

**EFFECT OF ENVIRONMENTAL STRESSES ON FISH
HAEMATOLOGY WITH SPECIAL REFERENCE TO A FRESH
WATER CATFISH *HETEROPNEUSTES FOSSILIS* (BLOCH.)**

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ABSTRACT

*Many environmental and physiological factors are known to influence fish haematology. These include stress due to capturing, transportation, sampling, age and sex. Piscine haematology is useful for assessing the health and general condition of the animal subjected to changing environmental conditions. Therefore, haematological studies have been widely used as means of assessing the state health of fishes. The present study was carried out on Catfish (*Heteropneustes fossilis*), this fish has a very wide range distribution. Exposure of fish to sub-lethal concentration of contaminants may impose physiological stress, resulting in a number of manifestations. In the present investigation, several haematological parameters of *Heteropneustes fossilis* altered as a result of exposure to various stresses. Parameters like TLC, MCV and MCH increased whereas TEC and MCHC altered under stress condition. Keywords: *Heteropneustes fossilis*, Hematology, environmental stress, Fish health.*

Abbreviations

TEC, Total Erythrocyte Count

TLC, Total Leukocyte Count

Hb, Hemoglobin

PCV, Packed Cell Volume

MCH, Main Corpuscles Hemoglobin

MCHC, Main Corpuscles Hemoglobin Concentration

MCV, Main Corpuscles Volume

ESR, Erythrocyte Sedimentation Rate

DLC, Differential Leukocyte Count

INTRODUCTION

Heteropneustes fossilis commonly known as singhi, contributing to about 15 % of inland landings and has potentials of mono and polyculture. It is much utilized for food and is known for medicinal properties in many parts of its range. The fish is formerly belongs to threatened

category but now it falls under vulnerable category. This change is mainly due to the reason of over exploitation, habitat loss and degradation (especially from pollution and dams).

The aquatic environment, where fish and other organisms live, is subjected to different types of pollutants which enter water bodies through industrial, domestic and agricultural discharge systems thereby introducing stress to living creatures. Stress is a general and non-specific response to any factors disturbing homeostasis. Stress reactions involve various physiological changes including alteration in blood composition and immune mechanisms (Witeska, 2004). Additionally, Stress caused by the aquaculture environment obstructs normal growth and immune response of fish and makes them susceptible to diseases. Stress disturbs the fine internal balance, homeostasis and has further detrimental effects on behavior, growth, reproduction, immune function and disease tolerance (Goos and Consten, 2002; Chen *et. al.*, 2004; Morales *et. al.*, 2005). Fish have developed physiological and biochemical adaptations to cope with these constraints that minimize or eliminate the deleterious effects, which is called stress response.

Environmental changes which include natural as well as anthropogenic changes have serious impact on fish population, physiology, its haematology and eventually on overall fisheries. In addition to producing extensive stress to the fishes these natural changes (temperature, starvation, pH, oxygen concentration, parasitic infestation etc.) and anthropogenic (industrial and domestic effluents) which changes water bodies constantly upset the homeostasis of fishes. For mirroring the health status of fishes haematological parameters such as TEC, TLC, PCV, Hb and red cell indices (MCV, MCH, MCHC) along with blood corpuscles morphology distinguish quickly than any other reacting parameters and react magnificently in the circumstances of stress or any uneven changes in the environment.

Changing environmental factors have potential to change the blood constituents of an organism. Because of alterations in environmental factors blood act as indicator while evaluating the toxic potential of the contaminants. An organism has ability to keep all blood parameters relatively stable under normal conditions but in stress condition they have aptitude to alter these values. Any dysfunctions of blood can have severe effects on the physiological activities of an organism. Over and above certain physiological dysfunctions in the body are reflected as alterations in blood constituents, which can be used as diagnostic indicators. Additionally, blood forms a valuable tool for the investigation of physiological change in animals caused by pollutants. Studies have shown that when the water quality is affected by

contaminants, any physiological changes will be reflected in the values of one or more of the haematological parameters (Van Vuren, 1986).

MATERIALS AND METHODS

Fresh water fishes were purchased from the Pheet Bazar of BHEL Haridwar, brought alive in plastic bags and transferred to the aquarium and maintained under laboratory condition for the purpose. The fish were rested for acclimation of at least for 72 hrs. To make various estimations from blood samples fishes were handled, anaesthetized, blood drawn, and processed for making studies on different selected parameters including slide preparations following the methods describe by Tandon & Joshi (1976). Stained blood smears (mainly by Giemsa stain) were examined under a microscope using a 100x oil immersion objective. Images were taken with the help of digital camera. For this paper only differential blood cell counts of erythrocytic series have been made along with a comparative blood picture for healthy & diseased fishes. The first 100 red and white corpuscles seen on each slide were counted and listed as either erythroblast or erythrocytes.

RESULT AND DISCUSSION

The results of the present study corroborates that the values of TEC, TLC, Hb and PCV are closely related with breeding activity and gonad maturation. Mishra et al. (2018) found that juveniles of *H. fossilis* have higher values of TEC, Hb, MCH and MCHC; which can be attributed to high metabolic activities. In the present study, majority of the peripheral haematological parameters (TEC, TLC, TTC, Hb, PCV, MCH and MCHC) tended to have higher values during the warm, pre monsoon period and lower values during the rainy and winter period. This is in conformity with the finding of previous workers who reported remarkably higher values in premonsoon period and lower values during the rainy season (Joshi and Tandon, 1977; Siddiqui and Naseem, 1979; Joshi, 1989). The temperature also affects the hematological values of the fishes in the present study the values of DEC and DLC were varies with the change in the temperature. In the present examination, leukocyte proportions were affected by temperature. A stressed leukogram of fish reveals decrease in small lymphocytes and increase in percentages of neutrophils. McLeay (1975a) institute that the percentage of small lymphocytes was positively correlated with temperature, in contrast with our findings. In fishes, an elevation of neutrophil may be witnessed on occurrences of chronic stress (Adams, 2002). The pollutants exposure decreases the values TEC, Hb and PCV along with the red cell indices (MCH, MCV and MCHC) also decreases in *H.fossilis* at higher concentrations and showed incidence of anemia within toxic condition. Rani, R (2002)

study on the toxic effect of linear alkyl benzene sulphonate (LAS) in *Heteropneustes fossilis* also corroborates with the present findings. The starvation affects TEC and Hb. Reduction in above parameters showed those longer days of starvation were end results in severe anemia in *H. fossilis*. In agreement with increase in days of starvation the values of TEC and Hb declined respectively. Liver damage May be the cause of decreased values of TEC due to the instability of erythrocyte membrane. As a consequence the demand of erythrocytes increased in the circulation (Abdel-Hamid *et. al.*, 2006). Diseases and infections caused extensive destruction to the fish culture. These infections normally cause alterations in the peripheral blood picture of fishes. Decrease of RBCs, haemoglobin as well as PCV values have been found in the present study. Identical changes in haematology were established in the results of Wobeser, G (1973) during an outbreak of redmouth disease in rainbow trout (*Salmo gairdneri*). Almost similar results were found during the present investigation. It should be revealed by a decline in the erythrocyte indices, MCH, MCHC and MCV, because younger and immature cells are smaller and enclosed with less haemoglobin.

The fish hematological parameters such as RBC, WBC, Hb and PCV values etc., are known to be influenced by many aspects, include environmental factors (Pandey, 1977), seasonal conditions different period of reproductive cycle and chemical stress (Joshi and Tondon, 1976), Studies have been made on the effect of various toxicants on freshwater fishes in relation to the hematological changes (Wells *et. al.*, 2005). Since, hematological alternations appear before the symptomologic and histopathologic changes, the study of blood components in the fishes are important from the diagnostic point of view. The hematological effects have been reported in fishes due to various toxicants by several investigators (Pandey *et. al.*, 1977). The fish hematology under pesticidal and other chemical stress has also been studied (Hicky, 1976; Mahajan *et. al.*, 1979; Srivastava and Roy, 1978). The fishery biologists are increasingly using the hematological parameters of fish as base for the indicators of disease or stress in fish and its pathological state.

Hematopoietic tissue which is located mainly in the spleen and kidney is responsible for production of blood in Teleost fishes (Fange, 1992). The cells of erythrocytic and leucocytic series arise from the haemocytoblast. Fish erythrocytes are ellipsoidal nucleated cells of different sizes. The size of erythrocytes depends on fish species, nutritional status, activity, seasons, habitat conditions like water temperature and dissolved oxygen as well as other physiological and environmental factors. There is presence of immature cells in the circulation of fish's blood because the barrier connecting haemopoietic tissue and blood

circulation is not very strong. Erythrocytes are most abundant types of cells in the blood. Joshi (1987) has described a variety of erythrocytic cells in circulation namely erythroblast, normocytes, microcyte, macrocyte and crenated erythroblast besides anisocytes and poikilocytes.. Erythrocytes show structural as well as behavioral changes quite conspicuously, therefore can be trusted to monitor fish health and their environment too. (Joshi *et. al.*, 2015 and Mishra *et.al.*, 2018).

For health parameters of fish, hematology functions as most important diagnosing tool (De Pedro *et. al.*, 2005; Martins *et. al.*, 2008), and alteration in percentages of blood cells possibly pinpointing a infection or an exposure to chemicals (De Pedro *et. al.*, 2005). Due to changing environmental conditions and increase in the pollution level it is necessary to analyze hematological parameters of animals (Ranzani-Paiva and Godinho, 1985), infections (Martins *et. al.*, 2008), stress and parasitism (Fazio, F, 2019) and seasonality (De Pedro *et. al.*, 2005). The number of leukocyte varies according to the environmental changes (Lea Master *et. al.*, 1990), nutritional state (Barros *et. al.*, 2002), the existence of infectious diseases (Martins *et. al.*, 2008) and parasitism (Fazio, F, 2019). The seasonal changes have a key role in fish physiology and immunity.

Changing environmental condition and increase in the level of pollution have a greater impact on health and well being of animals. With increasing pollution load animals tolerate environmental stress and further it moderates overall performance of organism including fishes. These changes are also responsible for alterations in the haematology of fishes (Hickey 1982), malnutrition (Casillas and Smith 1977), sex (Siddique and Naseem 1979; Orun, I, 2003), fish size (Martinez, F. J., 1994), seasonal variations and reproduction efficiency (Cech *et. al.*, 1996). The values of blood parameters rise at certain age after that it depends on the biological requirement of the fishes. Environmental temperature has a major role in seasonal variation as aquatic vertebrates like fishes (Joshi *et. al.*, 1980; Orun *et. al.*, 2003).

CONCLUSION

Blood parameters are considered good physiological indicators of the whole body conditions and therefore can be used in diagnosing the structural and functional status of fish exposed to toxicants (Adhikari *et. al.*, 2004, Seriani *et. al.*, 2009). In addition, haematological studies are procedures frequently and routinely applied in the diagnosis of diseases in aquaculture (Ranzani-Paiva *et. al.*, 2000). Fish blood parameters have been increasingly employed in environmental monitoring programs to indicate physiological changes due to toxicants (Seriani *et. al.*, 2009; Zutshi *et. al.*, 2009). However, the knowledge on the fish hematology

still needs to be expanded, to provide data for different species (Ranzani-Paiva *et. al.*, 2000; Zutshi *et. al.*, 2009, Seriani *et. al.*, 2009, Vijayakumari and murali, 2012).

It may be concluded that all the haematological parameters influenced with any kind of internal as well as external factors such as reproduction, temperature, water qualities like pH, hardness, DO in addition to various infections, or pathogens. This study might be useful approach to monitor health status of fishes in general and *H. fossilis* in particular in a view of fact that haematological parameters definitely serve as early detection of stress in the water bodies or any pathological condition of the fishes. Fish haematological parameters used as key to distinguish between the populations and provide reliable information on existing and changing environmental states on the aquatic water bodies for better management practices.

REFERENCES

- Adhikari, S., Sarkar, B., Chatterjee, A., Mahapatra, C. T., & Ayyappan, S. (2004). Effects of cypermethrin and carbofuran on certain hematological parameters and prediction of their recovery in a freshwater teleost, *Labeo rohita* (Hamilton). *Ecotoxicology and Environmental safety*, 58(2), 220-226.
- Adams, S. M., Greeley, M. S., Law, J. M., Noga, E. J., & Zelikoff, J. T. (2003). Application of multiple sublethal stress indicators to assess the health of fish in Pamlico Sound following extensive flooding. *Estuaries*, 26(5), 1365.
- Abdel-Tawwab, M., Khattab, Y. A., Ahmad, M. H., & Shalaby, A. M. (2006). Compensatory growth, feed utilization, whole-body composition, and hematological changes in starved juvenile Nile Tilapia, *Oreochromis niloticus* (L.). *Journal of Applied Aquaculture*, 18(3), 17-36.
- Barros, M. M., Lim, C., & Klesius, P. H. (2002). Effect of soybean meal replacement by cottonseed meal and iron supplementation on growth, immune response and resistance of Channel Catfish (*Ictalurus punctatus*) to *Edwardsiella ictaluri* challenge. *Aquaculture*, 207(3-4), 263-279.
- Casillas, E., & Smith, L. S. (1977). Effect of stress on blood coagulation and haematology in rainbow trout (*Salmo gairdneri*). *Journal of Fish Biology*, 10(5), 481-491.
- Chen, R., Lochmann, R., Goodwin, A., Praveen, K., Dabrowski, K., & Lee, K. J. (2004). Effects of dietary vitamins C and E on alternative complement activity, hematology, tissue composition, vitamin concentrations and response to heat stress in juvenile golden shiner (*Notemigonus crysoleucas*). *Aquaculture*, 242(1-4), 553-569.

- Cech Jr, J. J., Bartholow, S. D., Young, P. S., & Hopkins, T. E. (1996). Striped bass exercise and handling stress in freshwater: physiological responses to recovery environment. *Transactions of the American Fisheries Society*, 125(2), 308-320.
- De Pedro, N., Guijarro, A. I., López-Patiño, M. A., Martínez-Álvarez, R., & Delgado, M. J. (2005). Daily and seasonal variations in haematological and blood biochemical parameters in the tench, *Tinca tinca* Linnaeus, 1758. *Aquaculture research*, 36(12), 1185-1196.
- Fazio, F. (2019). Fish hematology analysis as an important tool of aquaculture: a review. *Aquaculture*, 500, 237-242.
- Fange R. (1992). Fish Blood Cells. In: Hoar WS, Randall DJ, Farrell AP, editors. Fish Physiology, Vol12B:1-54. San Diego, CA: Academic Press Inc.
- Goos, H. T., & Consten, D. (2002). Stress adaptation, cortisol and pubertal development in the male common carp, *Cyprinus carpio*. *Molecular and Cellular Endocrinology*, 197(1-2), 105-116.
- Hickey Jr, C. R. (1982). Comparative hematology of wild and captive cunners. *Transactions of the American Fisheries Society*, 111(2), 242-249.
- Hicky, C. (1976). Fish haematology its use and significance. N.V. Fish. Care .J. 23(2) : 170-175.
- Joshi, B.D. & Tandon R.S. (1976) Seasonal variations in hematologic values of fresh water fishes. 1 . Herteropneutus fossilis and *Mystus vittatus*. *Comp. Physio. Ecol.* Vol. 2, No. 1: 22-26.
- Joshi, B. D. (1987). Cyto-morphological classification and key to the identification of normal circulating blood corpuscles of freshwater teleosts. *Himalayan. Journal of Zoology*.1:98-113.
- Joshi, B.D., Joshi, N , Mishra, I, “Differncial Blood Cell Counts of the Fish Herteropneustes fossilis (Bloch) infected with Trypanosomes” *J. Env. Bio-Sci*, 2014: Vol. 28 (1): 77-80.
- LeaMaster, B. R., Brock, J. A., Fujioka, R. S., & Nakamura, R. M. (1990). Hematologic and blood chemistry values for *Sarotherodon melanotheron* and a red hybrid tilapia in freshwater and seawater. *Comparative Biochemistry and Physiology Part A: Physiology*, 97(4), 525-529.

- Mishra, I, Joshi, B. D. and Joshi, N. (2018) "Sex related variation in some hematological values of a fresh water catfish *Heteropneustes fossilis* (Bloch.) " *J. Env. Bio-Sci*, Vol. 32 (2): 331-336.
- Morales, A. E., Cardenete, G., Abellán, E., & García-Rejón, L. (2005). Stress-related physiological responses to handling in common dentex (*Dentex dentex* Linnaeus, 1758). *Aquaculture Research*, 36(1), 33-40.
- Mahajan, C. L., & Dheer, J. S. (1979). Seasonal variations in the blood constituents of an air-breathing fish, *Channa punctatus* Bloch. *Journal of Fish Biology*, 14(4), 413-417.
- Martins, M. L., Mouriño, J. L. P., Amaral, G. V., Vieira, F. N., Dotta, G., Jatobá, A. M. B., ... & Buglione-Neto, C. C. (2008). Haematological changes in Nile tilapia experimentally infected with *Enterococcus* sp. *Brazilian Journal of Biology*, 68(3), 657-661.
- Martinez, F. J., Garcia-Riera, M. P., Ganteras, M., De Costa, J., & Zamora, S. (1994). Blood parameters in rainbow trout (*Oncorhynchus mykiss*): simultaneous influence of various factors. *Comparative Biochemistry and Physiology Part A: Physiology*, 107(1), 95-100.
- McLeay, D. J., & Gordon, M. R. (1977). Leucocrit: a simple hematological technique for measuring acute stress in salmonid fish, including stressful concentrations of pulpmill effluent. *Journal of the Fisheries Board of Canada*, 34(11), 2156-2163.
- Muramoto, S., Aoyama, I., Hashimoto, K., & Kungolos, A. (1996). Distribution and fate of surface active agents in river and lake water, affected by domestic and agricultural wastewater, in an area in Japan. *Journal of Environmental Science & Health Part A*, 31(4), 721-729.
- Orun, I., Dorucu, M., & Yazlak, H. (2003). Haematological parameters of three cyprinid fish species from Karakaya Dam Lake, Turkey. *Online J. Biol. Sci*, 3, 320-328.
- Pandey, K. C., & Pandey, A. K. (1977, June). Hematology of a cat fish *Rita rita* (Ham). In *Proceedings of the Indian Academy of Sciences-Section B* (Vol. 85, No. 6, pp. 369-377). Springer India.
- Pandey, K.C. and A.K. Pandey, (1977). Haematology of a catfish *Rita rita* (Ham.) *Proc Ind. Acad. Sci.*, 85b:369-377.
- Ranzani-Paiva, M. J. T., Silva-Souza, A. T., Pavanelli, G. C., & Takemoto, R. M. (2000). Hematological characteristics and relative condition factor (Kn) associated with parasitism in *Schizodon borellii* (Osteichthyes, Anostomidae) and *Prochilodus lineatus*

- (Osteichthyes, Prochilodontidae) from Paraná river, Porto Rico region, Paraná, Brazil. *Acta Scientiarum*, 22(2), 515-521.
- Rani, R., Trivedi, S. P., Singh, P., & Singh, R. K. (2002). Haematotoxic effect of linear alkyl benzene sulphonate (LAS) in fish, *Heteropneustes fossilis*. *Journal of environmental biology*, 23(1), 101-103.
- Seriani, R., Ranzani-Paiva, M. J. T., Silva-Souza, A. T. and Napoleao, S. R. (2011). Hematology, micronuclei and nuclear abnormalities in fishes from SaoFrancisco, Minas Gerais state, Brazil. *Acta Scientiarum Biological Sciences*, Maringa. 33(1): 107-112.
- Srivastava, A. K., Roy, D. (2015). Effects of malachite green (Triarylmethane dye) and Pyceze (Bronopol) on the hematological parameters of a freshwater catfish *Heteropneustes fossilis* (Bloch). *International Journal of Fisheries and Aquatic Studies*. 2(6): 119-122.
- Siddiqui, A. Q., & Naseem, S. M. (1979). The haematology of Rohu, *Labeo rohita*. *Journal of fish Biology*, 14(1), 67-72.
- Tondon, R. S. and Joshi, B. D. (1976) Total red and white blood cells of 33 species of fresh water Teleosts., *Z. Tierphysio., Tierenahrg. U . Futtermittelkde.* 37: 293-297.
- Vijayakumari, K. N. and Murali, D. (2012) Peripheral haematology of *Puntius filamentous* (Valenciennes) in relation to sex, maturity, standard length and season. *Indian. J. Fish.* 59(3): 125-130.
- Van Vuren, J.H.J. (1986). The effects of toxicants on the haematology of *Labeo umbratus* (Teleostei; cyprinidae). *Comp. Biochem. Physiol.*, 83C: 155-159.
- Witeska, Ì.,(2004). The effect of toxic chemicals on blood cell morphology in fish. *Fresenius Environmental Bulletin*, 13 (12A): 1379-1384.
- Wells, R. M., Baldwin, J., Seymour, R. S., Christian, K., & Brittain, T. (2005). Red blood cell function and haematology in two tropical freshwater fishes from Australia. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, 141(1), 87-93.
- Wobeser, G. (1973). An outbreak of redmouth disease in rainbow trout (*Salmo gairdneri*) in Saskatchewan. *Journal of the Fisheries Board of Canada*, 30(4), 571-575.
- Zutshi, B., Raghu Prasad. S. G. and Nagaraja, R. (2010). Alteration in hematology of *Labeo rohita* under stress of pollution from lakes of Banglore, Karnataka, India. *Environ. Monit. Assess.* 168: 11-19.