , blood biochemical , enzymes and electrolyte Concentration of the fish *N. notopterus* exposed to acidic medium..

Sl.No.	Ex- group/ parameters	Control	Acidic medium
1	Haemoglobin (Hb) g/dl	8.70 ± 0.74	10.52 ± 0.91 **
2	Haematocrite (Hct) %	21.5 ± 2.21	27.5 ± 1.87 **
3	Glucose	48.23 ± 8.87	79.66 ± 6.22 ***
4	Protein	5.82 ± 0.79	4.17 ± 0.39 **
5	BUN	6.97 ± 0.59	2.20 ± 0.57 ***
6	Creatinine	2.44 ± 0.37	0.69 ± 0.08 ***
7	Cholesterol	253.5 ± 29.21	291.98 ± 74.82 *

8	Triglycerides	302.96 ± 65.09	56.30 ± 15.21***
9	HDL	67.32 ± 18.45	38.13 ± 2.39 ^{NS}
10	LDL	41.54 ± 12.38	242.25 ± 74.45***
11	SGOT	15.65 ± 0.69	2.02 ± 0.50***
12	SGPT	16.94 ± 0.26	2.14 ± 0.53***
13	ALP	59.55 ± 6.64	71.29 ± 8.18**
14	Sodium (Na)	74.13 ± 13.07	82.69 ± 7.21 ^{NS}
15	Potassium (K)	14.96 ± 1.59	14.58 ± 1.91 ^{NS}
16	Calcium (Ca)	9.01 ± 0.68	9.40 ± 0.30 ^{NS}

Each value is expressed as mean ± SD, N = 6 ,NS = Not significant, * = significant P = < 0.05, ** = significant P = < 0.01, *** = significant = P < 0.001

3. OBSERVATIONS

The results of the experiment on exposure to acidic pH at 06 on the hematology and blood biochemistry of the fish, *N.notopterus* is presented in the table-1. No mortality was observed throughout the experiment; in spite of acidic condition. Higher hemoglobin values were recorded in the fish exposed to acidic condition compared to the control fishes. A significant difference (t = 3.79 p = < 0.01) found for hemoglobin in the fish exposed to acidic medium. Hematocrite values were also found higher in the fish exposed to acidic medium.

The values of biochemical parameters obtained in the fish exposed to acidic medium and control fish, *N.notopterus* is presented in the Table – 1. Glucose values were found higher in exposed to acidic medium fishes whereas serum protein was found lower compared to the control group. Blood urea nitrogen (BUN) values were found lower in exposed to acidic medium fishes compared to the control fishes. Serum creatinine values were found lower in exposed to acidic medium fishes. Total cholesterol values were found higher in exposed to acidic medium fishes. Triglyceride values were found lower in exposed to acidic medium fishes. High density lipoprotein values were found similar in both exposed to acidic medium fishes and in the control group. Low density lipoprotein values were found higher in exposed to acidic medium fishes. The values of blood enzymes obtained in exposed to acidic condition fish and control fish *N.notopterus* are given in the Table – 1. The enzymes, SGOT and SGPT values were found lower in exposed to acidic medium fishes. The enzyme ALP values were found higher in fish exposed to acidic medium fishes.

The values of blood electrolytes obtained in acidic medium exposed and control fish, *N.notopterus* are given in the Table-1. The blood electrolyte sodium values were found higher. The blood potassium values were found to be similar whereas calcium values were found higher.

4. DISCUSSION

The response of fish to short term exposure to acidic medium was studied through assessing different hematological and blood biochemical parameters. Changes in the pH and redox-potential of the aquatic environment are of great concern to all stock holders, following the declining catch of fish species which had often times been attributed to altered water quality especially changes in pH (Spiff and Horsefall, 1998). Some studies have implicated nutrient enrichment, increased heavy metals, and presence of pesticides to the reduced pH of the aquatic medium (FAO, 1997; Sadler and

Lynam, 1987). Physical (movement of body, fins, opercular bones) and physiological (hematological parameters) activities have been used as indicator of fish responses to its externalities (Casillas and Smith, 1977). It is consequently crucial to use these parameters in the monitoring of fish response to increasingly acidic pH levels. The pH of the water is one of the most important water quality characteristics influencing the health of fishes. Fishes residing for a prolonged period in aquatic habitat with acidic pH either below 6.5 or alkaline above 9.0 is subjected to stress (Acharya *et al.*, 2005). Water acidification has become one of the most important environmental factors affecting fish (Kossakowski Korwin, 1988). Dheer *et al.*, (1987) reported mortality, loss of weight, increase in blood glucose and liver glycogen levels at low (acidic) pH in *Channa punctatus*.

Increase in the hemoglobin and hematocrite levels observed in the present study indicate haemoconcentration in the blood of fish *N. notopterus*. Concentration of plasma volume may have increased the hemoglobin concentration. Increase in the haematocrite might be due to cellular swelling (Sovio and Nikinmaa, 1981). Decrease in the pH in the aquatic environment cause acidosis which causes decrease in oxygen carrying ability of hemoglobin (Root effect) and its affinity to oxygen (Bohr Effect). Thus impairment of oxygen uptake or delivery appears to be a key toxic mechanism of lower pH (Spry *et al.*, 1981). With these disturbances, elevation in hematocrite level may include increase in erythropoiesis in response to tissue hypoxia or transfusion of cells from spleen (Milligan and Wood, 1982).

Blood glucose concentration has been widely employed to assess the extent of stress in fish (Wendelaar Bonga, 1997). Donaldson, (1981) suggests that this parameter may be particularly useful for determining stress levels in fish exposed to two or more sub lethal stressors. On the basis of the results presented here, the use of glucose as indicator of sub lethal stress under acidic condition is valid. The increase in glucose values was previously recorded by Kroglund & Finstad, (2003) for Atlantic salmon, *Salmo salar*, exposed to moderately acidic water (pH 5.8). Similar result was also obtained by Royset *et al.*, (2005) for brown trout, *Salmo trutta*, exposed to aluminum in acid fresh waters. Likewise, consistent results have been reported by Poleo and Hytterød, (2003) under alkaline conditions (pH 9.5). The depletion of liver glycogen (glycogenolysis) and the rise in blood glucose levels were reported in *T. zillii* as a consequence of water pollution (Abdelmeguid, *et al.*, 2002). Several studies on fish intermediary metabolism under different stress conditions show the importance of hormonal control in the mobilization of energy stores (Dugan and Moon, 1998). According to Vijayan *et al.*, (1997) cortisol contributes directly and/or indirectly to glucose production in stressed tilapia (*Oreochromis mossambicus*) following 24 h, probably due to gluconeogenesis from substrates, including lactate and amino acids. This may account for the high glucose levels and hyperglycemia of the fish, *N. notopterus* exposed to acidic pH. However, further studies on hormones, such as catecholamines, insulin, and cortisol, are necessary to confirm this hypothesis.

Blood serum protein is a fairly labile biochemical system, precisely reflecting the condition of the organism and the changes happening to it under influence of internal and external factors (Shalaby, Khatlab and Abdel-Rahman, 2006). Thus, the influence of toxicants on the total protein concentration of fish has been taken into consideration in evaluating the response to stressors and consequently the increasing demand for energy.

The blood analysis of the fish revealed that BUN and creatinine were significantly decreased in acidic medium exposed fishes. This decline in creatinine and BUN might be induced by glomerular insufficiency, decreased muscle tissue catabolism or the impairment of carbohydrate metabolism (Murray *et al.*, 1990). The cholesterol contents were increased significantly than the control. Triglycerides and cholesterol are known to participate in the rise of total lipid. The rise of these energy reserves in response to pollution could be due to the fact that excess energy reserves (as glucose, triglycerides and cholesterol) are required by organisms to mediate the effects of stress (Lee *et al.*, 1983). Since homeostasis of lipids is one of the principal liver functions, any change in serum triglyceride concentration is used as an indicator of liver dysfunction (Kaplan *et al.*, 1988.). The triglyceride levels were decreased in the present study.

A significant decrease in the activities of serum SGOT and SGPT in the acidic medium exposed as compared with control fish SGOT and SGPT belong to the plasma non-functional enzymes which are normally localized within the cells of liver, heart, gills, kidneys, muscle and other organs. Their presence in blood plasma may give information on tissue injury or organ dysfunction (Wells *et al.*, 1986). In the present study on exposure to acidic medium to the fish, *N. notopterus* the decrease in SGOT, SGPT, and increase ALP has been observed and this may be because of the degeneration and destruction of the tissues. Therefore, the decrease or increases of these enzymes in plasma are indicative of liver damage and thus alterations in liver function.

The blood electrolytes such as sodium (Na^+), potassium (K^+), calcium (Ca^{++}), magnesium (Mg^{++}), phosphorus (P^+) are commonly used to determine the physiological characteristics, toxicity and health status of fish (Percin *et al.*, 2010). The monovalent ions namely sodium (Na^+), potassium (K^+), and chloride (Cl^-) play an important role in osmoregulation and homeostasis. The calcium (Ca^{++}) serves as number of functions in fish, it combines with phosphorus (P^+) for deposition to the bone. It is possible thus bones serve as a reservoir of calcium for plasma and tissues. Additionally Ca^{++} appears to be important in the reproduction and mitochondrial functions. It is generally recognized that Ca^{++} has an important role in osmoregulation (Wurst and Stickney, 1989). The impact of acidification (Low pH 6.0) on serum electrolytes (Na^+ , K^+ , and Ca^{2+}) in a freshwater fish *N. notopterus* was studied for a 3 day (short term) exposure period, while control groups were maintained at neutral pH (7.3). During exposure periods, plasma Na^+ levels were increased and on the other hand, plasma, K^+ and Ca^{2+} levels were remain unchanged in the fresh water fish *N. notopterus*.

The impact of acidification (Low pH 5.0) on plasma electrolytes (Na^+ , K^+ , Cl^- , Ca^{++} , and Mg^{2+}) in a freshwater fish *Cyprinus carpio* was studied for a 35 day (long term) exposure period by Ramesh *et al.*, (2009) and reported that the loss of plasma Na^+ , Cl^- , and Ca^{++} indicates the displacement of Ca^{++} from tight junctions. The increased plasma K^+ ion might have resulted from acidosis, because intracellular K^+ is released from muscle and suggested that the elevated level of plasma Mg^{2+} might be due to inhibition of active transport of magnesium across the kidneys resulting in the accumulation of this ion in the plasma. Ionic alteration takes place upon exposure to acidic pH (long term) since the period of exposure and pH levels can be considered as a potential tool for detecting environmental stresses caused by acidification.

ACKNOWLEDGEMENT

The author Raghavendra S. Kulkarni is grateful to University Grants Commission, New Delhi for selecting Emeritus fellowship (2014-15 batch/ starting from feb.2015) No.F-6-6/2014-15/EMERITUS-2014-15-GEN-3514 (SA-II) DT.30-04-2015

REFERENCES

- [1] Abdelmeguid, N., Kheirallah, A. M., Abou-Shaban, Adham, K and Abdel-Moneim, A. (2002). Histolochemical and biochemical changes in liver of *Tilapia zilli*, as a consequence of water pollution. *J. Biol. Sci.*, 2: 224-229.
- [2] Acharya, K., Jack, J.D. and Bukaveckas, P.A. (2005). Dietary effects on life history traits of riverine *Bosmina*. *Freshwater Biol.*, 50: 965–975.
- [3] Baker, F. J. and Silverton, R.E. (1976). The theory of staining. Introduction to Medical Laboratory Technology 6th ed. Butterworths, pp. 385 –391.
- [4] Castellini, M.A. and Rea, L.D. (1992). The biochemistry of natural fasting at its limits. *Experientia.*, 48: 575-582.
- [5] Dheer, J.M.S., Dheer, T.R. and Mahajan, C.L. (1987). Haematological and haematopoietic response to acid stress in an air-breathing freshwater fish, *Channa punctatus* Bloch. *J. Fish Biol.*, 30 (5): 577-588.

-
- [6] Donaldson, E.M. (1981). The Pituitary Interregal Axis as an Indicator of Stress in Fish In: Pickering, A.D. (Ed.) Stress in fish. Academic Press, London, pp: 11-47.
 - [7] Dugan, S.G. and Moon, T.W. (1998). Cortisol does not affect hepatic α - and β -adrenoceptor properties in rainbow trout (*Oncorhynchus mykiss*). *Fish Physiol. Biochem.*, 18: 343–352.
 - [8] FAO, (1998). Aquaculture production statistics 1987-1996. Fish Cir. 815 FAO: Rome. P. 96.
 - [9] Kaplan, A., Ozabo, L.L., and Ophem, K.E. (1988). Clinical Chemistry: Interpretation and Techniques. 3rd edn. Lea & Febiger, Philadelphia.
 - [10] Kossakowski Korwin, M. (1988). Larval development of carp, *Cyprinus carpio* in acidic water. *J. Fish. Biol.*, 32: 17-26.
 - [11] Kroglund, F., and Finstad, B. (2003). Low concentrations of inorganic monomeric aluminum impair physiological status and marine survival of Atlantic salmon. *Aquaculture*, 222: 119-133.
 - [12] Lawrence, M. S. (1986). Amino acids and Proteins. In: *Textbook of Clinical Chemistry*. Tietz, N W (editor) W B Saunders Company, US p. 519-618.
 - [13] Lee, R.M., Gerking, S.D. and Jezierska, B. (1983). Electrolyte balance and energy mobilization in acidstressed rainbow trout (*Salmo gairdneri*) and their relation to reproductive success. *Environment and Biology of Fishes*, 8: 115-123.
 - [14] McWilliams, P. G. and Potts, W. T. W. (1978). The effects of pH and calcium concentrations on gill potentials in the brown trout, *Salmo trutta*. *J. comp. Physiol.*, 12: 277—286
 - [15] Milligan, C.L. and Wood, C.M. (1982). Disturbances in hematology, fluid volume distribution and circulatory function associated with low environmental pH in the rainbow trout (*Salmo gairdneri*). *J. Exp. Biol.*, 99: 397-415.
 - [16] Murray, R.K., Granne, D.K., Mayes, P.A. and Rodwell, V.W. (1990). Harper's Biochemistry. 23rd ed., Appleton and Large publishers, Norwalk, Connecticut/ Los Altos, California.
 - [17] Percin, F., Sibel, K., Kursat, F and Sahin, S. (2010). Serum electrolytes of wild and captive Bluefin Tuna (*Thunnus thynnus* L.) in Turkish Seas **J. Anim. Vet. Adv.** 9(16): 2207-2213.
 - [18] Poleo, A. and Hytterod, S. (2003).The effect of aluminium in Atlantic salmon with special emphasis on alkaline water. *Journal of Inorganic Biochemistry*, 97: 89–96.
 - [19] Ramesh, M., Srinivasan, R. and Saravanan, M. (2009). Effect of atrazine (herbicide) on blood parameters of common carp *Cyprinus carpio* (Actinopterygii: Cypriniformes). *Afr. J. Environ. Sci. Technol.*, 3(12): 453-458.
 - [20] Royset, O., Rosseland, B.O., Kristensen, T., Kroglund, F., Garmo, O.A. and Steinnes, E. (2005). Diffusive gradients in thin films sampler predicts stress in brown trout *Salmo trutta* L. exposed to aluminum in acid fresh waters. *Environ Sci Technol.*, 15(4), 1167-1174.
 - [21] Shalaby, A.M., Khattab, Y.A. and Abdel-Rahman, A.M. (2006). Effects of garlic *Allium sativum* and chloramphenicol on growth performance, physiological parameters and survival of Nile tilapia *Oreochromis niloticus*. *J. Venom. Anim. Toxins incl. Trop. Dis.*, 12(2), 172-201.
 - [22] Soivio, A. and Nikinmaa, M. (1981). The swelling of erythrocytes in relation to the oxygen affinity of the blood of the rainbow trout *Salmo gairdneri* Richardson. In: Stress and fish. (ed. A.D. Pickering), pp. 103-119. Academic Press, London.
 - [23] Spiff, A. I. and M. N. Horsefall. (1998). Principles of environmental chemistry. Metroprints Ltd. Port Harcourt. 82 p.

-
- [24] Spry, D. J., Wood C. M. and. Hodson. P. V (1981). The effects of environmental acid on freshwater fish with particular reference to the soft water lakes in Ontario and the modifying effects of heavy metals. A literature review. *Can. Tech. Rep. Fish. Aquat. Sci.*, 999: 144 pp.
- [25] Vijayan, M.M., Pereira, C., Grau, E.G., and Iwama, G.K. (1997). Metabolic responses to confinement stress in tilapia: the role of cortisol. *Comp. Biochem. Physiol.*, C, 116, 89-95.
- [26] Wells, R. M G., R. H. Mc Intyre, A. K. Morgan, and P. S. Davie. (1986). Physiological stress responses in big gamefish after capture: observations on plasma chemistry and blood factors. *Comp. Biochem. Physiol.*, 84:565–571.
- [27] Wurst, W.A. and R.R. Stickney, (1989). Responses of red drum (*Sciaenopso cellatus*) to calcium and magnesium concentrations in fresh and salt water. *Aquaculture*, 76: 21-35.