Hematological Characterization of *Triplophysa marmorata* Heckel

DISSERTATION

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MASTER OF PHILOSOPHY
In
ZOOLOGY

By

Maryum Meraj

Under the Joint Supervision

Prof. (Dr.) A.R Yousuf
Supervisor
Professor Centre of Research for Development
P.G. Department of Environmental Science
University of Kashmir, Sgr.

Dr. Farooz Ahmad Bhat
Co-Supervisor
Asst. Prof. (SS), Faculty of Fisheries,
SKUAST-K.

Centre of Research for Development
Faculty of Biological Science
The University of Kashmir
Srinagar - 190 006, Kashmir
(NAAC Accredited Grade 'A' University)
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Centre of Research for Development
University of Kashmir
Srinagar – 190 006, Kashmir

No:
Date:

Certificate

This is to certify that the dissertation entitled **"Hematological characterization of *Triplophysa marmorata* Heckel"** submitted to the University of Kashmir for the award of the **Degree Masters of Philosophy in Zoology**, is the original research work of **Ms. Maryum Meraj,** a bonafide M. Phil. Research Scholar of the Centre, carried out under my supervision. The dissertation has not been submitted to this University or to some other University so far and is submitted for the first time. It is further certified that this dissertation is fit for submission for the degree of Masters of Philosophy (M. Phil.) in Zoology and the candidate has fulfilled all the statutory requirements for the completion of the M.Phil. Programme.

Prof. (Dr.) A.R Yousuf
Supervisor
Professor Centre of Research for Development
P.G. Department of Environmental Science
University of Kashmir, Sgr.

Dr. Farooz Ahmad Bhat
Co-Supervisor
Asst. Prof. (SS), Faculty of Fisheries,
SKUAST-K.

Prof. (Dr.) A.N.Kamili
Director Centre of Research for Development
Head P.G. Department of Environmental Science
University of Kashmir, Sgr.

DEDICATED

TO

MY

BELOVED

PARENTS

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Maryum Meraj

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Abbreviations

Abbreviation	Full Form
Hb	Hemoglobin concentration
PCV	Packed cell volume
RBC	Red Blood Cell
WBC	White Blood Cell
TLC	Total Leucocyte Count
TRBC	Total Red Blood Cell
ESR	Erythrocyte Sedimentation Rate
MCH	Mean Cell Hemoglobin
MCV	Mean Cell Volume
MCHC	Mean Cell Hemoglobin concentration
Fig.	Figure
SD	Standard Deviation
%	Percentage

Chapter -1

Introduction

The valley of Kashmir lies between 33° 20' and 34° 54' N latitudes and 73° 55' and 75° 35' E longitudes, surrounded by the lofty mountains of the Pir-Panjal Range in the south-west and the Greater Himalayan Range in the North East making it an elliptical bowl shaped. The highest mountain peaks enclosing the valley have an elevation of more than 5300 m.a.s.l on the great Himalayan side and more than 5500 m.a.s.l on the Pir Panjal side. The Valley has a temperate climate and is known for its seasonality. It enjoys a unique position in harboring rich and diverse types of aquatic habitats, occupying 6% of its total land area (Zutshi and Gopal, 2000). These water bodies are of immense importance for providing potable water and also ensuring stability of the microclimate of the area, ground water recharge, and generating employment by boosting tourism, fisheries and recreation.

The lentic waters include Wular lake, Dal lake, Manasbal lake, Nilnang and many other lakes, while the lotic habitats include numerous streams like Sind, Lidder, Veshau, Dudhganga, etc., all being directly or indirectly connected with the River Jhelum (also called Vitasta / Vyeth), the lone natural drainage system of the valley. These aquatic habitats harbour a number of native fishes like *Schizothorax* spp., *Glyptothorax* spp., *Triplophysa* spp., etc., as well as the exotic fishes like *Onchorhynchus mykiss*, *Salmo trutta fario, Cyprinus carpio, Carassius carassius*, etc., each adapted to a particular habitat.

The importance of the water bodies as well as the fish fauna inhabiting them can hardly be overemphasized. However, during the past few decades there has been disproportionate urbanization that has resulted in severe imbalance in biotic and abiotic environment. The anthropogenic pressure on water bodies of the valley has increased tremendously and this has in turn affected the various fish populations especially *Triplophysa* and *Schizothorax*, some species of which are endemic to the valley (Kullander *et al.*, 1999). Aquatic pollution is one of the main reasons which has synergistically worked with several other man-induced factors and led to the decreasing trend in the population of many species, influencing significant changes in the fish species composition in aquatic habitats of Kashmir.

Since changes in various environmental parameters result in a new steady state of physiological reactions, patterns of these reactions are expected to provide some indication that the organism is faced with. Fishes can be used as a measure of environmental health, as they are in direct contact with their environment and are as such susceptible to any change that may occur in it. It is expected that such changes would be reflected in the physiology of the fish and particularly in the values of hematological parameters (Blaxhall,1972).Blood is therefore recognized as a potential index of fish response to water quality (Hickey, 1982), as it can be used to ascertain the effect of pollutants in the environment. Blood parameters have been commonly used to observe and follow fish health, since variations in blood tissue of fish are caused by environmental stress (Shah and Altindag, 2005; Bhaskar and Rao, 1985).Blood parameters in fish have been studied to elucidate physiological adaptation and to assess the health of fishes (Vazquez and

Guerrero, 2007).Bouck and Ball (1966) stated that hematology may be a useful tool in monitoring stress levels of aquatic pollution on fish. Hematological parameters are increasingly used as indicators of the physiological stress response to endogenous and exogenous changes in fish (Adams, 1990; Santos and Pacheco, 1996; Cataldi *et al.*, 1998).

A variety of studies carried out during the past few years suggest that hematological parameters may, under specific circumstances, provide the fisheries biologists with useful indices of dietary sufficiency, pathological status and physiological response to environmental stress (Dewilde and Houston, 1967).Like in humans, hematological studies have also been used as a means for diagnosis of fish disease.

Kashmir loach (Genus *Triplophysa* of sub family Nemachilinae and Family Balitoridae), locally known as ‘Ara gurun’, is a small fish having elongated and scale-less body, with eyes high on head, and inferior mouth having two rostral, and one maxillary pair of barbells.

The Kashmir loaches normally live among pebble and shingle at the bottom of clear rocky streams but some drift into lakes among the hills and become secondarily modified for life in deeper waters (Hora, op. *cit.).*The comparatively bigger size of the loaches of the high altitudes may be due to the plentiful aquatic insect life and other food organisms. The *Triplophysa* species of Kashmir occurs in the colder tributaries and are usually found in running water, in small stream flowing from the Kashmir waterworks reservoir to the trout farm at Harwan (in Srinagar),Veerinag and Kokernag springs. These are also found in Sind River, Wular Lake and Jhelum but the size is smaller in Jhelum compared to other water bodies.

The genus is represented by two species, viz., *Triplophysa marmorata* and *T.kashmirensis* in the valley (Kullander *et al.,* 1999). Out of the two, *T.marmorata* (Heckel) is more widely distributed in the region.

It has an elongated scale-less body (maximum length, 120mm), with eyes high on head, mouth inferior. Two rostral and one maxillary pair of barbells are present. The sides and head are marked with brownish or grayish blotches of different sizes also scattered over the dorsum.

So far the researchers have paid little attention towards this ecologically as well as economically important fish of the valley and the few reports available are restricted to its morphology. Since most of the water bodies where from the species has been reported are experiencing cultural eutrophication, it was thought worthwhile to have a study on the hematology of the fish from three different types of habitat, viz., a lentic habitat (Wular Lake) and a slow flowing lotic habitat (Telbal Stream near its mouth) and a fast flowing lotic habitat (Sind stream).The data collected during the study (March to December, 2012)is presented in the form of the present dissertation. In the following pages first the important ecological parameters of the water bodies, from where the fish were collected are described. After describing the ecological set up of the water bodies the hematological features of the fish in the different habitats are given in detail. This is followed by a discussion on the observations made on various blood parameters in the light of the available literature. At the end there the concluding remarks on the hematological peculiarities of the fish in response to changed habitats are given.

Chapter -2

Study Area and Study Sites

The valley of Kashmir, situated in the midst of the Himalaya, is characterized by a vast array of fresh water bodies, represented by breathtaking lakes, mountain tarns, snow and spring-fed streams and rivers, numerous wetlands famous for migratory birds and a number of springs. The Lakes of Kashmir exhibit various degrees of trophic evolution and grade from oligotrophic (Alipathar, Tarsar, Marsar, Kishansar, Vishansar, etc.) through mesotrophic (Nilnag, Manasbal) to eutrophic waters (Dal, Anchar,Wular, Khushalsar, etc). The lotic habitats of the region include the River Jhelum and the streams (Bringhi, Vishau, Romshu, Sind, Madhmati, Erin, etc.) that join it in the valley. Among the wetlands Hokarsar, Mirgund, Malgam, etc., are famous for many resident and migratory birds, the first one being a notified Ramsar site. Among springs Verinag, Achhabal, Beehama, Kokarnag, etc. are famous for the native snow trout. All these aquatic habitats are inhabited by a number of fish species, including *Triplophysa marmorata*. In order to have an insight in to the biology and physiology of this fish in different freshwater habitats of the Kashmir Himalaya it was decided to study the general hematology of the fish in three typical habitats, viz., fast flowing lotic (Sind stream), slow flowing lotic (Telbal stream close to its mouth) and lentic (Wular Lake) habitats, in which it occurs in the valley.

2.1) WULAR LAKE

The Wular Lake is the largest freshwater lake of the Kashmir valley and has been designated as a Ramsar site in 1992. It is situated in northwest of Srinagar at distance of 35km. At the turn of the 19^{th} century the area of lake was reported to be 217.8 km^2, which has got reduced to 86.71 km^2 by the start of 21^{st} Century (Anonymous 2007). The shrinkage in lake area was mainly due to continuous siltation brought about by various tributaries (Erin, Madhumati, Ashtung) besides river Jhelum. The water body is very shallow, with the maximum depth of 5.8m. The lake is surrounded by high mountainous ranges on the northeastern and northwestern sides, which drain their runoff through various *nallas*, prominent being Erin and Madhumati. Geographically the lake is located at an elevation of 1580m (a.m.s.l) between the coordinates of 34° 15' - 34° 25' N Latitude and 74° 33' - 74° 44' E longitude. The Lake is an important habitat for the fish fauna of the region. The dominant fish species found in the Wular are: *Nemacheilus* sp., *Cyprinus carpio, Barbus conchonius, Gambusia affinis, Crossocheilus diplochilus, Schizothorax curvifrons, S. esocinus, and S. niger*. Two species of *Triplophysa*, viz., *Triplophysa marmorata* and *T. kashmiriensis* have also been reported from this water body (Kullander *et al*, 1999). Fish for the present study was collected from the Ningli area of the lake (Plate 1-2).

A VIEW OF WULAR LAKE NEAR STUDY SITE IN NINGLI AREA

Plate 1

Plate 2

2.2) SIND STREAM

Sind stream is a snow fed stream, originating from Zojila Mountains. It gets augmented with waters coming down from the mountains on left and right sides, flowing towards the west. It is joined by water from Amarnath, Kolahai and Panjtarni snow fields. On its way from Zojila mountain it receives several streams like Nilgrar, Wangat Nalla and Brahamsar. After flowing through Ganderbal it spreads out near Harran and branches into a number of channels over an extensive deltaic region. One of its branches escapes into the Anchar Lake, while the others merge with Jhelum at Shadipora (Raza *et al*., 1978) and then gradually becomes sluggish and is navigable. Sind is a fast flowing torrential river in its upper and middle reaches, while in the lower reaches its velocity reduces. Fish specimens from this stream were collected near Haripora (Plate 3- 4) in close proximity to Wayil Bridge. The proposed site seems to be a congenial habitat for the *T.marmorata* as most of the samples were collected with a good degree of ease.

A VIEW OF SIND STREAM NEAR STUDY SITE HARIPORA AREA

Plate 3

Plate 4

2.3) TELBAL STREAM

The Telbal stream (also called Dagwan nalla) is the main tributary of Dal lake and harbours a number of fish species belonging to genera *Schizothorax*, *Triplophysa* and *Salmo*. The stream has its origin in the high altitude Marsar Lake (12500ft) and snow melt slopes of Harwan, Barzawas and Mahadeo mountains. The stream traverses through the Dachigam National Park and enters the Dal Lake on its north side at the place called Hanzheul. About 2km away from its mouth it receives one of its major tributaries, Dara stream at Wangund. Fish for the present study were collected from the Dagi-Mohalla area of the Telbal stream (Plate 5).

Plate 5: A view of Telbal stream near study site Dagi-mohalla

Chapter -3

Review of Literature

Fish blood is a patho- physiological indicator of the whole body function and therefore blood parameters are important in diagnosing the structural and functional status of fish hence are recognized as a potential index of fish response to the changes (if any) occurring in their environment. For quite some time the impact of water quality on the fish and other aquatic organisms has been the subject of research throughout the world and numerous publications are available on the effects of water pollution on the hematology as well as physiology of freshwater fishes. Since the volume of literature pertaining to fish hematology and its role as an indicator of the health of fish environment vis-à-vis water pollution is quite extensive and a listing of all the studies is beyond the scope of the dissertation, an attempt has been made to give a review of important works in the field in the following pages. It may be pointed out that most of the studies during early and mid-twentieth century were focused on the characterization of the fish hematology and the use of fish blood as an indicator of the conditions of the environment surrounding the fish started only in the late twentieth century.

Hall and Gray (1929) made a study on concentration of hemoglobin of the blood of marine fishes. The study revealed that the more active species appear to have the highest hemoglobin concentration and the less active species have the lowest concentration. Das (1958) reported haematological parameters from three Indian major *Carps, Labeo calbasu, Catla catla* and *Cirrhinus mirgala.* Pillay (1958) made routine investigations on the blood of *Hilsa ilisha* from river Hoogly.

Hesser (1960) reported the use of haematological parameters in assessment of fish physiology. Larson and Sniesko (1961) compared various methods for determination of hemoglobin in trout blood. Watson *et al*. (1963) carried out the haematological parameters of gold fish, *Carassius auratus.* Wenreb (1963) made an electron microscopic study on the blood cells of some Teleosts. Cairns and Scheiser (1964) worked on the hematology of pumpkin seed sunfish *Lepomis gibbossus* (linn.) and reported values for hemoglobin concentration and total blood cell counts. Smirnova (1965) reported total leucocyte count (TLC) in fish blood to exhibit a direct correlation with their feeding status.

Bouck *et al*. (1966) was among the pioneers who suggested that fish hematology could be a useful tool in monitoring stress levels of aquatic pollution on fish. Fry (1969) studied the physiological stress on fishes caused by Lake Eutrophication. It was concluded that reduction of the oxygen content of water is the most pressing source of stress for fishes in eutrophic lakes and that almost all other stresses are incidental or aggravated by that primary one. Enomato (1969) found a decrease in Lymphocyte number during oxygen deficient conditions, but there was no difference in numbers before and after feeding, and numbers did not vary between heart blood and tail cut blood.

Eddy and Morgan (1969) found a mean increase in the hemoglobin concentration from 5.3 to 7.6 g/100ml between control Rainbow trout and a group acclimatized to high levels of free carbon dioxide. Gelineo (1969) studied concentration of hemoglobin in 53 species of freshwater and marine fish and observed an average of 8.6% haemglobin. He reported that fresh water, active and male fish have higher concentration of haemoglobin than marine inactive and female fish related to them.

Cameron (1970) studied blood parameters of pinfish *Lagodon rhomboids*, in response to high temperatures and reported increased hemoglobin and erythrocyte count, with a decreased hematocrit.Bali (1971) devised a new method for multiple blood sampling in fish. He developed an improved technique of drawing blood samples from *Cyprinus carpio* by inserting a 24 bore sterile hypodermic needle fixed with 2ml sterile syringe containing 1ml of dry EDTA into the gill filaments. Yashnovich and Leonenbo (1971) while studying the hematology of fish found changes in blood volume and total hemoglobin content due to age and season in *Hypothalmichthys molitrix* (val), *Ctenopharyngodon idella* L. and *Cyprinus carpio L.*

Blaxhall (1973) depicted error in hematocrit value produced by inadequate concentration of EDTA and reported a rise in hematocrit value of blood from rainbow trout *Salmo gairdneri (Richardson*) after collection in dipotassium EDTA and storage. Blaxhall and Daisley (1973) described routine hematological methods for examining fish blood, including hemoglobin estimation, PCV, Erythrocyte counts, ESR, TLC and DLC's and cytochemical stained blood cells and suggested that these could be used as possible means of assessing the fish health. McCarthy *et al*. (1973) studied 12 hematological parameters of Rainbow trout from Kamploops. Their results showed that ESR, Hb, PCV, TEC, Total protein, Erythrocyte diameter and DLC fell within limited ranges, whereas TLC and Glucose levels were more greatly spread. Hussein *et al*. (1974) made a hematological study of healthy *Anguilla vulgaris* and *Mugil cephalus*. They found that the average RBC counts, hematocrit values and hemoglobin content for the mullet, *M. cephalus* were always higher than those of the eel, *A. vulgaris* throughout the whole experimental period. They observed no clear seasonal variations for both species in ESR and specific gravity whereas erythrocyte count, hematocrit values and hemoglobin content were found to be higher in summer and lower in winter.

Denten and Yousuf (1975) concluded experimentally that water temperature was not responsible for variation in hematology of rainbow trout. Instead, diet, metabolic adaptations and activity were suggested as probable cause of seasonal changes in the hematology of fish. Larson *et al*. (1976) made comparative study of some haematological and biochemical blood parameters in fishes from the Skagerrak. Interspecies variations as well as variations within some species were observed. The hemoglobin values for all species showed a positive correlation to the corresponding haematocrit values. Tandon and Joshi (1976) studied total red and white blood cell count of 33 species of fresh water Teleosts. The fishes showed a wide range of variations in their total red and white blood cell counts, both of which were reported to be affected by size of the fish, their general habits, feeding habits and metabolic needs.

Buckley (1977) reported haemolytic anaemia with pathological changes in erythrocytes, reduction in packed cell volume and Hb concentration in *Onchorhynchus kisutch* after exposure to chlorinated waste water. Atinkson and Judd (1978) stated that besides assessing the health of a fish, various blood parameters reveal physiological adaptations in their natural habitats and are also helpful in determining systematic relationship. Johansson- Sjobeck *et al.* (1978) reported an increase in percentage of neutrophils and monocytes and decreased percentage of lymphocytes in the circulating blood of *Anguilla Anguilla* exposed to handling stress.

Guakghua (1979) while working out the hematological parameters of *Crucian carp* collected during different seasons of the year reported that the hematology of the fish exhibits definite cyclic changes which are profoundly affected by the water temperature and its dissolved oxygen content and also the breeding phase for this fish. Rani and Rao (1979) studied the blood cell counts of marine fish, *Gerrs filamentosus* from Vishakhapatanum harbor. Siddiqui and Nasim (1979) made hematological observation on *Cirrhina mrigala* and recorded higher hemoglobin and erythrocyte concentration in males than in females.

Matkovics *et al*. (1981) found a quick decrease in haemoglobin content of *Cyprinus carpio* in response to a toxicant, paraquat.Mille *et al*. (1983) established normal ranges for diagnostically important haematological and blood chemistry characteristics of rainbow trout, *Salmo gairdneri*. Munkittrick and Leatherland (1983) measured hematocrit values in gold fish *Carassius auratus* as indicators of the health of the population. They found that hematocrit in male were larger than in females in all collections of the fish except spring catch.Wedemyer *et al*. (1983) studied physiological stress response in *Oncorhynchus kisutch* and found that leucocrit was a sensitive indicator of the physiological stress resulting from crowding population densities and to stress of handling and to temperature changes.

Kumar *et al*. (1984) worked on hematological changes in cold water fish *Schizothorax plagiostomus* (Heckel). They found that *S. plagiostomus* naturally infected with metacercareae of *Diplostomium tertare* had decreased total Erythrocyte count, PCV, Hb content and total leukocyte count relative to uninfected controls indicative of anemia. The fishes which were infected showed abnormal behavior and they were also lethargic.

Muragesen and Haniffa (1985) worked on the impact of textile mill effluent on haematology of *Anabas testudineus* and reported a significant decrease in RBC, haemoglobin and haematocrit content of the fish with increase in effluent concentration. Ralio and Nikinmaa (1985) found that hematological parameters of fish like erythrocyte and leucocyte count, hemoglobin, haematocrit and differential leukocyte counts greatly varies with factors such as physical stress and environmental stress due to water contaminants.

Chaudhury *et al.* (1986) investigated the sex related variations of some blood parameters of an exotic fish, *Sarotherodon mossambica*. They observed that the TEC, Hb, and PCV Values were highest in males compared to females. Sharma and Joshi (1986) worked on the blood of hill stream fish, *Tor putitora* of varying length group. Their study revealed that TEC, TLC, Hb, PCV and MCHC increased with increasing length and weight, whereas MCH and MCV values fell in higher length groups. Vuren (1986) carried out a study on the haematological parameters of *Labeo umbratus* exposed to four different toxicants and reported that detergent and fertilizer caused decrease in number of erythrocytes, haematocrit, mean corpuscular volume, and average corpuscular volume. Dheer (1986) studied haematological, haematopoitic and biochemical characteristics of *Channa punctatus* in response to thermal stress. All the parameters tested indicated deviation from the normal healthy conditions. Erythrocytic polycythemias accompanied by an increase in haemoglobin content and haematocrit values were indicative of thermal stress.

Bhaskar and Rao (1989) studied the influence of temperature, salinity, dissolved oxygen, and pH of water on nineteen blood characteristics of juvenile milkfish, *Chanos chanos* from three different brackish water fish farms and found no significant difference between fish grown under different farm conditions with regard to most blood values. Narain and Srivastava (1989) reported that sewage pollution induces anemic condition in *Heteropneustes fossilis*, with a significant decrease in haemoglobin, RBC count, MCHC and increase in PCV, ESR values. Rao *et al*. (1989) found that active, fast-moving fish (*Scomberomorous guttatus and Rastrelliger kanagurata)* had higher values of erythrocyte parameters to meet the high metabolic rate than the sluggish, predacious fish (*Arius maculates)* and bottom detritus feeder (*Liza parsia*). Tucker *et al.* (1989) studied nitrite induced anemia in channel catfish *Iclaturus punctatus*. It was observed that mean haematocrit and total haemoglobin concentration in fish exposed to the two highest nitrite concentrations indicate only moderate anemia, however, there was considerable variation among fish within these groups and some fish were anemic.

Lea Master *et al*. (1990) worked on hematology and blood biochemistry of *Sarotherodon melanotheron* and reported that number of white blood cells depends on the quality of aquatic environment. Raja *et al.* (1990) studied hematological values of fish *Oreochromis mossambicus* living in polluted water tank. Decrease in hemoglobin content and RBC count was observed, whereas leucocytes count was high when compared to control.

Aggarwal (1992) worked on hematological aspects of *Channa punctatus* (bloch) and found a significant dose dependent elevation in all the parameters except the blood glucose. Hoglund *et al.* (1992) studied the hematological variations in population of *Anguilla anguilla* naturally infected with *Anguillicola crassus* off the Swedish Baltic coast in an area receiving heated cooling water from a nuclear power station. Most variables showed no or only minor reactions to the infection, reduced lymphocyte numbers and increased granulocyte numbers were considered indicative of a humoral and cellular immune response. Allen (1993) determined the haematological parameters of O*reochromis aurens*. The results revealed that *O. aurens* parameters appear to be similar to those of *O. niloticus* and *O. mossambias*. Hemoglobin concentration was higher than latter species.

Martinez *et al*. (1994) studied the effect of the simultaneous influence of weight, temperature, density and O_2 concentration in the water on various blood parameters of *Onchorhynchus mykiss*. Multiple correlation and regression analyses showed a strong dependence of Ht, Hb and RBC on the factors considered the most influential of which was temperature. Rajyasree and Perviz (1994) studied the effect of urea on hematology of *Labeo rohita* fingerlings. Significant decrease in blood parameters was observed at higher concentrations.

Rauthan and Grover (1994) reported that blood parameters are altered by intrinsic as well as extrinsic factors. Studies on blood parameters of *Barilius bendelisis* during different seasons of year showed that total erythrocyte count, hemoglobin, packed cell volume, blood glucose values raised during summer months, whereas lowest values of all parameters were observed in winter months, when ambient temperature was quite low.

Roche and Boge (1996) made an investigation on classical stress indicators (cortisol, hemoglobin, and hematocrit) in sea bass (*Dicentrarchus labrax).* Hrubec *et al*. (1997) studied the effect of water temperature on haematological and serum biochemical analysis in hybrid stripped brass. Verdegem *et al.* (1997) studied influence of salinity and dietary composition on blood parameter values of hybrid red tilapia, *O. niloticus* and *O. mossambicus* under similar environmental conditions. Results showed that salinity influences all cellular blood parameters except haematocrit. Dietary composition influenced haematocrit.

Collazos *et al*. (1998) carried out seasonal variation in male and female tench *Tinca tinca* and found significant changes in red blood cell count and haematocrit in males comparing spring and summer with autumn and winter, whereas in females the RBC remained constant for all four seasons but the haematocrit decreased in autumn and winter compared to spring and summer. The results indicated marked seasonal variation in the blood of male and female *Tinca tinca*. Sahoo and Mukherjee (1999) worked out normal ranges for diagnostically important haematological parameters of laboratory reared rohu (*Labeo rohita*) fingerlings. The morphology of cells was also described. There was a wide variation in hematocrit and MCV of individual healthy fish.

Aysel *et al*. (2001) investigated some haematological parameters in spotted Barb *(Capoeta barrosi lortet*, 1894) and Roach (*Rutilus rutilus*, Linnaeus, 1758) living in Seyhan river (Adana city region). Basic haematological parameters and effects of seasonal differences on these parameters were determined in Roach and spotted barb which had adapted to water polluted by agricultural, industrial and domestic wastes. The results showed that there were deviations from standard values for most of the haematological parameters for *R. rutilus* indicating that *R. rutilus* individuals were affected by environmental stress factors more than those of *C. barrosi*. Orun *et al.* (2001) conducted a study to determine and compare blood parameter levels of *Alburnoides bipunctatus*, *Chalcalburnus mossulensis* and *Cyprinion macrostomus*. Their results indicated that blood parameter levels of all species in warm months were significantly different than those measured in cold seasons. The number of total leukocyte, neutrophil and monocyte levels was found to be higher in female fish, especially in reproductive season than in male fish. Levels of hemoglobin, haematocrit, and erythrocyte were high in male fish in an annual period. Yousuf *et al*. (2001) while giving an account on length-weight relationship of *Schizothorax niger* Heckel, in two water bodies of Kashmir which are almost similar in environmental condition, said that fish health, on which the length-weight relationship depends is greatly influenced by environmental factors.

Atamanalp (2002) studied hemoglobin, red blood cells and total white blood cell counts of *Capoeta capoeta* living in Tuzla stream and compared between hemoglobin, red blood cells, white blood cells of fish from polluted and non polluted areas. Homatowska *et al*. (2002) worked on hematological profile and cytomorphometry of circulating blood in the sun bleak, *Leucaspius delineates* (Heckel, 1843).Parameters like Red blood cell count, hemoglobin content, Hematocrit values of red blood cell indices, were determined. Red cell indices were found to be higher, and made it possible for the species to function normally even in less comfortable oxygen conditions of its natural habitat. The lymphocyte and monocyte count was lower.

Orun and Erdemil (2002) studied seasonal effects on blood parameters of *Capoeta trutta* (Heckel, 1843) from Karakaya Dam Lake (Malatya, Turkey). Analysis revealed that the values of blood parameters increase in spring-summer and decrease in autumn-winter period irrespective of age-weight-length variables. A significant ($P<0.05$) difference was found between male and female in reproductive period (spring-summer). Svetina *et al*. (2002) carried out a study on haematology and some blood chemical parameters of young carp till the age of three years, their results suggested that the investigated hematological and biochemical variables could be successfully utilized in monitoring the metabolic balance and health status of fish.

Guijarro *et al.* (2003) while studying seasonal changes in hematology of the *Tinca tinca* reported highest count of RBC, WBC, and hematocrit in summer compared to autumn and winter season. Among the WBC, Lymphocytes showed high % (35-40%) with similar values among seasons and both sexes. Monocytes were the least common cells in the blood of the *Tench* (6%). Orun *et al*. (2003) studied hematological parameters of three Cyprinid fish species *(Alburnoides bipunctatus F., C halcalburnus mossulensis and Cyprinion macrostomus)* from Karakaya dam lake, Turkey. The results from the study indicated that blood parameter levels of all species in warm months were significantly different than those measured in cold season. Siwicki *et al*. (2003) examined hematological and biochemical parameters of Pikeperch (*Sander Lucicoperca*).Results showed that Pikeperch have hematological and biochemical parameters similar to rainbow trout.

Bastuta and Sen (2004) investigated the changes in blood parameters of *Acanthobrama marmid* .The total number of erythrocytes, leucocytes, thrombocytes, hemoglobin, and hematocrit were determined. Ezeri *et al.* (2004) conducted a study on the haematological response of cultured and wild *Clarias gariepinus* to acclimation and observed a reduction in the values of Packed Cell Volume after acclimatizing the fish for seven days. Gabriel *et al.* (2004) studied the influence of sex, source (pond and wild) acclimation and health status on some blood parameters of *Clarias gariepinus*. Results from this study suggest that sex, source of fish and period of acclimation have some degrees of influence on the blood parameters of *C. gariepinus*. Jawad *et al.* (2004) analyzed the relationship between hematocrit and some biological parameters of the Indian shad, *Tenualosa ilisha*. They found hematocrit values to show a quadratic relationship to fish size (body length). Male fish was found to show higher hematocrit value than females.

Rehulka and Adamec (2004) studied 161 immature female rainbow trout to calculate reference haematology values for red cell counts, haematocrit values hemoglobin concentration. Multiple correlation indices obtained from the cage fish to determine the effects of time (day), water temperature, dissolved oxygen, oxygen saturation level of the water, chemical oxygen demand, biological oxygen demand and ammonia have shown that varying physical and chemical properties of water and availability of natural food may influence erythropoiesis in caged fish. Yousafzai (2004) while studying the toxicological effects of industrial effluents dumped in River Kabul on mahaseer, *Tor putitiora* at Aman Garh Industrial area, Peshawar, Pakistan found that the fish collected from polluted sites when compared with the control had lower hemoglobin. RBC counts, PCV and MCHC and higher WBC counts and MCV.

Adeyemo (2005) worked on the haematological and histopathological effects of cassava mill effluent in *Clarias gariepinus.* It was concluded that since cyanide is a potent respiratory poison, un-detoxified or insufficiently detoxified cyanide-containing liquid wastes could easily contaminate fish and ultimately extinguish aquatic life if discharged into aquatic environments. Aras *et al*. (2005) studied monthly fluctuations in hematology and serum biochemical data in *Leuciscus cephalus*. The reports showed that RBC, WBC Counts, Hb, Hct, MCV, MCHC and MCH observed highest values during month of May and minimum during January. Also, these parameters were affected by many exogenous and endogenous factors such as reproductive cycle, water temperature and metabolic rate.

Arnold (2005) established standardized haematological methods and reference intervals for cartilaginous fishes (sharks, skates, and rays). The study focused to validate complete blood composition methods for sandbar shark (*Carcharhinus plumbeus*). Results revealed that total white blood cell counts in diluents modified for elasmobranch blood, haemoglobin concentration by the cyanomethemoglobin method after removal of nuclei, and white blood cell differential percentages showed acceptable performance. Packed cell volume results were acceptable when tubes were centrifuged for at least five minutes. Total white blood cell counts by all three methods exceeded the acceptable error for manual counts of human cells. Cazenave *et al*. (2005) analyzed hematological parameters in healthy fish, *Corydoras paleatus* from an unpolluted area and found that normal blood parameters did not change according to maturation stages, sex or seasons, but fish captured from a site polluted by a sewage showed significantly higher values of Er, Ht, Hb, MCH and MCHC than those captured in an unpolluted area. De-Pedro *et al*. (2005) studied daily and seasonal variations in haematology and blood biochemical parameters in tench *Tinca tinca* .

Elahee and Bhagwant (2005) studied gill histopathology and haematological primary indices, including blood d-aminolevulinic acid dehydratase (d ALA- D) activity and nucleocytoplasmic ratio of erythrocytes, in three tropical marine fish species, *Scarus ghobban*, *Epinephelus merra*, and *Siganus sutor*, from the presumably contaminated lagoon of Bain des Dames, Mauritius. Concurrently, the non polluted region of Blue Bay/Pt d'Esny was used as a reference site for comparison of fish physiological responses and seawater quality. Bain des Dames fish showed high seawater mercury content (6.470.5 mg/L), traces of iron (70740 mg/L), and fluctuating biochemical oxygen demand values (0.48870.171 mg/L day_1). Gill histopathological analysis revealed lesions such as epithelial hyperplasia and inflammation. Similarly, a generalized increase in blood dALA-D activity (131.27–355.76 nmol PBG/ml RBC.h) was recorded. Fish from Bain des Dames showed species-specific haematological responses including normocytic macrocytic blood cells (*S. ghobban*), macrocytic anemia (*S. sutor*), and active erythropoiesis (*E. Merra*). Fagbenro *et al*.

(2005) analyzed the hematological profile of three fish species, in order to determine the reference values for some hematological indices of *Parachanna obscura, Malapterurus eectricus and Malapterurus minjiriya*. Fujimaki and Isoda (2005) carried out electron microscopic study on leucocytes from circulating blood of gold fish. The leucocytes were divided into eight types: neutrophil, eosinophil, large granular leucocytes (LGL), medium-sized granular leucocytes (MGL), small granular leucocytes (SGL), fine granular leucocytes (FGL), lymphocyte and monocyte. The existence of gold fish monocytes was demonstrated for the first time in the report.

Kori-Siakpere *et al.* (2005) carried out hematological studies of African snakehead, *Parachanna obscura* in order to establish a normal range of blood parameters which would serve as a base line data for assessment of the health status of the fish as well as reference point for future comparative surveys. Blood parameters such as erythrocyte, leucocyte and thrombocytes count, haemoglobin contents, Mean corpuscular hemoglobin concentration, hematocrit, etc were determined.

Valenzuela *et al.* (2005) carried out an experiment in trout (*Oncorhynchus mykiss*), exposed to acute hypoxia. Blood cell counts, and immature erythrocytes were studied to evaluate their relationship with degree of hypoxia, there was significantly increase in hematocrit as well as in total number of leukocytes. Results showed that oxygen concentration lower than normal may put at risk OR_S production and also non –specific defense mechanisms in trout. Vutkuru (2005) reported a significant decrease in RBC’s, hemoglobin and packed cell volume of *Labeo rohita* exposed to heavy metals.

Carvalho and Fernandes (2006) investigated the susceptibility of the neotropical freshwater fish *Prochilodus scrofa* to copper at two temperatures with low and high water pH. The reports showed that the use of copper sulfate to control algae and fish parasites should take into account the sensitivity of the species to copper and mainly the water pH of aquaculture ponds.

Das *et al.* (2006) exposed the fingerlings of catla (*Catla catla*), rohu *(Labeo rohita*) and mrigal (*Cirrhinus mrigala)* to acidic (pH 5.5, 6.5) and alkaline (pH 8.0, 8.5 and 9.0) water. Change in water pH either to acidic or alkaline conditions exerted stress in fish characterized by swelling of erythrocytes, production of immature erythrocytes, and reductions in the total erythrocyte counts, hemoglobin and serum protein content. There were also increases in total leukocyte counts and blood glucose. *Rohu* was found to be least affected to the stress of altered water pH, followed by *mrigal,* while *catla* was the most vulnerable to pH changes.

Gbore *et al*. (2006) investigated the effects of stress due to handling and transportation on haematology and plasma biochemistry in the fingerlings of two species of fish. The results indicated reduced values for all parameters examined (PCV, Hb, RBC) except for the leukocyte, Hb for *Tilapia zilli*, while the blood constants, increased for *C. gariepinus*. The changes in the Hb, leukocyte, MCHC were more significant ($p<0.05$) for fingerlings of *T. zilli* compared to those of *C. gariepinus*. It was concluded from the study that fingerlings generally are susceptible to stress but those of *T. zilli* are more susceptible to physical stresses than those of *C. garipinus*.

Romao *et al*. (2006) studied blood parameters and morphological alterations as biomarkers on the health of *Hoplias malabaricus* and *Geophagus brasiliensis*. Analysis of red blood cell count, microhaematocrit, hemoglobin concentration, white blood cell count and differential white blood cell count in blood smear were carried out to assess the influence of environment on fish health. The results showed variation at different sites /areas. Trumble *et al*. (2006) studied dietary and seasonal influences on blood chemistry and hematology in seals.

Vetansnik *et al*. (2006) carried out haematological study on *Carassius auratus* irrespective of sex, and found that ploidy level affected significantly ($P < 0.01$) the values of the erythrocyte count and Mean Corpuscular hemoglobin. The erythrocyte count decreased significantly ($P<0.01$) with increasing ploidy level. The index of hemoglobin followed the same trend of a decreasing mean value with increasing ploidy level. Mean corpuscular volume and Mean cell corpuscular hemoglobin increased with increasing ploidy level ($P<0.01$). Hematrocrit and Mean Corpuscular Hemoglobin concentration did not significantly differ from the ploidy level.

Adeymo (2007) worked on hematological profile of *Clarias gariepinus* exposed to lead and found reduction in the RBC count. Dube and Munshi (1973)noticed increase in the RBC counts and the hemoglobin percentage in blood of *Pleuronectes platessa* and *Anabas testudineus* respectively with increasing body weights. Pandey *et al*. (1976) in *H. fossilis* and Pandey *et al*.(1984;1985;1986) in *Channa punctatus*, also reported an increased RBC counts, Hb conc., PCV and WBC counts with the increase of body weights.

Gabriel *et al.* (2007) presented the Hematological characteristics of *sartherodon melanontheron* from the brackish water creek of Buguma. The highest range of parameters was recorded in thrombocytes, while the lowest was recorded in RBC.Significant differences ($p<0.05$) between males and females were observed in hemoglobin, hematocrit, red blood cells and thrombocytes. Garcia *et al* (2007) observed that the numbers of total leukocytes, lymphocytes and eosinophils decreased, while the numbers of neutrophils and monocytes increased in *Piaractus Mesopotamicus* when fed with diets supplemented with vitamin C and E, challenged by *Aeromonas hydrophila.*

Gupta *et al*. (2007) found a trend of increase in RBC counts and decrease in PCV (haematocrit) and MCV with increasing temperature was marked but the differences between control and experimental groups remained statistically non significant ($p>0.005$). Ishikawa *et al.* (2007) made a study on some hematological parameters in Tilapia, *Oreochromis niloticus* when exposed to various mercury concentrations. The study revealed that there were no significant differences among the mean hematological values at different mercury concentrations indicating that mercury up to a concentration of 0.02mg/l was not toxic to Tilapia.

Kapila *et al*. (2007) studied the impact of water pH on haematology and serum enzyme activities in *Schizothorax richardsonii* (Gray) and concluded that the fish thrived well in pH range of 6.0-9.0 but did not tolerate high acidic and alkaline conditions. Sahan *et al.* (2007) carried out a study of some hematological parameters in European eel, caught from agricultural, Industrial, domestic and slaughter house discharging region of Ceyhan River. Reports from the study showed that leucocyte values and neutrophil proportion were found increased by means of environmental stressors ($p<0.05$).

Vazquez and Guerrero (2007) carried out a study on morphological features of blood cells and hematological parameters in *Cichlosoma dimerus*. The study revealed that Thrombocytes are the most abundant blood cells after erythrocytes and can be easily recognized from lymphocytes by morphological features and size. Zexia *et al*. (2007) carried out morphological studies on peripheral blood cells of Chinese sturgeon, *Acipenser sinensis*. The erythrocyte and four main types of leucocyte- thrombocytes, lymphocytes, granulocytes (including neutrophils and eosinophils) and monocytes were identified in the peripheral blood.

Adam and Agab (2008) investigated the reference values for haematological and biochemical ranges for *Clarias gariepinus*. A correlation matrix was established to compare the degree of association among the biochemical and haematological indices. A positive correlation was observed. The blood glucose level was positively correlated with weight and length, whereas the total plasma was negatively correlated with hemoglobin. Red blood cell count was positively correlated with hemoglobin and negatively correlated with MCV and MCH.

Aras *et al*. (2008) worked on the monthly fluctuations in haematology and serum biochemical data in wild chub (*Leuciscus cephalus*) by measuring red blood cells, white blood cells, hemoglobin, haematocrit, MCH, MCV, MCHC, triglycerides, cholesterol, high density lipoprotein. The minimum values were obtained in cold months for red blood cells, haematocrit, MCV, and white blood cells and it was found that all studied parameters were affected by many endogenous and exogenous factors such as reproductive cycle, water temperature and metabolic rate.

Arnaudova *et al.* (2008) carried out a study on haematological indices of freshwater fish from Studen Kladenetsh Reservoir. Results showed that concentration of toxic substances in the water of reservoir exceeds acceptable level which resulted in a decrease in concentration of haemoglobin, hematocrit and red blood cell count, indicates anemic changes in the blood of fish .Nikolov *et al.* (2007) studied 11 parameters of the red blood cell count in three carp species: *Carassius gibelio* (L.), *Alburnus alburnus* (L.) and *Scardinius erythrophthalmus* (L.). The results were compared for individual species, as well as with data for other freshwater fishes.

Ramesh and Saravanan (2008) found haematological parameters like Red blood cells & Haemoglobin level to decrease in the chlorpyrifos treated fish, whereas White blood cells level increased. Singh *et al.* (2008) exposed freshwater fish, *Channa punctatus* to a sub-lethal concentrationof 0.36 mg l^{-1} of copper for different periods and found significant decrease in the hemoglobin (Hb) content from 10.73 to 6.60%, red blood cells (RBC) from 2.86 to 1.84 $x10^6$/ mm^3 and packed cell volume (PCV) from 31.00 to 23.33% at the end of 45^{th} day as compared to control. The white blood cells (WBC) number, however, increased from 60.00 to 92.48 $x10^3$/ mm^3.

Zaki *et al.* (2008) reported significant elevations in Red blood cells, Hemaglobin, Hct, and MCHC in the fish *Oreochromis niloticus* exposed to lead pollution. Adeyemo *et al.* (2009) conducted a study for the induction of acute handling and transport stress that could reproducibly affect haematological changes in African catfish, (*Clarias gariepinus*. Bruchell, 1822) and found no significant differences ($p < 0.05$) in the haematocrit, white blood cell, hemoglobin and eosinophil of the stressed fish relative to the baseline values. However, significant differences ($p<0.05$) were observed in the values of the neutrophil and lymphocyte of the stressed fish relative to the baseline data.

Jamalzadeh *et al*. (2009), while comparing the blood indices in healthy and fungal infected Caspian salmon, found white blood cells, neutrophil and eosinophil have higher values ($p<0.05$) in fungal infected fishes than healthy Caspian salmon and the other parameters like Hb , Hct, RBC, lymphocyte, monocyte were greater in healthy Caspian salmon. Olufemi and Owolabi (2009) worked on hematological and serum biochemical profile of *Synodontis membranaeca,* collected from the lake Jebba of Nigeria and collected data on RBC, Hb, PCV, MCH, WBC, monocyte, albumin, creatinin, uric acid, cholesterol, calcium, potassium, sodium and alanine.

Ovie *et al.* (2009) determined the toxicological effects of Potassium permanganate on hematological parameters of African Cat fish, *Clarias gariepinus* on exposing to sublethal concentrations (0.0, 2.0, 6.0 and 10.0 mg/L) of potassium permanganate for 12, 24, 48, 96 and 192 hr, and found Hb concentration and Hematocrit values were significantly ($P< 0.05$) decreased in all sub- lethal levels. Ramesh *et al.* (2009) studied hematological parameters of common carp, *Cyprinus carpio* exposed to lethal concentration of herbicide, Atrizine. The plasma glucose, hemoglobin, Red blood cells and plasma protein progressively decreased, whereas white blood cell count was enhanced. The differences in hematological and biochemical values were stastiscally significant ($p<0.05$).

Singh and Tandon (2009) studied the effect of river water pollution on hematological parameters of fish, *Wallago attu* (padhan) from river Suheli and river Gomti. The hemoglobin percentage and red blood cells were found to be greater in Suheli river fish as compared to the fish from the Gomti. The white blood cells and erythrocyte sedimentation rate were found to be lesser in the fish of river Suheli as compared to Gomti. The total leucocyte count and packed cell volume values were significantly higher in Suheli fish than that of Gomti fish. The study revealed that blood parameters are sensitive indicators of stress on fishes exposed to water pollutants.

Talevski *et al*. (2009) studied the anthropogenic influence on biodiversity of ichthyofauna and macrophyte vegetation from Ohrid and Lake Skadar from the results it was concluded that biodiversity of the fish populations and macrophytic vegetation from the both lakes is different and mostly depends on the ecological condition present in researched localities. Zhou *et al*. (2009) compared haematology and serum biochemistry between cultured and wild ecotypes of dojo loach *Misgurnus anguillicaudatus*. The results revealed that hemoglobin, cholesterol, total protein, creatinine and uric acid levels in the two ecotypes were significantly different. In addition red blood cell, were significantly higher in cultured individuals than in wild counterparts. In contrast, the white blood cell level in cultured fish was significantly lower than that in the wild one.

Adewoye (2010) found the haematological and biochemical changes in *Clarias gariepinus* exposed to *Trephosia vogelii* extract and reported an increase in the level of White Blood Cell (WBC) in treated fish. Akinrotimi *et al.* (2010) investigated the hematological characteristics of *Tilapia guineensis* from Bunguma creek Nigeria. Reports from the study showed that there were significant differences (P<0.005) in all parameters of blood between male and female fishes.

Ayoola (2010) studied that Fish *Clarius gariepinus* fed with poultry hatchery waste showed a slight decrease in hematological values of Packed cell volume (PCV), Hemoglobin (Hb), total red blood cells (RBC), Mean corpuscular value (MCV), Mean corpuscular hemoglobin(MCH) ,Mean corpuscular hemoglobin concentration (MCHC), as compared to the values of fish fed on the control diet. Ikechukwu and obinnaya (2010) worked on hematological profile of the African lungfish, *Protopterus annectens*. Blood parameters such as erythrocyte, leucocyte and thrombocytes count, hemoglobin contents, Mean corpuscular hemoglobin concentration, blood osmalality, pH, hematocrit, etc were determined during the various reproductive stages of *P. annectens*.

Kayode and Shamusideen (2010) carried out haematological studies of *Oreochromis niloticus* exposed to diesel and drilling fluid in Lagos, Nigeria. Diesel and drilling fluid evoked significant changes in the haematological parameters of the fish.Ololade and Oginni (2010) studied the toxic stress and hematological effects of nickel on *Clarias gariepinus* fingerlings. All the blood parameters (erythrocyte, leucocytes, hematocrit and haemoglobin count) were reported to decrease with increasing concentration of toxicant. Satheeshkumar *et al.*

(2010) studied the haematological and biochemical parameters of wild marine teleost fishes from Vellar estuary, southeast coast of India. The haematological parameters such as red blood cell count (RBC), white blood cell count (WBC), haematocrit (HCT), hemoglobin (HB), mean cell volume, mean cell hemoglobin and mean cell hemoglobin concentration, RBC/WBC ratio, erythrocyte sedimentation rate (ESR) were determined. Statistical analysis confirmed that differences in haematological parameters between all the species were ($P<0.01$) significant. The result revealed that RBC, RBC/WBC ratio, HCT, HB, ESR was significantly correlated at $P<0.05$ level. RBC/ WBC level was more due to the decrease in WBC during the study. These differences were attributed to the physiological acclimatization of the fish to their living conditions and feeding regime, which influences the energy metabolism and consequently, the health of the fish.

Singh and Srivastava (2010) studied haematological parameters, such as erythrocyte and leucocyte count, erythrocyte indices and thrombocyte number vis-a-vis coagulation of blood as bioindicators of toxicosis in fish. Srivastava and Choudhary (2010) studied the influence of artificial photoperiod on the blood cell indices of *C. batrachus* (Linn.) and concluded that exposure to continuous light elicits stress responses in the leukocyte profile of this nocturnal fish. Zarejabad *et al*. (2010) studied the effect of environmental temperature changes on haematological and biochemical parameters of *Hoso hoso* juveniles. The results showed that hematocrit, calcium and eosinophil were affected by temperature. Increasing temperature led to significant increase in haematocrit, calcium and eosonophil, but WBC lymphocyte, cortisol and glucose concentration decreased slightly ($p<0.05$).

Zutshi *et al*. (2010) conducted a study to investigate the hematological parameters of wild population of rohu, *Labeo rohita* (Ham) sampled from Hebbal (receiving a storm Water drain) and Chowkalli lake (receiving domestic sewage and industrial effluents from various sources and was more polluted than Hebbal lake) of Bangalore. Reports from the study showed that severe anemia can be marked by a significant decrease in RBC count ($p < 0.5$), hemoglobin content, and PCV and MCHC values, whereas an increase in leukocyte count and MCV values were observed in fish from Chowkalli Lake. The variation in values of different parameters can be attributed to exposure of fish to various types of Pollutants present mainly in the Chowkalli Lake which receives heavy metals, synthetic detergents, petroleum products, and other acid and alkali substances from the nearby local industries.

Adedeji and Adegbile (2011) worked on the hematology of Bagrid catfish (*Chrysichthys nigrodigitatus*) and the African catfish (*Clarias gariepinus*) comparatively. The study revealed that there was significant difference in RBC, Hb, MCV, and Lymphocytes values of the two fish. Farell (2011) studied cellular composition of the fish blood and reported that Hct of most of the fishes ranges between 10-40%. Gabriel *et al*. (2011) investigated the haematological characteristics of Bloody cockle (*Anadara senilis).* A total of two hundred and forty (240) were sampled from Andoni flats during low tide. The highest range of the parameters was recorded in platelets, while the lowest was observed in RBC. Significant differences ($P < 0.05$) were observed between the four size groups in all the parameters studied. Innocent *et al.* (2011) studied the haematology of *Cirrhinus mrigala* fed with Vitamin C supplemented diet and reported that supplementation of feed with Immunostimulant

(Vitamin C) improved the total leucocyte counts and granulocyte population, which nonspecifically helped to minimize infection induced stress, improved resistance against infection and faster recovery from stress.

Pradhan *et al*. (2011) worked on seasonal changes in blood parameters of *Catla catla* and found that the hemoglobin concentration of fish was higher in summer season and lower in autumn season; similar trend was also reported in Total erythrocyte count, Total leucocyte count, PCV and MCV values. Siakpere and Ikomi (2011) studied the haematological alterations produced on exposure of *Parachanna africans* to the sublethal concentration of cadmium (Cd^{2+}).They found that red blood cell count, haemoglobin concentration, haematocrit the mean corpuscular haemoglobin and the mean corpuscular volume levels decreased, but the level of the mean corpuscular haemoglobin concentration increased with an increase in exposure concentration of cadmium. Kori-Siakpere and Oboh (2011) concluded that sublethal concentration of tobacco leaf dust have deleterious effects on the haematological parameters of *C. gariepinus* and cautioned that the use of this toxicant in fish ponds needs proper control to avoid reduction in fish production and aquatic fauna.

Ada *et al*. (2012) studied the haematological, biological and behavioral changes in *Oreochromis niloticus* juveniles exposed to Paraquat herbicide. Haemoglobin, mean cell haemoglobin, mean cell haemoglobin concentration and erythrocyte sedimentation rate were observed to be negatively related to concentration of Paraquat, whereas packed cell volume, white blood cell count (WBC), red blood cell count (RBC) showed positive relationship. Al-Ghanim (2012) carried out a study to investigate the toxic effects of malathion in *Oreochromis niloticus* and found that erythrocyte count, haematocrit value and haemoglobin content of Nile tilapia decreased with increased pesticide concentrations, while total production, net returns and profitability of reared fish decreased with increase in concentrations of pesticides.

Ekanem *et al.* (2012) while investigating the effect of anticoagulants, EDTA and Heparin on the hematological parameters of *Oreochromis niloticus* found blood films with heparin gave blood pictures that were closer to normal (control) than EDTA. The results from the study showed that EDTA is not the best anticoagulants for routine hematological analysis; heparin has proved to be the preferred anticoagulant for fish hematology. Francesco *et al.* (2012) while carrying out comparative study on the hematology of Indian and Italian Grey mullet (*Mugil cephalus*) found that physico-chemical factors of water influences the ranges of hematology of fish within same species, suggesting that blood parameters may therefore be a valuable in monitoring the effects of habitat changes on fish biology and fish culture practices.

Ghanbari and Jami (2012) examined the effects of water pH changes on certain hematological parameters of fingerlings of common carp (*Cyprinus carpio)* exposed to acidic (pH 5.5 and 6.5) and alkaline (8.5 and 9.0) water for 21 days. The results showed that this exposure affected the hematology of carp due to stress, also there was significant reductions ($p<0.005$) in TEC, Hb content. Ahmed *et al.* (2012) conducted a study to determine hematological parameters of *clarias gariepinus* from White Nile and Blue Nile River. The results showed that there was no significant difference in any parameter between samples from two rivers at ($p<0.05$) except Hb concentration.

Summarwar (2012) compared hematological parameters of *Clarias batrachus* from two sites of Pushkar Lake (polluted) and Bisalpur reservoir (control).The parameters showed adverse effect of pollution on hematological parameters of fish inhabiting Pushkar reservoir. The fish from Pushkar Lake when compared with those from Reservoir had lower Hb (38% and 12% respectively), RBC count (35% and 11%), PCV (21% and 7% respectively) and higher WBC counts (118% and 85% respectively).

WORK DONE IN KASHMIR

Gadru (1987) carried out a study on the Hematology of some of the fishes of Kashmir valley and found that *Botia birdi* has higher hemoglobin concentration and PCV value compared to other species.

Abida (2003) studied hematology of some helminth infected fishes of Wular Lake. It was observed that Hb, PCV and RBC content decreased due to infection resulting in Anemia, the severity of which depended upon the intensity of infection, while as Total leucocyte count, MCV and MCH value increased due to helminthic infection as required by the immune mechanism.

Qadri (2004) conducted a study to investigate the hematological parameters of three species of Schizothorax Heckel sampled from Sind stream (least polluted) and Dal lake (polluted water body).Reports from the study showed significant decrease in RBC count, Hemaglobin and Haematocrit value, while an increase in Total leucocyte count and MCV values were observed in fish from Dal Lake. The variation in values of different parameters can be attributed to exposure of fish to various types of pollutants like increasing concentration of nitrite in Dal Lake which leads to fluctuation in blood parameters.

Shah *et al.* (2009) while studying the impact of Helminth parasitism on Hematology of fishes from Anchar Lake reported that helminth infection produces macrocytic anemia with decreased RBC, increased Total leucocyte count and DLC content in fish.

Shahi (2009) while studying the hematology of fishes of Kashmir valley from two different habitats find significant decrease in RBC count, Hb and Ht value of fishes from Anchar Lake in comparison to those from River Jhelum due to higher concentration of Nitrite in Anchar Lake than in River Jhelum. Tramboo (2010) carried out a study on antioxidants of fish with special reference to their use as biomarkers of aquatic pollutants.

Mir (2011) studied the influences of organophosphate pesticides on reproductive activities of female common carp, *Cyprinus carpio communis*. Sareer (2011) compared hematobiochemical parameters of Schizothorax spp. From two sites of Lidder stream, upper Lidder (control) and lower Lidder (polluted).The parameters showed effect of pollution on hematological parameters of fish inhabiting lower Lidder. The fish from Lower Lidder when compared with those from upper Lidder had lower Hb, RBC count, PCV and higher WBC counts in all the three species of Schizothorax.

Habiba (2012) while exposing *Schizothorax niger* to various concentrations of Butachlor observed that both RBC count and Hb value were significantly higher in response to Butachlor exposure of 1.3 mg/l and 0.9mg/l respectively than the control groups. It was also reported that LC50 value of Butachlor was unexpectedly high for *S.niger* Heckel i.e 1.8mg/l, indicating that Butachlor is moderately toxic to the fish.

Khurshid and Fayaz (2012) carried out a study on the impact of helminth parasitism on hematological profile of Schizothorax spp. and Cyprinus Spp of Shallabugh wetland .Reports from the study showed significant decrease in RBC count and Hb value while there was an increase in WBC count which may be associated with the defense mechanism and immunological responses against infectious diseases caused by helminth parasites.

Ulfat *et al.* (2012) worked on the hemato biochemical parameters of common Carp in Kashmir. The reports from the study revealed that the total red blood cell count (RBC) was high in summer and low in winter, similarly the total white blood cell count (WBC) was high in spring and lowest in summer.

A perusal of the above review clearly reveals that not much work has so far been undertaken on the haematology of fish occurring in Kashmir Himalaya and the impact of the eutrophication and/or pollution of different waters of the region of the fish have also not received due attention. In this scenario the present work was undertaken.

Chapter -4

Material and Methods

The present work deals with the hematology of *Triplophysa marmorata* (Heckel). The fish is widely distributed in the freshwaters of Kashmir Himalaya occurring in both lotic and lentic habitats. For the present study the fish was collected from Wular Lake, Sind stream, and Telbal stream. The methods used for the detailed study of fish hematology are presented below under five broad heads. In order to have an idea about the ecology of the habitats from where the fish were collected, important physico-chemical parameters of water were also analyzed. The sampling of the water was done at the time of fish collection itself.

Fish Haematology

4.1. Collection of fishes

4.2. Sampling of blood

4.3. Hematology of *Triplophysa marmorata* Heckel

4.4. Physico-chemical analysis of water.

4.5. Statistical analysis of the results

4.1) Collection of fishes

For the collection of the fish a thorough survey of the study area was done. Fishes were collected with the aid of local fisherman from Wular Lake at Ningli, Telbal stream just above its entry in to the Dal lake (near Dagi Mohalla) and with the help of battery-powered electro fisher, and collected in a hand-held seine or dip net in Sind stream at Haripora area. The blood samples were taken either from live specimens on the spot or live fish specimens were brought from the site to the laboratory in plastic buckets. To overcome the physiological stress during transportation fish were transferred into large containers or glass aquaria and allowed to acclimatize for about two to three hours in the laboratory before blood samples were taken.

4.2) Collection of blood samples

The fishes collected from the study sites were either bled alive or narcotized, using 40% ethanol, which was applied with a cotton tampon. The tampon was put on the gills under the opercular cover as recommended by Lucky (1977). The length and the weight of fishes were recorded before the collection of the blood. The blood was mainly collected by severing the caudal peduncle. Care was taken to prevent the blood from coming in contact with water. Blood was also drawn out with a syringe from the heart by stabbing body wall exactly in midline from the posterior margin of opercular cover and directed dorso-caudally at an angle of 45^{o} (Lucky, 1977). The collection of blood was completed within 24 hrs of the capture of the fish. Part of the blood sample was used directly to make smears on clean and dry slides for staining. For determining haematocrit, samples were collected in glass vials containing EDTA as anticoagulant at an approximate concentration of 5mg/ml of blood (Blaxhall & Daisley, 1973).

Plate 6: *Triplophysa marmorata*

4.3) Hematological parameters

Blood parameters are considered patho-physiological indicators of the whole body and therefore are important in diagnosing the structural and functional status of fish exposed to toxicants.

4.3.1) Estimation of Hemoglobin concentration:

The hemoglobin was estimated by Cyanomethemoglobin method recommended by International Committee for Standardization in Hematology (ICSH 1978).In this method, the alkaline solution, Ferricyanide converts hemoglobin ferrous (Fe^{2+}) iron to the ferric (Fe^{3+}) state to form methemoglobin.Methemoglobin reacts with potassium cyanide to form Cyanomethemoglobin. The color developed was measured spectrophotometrically at 540 nm (Wharton and McCarty, 1972; Blaxhall and Daisley, 1973).

Drabkin's solution used was prepared by mixing the following reagents in the given proportion:

Sodium bicarbonate : 1.0 g.

Potassium cyanide : 0.05 g.

Potassium ferricaynide : 0.2 g.

Distilled water : 1000 cc.

Calculation:

$$Hb\ (g/100ml) = \frac{A_{540}\ \text{test sample} \times 15.06\ (\text{Std .conc .as stamped on the vial} \times 0.251\ )}{A_{540}\ \text{standard}}$$

4.3.2) Total erythrocyte count

Red blood cell count estimates the total number of red blood cells in a cubic millimetre of blood. An improved Neubaurs chamber was used for counting RBC (Baker and Silverson, 1982). The Hayem's dilution fluid which was used had following composition:

Mercuric Chloride ($HgCl_2$) : 0.5g

Sodium Chloride (Nacl) : 1.0g

Sodium Sulphate (Na_2SO4) : 5.0g

Distilled water (H_2O) : 200ml

Blood was drawn up to the 0.5mark in the RBC Pipette. The tip of the pipette was cleared and RBC dilution fluid was drawn up to 101mark.The resulting solution was shaken for 3 minutes. The first few drops of the solution were discarded and then chamber was loaded by one or two drops of blood solution. RBC was counted under a compound microscope, using 10x (eye piece) and 40x (objective).After counting the RBCs in smallest 80 squares, calculations were made.

Calculation

$$\text{RBC count} = \frac{\text{Number of cells counted x dilution factor x depth of chamber}}{\text{Area counted}}$$

Where dilution factor is one in 200, depth is 1/10mm and area counted = 80/400 = 1/5 sq.

$$\text{RBC count} = \frac{\text{Number of cells counted} \times 200 \times 10}{\frac{1}{5}}$$

RBC /cu.mm. = number of cells counted x 10,000

4.3.3) Total leucocyte count:

A white cell count (TLC) estimates the total number of white cells in a cubic millimeter of blood. WBC diluting fluid or Turk' fluid contains a weak acid to lyse the red blood cells and Gentian violet stain for staining the nucleus of White blood cells. This was done in the same manner as the RBC count was done. Turk's WBC dilution fluid was used which had the following composition:

Glacial acetic acid (CH_3COOH) : 1.5 ml

1% aqueous solution of Gentian violet : 1ml

Distilled water : 100ml

Neubaur's haemocytometer (Baker and Silverson, 1982) was used for counting leucocytes. The blood was sucked up in the WBC Pipettes up to the 0.5mark and then WBC dilution fluid was drawn up to the 11 mark of pipette. Solution was mixed gently and bubbling was avoided. The Neubaur's chamber was charged by the resulting mixture. The cells were counted under a compound microscope, using 10x (eye piece) and 40x (objective).

$$\text{TLC} = \frac{\text{Cells counted} \times \text{blood dilution} \times \text{chamber depth}}{\text{Area of chamber}}$$

$$\text{TLC} = \frac{\text{Cells counted} \times 20 \times 10}{4}$$

TLC/cu.mm = Cells counted × 50

4.3.4) Differential Leucocytes count

A thin blood film was made by spreading a blood drop evenly on clean grease free slide using smooth edged spreader. Giemsa's and Leishman's stains were employed for the staining of blood films. For Giemsa's staining the blood smears were prefixed with acetone free methanol. The stains used had following composition.

Giemsa's stain

Giemsa powder : 0.3g

Glycerin : 25.0ml

Acetone free methyl alcohol: 25.0 ml

Leishman's stain:

Powdered Leishman's stain : 0.15 g

Acetone fee methyl alcohol : 133ml

4.3.5) Hematocrit or Packed cell volume:

This was obtained by centrifuging blood (containing 5mg/ml EDTA) in a graduated tube until corpuscles were packed down to a constant volume. The volume of packed cell was then expressed as a percentage of the original volume of blood. With the aid of capillary pipette a Wintrobe's haematocrit tube was filled to the 100 mark with the anticoagulated blood and centrifuged for 5-10 min at ~ 7,000 RPM. As the original column of blood in the tube is 100 mm long, the volume of packed cell is read directly as percentage. The analysis was done according to England *et al.* (1972).

4.3.6) Erythrocyte Indices:

Wintrobe (1974) introduced calculation for determining the size, content and Hemoglobin concentration of red cell. These erythrocyte indices were found very useful in the morphological characterization of anemia.

Mean cell volume (MCV):

The MCV is the average volume of red cells and was calculated from the haematocrit (Hct, Packed cell volume) and Red cell count (TRBC).

$$\text{MCV} = \frac{\text{PCV (\%)} \times 10 \text{ cubic microns}}{\text{RBC (millions /}\mu\text{l)}}$$

Mean cell Hemoglobin (MCH):

The MCH is the content (weight) of the Hb of the average red cell. It was calculated from the Hb concentration and red cell count.

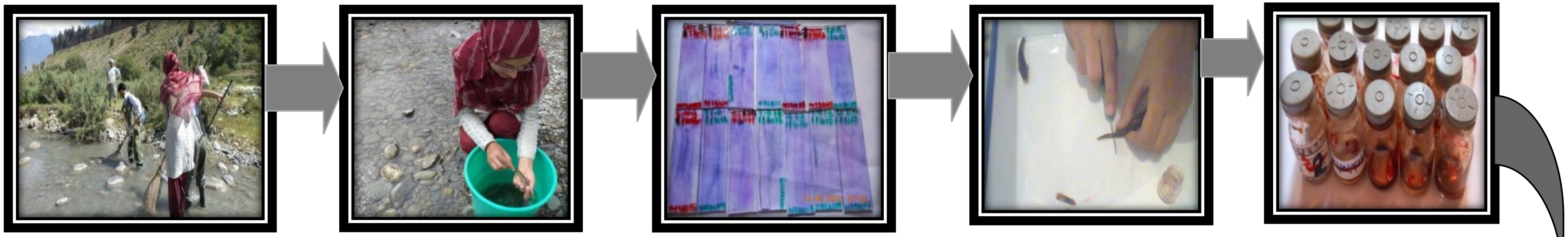
$$\text{MCH} = \frac{\text{Hb (g/dl)} \times 10 \text{ micrograms}}{\text{RBC (millions /}\mu\text{l)}}$$

Mean cell Hemoglobin concentration (MCHC):

The Mean hemoglobin concentration in g% for 100 ml erythrocytes was calculated by following formula:

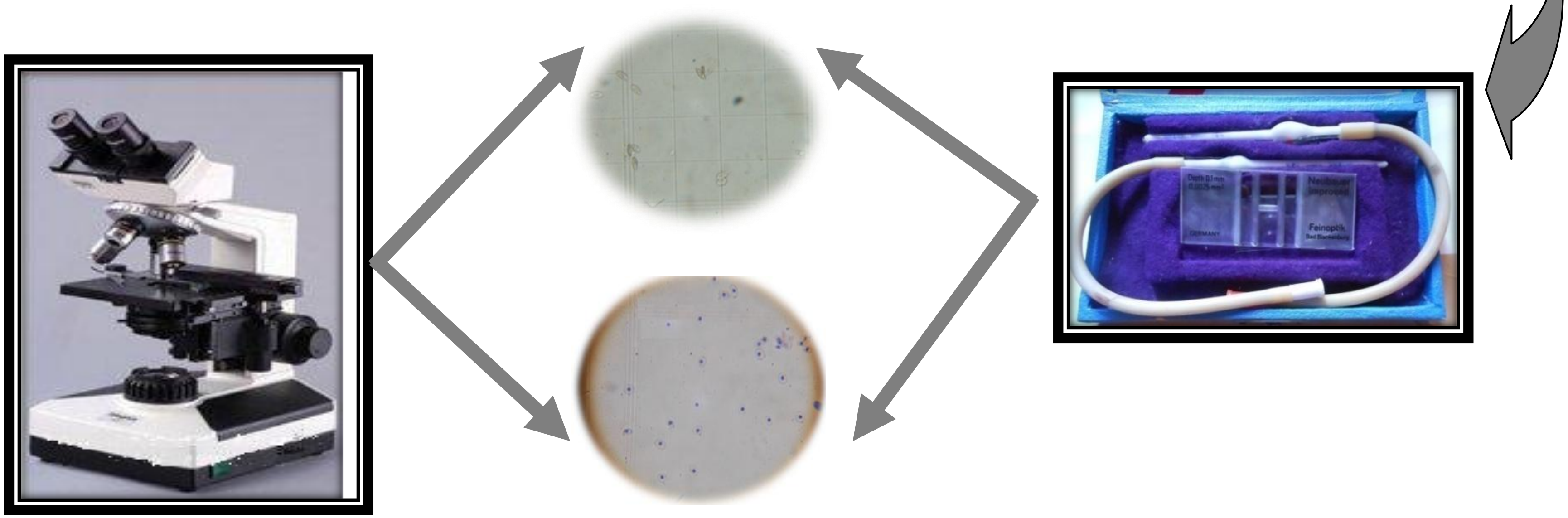
$$\text{MCHC} = \frac{\text{Hb (g/dl)} \times 100 \text{ ml}}{\text{PCV (\%)}}$$

Procedure involved in carrying out the Hematological Study of *T. marmorata*



Collection of Fishes with the help of Electrofisher and taking Blood Samples for making DLC Slides

Collection of Blood in EDTA Vials Severing the Caudal Peduncle of *T.marmorata*



Neubar`s Haemocytometer used for counting RBC`s and WBC`s

4.4) PHYSICO-CHEMICAL ANALYSIS OF WATER

For physico-chemical analysis, water samples were collected from sites namely Ningli, Haripora and Telbal stream from March 2012–December 2012. The water samples were collected in polythene bottles just below the surface of water during morning hours. Temperature was recorded on spot. DO was fixed in stoppered glass bottles on spot. Analysis of other parameters was done in the laboratory within 24 hrs in accordance with APHA (1998) and CSIR (1974). The methods are listed in the following Table (4.1).

Table: 4.1

S No	Parameter	Method	References
1	Temperature	Celsius thermometer	-
2	pH	Digital pH meter	-
3	Dissolved oxygen	Azide modification of Winkler's method	APHA (1998)
4	Free CO_2	Titrimetric method	APHA (1998)
5	Total Alkalinity	Titrimetric method	APHA (1998)
6	Ammonical Nitrogen	Phenate method	APHA (1998)
7	Nitrate Nitrogen	Salicylate method	CSIR (1974)
8	Total Phosphorus	Stannous chloride method	APHA (1998)
9	Ortho Phosphorus	Stannous chloride method	APHA (1998)

4.5) Statistical Analysis

The whole data was fed into a micro soft excel 2007. A computer program (SPSS 12.0 for windows) was used for data analysis. The descriptive data was given as a mean ± standard deviation (S.D).

Chapter -5

Results and Discussion

Blood analysis is crucial in many fields of Ichthyological research and fish farming and in the area of toxicology and environmental monitoring. It is an indicator of physiological or pathological changes in fishery management and disease investigations (Adedeji *et al.*, 2009). Hematological studies have often been used in fishery research to comprehend the adaptations of fish to its environment as a single drop of blood is sufficient to get information regarding their physiological status as well as the health status (Atkinson and Judd, 1978). The fish hematology is emerging as a useful tool in monitoring fish health. The blood of *T. marmorata*, as that of any other vertebrate, comprises plasma and cellular components. The plasma consists of water (about 97%), dissolved salts, electrolytes and hormones. The cellular components include erythrocytes (RBC), leucocytes (WBC) and thrombocytes. The variation in the values of different components forms the basis of diagnosis for the health of the fish. The hematological profile of *T. marmorata* was investigated from March, 2012 to December, 2012. During this period, various physico-chemical parameters too were analyzed. The main aim of the present study was to describe the hematology of the loach in three different types of habitat, viz., (i) a fast flowing and (ii) a slow flowing lotic habitat and (iii) a lentic habitat.

5.1) Ecological Features of the water bodies

5.1.1 WATER TEMPERATURE

The water temperature of the three water bodies at the time of fish collection ranged from 9°C to 26 °C. The minimum temperature of 9°C was observed at Wular Lake (Table 5.1) during spring season and the highest of 26 °C was observed at Sind stream (Table 5.2) during summer season. The mean water temperature recorded in Wular Lake was 16.33°C while it was 20.66°C in Sind stream and 17.00°C in Telbal (Table 5.4).

5.1.2 pH

The pH of the three water bodies ranged from 7.4 to 8.3. The minimum pH of 7.4 was observed in Wular Lake during the autumn season (Table 5.1) and maximum pH of 8.3 was observed in the same season (Table 5.3) at Telbal. The mean pH in the Wular Lake was 7.73, while it was 8.1 in Sind stream, and 8.0 in Telbal (Table 5.4).

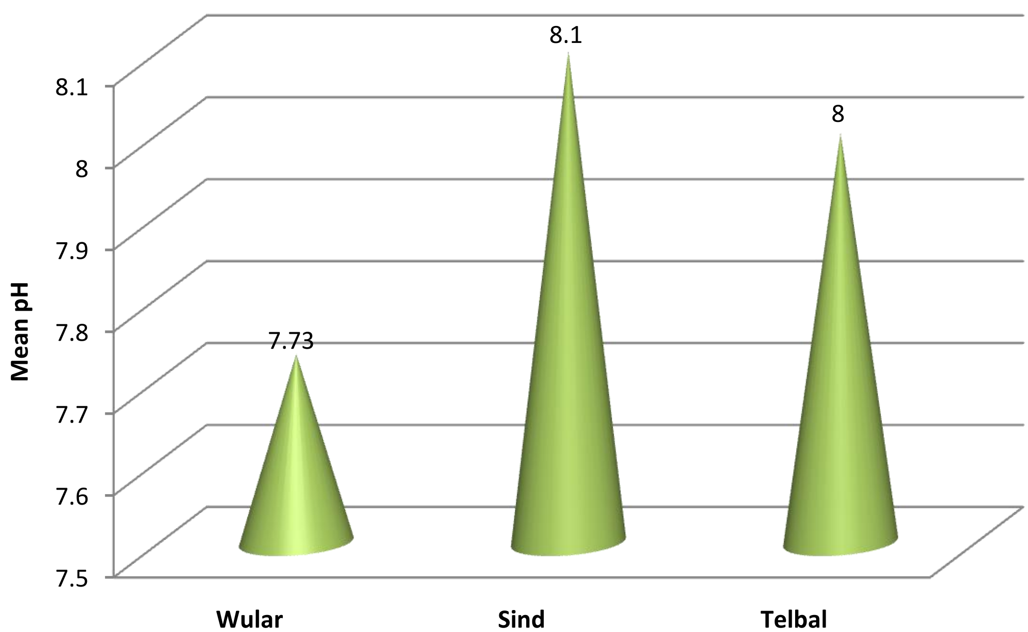


Fig 5.1: Mean variations in the pH values of the three water bodies

5.1.3 DISSOLVED OXYGEN (mg/l)

The lowest concentration of dissolved oxygen (7 mg/l) was seen in Wular Lake i.e., (Table 5.1) in the summer season, while the highest concentration (8.8 mg/l) was seen at Sind (Table 5.2) in the spring season. The mean concentration of dissolved oxygen was 7.56 mg/l in Wular Lake; while it was 8.13mg/l in Sind stream and 7.43mg/l in Telbal (Table 5.4).

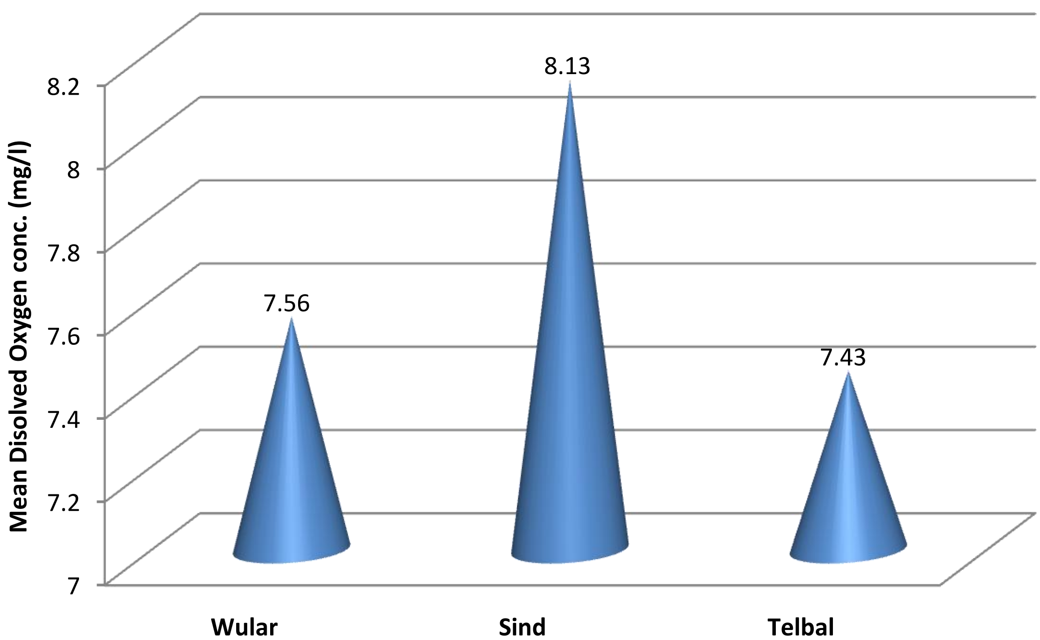


Fig. 5.2: Mean variations in the Dissolved oxygen values of the three water bodies

5.1.4 Free CO_2 (mg/l)

The free CO_2 concentration in three water bodies ranged from 7 mg/l to 14 mg/l. The mean concentration of free CO_2 was 12 mg/l in Wular Lake, while it was 8.33mg/l in Sind stream and 12.66 mg/l at Telbal. (Table 5.4).

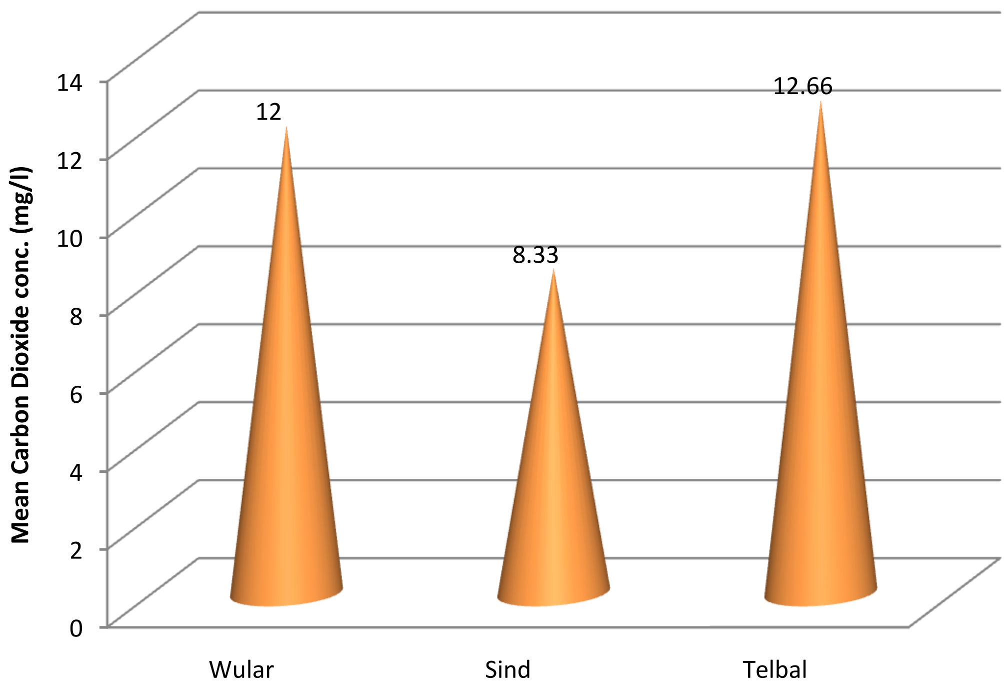


Fig. 5.3: Mean variations in the free carbon dioxide values of the three water bodies.

5.1.5 Total Alkalinity (mg/l)

The total Alkalinity in the three waters ranged from 50mg/l to 175 mg/l. The mean concentration of total Alkalinity was 139mg/l in Wular Lake, in Sind stream was 66mg/l, while in Telbal it was 161 mg/l (Table 5.4).

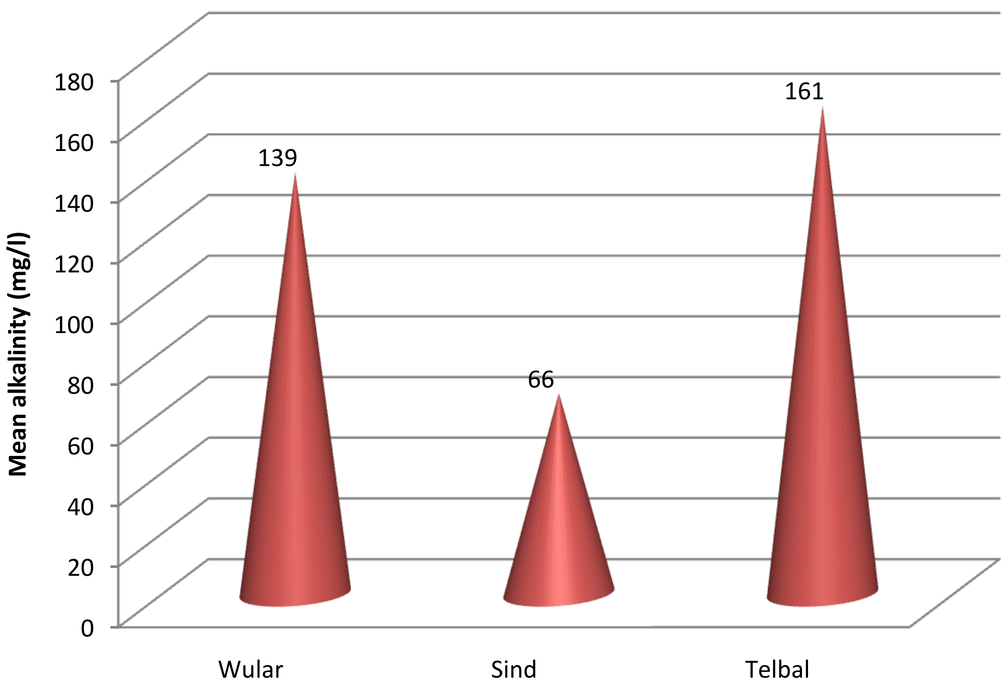


Fig 5.4: Mean variations in the Total Alkalinity values of the three water bodies

5.1.6 Ammonical Nitrogen (µg/l)

The lowest concentration of Ammonical nitrogen (17 µg/l) was recorded at Sind in summer (Table 5.2), while the highest concentration of 102µg/l was recorded at Wular Lake in spring (Table 5.1). The mean concentration of Ammonical nitrogen was 90.33µg/l in Wular Lake, 22µg/l in Sind stream, while at Telbal stream it was 60.33µg/l (Table 5.4).

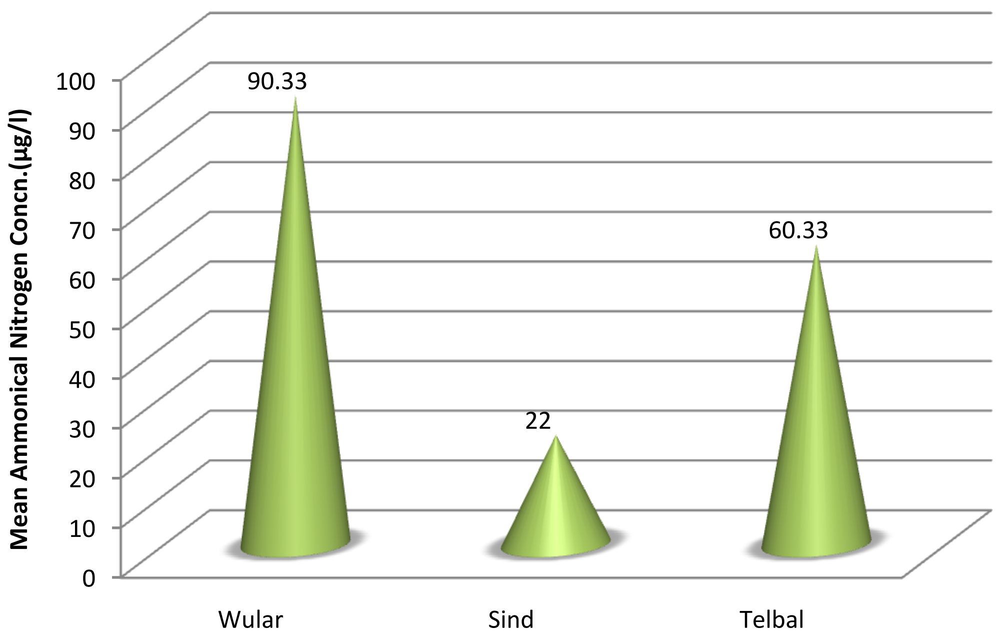


Fig. 5.5: Mean variations in the Ammonical nitrogen values of the three water bodies

5.1.7 Nitrate Nitrogen (µg/l)

Nitrate Nitrogen content was lowest (119µg/l) at Sind stream in the spring (Table 5.2), while the highest content of 230µg/l was recorded at Wular Lake in autumn (Table 5.1). The mean concentration of Nitrate nitrogen was 193.33µg/l in Wular Lake, 153µg/l in Sind stream, while it was 167µg/l at Telbal (Table 5.4).

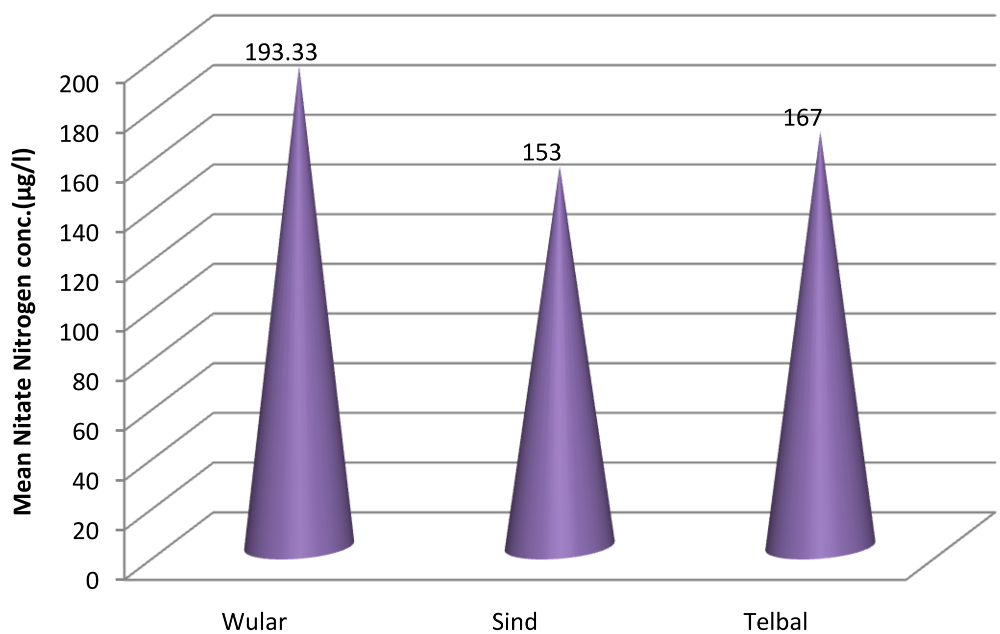


Fig. 5.6: Mean variations in the Nitrate nitrogen values of the three water bodies

5.1.8 Ortho phosphorus (µg/l)

The ortho phosphorus concentration was lowest (15µg/l) at Sind stream in spring (Table 5.2), while the highest concentration (123µg/l) was recorded at Telbal in summer (Table 5.3). The mean concentration of ortho-phosphorus was 46.66µg/l in Wular Lake, 26.33µg/l in Sind stream, while it was 87µg/l in Telbal (Table 5.4).

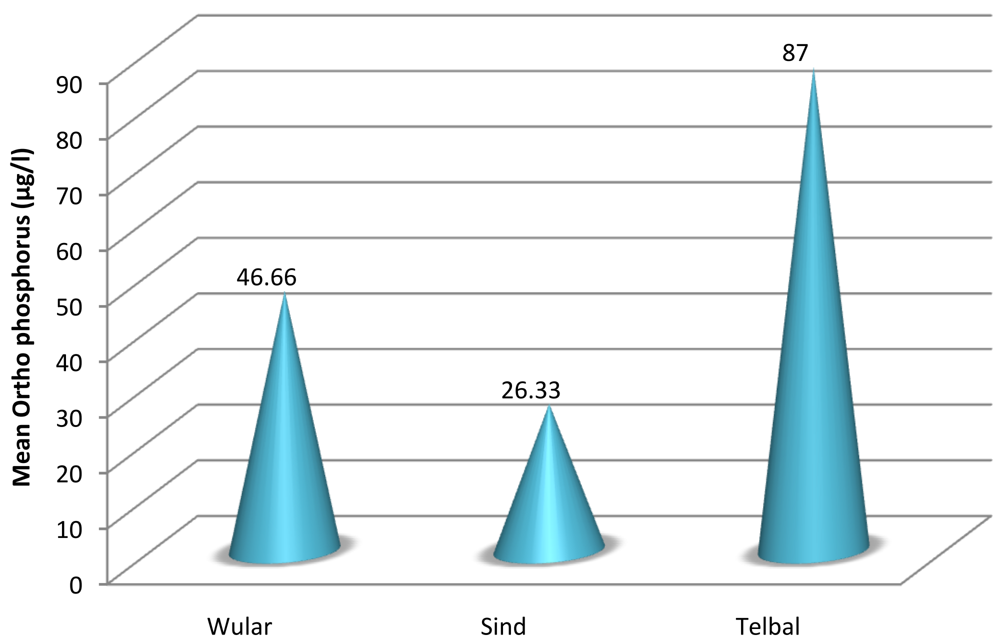


Fig. 5.7: Mean variations in the Ortho phosphorus values of the three water bodies

5.1.9 Total phosphorus (µg/l)

The total phosphorus concentration was lowest (30µg/l) at Sind stream in spring season (Table 5.2), while the highest concentration of 243µg/l was recorded at Wular Lake in autumn season (Table 5.1). The mean concentration of total phosphorus was 205.66 µg/l in Wular Lake and 44µg/l in Sind stream, while it was 166.33µg/l in Telbal (Table 5.4).

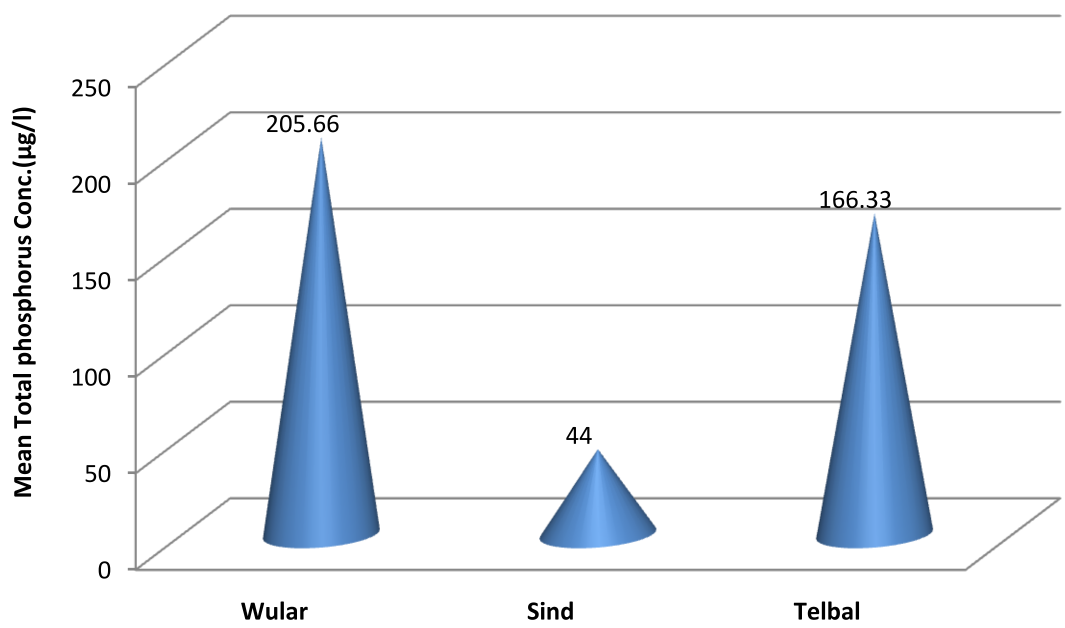


Fig. 5.8: Mean variations in the Total phosphorus of the three water bodies

On the whole, Free CO_2, Total alkalinity, Ammonical nitrogen, Nitrate nitrogen, Ortho-phosphorus and Total phosphorus was comparatively higher in Wular Lake and Telbal stream than Sind Stream. Discharge of untreated Sewage and agricultural runoff is the main source of nutrient load in these waters.

Table 5.1

Some important physico-chemical parameters of the Wular Lake

Parameters	Spring	Summer	Autumn
Water Temp. °C	9	23	17
pH	8.1	7.7	7.4
Dissolved Oxygen(mg/l)	7.6	7.0	8.1
Free Co_2 (mg/l)	12	14	10
Alkalinity(mg/l)	160	125	132
Ammonical-Nitrogen(µg/l)	102	80	89
Nitrate-Nitrogen(µg/l)	150	200	230
Orthophosphorus(µg/l)	31	44	65
Total phosphorus(µg/l)	183	191	243

Table 5.2

Some important Physico-chemical parameters of Sind Stream

Parameters	**Spring**	**Summer**	**Autumn**
Water Temp. °C	12	26	24
pH	8	8.2	8.1
Dissolved Oxygen(mg/l)	8.8	8.4	7.2
Free Co_2 (mg/l)	7	8	10
Alkalinity(mg/l)	50	78	70
Ammonical-Nitrogen (µg/l)	23	17	26
Nitrate-Nitrogen (µg/l)	119	130	210
Orthophosphorus(µg/l)	15	26	38
Total phosphorus(µg/l)	30	44	58

Table 5.3

Some important physico-chemical parameters at Telbal

Parameters	**Spring**	**Summer**	**Autumn**
Water Temp. ^oC	15	20	16
pH	8.1	7.6	8.3
Dissolved Oxygen(mg/l)	7.8	7.1	7.4
Free Co_2 (mg/l)	11	14	13
Alkalinity(mg/l)	140	168	175
Ammonical-Nitrogen (µg/l)	49	35	97
Nitrate-Nitrogen (µg/l)	126	155	220
Orthophosphorus(µg/l)	72	123	66
Total phosphorus(µg/l)	167	194	138

Table 5.4

Some important physico-chemical parameters (Mean ± S.D) of the three Water Bodies

Parameters	**Wular**	**Sind**	**Telbal**
Water Temp. ^{o}C	16.33±7.02	20.66±7.57	17±2.64
pH	7.73±0.35	8.1±0.1	8.0±0.36
Dissolved Oxygen(mg/l)	7.56±0.55	8.13±0.83	7.43±0.35
Free Co_2 (mg/l)	12±2	8.33±1.52	12.66±1.52
Alkalinity(mg/l)	139±18.52	66±14.42	161±18.52
Ammonical-Nitrogen (µg/l)	90.33±11.06	22±4.58	60.33±32.51
Nitrate-Nitrogen (µg/l)	193.33±40.41	153±49.66	167±48.13
Orthophosphorus (µg/l)	46.66±17.15	26.33±11.50	87±31.32
Total phosphorus (µg/l)	205.66±32.57	44±14	166.33±28.00

5.2) Hematology of *T. marmorata*

The blood of Fish from three water bodies was screened for various hematological parameters. Before the collection of the blood sample, the length and weight of fish were also noted.

5.2.1 Length of fish (mm)

The mean length of *T. marmorata* collected from Wular Lake was found to be 90.0±6.20 (lowest) and 124.3±5.8 mm (highest) of Sind, while it was 93.10± 15.0mm from Telbal (Table 5.8 and Fig 5.9).

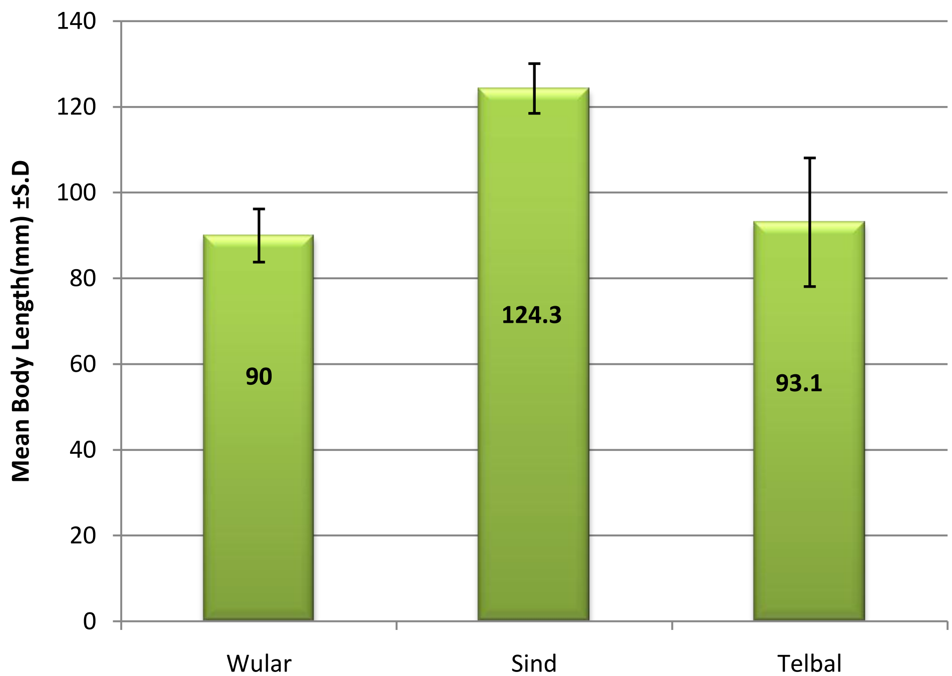


Fig 5.9: Length of *T. marmorata* from the three water bodies

5.2.2 Weight of Fish (g)

The fish samples collected during the present study varied in mean weight from 6.43g in wular lake to 13.68 g in Sind stream .The mean weight of the fish collected from Wular Lake was found to be 6.43±0.86g, while that for the fish collected from Sind was 13.68±2.28g and from Telbal it was 6.63± 2.16g (Table 5.8 and Fig 5.10). The data clearly showed that fish from the Sind were the heaviest of all.

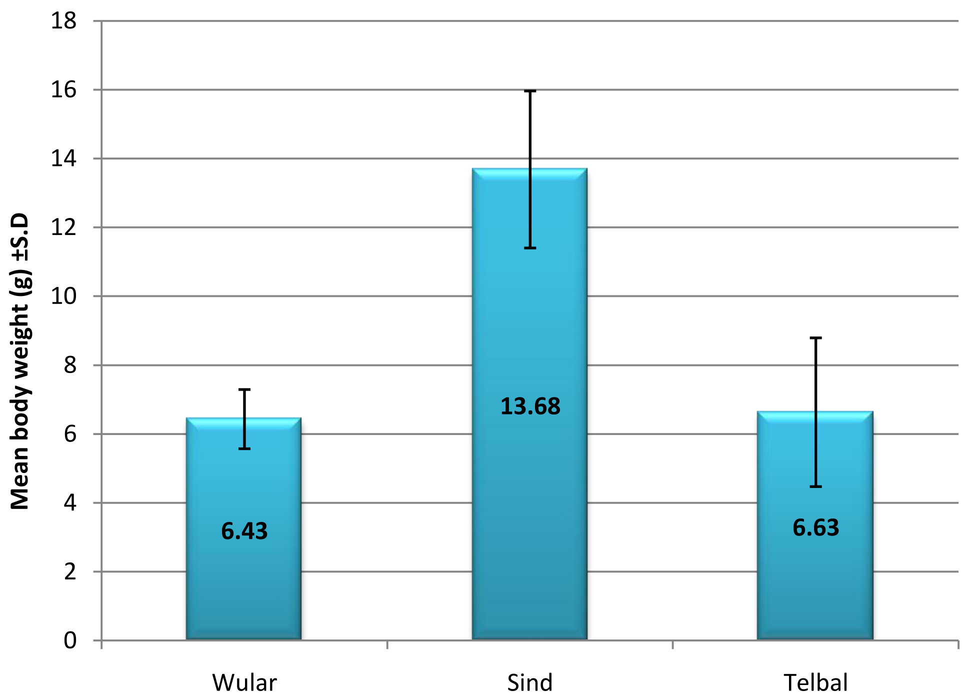


Fig 5.10: Mean Weight of *T. marmorata* from the three water bodies

5.2.3 Hemoglobin (g/dl):

The mean hemoglobin value in the fish varied from 9.65±0.91 (lowest) in case of samples from Telbal stream to 10.43±0.10 (highest) in case of the fish from Sind stream(Table 5.8 and Fig 5.11). The differences in the Hb value of the fish from three water bodies were not statistically significant (ANOVA: F= 2.83; p=0.09 i.e. p>0.05).

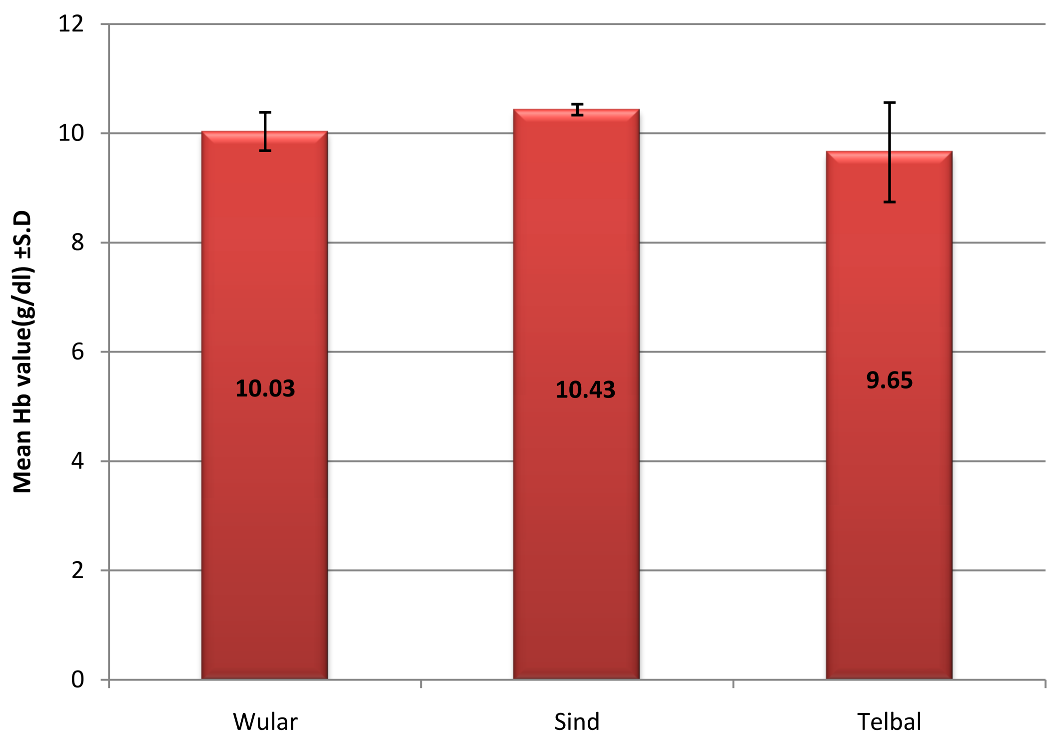


Fig 5.11: Hemoglobin content of *T. marmorata* from the three water bodies

5.2.4: RBC COUNT ($10^6/mm^3$)

The RBC value of the fish from Wular Lake was found to be comparatively lower than the RBC value of fish from Sind stream and Telbal (Table 5.8).The mean RBC value in *T. marmorata* from Wular Lake was 1.14±0.02, while it was 1.37±0.01 in case of those from Sind stream and 1.19±0.01 in fish from Telbal (Table 5.8 and Fig 5.12). The differences in RBC value of the fish from the three water bodies were statistically significant (ANOVA: F=303.32; p=0.000 i.e. p<0.001).

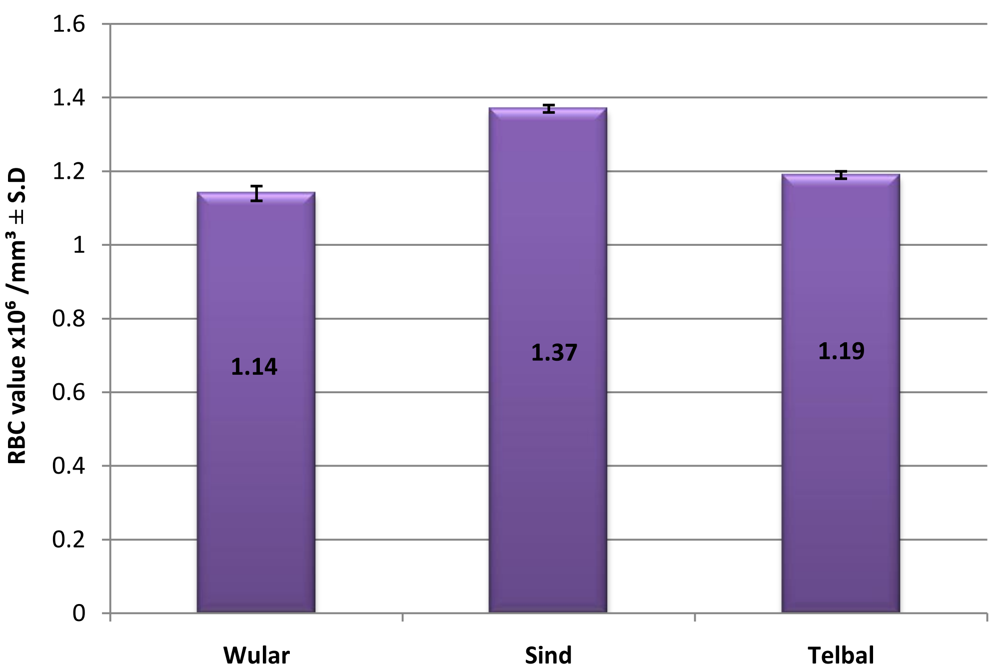


Fig 5.12: RBC count of *T. marmorata* from the three water bodies

5.2.5 Packed cell volume (PCV) (%)

PCV or Haematocrit (Hct) expresses the volume of RBCs in 100 ml of whole blood. Any deviation from its normal values can lead to various pathological conditions. The mean packed cell volume in the samples from Wular Lake was found to be 21.09±0.81 (lowest), while it was 26.61±0.55 (Highest) in those from Sind stream and 21.54±0.79 in those from Telbal (Table 5.8 and Fig 5.13). The differences in PCV value of the fish from three water bodies were statistically significant (ANOVA: F= 105.42; p=0.000 i.e. $p<0.001$).

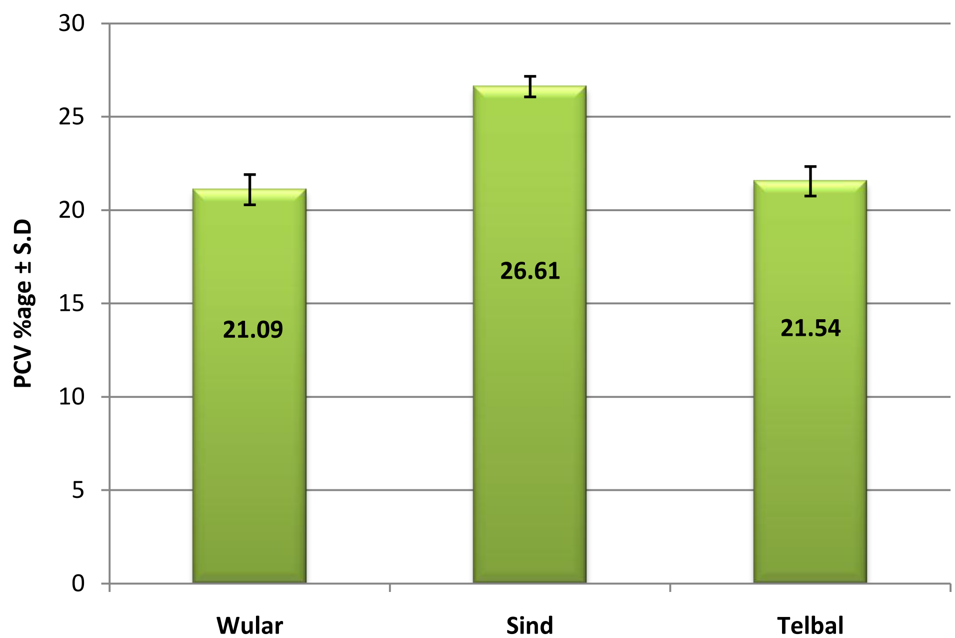


Fig 5.13: Packed cell volume of *T. marmorata* from the three water bodies

5.2.6 WBC: ($10^4/mm^3$)

Leucocytes or white blood cells (WBCs) are the defense cells of the body which provide protection to the organism against environmental as well as anthropogenic stress. The total number of leucocytes per cubic millimetre (TLC) is a diagnostic feature of many diseases. Rise in WBC count from normal range is called leucocytosis, while their fall represents leucopenia. The WBC count of fish caught from Wular Lake was higher than the fish from Sind stream as well as those from Telbal. The mean WBC count in the fish from Wular Lake was 55.10±2.51. In case of the fish from Sind stream it was 40.34±1.07 of Sind stream, while in case of fish from Telbal it was 40.79±0.56 (Table 5.8 and Fig 5.14). The differences in WBC value of the fish from three water bodies were statistically significant (ANOVA: F= 162.77; p=0.000 i.e. p<0.001).

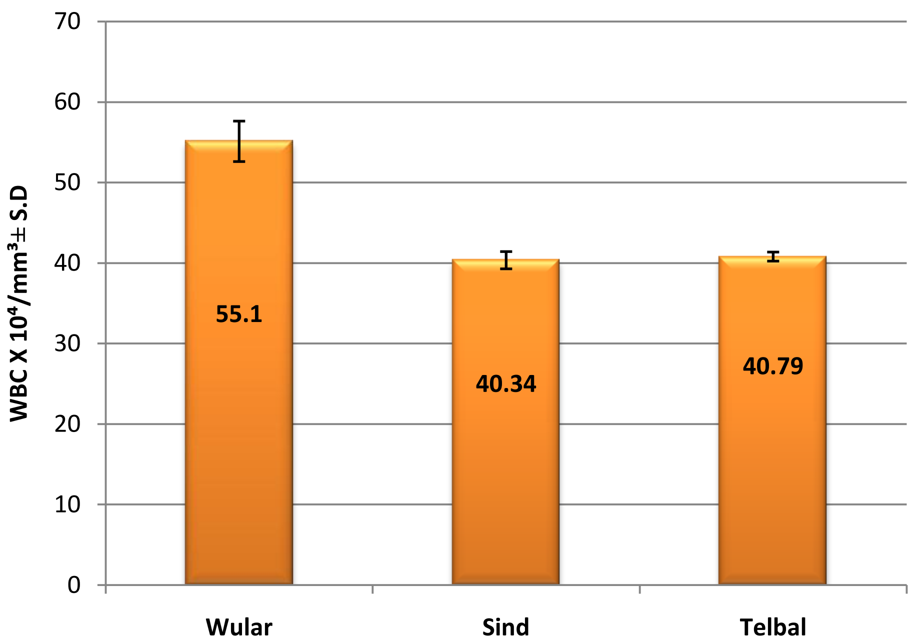


Fig 5.14: WBC count of *T. marmorata* from the three water bodies

5.2.7 Mean Corpuscular Volume (MCV μm^3)

MCV is the measure of average volume of RBCs in the whole blood. While increase in MCV indicates red blood cell swelling (macrocytosis), its decrease depicts RBC shrinkage (microcytosis). The mean MCV value in the fish from Wular Lake was 178.87±13.22, while in case of those from Sind stream it was 194.27±1.49 and in case of Telbal it was 177.71±6.31 (Table 5.8 and Fig 5.15). The differences in MCV value of the fish from three water bodies were statistically significant (ANOVA: F=7.095; p=0.007 i.e. $p<0.01$).

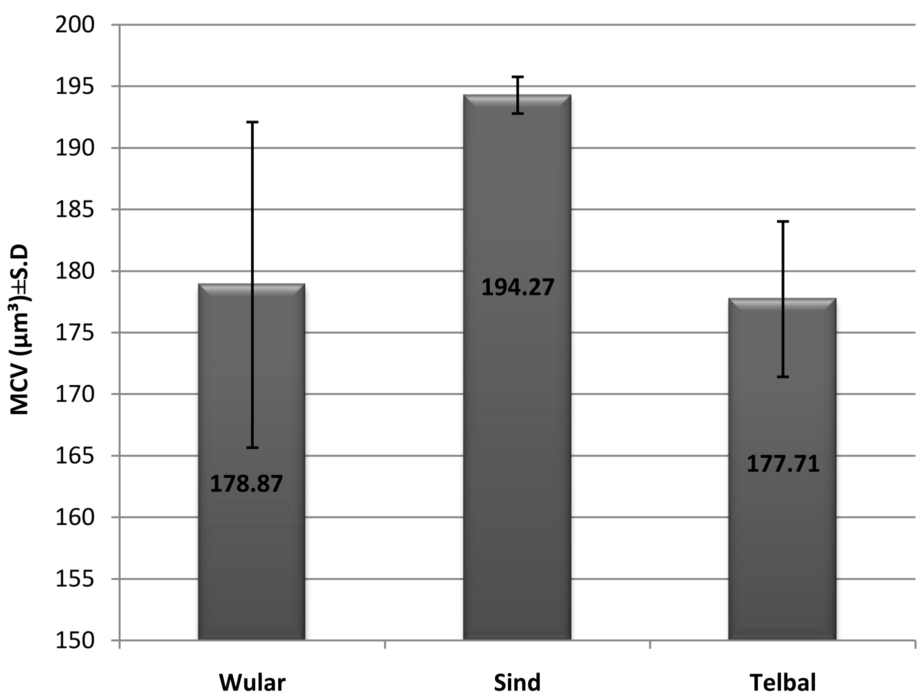


Fig 5.15: MCV value of *T. marmorata* from the three water bodies

5.2.8 Mean Corpuscular Hemaglobin (MCH µg)

MCH is the average hemoglobin content in a red blood cell. The MCH value of *T. marmorata* was higher in Wular Lake compared to Sind stream and Telbal (Fig 5.16). The mean MCH value was 88.16±1.94 in *T. marmorata* of Wular Lake and 75.89±0.72 in that from Sind stream, while it was 80.10±8.80 in case of fish from Telbal (Table 5.8 and Fig 5.16). The differences in MCH value of the fish from three water bodies were statistically significant (ANOVA: F=8.53; p=0.003 i.e. $p<0.01$).

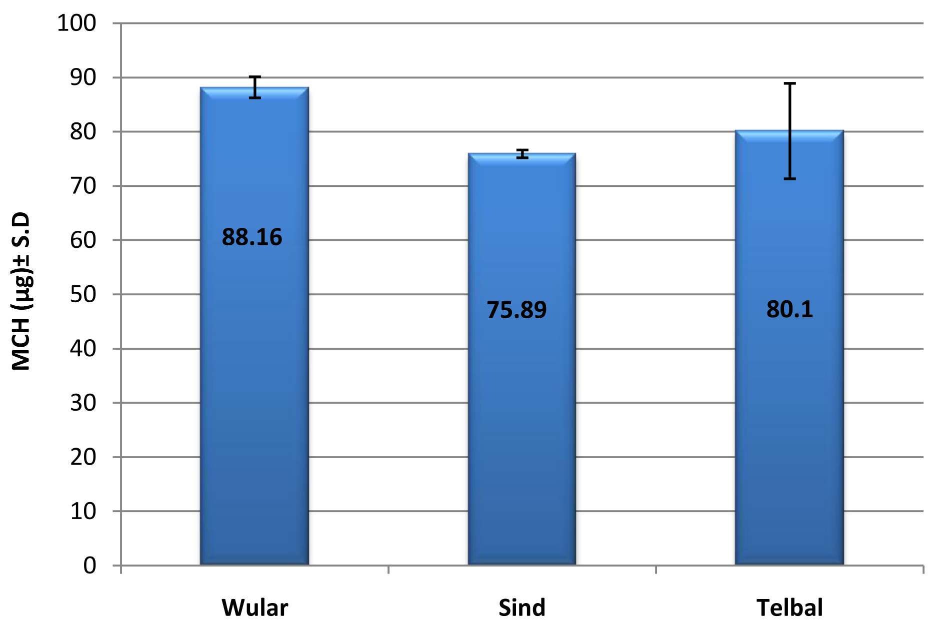


Fig 5.16: MCH value of *T. marmorata* from the three water bodies

5.2.9 Mean Corpuscular Hemoglobin Concentration (MCHC %)

MCHC is the measure of average concentration of hemoglobin in a given volume of blood. The mean MCHC value of *T. marmorata* was highest in case of fish from the Wular Lake as compared to Sind stream and Telbal (Fig 5.17).The mean value was 46.08±2.42, 39.16±0.58 and 43.90±3.14 in case of Wular lake, Sind stream and Telbal respectively (Table 5.8 and Fig 5.17). The differences were statistically significant (ANOVA: $F=14.001$; $p=0.000$ i.e. $p<0.001$).

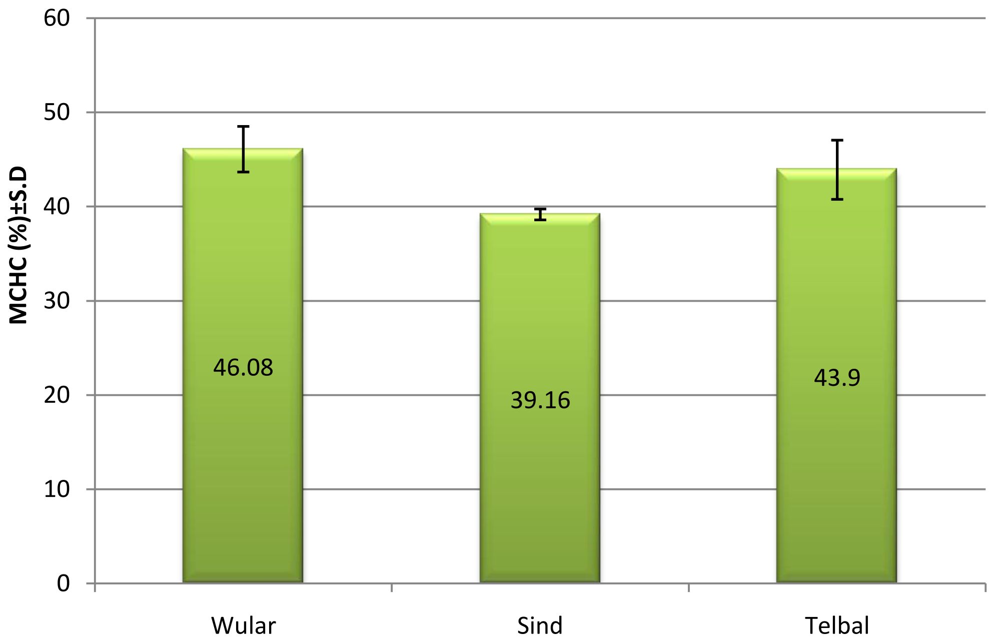


Fig 5.17: MCHC value of *T. marmorata* from the three water bodies

5.2.10 DLC

Lymphocytes are the most abundant and constitute about 64-71% of the Total Leucocytes and provide protection against entry of various antigens by production of antibodies. Any increase (lymphocytosis) or decrease (lymphophenia) in their normal values is indicative of infection. The mean Lymphocyte content was 64.16±4.62 in the fish from the Wular Lake and 71±5.86 of Sind stream, while it was 68±6.54 in the fish from the Telbal stream (Table 5.8 and Fig 5.18). The differences in lymphocyte value of the fish from three water bodies were statistically insignificant (ANOVA: $F=2.142$; $p=0.152$ i.e. $p>0.05$). Neutrophils constitute about 25-31% of total leucocytes in the fish. These are the chief phagocytic cells of the body and engulf foreign particles by phagocytosis. The neutrophil content in case of Wular fish was found to be the highest. The mean neutrophil content was 30.5±3.93 and 25.33±3.88 and 26.5±6.59 in fish from Wular, Sind and Telbal respectively (Table 5.8 and Fig 5.18). Monocytes, eosinophil and basophil contributed about 4 to 6% of the total WBC count.

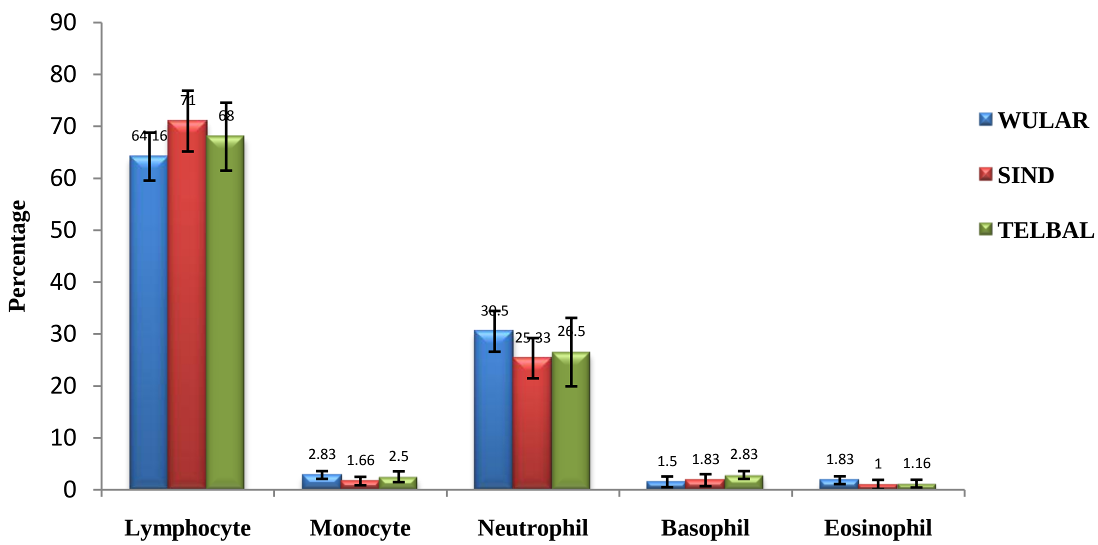


Fig 5.18: DLC of *T. marmorata* from the three water bodies

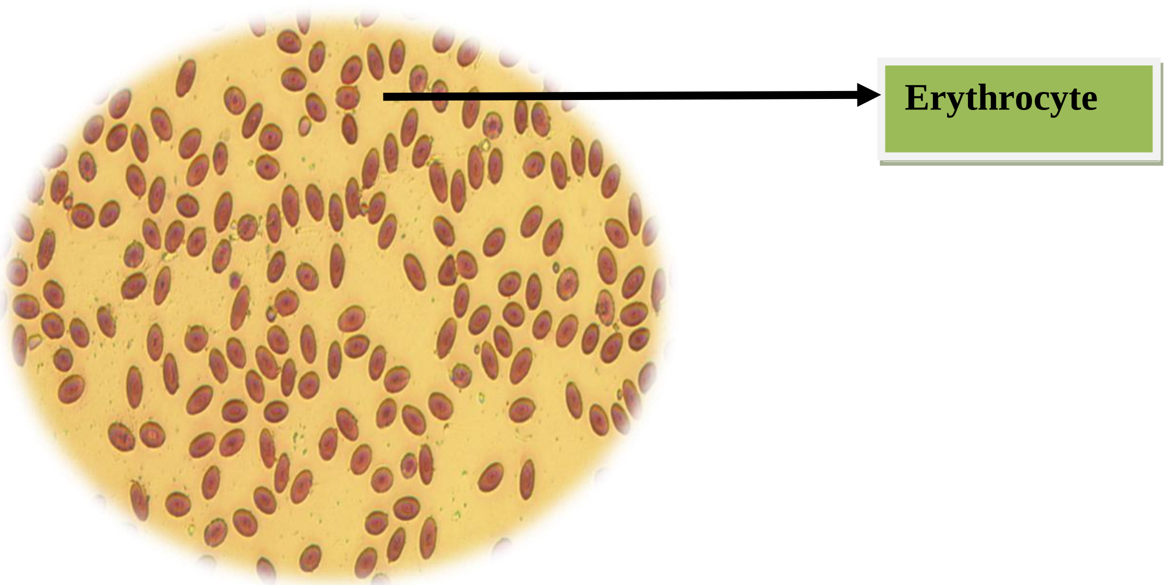


Plate 7: Erythrocyte

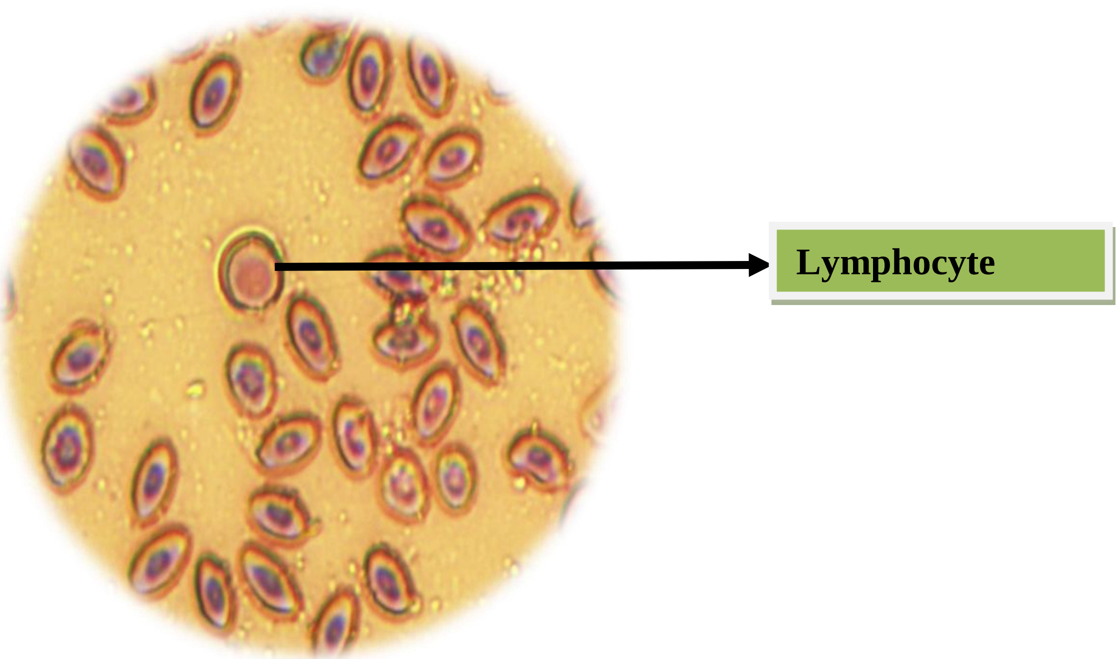


Plate 8: Lymphocyte

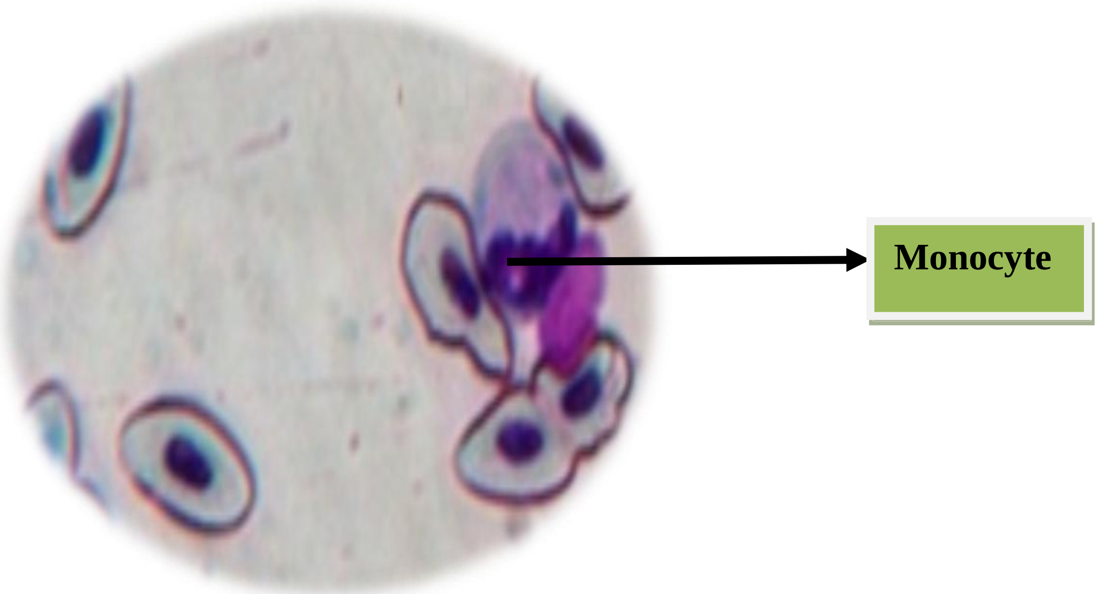


Plate 9: Monocyte

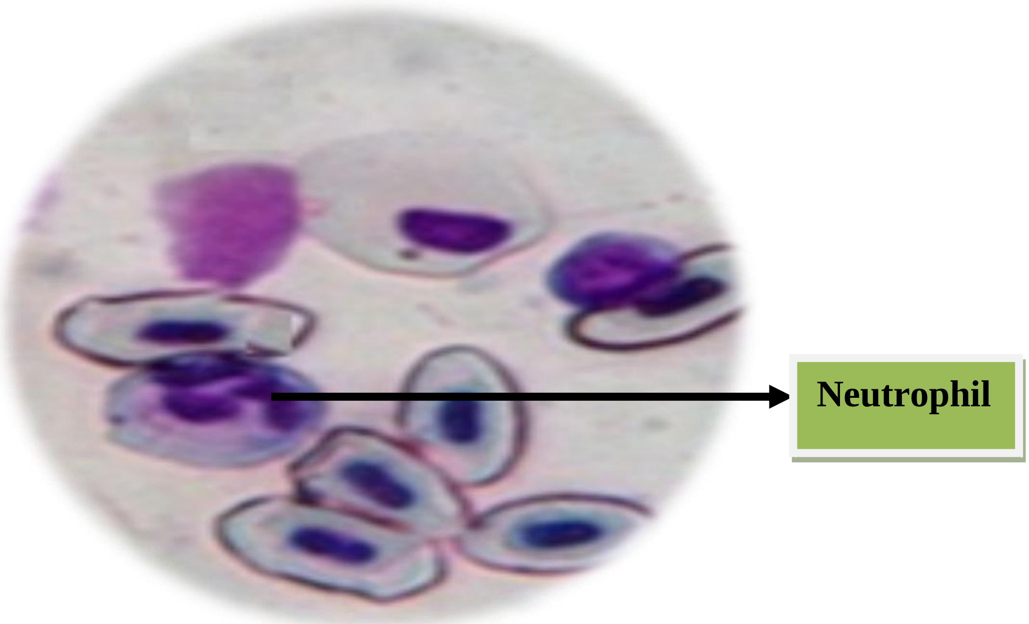


Plate 10: Neutrophil

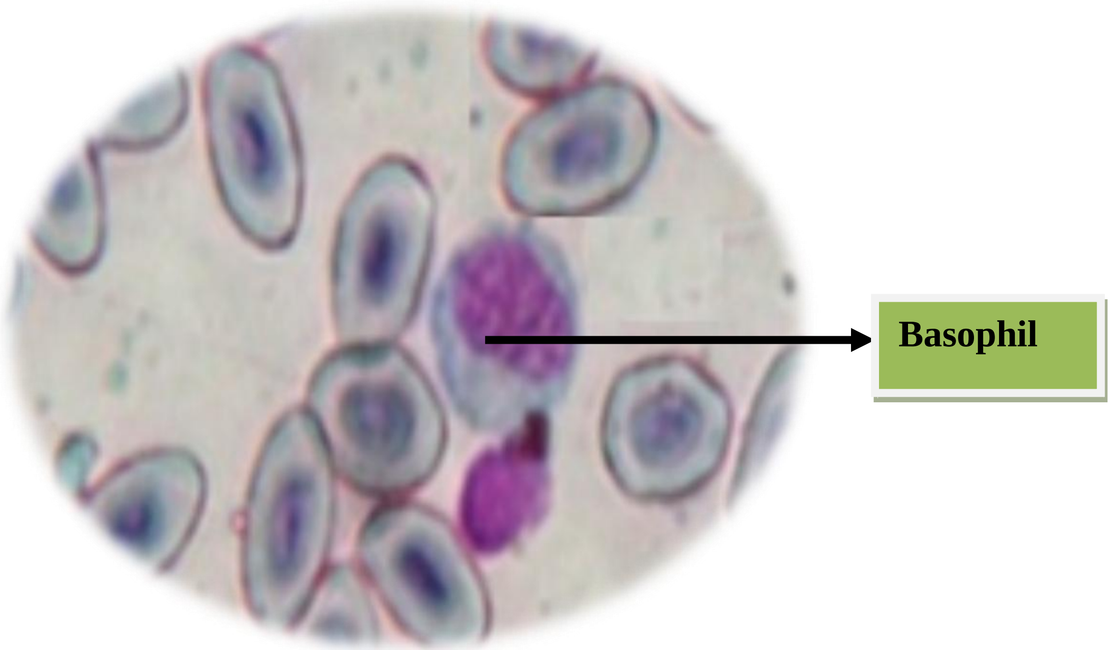


Plate 11 : Basophil

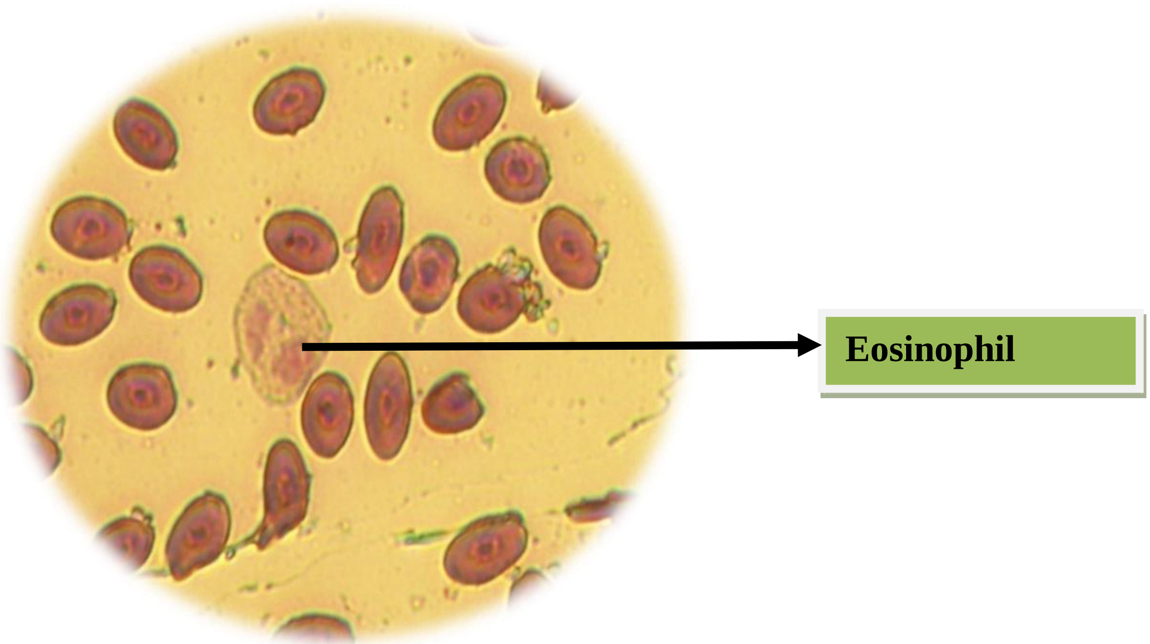


Plate 12 : Eosinophil

Table 5.5

Hematological parameters of *T. marmorata* from Wular Lake

Parameters	Range	Mean±S.D
Body Length(mm)	80 — 97	90±6.2
Body Weight(g)	5.06 — 7.35	6.43±0.86
Hb(g/dl)	9.5 —10.52	10.03±0.35
RBC(10^6/mm^3)	1.11—1.17	1.14±0.02
PCV (%)	19.9 —22.1	21.09±0.81
WBC(10^4/mm^3)	51.92—58.32	55.10±2.51
MCV(μm^3)	160.2—199.09	178.87±13.22
MCH(µg)	85.58—90.52	88.16±1.94
MCHC (%)	42.98—48.54	46.08±2.42
DLC Lymphocyte (%)	59—70	64.16±4.62
Monocyte(%)	2—4	2.83±0.75
Neutrophil(%)	25—35	30.5±3.93
Basophil(%)	0—3	1.5±1.04
Eosinophil(%)	1—3	1.83±0.75

Table 5.6

Hematological parameters of *T. marmorata* from Sind stream

Parameters	**Range**	**Mean±S.D**
Body Length(mm)	115—130	124.3±5.8
Body Weight(g)	11.2—16.5	13.68±2.28
Hb(g/dl)	10.3—10.6	10.43±0.10
RBC($10^6/mm^3$)	1.35—1.39	1.37±0.01
PCV (%)	25.8—27.5	26.61±0.55
WBC($10^4/mm^3$)	38.9— 41.8	40.34±1.07
MCV(μm^3)	192.21—196.36	194.27±1.49
MCH(µg)	74.6—76.61	75.89±0.72
MCHC (%)	38.25 —40.1	39.16±0.58
DLC Lymphocyte (%)	64 —78	71±5.86
Monocyte (%)	1 —3	1.66±0.81
Neutrophil (%)	20 —30	25.33±3.88
Basophil (%)	0 —3	1.83±1.16
Eosinophil (%)	0 —2	1.00±0.89

Table 5.7

Hematological parameters of *T. marmorata* from Telbal stream

Parameters	Range	Mean±S.D
Body Length(mm)	69 —115	93.1±15.0
Body Weight(g)	5.3—11.02	6.63±2.16
Hb(g/dl)	8.28—10.63	9.65±0.91
RBC($10^6/mm^3$)	1.18—1.21	1.19±0.01
PCV (%)	20.52—22.8	21.54±0.79
WBC($10^4/mm^3$)	39.9—41.5	40.79±0.56
MCV(μm^3)	171—184.27	177.71±6.31
MCH(µg)	69—89.32	80.10±8.80
MCHC (%)	40.35—48.87	43.90±3.14
DLC Lymphocyte (%)	60—76	68±6.54
Monocyte (%)	1—4	2.5±1.04
Neutrophil (%)	20—35	26.5±6.59
Basophil (%)	2—4	2.83±0.75
Eosinophil (%)	0—2	1.16±0.75

Table 5.8

Mean values of the Hematological parameters of *T. marmorata* from Wular Lake, Sind stream and Telbal stream

Parameters	Wular	Sind	Telbal
Body Length(mm)	90.0±6.20	124.3±5.8	93.1±15.0
Body Weight(g)	6.43±0.86	13.68±2.28	6.63±2.16
Hb(g/dl)	10.03±0.35	10.43±0.10	9.65±0.91
RBC($10^6/mm^3$)	1.14±0.02***	1.37±0.01***	1.19±0.01***
PCV (%)	21.09±0.81***	26.61±0.55***	21.54±0.79***
WBC($10^4/mm^3$)	55.10±2.51***	40.34±1.07***	40.79±0.56***
MCV(μm^3)	178.87±13.22**	194.27±1.49**	177.71±6.31**
MCH(µg)	88.16±1.94**	75.89±0.72**	80.10±8.80**
MCHC (%)	46.08±2.42***	39.16±0.58***	43.90±3.14***
DLC Lymphocyte (%)	64.16±4.62	71±5.86	68±6.54
Monocyte (%)	2.83±0.75	1.66±0.81	2.5±1.04
Neutrophil (%)	30.5±3.93	25.33±3.88	26.5±6.59
Basophil (%)	1.5±1.04	1.83±1.16	2.83±0.75
Eosinophil (%)	1.83±0.75	1.00±0.89	1.16±0.75

Data is presented as Mean ± S.D. Data was analyzed using one way ANOVA. The values were considered significant for * $p < 0.05$, **$p<0.01$ and ***$p<0.001$

DISCUSSION

As no considerable work has been done on the various biological aspects of the presently selected loach (*T. marmorata*), the present study is expected to help not only understand the hematogical peculiarities of the fish but also throw some light on the adaptability of the fish to its habitat. The area of Wular Lake wherefrom the fish was collected is shallow macrophyte infested lentic water, while the study area in the Telbal Nalla is characterized by slow unidirectional flow of water, with sandy and silty bottom. The study area in the Sind Nalla, though not torrential, did show fast flow of water. Here the bottom was characterized by sand gravel and rubble. It can safely be concluded that the species is adapted to different habitats having diverse physical features. A comparison of the data on the length and weight of the fish in the three habitats revealed that the Sind Nalla provided the best habitat for the fish as the specimens collected from here were bigger and healthier from either of the other two habitats. This preference is not only influenced by the physical features of the waters concerned but also by the water chemistry of the three habitats. A comparison of the three water bodies showed the least oxygen concentration in the lentic water body. Due to continuous fast current, especially in case of Sind Nalla, the running waters contain relatively higher concentration of oxygen than the lentic water of Wular (Vass *et al.*, 1977 and Qadri *et al*., 1981). The higher value of the free carbon dioxide content has been related to the eutrophication/pollution of water (Todda, 1970 and Coole, 1979). This is also confirmed by our data from Wular Lake and Telbal Nalla, which show much higher concentration than recorded in the Sind Nalla. Same was true with respect to pH value as well as total alkalinity. Level of phosphorus and inorganic nitrogen in Wular Lake and Telbal was much higher than the Sind Nalla.

On the basis of physico-chemical limnology it can be said that Sind stream, which is comparatively free from pollution, as compared to the Wular and Telbal is best suited for *Triplophysa marmorata*.

HEMATOLOGY OF FISH

A significant quantity of most of the chemicals employed by human beings in agricultural practices finds its way into rivers, lakes and ponds. Majority of them have been found to be highly toxic not only to fishes but also to the organisms which contribute to the food chain of fishes (Anees, 1975). In India more than 70% of the chemical formulations used in agricultural practices are believed to affect non target organisms and find their way to fresh water bodies and polluting them in the process (Bhatnagar *et al*., 1992). The fishes are an important food source for human beings and their flesh contains a high percentage of proteins, calcium and phosphorus. A major part of the world's food is supplied from fish source and it is very essential to secure the health of fishes (Tripathi *et al*., 2002). Being at the mercy of their environment, any change in the quality of their environment is bound to affect their health vis-à-vis the fishery resource of a water body. This being so it is pertinent to have an insight in to the health of the fish and the factors responsible for it.

The blood is the most important fluid in the body and its composition often reflects the total physiological condition of an organism. Hematological parameters are nowadays not only used for clinical diagnosis of physiology but also help in addressing the effects of toxic substances on the fish (Wendelaar, 1997). Studies have shown that when the water quality is affected by toxicants, any physiological changes occurring in the fish are reflected in the values of one or more of the hematological parameters (Van Vuren, 1986). Blood cell responses are important indicators of changes in the internal and/or external environment of animals. Ralio and Nikinmaa (1985) reported that the blood parameters of diagnostic importance like erythrocyte and leucocyte counts, hemoglobin, haematocrit and differential leukocyte counts would readily respond to incidental factors such as physical stress and environmental stress due to water contaminants.

Hematological studies on fishes have assumed greater significance due to the increasing emphasis on pisciculture and greater awareness of the pollution of natural freshwater resources. The determination of hematological parameters of freshwater fishes gives an idea of their physiological status and the influence of various environmental factors (Ramaway and Reddy, 1978). The determination of blood parameters, and in particular those of the red blood cell count, are used for assessing fish health (Bhaskar and Rao, 1985). Such studies have generally been used as an effective and sensitive index to monitor physiological and pathological changes in fishes (Iwama *et al*., 1976; Chekrabarty and Banerjee, 1988). A comparison of the various hematological data of the fish collected from three different water bodies showed significant variations which are clearly related to the ecological set up.

HEMOGLOBIN

Hemoglobin is a conjugated protein formed of four heme groups and a protein called globin. Each heme molecule contains an iron poryphyrin ring. Hemoglobin serves to transport oxygen from gills to different tissues of the fish in the form of oxyhemoglobin and carbon dioxide from tissue to the gills in the form of carboxyhemoglobin. The hemoglobin value of *T. marmorata* decreased at Wular Lake and Telbal compared to Sind stream. According to Pamila *et al*. (1991) the reduction in hemoglobin content in a fish exposed to pollutant could be due to the inhibitory effect of those substances on the enzyme system responsible for synthesis of hemoglobin. The pollutant entering into fish system is slowly eliminated (Newman and Mitz, 1988; James and Sampath, 1996 and James *et al*., 1996), and hence the blood parameters get affected on account of pollutant toxicity. The low Hb value in Wular and Telbal water bodies may also be associated with less active fishes. Similar results were reported by Engel and Davis (1964) and Rambhaskar and Srinivasa Rao (1986). Eisler (1965) suggested that there was a correlation between hemoglobin concentration and activity of fish. The more active fishes tend to have higher hemoglobin values than the more sedentary ones. Consequently, *Pleuronectes annectens* being a relatively quiet and sedentary species (Okafor, 2006) has a slightly lower hemoglobin concentration than more active African teleosts such as *Clarias buthupogon* whose mean hemoglobin concentration is as high as 9.88g/dl (Kori-Siakpero and Egor, 1997). The high hemoglobin content in *T. marmorata* from Sind stream may be related to its preferred environmental conditions.

RBC

The count of red blood cells is quite a stable index and the fish body tries to maintain this count within the limits of certain physiological standards using various physiological mechanisms of compensation. Any alteration in the number (quantitative) or morphology (qualitative) of RBCs from normal values can cause various pathological disorders in fish under stressful conditions**.** The RBC value of *T. marmorata* decreased significantly ($P<0.001$) at Wular Lake and Telbal compared to Sind stream. A fall in RBCs count, Hb% and PCV%, in the fishes, due to water pollution, has been reported along with acute anaemia (Singh, 1995). According to Singh *et al*. (2002) the discharge of waste may cause serious problems as they impart odour and can be toxic to aquatic animals. The organic wastes present in Wular Lake and Telbal stream seem to cause stress in the fish and as such seem to be responsible for the changes in the hematological parameters. Decreased RBC, Hb, and PCV in the fish at Wular Lake and Telbal stream may be due to anemic condition in fish. Similar results with reduction in RBC's and Hb% content in fishes exposed to different pollutants have been reported previously by Goel *et al*. (1985) and Goel and Sharma (1987) . Lowering of TEC count coupled with low Hb content may be due to destructive action of pollutants on erythrocytes as a result of which the viability of the cells may be getting affected (Karuppasamy, 2000). Buckley *et al.* (1976) also showed that prolonged reduction in Hb content is deleterious to oxygen transport in catfish exposed to Cassava mill effluent. This could be ascribed as pathological condition in fish exposed to toxicants. Hemolysis occurs in response to toxicity that leads to alteration in the selective permeability of the membrane (Das *et al*., 1987). All these reports are in agreement with the present study of reduction in TEC count and Hb content of fish from Wular Lake and Telbal due to the inhibition of aerobic glycolysis curtailing synthesis of iron and hemoglobin via the lowered energy status in fish.

PCV

PCV or Haematocrit is an important tool for determining the amount of plasma and corpuscles in the blood (measurement of packed erythrocytes) and is used to determine the oxygen carrying capacity of blood (Larsson *et al*., 1985). The haematocrit reading is valuable in determining the effect of stressors on the health of fish (Munkittrick and Leatherland, 1983). Baxhall and Daisely (1973) have reported the possibility of using haematocrit as a tool in aquaculture and fishery management for checking anaemic condition. Reported values for fish haematocrit are usually between 20% and 35% and scarcely attain values greater than 50% (Clark *et al*., 1979). The mean haematocrit values of *T. marmorata* from the three water bodies were within the range of 21 – 26%. PCV decreased significantly ($P < 0.001$) in Wular Lake and Telbal stream in comparison to Sind stream. Joshi *et al*. (2002) and Banerjee and Banerjee (1988) have suggested that pollutant exposure decreases the TEC count, Hb content, and PCV value due to impaired intestinal absorption of iron. PCV values always decrease when a fish loses appetite or becomes diseased or stressed. Similar results were obtained by Larsson *et al*. (1985) after exposure of fish to $KMnO_4$, indicative of anaemia and haemodilution, possibly due to gill damage or/and impaired osmoregulation .The exposure of *Channa punctatus* to sub-lethal concentration of copper significantly decreased Hb, RBC count and PCV% values leading to anaemia. The anaemia might have led to a fall in the red blood cell count, hemoglobin concentration, and haematocrit volume. Anaemia, under copper induced stress, may also be due to blood cell injury and disrupted hemoglobin synthesis (Mckim *et al*., 1970; Gross *et al*., 1975). Anemia of this type characterized by reduced RBC count, hematocrit and hemoglobin contents have also been reported by several workers after insecticide feeding (Mandal *et al.*, 1986; Ali, 1989).

WBC AND DLC

In this study there was an increase in WBC quantity and leukocyte cell proportions (neutrophil, monocyte) in the fish specimens from Wular Lake and Telbal stream. Increase in WBC count can be correlated to direct stimulation for its defense from diseases due to the presence of polluted substances. Similar results were obtained by Sahan and Cengizler (2002) on carp caught from different regions of Seyhan River. Increased levels of TLC have been reported in *Channa punctatus* exposed to lead (Hymavathi and Rao, 2000) and *Clarias batrachus* exposed to mercuric chloride (Joshi *et al*., 2002). Leukocytosis is directly proportional to severity of stress condition in maturing fish and is a result of direct stimulation of immunological defense due to the presence of pollutants in water bodies. This is in conformity with the report by Saravanan and Harikrishnan (1999) in freshwater fish, *Sarotherodon mossambicus*, when exposed to sublethal concentration of copper and endosulfan and by Nanda (1997) in respect of *Heteropneustes fossilis* during nickel intoxication. This may be attributed to alteration in blood parameters and direct effects of various pollutants. These observations are also in good agreement with those of Karuppasamy *et al*. (2005) and Hardikar and Gokhale (2000). In fish, any infestation with any organism activates the cellular and humoral immune system. This is followed by changes in circulating antibodies and percentages and absolute number of the different WBC (Boon *et al*., 1990). Weinreb (1958) used leukocyte count changes as a means of assessing the systematic response of the rainbow trout, *Salmo gairdneri richardsoni*, to various injections. Mishra and Srivastava (1980) also reported an increase in leucocytes count of fish due to pollution. Some of the most common causes of pollutant toxicity are inflammatory lesions associated with tissue damage, anemia and neoplasia.

The lymphocytes are reported to be responsible for immune response, while neutrophils are reported to show the greatest sensitivity to change in the environment. Their characterization and identification is, therefore, of significance for assessing the changes in the physiological state of fishes. The percentage of these cell types generally decreases during acute exposure to copper (Nussey *et al*., 1995and Svobodova *et al*., 1994), and in situations of chronic copper exposure, the neutrophil percentage has been reported to increase (Dick and Dixon, 1985). In the present study, the increases in WBC and neutrophil quantities in the samples collected from Wular Lake and Telbal seem to be a response of cellular immune system to pollution (Palikova and Navratil, 2001; Şahan and Cengizler, 2002and Saravanan *et al.*, 2003).

ERYTHROCYTE INDICES

The TEC, Hb and PCV and similar other indices based on blood parameters provide vital clues about the overall health of the fish vis-à-vis the condition of the fish environment. MCHC, MCH, and MCV have a particular importance in the diagnosis of anemia in most animals (Coles, 1986). The statistically significant decrease in MCV coupled with low hemoglobin content in the Wular lake and Telbal stream indicate that the red blood cells have shrunk, either due to hypoxia or microcytic anemia; microcytosis been due to the decrease in the haematocrit values. A significant decrease in MCV value observed in fish is in agreement with that reported in *Heteropneustes fossilis* exposed to deltamethrin (Kumar *et al.*, 1999). The fluctuation in the MCH values clearly indicates that the concentration of hemoglobin in the red blood cells were much lower in the fish collected from Wular lake and Telbal stream than in the Sind stream, thus indicating an anaemic condition.

Chapter -6

Conclusion

- ❖ The study was initiated with an objective to assess the impact of environment on the hematology of *Triplophysa marmorata*.
- ❖ Fish were collected from three different habitats, viz., (i) a fast flowing clean and (ii) a slow flowing moderately polluted lotic habitat and (iii) an eutrophic lentic habitat.

The key findings of the present study are as under.

- ➢ Hemoglobin content showed higher values in the fish from the Sind stream.
- ➢ TEC count showed significant decrease in fish due to the inhibition of aerobic glycolysis, curtailing synthesis of iron and hemoglobin via the lowered energy status in Wular Lake and Telbal stream.
- ➢ WBC count showed significant increase in fish faced with polluted environment in the Wular Lake and the Telbal stream.
- ➢ On the basis of physico-chemical limnology it was evident that the Sind water is least polluted and hence best suited for *T. marmorata*, while the other two water bodies (Wular Lake and Telbal Stream) are comparatively less suited for the fish.
- ➢ Large and Heavier specimens of fish were recorded in Sind stream, which can be attributed to favorable habitat, i.e., availability of more food and comparatively pollution free water.
- ➢ Hematological parameters of fish from three water bodies showed varied trends in relation to stress effects of environmental factors. On the whole it may be concluded that:
    - The hematology of the fish is influenced by the environment in which it lives and

- Blood parameters can be used for assessing the quality of the fish's environment.
- Stress due to various pollutants present in water bodies (Wular Lake and Telbal Stream) caused hematological disturbances, thereby affecting immune system.

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