ICHTHYOFAUNA OF LIDDER STREAM WITH EMPHASIS ON HAEMATOBIOCHEMICAL STUDY OF *SCHIZOTHORAX* SPP.

DISSERTATION

Submitted in Partial Fulfillment of the Requirements for the Award of the Degree of

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In

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By

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Certificate

This is to certify that the dissertation entitled **"ICHTHYOFAUNA OF LIDDER STREAM WITH EMPHASIS ON HAEMATOBIOCHEMICAL STUDY OF *SCHIZOTHORAX* SPP."** submitted to the University of Kashmir for the award of the Degree ***MASTERS OF PHILOSOPHY IN ZOOLOGY***, is the original research work of ***Ms. Farhana Sareer***, 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.

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Farhana Sareer

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Abbreviations

Hb	Haemoglobin
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 Haemoglobin
MCV	Mean Cell Volume
MCHC	Mean Cell Haemoglobin concentration
Fig	Figure
SD	Standard Deviation
%	Percentage
Phg	Photograph
Phgs	Photographs

CHAPTER NO – 1

INTRODUCTION

Jammu & Kashmir, lying between six mountain ranges and covering an area of 2,22,236 sq. kms. is located between 32° 17' and 36° 58' North latitude, and between 37° 26' and 80° 30' East longitude. The state commonly known as Kashmir is bounded in the north by Afghanistan and China, in the east by China, in the south by the state of Himachal Pradesh and the state of Punjab in India, and in the west by the North-West Frontier Province and the Punjab Province of Pakistan. Jammu and Kashmir geographically comprises of three regions: the foothill plains of Jammu; the lakes and blue valleys of Kashmir rising to alpine passes, the high altitude plains and starkly beautiful mountains of Ladakh which lies beyond narrow passes. Kashmir is rightly said to be the nature's grand finale of beauty. In this masterpiece of earth's creation seasons in strong individuality vary with one another in putting up exquisite patterns of charm and loveliness. This state holds the glorious history of the valiant kings, the placid lakes, the greenery of the forests and the amazing rivers. The sunny gardens, romantic house boats and Lakes add to the beauty of the region. The state of J&K measures about 425 kms from north to south and extends over 520 kms from east to west.

Nature has bestowed the valley of Kashmir, with plenty of gifts like snow clad mountains, vast meadows full of flowers, thick forests, small mountain tarns and valley lakes, numerous serpentine rivers etc. The valley is famous throughout the world for its waters both lentic and lotic. The lotic habitats include numerous streams like Lidder, Veshu, Dudhganga, Sindh etc., spread throughout the valley forming tributaries of the river Jhelum that flows through the valley from south to northwest direction. All these streams harbour a number of indigenous fishes like *Schizothorax* spp., *Glyptothorax* spp., *Triplophysa* spp., etc as well as the exotic Trout i.e.*Onchorhynchus mykiss* and *Salmo trutta* form. Inspite of the fact that most of these streams are an important fishery resource of the valley, not much is known about the ecology of fishes of these habitats.

The fish fauna of the high altitude waters of the valley of the Kashmir lakes, rivers and springs comprise chiefly of indigenous fishes and exotic species. The earliest report on the fishes of Kashmir is that of Heckel (1839), who described sixteen species of fishes from the valley, thirteen of them belonging to family Cryprinidae. Since then a number of workers have reported on the ichthyofauna of the region (e.g., McClelland, 1839; Heckel, 1844; Day, 1876; Chaudhury, 1909; Hora, 1936; Mukerji, 1936; Das and Subla, 1963, 1964, 1969; Yousuf, 1996 and Kullander et al., 1999) but all the water bodies have not been explored as yet and there is probability that the number of species may have increased.

The lidder as well as its tributaries and distributaries are known to harbour a number of fish species. However, except for a few reports (Yousuf and Shah, 1988 and Bhat *et al.*, 2004) not much is known about the Ichthyofauna of this important tributary of the jehlum. In view of the importance of the stream as well as the fish fauna inhabiting it, it has been proposed to study the haematobiochemistry of the indigenous fish Schizothorax reported to be occurring in the stream. During the past century the anthropogenic pressure on water bodies of the valley has increased tremendously and this has in turn affected the fish population especially the Schizothoracine group some of

which are endemic to the valley. While looking for the possible reasons for the decreasing trend in the Schizothoracine population, one is struck with several factors including pollution which seem to have worked synergistically to influence the changes in the fish species composition in aquatic habitats of Kashmir. Since changes in various environmental parameters results in a new steady state of biochemical and physiological reactions, changes in these reactions are expected to provide some indication that the organism is faced with. 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.). In addition measurements of blood serum chemistry parameters are further commonly used as diagnostic tool in fish toxicology and biomonitering (Mc.Donald and Milligan, 1992; Folmar et al, 1993). Intergeneric and interspecific variations also occur in the electrophoretic pattern of serum and plasma proteins of fish as shown in the works of Moore (1945), Chandreshaker (1959), Yamashita(1969), Einszporn Orecka (1970), Perrier *et al*. (1973), Harris (1974), Baron (1975), Nakagawa (1978) and Bradley and Rourke (1984).

Fish 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 and biochemical parameters (Blaxhall, 1972).Hematological values of fish have also been used as probes in connection with pollution and its effects. Recently pesticide pollution of the aquatic environment has received widespread attention. Bouck and Ball (1966) stated that hematology may be a useful tool in monitoring stress levels of aquatic pollution on fish. 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 (e.g. Dewilde & Houston, 1967). Like in humans, haematological studies have

also been used as a means for diagnosis of fish disease. Katz (1950) stated that blood counts held evaluate diets because the number of erythrocytes responds more quickly to some dietary deficiencies that do the condition factor or growth rate, high mortalities attributed in part to inadequate diets could be averted by alert biologists who might interpret the observed anaemia's as indicative of poor nutrition. Hesser(1960) recognized the use and reliability of hematology in human medicines and sought to adopt these techniques in fish hematology as an aid in the diagnosis of fish disease. Many workers have stressed the need for the establishment of normal hematological values with a view to the diagnosis of disease (Hesser,1960;Larson & Sneiszko ; Summerfelt , 1967; Blaxhall, 1972;Alexander *et al* .,1980;Campbell & Murra,1990) and in connection with pollution and its effects (Mawdeseley-Thomas,1971). Besides assessing the health of a fish various blood parameters reveal physiological adaptation of their natural habitat and are also useful in determining systematic relationship (Atkinson and Judd, 1978) numerous studies have shown marked interspecific variations in total Erythrocyte and total Leukocyte counts, Hemoglobin content, hematocrit and erythrocyte size of fishes .

The Schizothoracinae are a specialized group of fishes, dominant of the torrential mountain streams of the Himalaya and Central Asia. Heckel (1838) described 10 species of *Schizothorax* from Kashmir. *Schizothorax* Heckel is elongate, laterally compressed with short head and lateral eyes which are not visible from below. The third dorsal fin ray is thickened and has strong serrations along the posterior margin. Lateral line is along middle of side, often slightly irregular, complete. Maxillary and rostral barbells are short. Snout tubercles are often present and caudal fin is forked. Anal fin is slightly longer in females than in males of comparable size. Lateral line scales are distinctively larger than other scales on the side. Scales of the abdominal mid line from about tips of pelvic fin are enlarged, deep and non-imbricating across ventral mid-line, leaving a naked area exposing the

genital opening. Color is usually dark grey on dorsum, lighter on sides, underside witish.(Kullander etal.,1999)

Three species of Genus *Schizothorax Heckel,* were selected for the present study. These included:

1. *Schizothorax plagiostomus.*
2. *Schizothorax esocinus.*
3. *Schizothorax labiatus.*

S. plagiostomus, locally known as Khont, is distinguished remarkably by elongate body, with projecting snout. Mouth distinctively inferior, wide and lower jaw very deep, short and with a sharp keratinized antero-ventral cutting edge. Lower lip fold is expanded and papillose. A series of enlarged scales are found along the base of its anal fin. It has minute scales, 89-99 in the lateral line. The fish is typically lotic water species being distributed in fast flowing streams of Kashmir. (Kullander etal.,1999)

S. esocinus, locally known as churru, differs from all other Kashmir valley Schizothorax in much longer jaws, without enlarged lips or tuberculate pads. The fish is silvery with numerous dark, small irregular spots on back and flanks of body. The fins are silvery grey with similar dark spots which are more numerous at base. Also the color pattern is distinctive with light ground color and contrasting black spots in most specimens. Its body is streamlined, mouth wide, horse-shoe shaped, cleft is very deep, lips are thick fleshy, lower labial fold is interrupted in middle. Barbells are two pair, rostral pair about 1.5 times longer than eye diameter and maxillary pair slightly shorter. Dorsal fin is inserted slightly nearer to base of caudal fin than to snout tip. Scales are very small, about 104 in lateral line. (Kullander etal.,1999)

S.labiatus, (Mcclelland &Griffith,1842) locally khown as chush, is distinguished by elongate, fusiform, body with a prognathous upper jaw, a lower jaw with wide lip folds usually separated by a distinct raised pad. S labiatus has a rounded lower jaw, with a narrow cornified margin, no keratinized cutting edge, and lips restricted to wide lateral flaps and a more

or less well developed median thickening, without enlarged papillae. (Kullander etal.,1999)

Blood parameters are considered pathophysiological indicators of the whole body (intrinsic and extrinsic eco-physiological conditions) and therefore are important in diagnosing the structural and functional status of fish exposed to pollutants. Haematobiochemical studies have been employed as aid in assessing the health of fish exposed to various pollutants. Routine piscine hematological studies have now acquired a status of an essential laboratory diagnostic subject. A single drop of blood is sufficient as indicator regarding fish health. Just one ml of blood is sufficient enough to let us know the state of ambient environment, physiological requirements of fish at a particular stage of development, effect of seasonal variations, effect of sex ,size,gonadal stage of maturity and various types of internal pathological conditions. Fish blood fully reflects the minutest of changes in its internal and external milieu, within very short period of time. Even the stress caused due to thermal, transportation stress, oxygen stress etc. can be fully reflected by certain parameters. The present study will open the new area of research in fisheries and it will definitely help to improve the fish health. Besides investigating the distributional pattern of the fish in the stream, an attempt was made to study its haematobiochemical parameters and check the impact of environmental characteristics on these features.

CHAPTER NO –2

STUDY AREA AND STUDY SITES

2.1: Description of Lidder River and Study Sites:

Lidder River, an important tributary of the river Jhelum, has its origin from the high altitude glacier fed lakes Sheshnag and Tarsar and the Kolhai glaciers. The Sheshnag lake, one of the base camps for the Amarnath Yatries, about 30 km from Pahalgam, gives rise to the east Lidder which joins at Pahalgam with the west Lidder. The latter gets its waters from the Tarsar lake and Kolhai glaciers and flows through Lidderwat and Aaru torrentially and after covering a distance of about 30 km before meeting the East Lidder. From Pahalgam the combined river flows through Lidder Valley in south-west direction and after traversing a distance of about 35 km, is divided into a number of branches below Mattan, which join the river Jhelum at different places at Gour and Adura.

After the confluence of East and West Lidder, the Lidder gets additional water from some small tributaries on its left and right banks. Some of the important ones are; Sarbal nallah, Langniah nallah, Overa nallah and Aayono nallah. From Pahalgam below upto Nambal village it flows torrentially and below Nambal its speed slows down. All along from its origin upto mouth its bottom is totally rocky with gravel and sand. The river has a number of human settlements along its course, important ones being Aaru, Frislun, Aathnadan, Larkipora, Hera Pahalgam and Bona Pahalgam, Liddroo, Lamad, Veerseran, Ladi, Dehwatoo, Moveera, Dalseer, Kullar, Wullarhama, Sallar, Katsoo, Budroo, Batkote, Ganeshpora, Aishmuqam, Karshangam, Siligam, Nambal, Seer, Hutmurrah, Bumzoo, Akura, Nanil-Matipora etc. The river receives agricultural effluents containing fertilizers, pesticides and domestic sewage along its course.

For the present study two sites were selected for the collection of samples, both being located along the course of the combined Lidder.

Site I: Loripora(Pahalgam)

Elevation: 2162 m (a.s.l)

Latitude: 34°01' 16. 7″N

Longitude: 75°19' 02.5″E

This site is located about 2km below Pahalgam, a little above Langanbal bridge, where the two roads of Alaqa Dachnipora and Khowerpora meet.In this area the Lidder is highly torrential in nature.

Phg-1: A view of Lidder Stream at Site-1

Site II: Akura (Mattan)

Elevation: 1605 m (a.s.l)

Latitude: $33^{o}46'13.3''N$

Longitude: $075^{o}12'13.1''E$

This site is located near the Akura bridge and about 25 km below the site I and about 4 km before the confluence of Lidder with the Jhelum river. The velocity of water here is comparatively slower and the bottom includes boulders, gravel and sand. The site II receives the whole runoff and effluent of alaqa Khawerpora and a little from the alaqa Dachnipora.

Phg-2: A view of Lidder Stream at Site-II

CHAPTER NO –3

MATERIAL

&

METHODS

The present work deals with the haematology and biochemistry of Schizothorax spp. of Lidder stream and incorporates the data of hematology and biochemistry of the fish as well as Physico-chemical parameters of the concerned water body. The methods used for the study are described under the following six broad heads:

4.1 Survey of fish, their distribution pattern and collection.

4.2 Sampling of blood.

4.3 Haematology of schizothorax spp.

4.4 Biochemistry of schizothorax spp.

4.5 Physico-chemical analysis of water.

4.6 Statistical analysis of the results

4.1) Survey of fish, their distribution pattern and collection.

A through survey of the study area was done to collect the fishes. Distribution pattern of the fish was carried out randomly. Four sites were selected along the course of Lidder. In each habitat fish were stunned, by the aid of local fisherman using cast net or by fishing downstream with a battery-powered pulsed DC backpack electrofishing machine, and collected in a hand-held seine or dip net. The combined length of the sites sampled at each location varied between 45 and 50 m. Fish were identified, measured,

counted, and average distribution pattern was worked out. The blood samples were taken either from live specimen on the spot or the 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 and allowed to return to normal conditions before blood samples were taken.

Three fish taxa namely, *Schizothorax plagiostomus* , *Schizothorax esocinus* and *Schizothorax labiatus* were included for the present investigations.

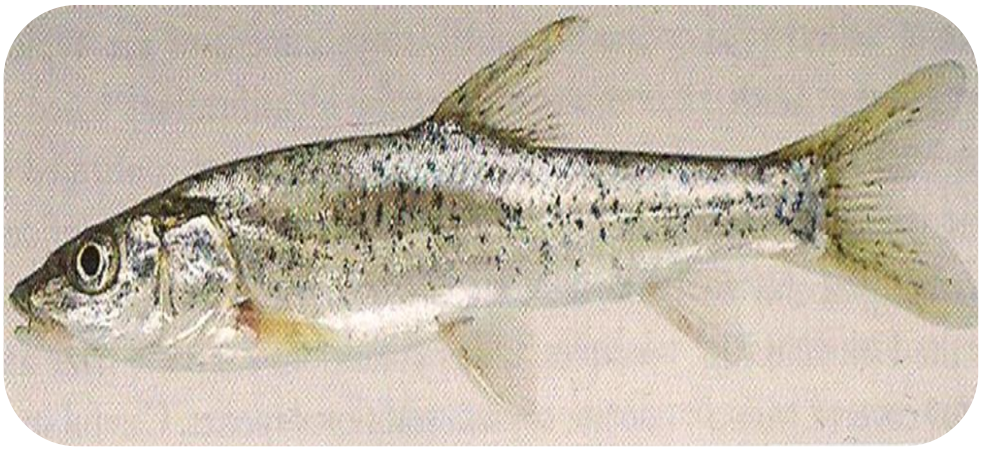
4.2) Collection of blood samples

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 & weight of the fishes were recorded after the collection of the blood. Blood was drawn out with a syringe from the heart by stabbing body wall exactly in midline from the posterior margin of opercular cover & directed dorso-caudally at an angle of 45^0 (Lucky, 1977). The blood was also collected by severing the caudal peduncle. Care was taken to prevent the blood from coming in contact with water. 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).

Phg.3

Phg.4



Phg.5

Phgs:3-5: *Schizothorax plagiostomus* (phg-3), *Schizothorax labiatus* (phg-4) and *Schizothorax esocinus* (phg-5).

4.3) Hematological parameters

4.3.1) Estimation of Haemoglobin concentration:

The haemoglobin was estimated by Cyanomethemoglobin method (ICSH, 1978). In this method, Ferricyanide present in the Drabkins solution converts ferrous (Fe^{2+}) iron of haemoglobin to the ferric (Fe^{3+}) state to form methemoglobin. Methemoglobin reacts with potassium cyanide to form Cyanomethemoglobin. The colour 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 gm.
Potassium cyanide	0.05 gm.
Potassium ferricyanide	0.2 gm.
Distilled water	1000 cc.

Calculation:

$$\text{Hb (g/100ml)} = \frac{A_{540}\ \text{test sample}}{A_{540}\ \text{standard}} \times 15.06 \text{ (Std. conc. as stamped on the vial x0.251)}$$

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 ($HgCl_2$)	:	0.5gm
Sodium Chloride (Nacl)	:	1.0gm
Sodium sulphate (Na_2SO4)	:	5.0gm
Distilled water (H_2O)	:	200ml

Blood was drawn upto the 0.5mark in the RBC Pippette. 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 by using 40x, after counting the RBC's 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.

$$\text{RBC count} = \frac{\text{Number of cells counted x 200 x10}}{1/5}$$

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 (CH3COH) : 1.5 ml

1% Aqueous solution of Gentian violet : 1ml

Distilled water : 100ml

This fluid contains two things, weak acid which lyse the RBC cells and stain which gives colour to the nucleus of WBC.

Neubaur's haemocytometer (Baker and Silverson,1982) was used for counting leucocytes. The blood was sucked up in the WBC Pipettes upto the o.5mark and then WBC dilution fluid was drawn upto 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 40x objective lens.

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

$$TLC = \frac{\text{Cells counted x 20 x10}}{4}$$

TLC/cu.mm.= Cells counted x 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 : 0.3gms

Glycerine : 25.0ml

Acetone free methyl alcohol: 25.0 ml

Leishman's stain:

Powdered Leishman's stain : 0.15 gm

Acetone fee methyl alcohol : 133ml

4.3.5) Estimation of ESR

This is the rate at which erythrocytes sediment by their own weight when blood containing anticoagulant is held in a vertical column. It is expressed as the fall of RBC's in mm at the end of first hour.

The estimation of ESR was done by Wintrobe's method .The wintrobe's tube is about 11 cm long with a bore diameter 2.5mm and the 10cm of the tube is graduated. The graduations are from zero (top) to hundred (bottom) for ESR and Zero (bottom) to hundred (top) for PCV. Blood containing EDTA was used for the estimation of ESR. Wintrobe's tube was filled upto zero mark on top and the tube was kept vertically in ESR stand for one hour. After an hour reading was taken from the tube directly.

4.3.6) 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).

4.3.7) Erythrocyte Indices:

Wintrobe (1974) introduced calculation for determining the size, content and Haemoglobin concentration of red cells which have been found very useful in the morphological characterization of anemia.

- Mean cell volume (MCV):

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

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

- Mean cell Haemoglobin (MCH):

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

$$MCH = \frac{Hb\ (gms/\ dl)\ x\ 10\ micro\ grams}{RBC\ (millions/\mu l)}$$

- Mean cell Haemoglobin concentration (MCHC):

The Mean haemoglobin concentration in g% for 100 ml erythrocytes was calculated by the formula:

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

4.4 Biochemical parameters

4.4.1 Estimation of total Protein

Total protein content of the serum of fish was estimated by biuret method which is based on the principle that proteins and peptides containing at least two adjacent peptides bonds which react with cupric ions in alkaline solution forming violet coloured complex having absorption maximum at 550nm

Table:4.1

Reagent	**Blank**	**Test**	**Standard**
Total Protein	3ml	3ml	3ml
Distilled water	50 ul	-	-
Serum	-	50 ul	-
Standard	-	-	50ul

The tubes labelled as Standard ,Blank and Test were mixed well and incubated at 37 °C for 10 minutes and then the absorbance of all the tubes was measured at 550 nm against the blank.

Calculation;

Total protein in g/dl =(absorbance of test /absorbance of sample) x 6 gm/dl.

4.4.2 Albumin estimation

The serum albumin was estimated by BCG method which is based on the principle that serum albumin can bind with certain dyes such as bromocresol green, forming coloured complex. The blue green complex has maximum absorption at 630nm.The concentration of albumin in serum is estimated by comparing the colour intensity of test to the known albumin at 630nm.

Table: 4.2

Reagent	Test	Blank	Standard
Albumin	3.0ml	3.0ml	3.0ml
Serum	0.02ml	-	-
Standard	-	-	0.02ml
Distilled water	-	0.02ml	-

The tubes were mixed well and then allowed to stand for five minutes at room temperature. The absorption of all the test tubes was measured at 630nm against blank.

Calculation

Serum albumin = (absorption of test/absorption of standard) x 4gm/dl.

4.4.3) Globulin estimation

Globulin was estimated by subtracting the value of total albumin from the value of total protein as:

Globulin in g/dl =(total protein in g/dl – albumin in g/dl)

4.5 PHYSICO-CHEMICAL ANALYSIS OF WATER

For physico-chemical analysis, 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 bottles on spot. Analysis

of other parameters was done in lab within 24 hrs in accordance with APHA (1998) and CSIR (1974). The methods are listed in the following Table 1:

Table: 4.3

SNO.	Parameter	Method	References
1	Temperature	Celsius thermometer	-
2	pH	Digital pH meter	-
3	Conductivity	Conductivity meter	-
4	Dissolved oxygen	Azide modification of Winkler's method	APHA (1998)
5	Ammonical Nitrogen	Phenate method	APHA (1998)
6	Nitrate Nitrogen	Salicylate method	CSIR (1974)
7	Nitrite Nitrogen	Modified Griess – Illosvay method	APHA (1998)
8	Total Phosphorus	Stannous chloride method	APHA (1998)
9	Ortho Phosphorus	Stannous chloride method	APHA (1998)

4.6 Statistical Analysis

The whole data was fed into a micro soft excel 2003. A computer program (SPSS 10.05 for windows) was used for data analysis. The descriptive data was given as a mean ± standard deviation (SD).

CHAPTER NO –4

REVIEW OF LITERATURE

Fishes live in very intimate contact with their environment and are therefore very susceptible to physical and chemical changes in it, which may be reflected in their blood components. Blood is therefore recognized as a potential index of fish response to water quality, and can be used to ascertain the effects of pollutants in the environment. Hesser (1960) framed out methods for routine fish haematology,while Larson and Sneiszko (1961) compared various methods of determination of hemoglobin in trout blood. Bouck *et al*. (1966) stated that hematology may be useful tool in monitoring stress levels of aquatic pollution on fish.

Gelineo (1969) studied concentration of hemoglobin in 53 species of fresh water and marine fish and reported that freshwater, active and male fish recorded a higher concentration of hemoglobin than marine inactive and female fish.

Ashman and Barber (1970) prepared a dual diluter for haematology. They proposed a diluter, which was accurate, simple to use, reliable and saved a considerable amount of time. Burck (1970) studied the various methods used for determining haematocrit and pointed out that the design of the centrifuge should include a flat bottom for greater accuracy. He also reported that the

elimination of error caused by plasma trapped between cells is possible by a serial dilution procedure using Cr^{51}-EDTA.

Avtalion *et al*. (1974) gave a new method for determination of blood volume in fish. This method was based upon RBC dilution which occurs in vivo after bleeding quantities of blood varying from 20-50% of the presumed blood volume. Bali (1971) devised a new method for multiple blood sampling in *Cyprinus carpio* by inserting a 24 bore sterile hypodermic needle fixed with 2ml sterile syringe containing 1ml of dry EDTA into the gill filaments. Hutin (1971) devised an anticoagulant formula for haematological tests (haemogram) which allowed good preservation of the formed elements of the blood in sample. With this solution consisting of sequestrene (10g), formol (1ml), glycocoll (0.80g) and distilled water it was possible to make smears 24 hours and even 72 hours after sampling. Yashnovich and Leonenbo (1971) investigated 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 (1972) reviewed some selected literature regarding the use of haematological techniques for assessing the health of fresh water fish. He compared the techniques already widely used in human pathology for the assessment of health and for aid in diagnosis of various diseases and condition in fresh water fish. In a later study (Blaxhall,1973) he reported an error in haematocrit value produced by inadequate concentration of EDTA and observed a rise in haematocrit value of blood from rainbow trout, *Salmo gairdneri* (Richardson) after collection in di-potassium EDTA. Blaxhall and Daisely (1973) described routine hematological methods for examining fish blood which included hemoglobin estimation, PCV, erythrocyte counts, ESR, TLC and DLC's and cytochemical staining. They gave description of stained blood cells as well as the range and mean value for these tests on brown trout *Salmo trutta* (L). These methods were suggested as possible means of assessing the fish health. Haider (1972) made haematological

observation on rainbow trout, *Salmo gairdnari* and formulated an erythrocyte distribution curve.

Hussein *et al.* (1974) made a haematological study of healthy *Anguilla vulgaris* and *Mugil cephalus*. They studied the seasonal variations of the cellular blood constituents, erythrocyte counts, size of the erythrocytes and the erythrocyte nuclei, the number of leukocyte and DLC, haematocrit values, hemoglobin content, ESR and specific gravity. They found that the average RBC counts, haematocrit 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, haematocrit values and hemoglobin content were found to be higher in summer and lower in winter. Krishnamoorthy & Shakunthala (1974) studied haematological and respiratory parameters in frogs. Seasonal variation in RBC count was found, the count being higher in January than in September. Females showed higher counts than males. Cold- acclimation resulted in elevation of counts in all seasons and in both sexes, and was accompanied by a rise in hemoglobin content. Denten and Yousuf (1975) observed seasonal changes in the haematology of *Salmo gairdneri.*

John (1976) studied the relationship between haematological variables and body weight (W) in the American plaice. Haematocrit, hemoglobin concentration, and cell volume and numbers were directly correlated with W, indicating that small fish have low blood oxygen solubility in spite of a high weight specific oxygen consumption. Mean cell hemoglobin content was found to be positively correlated while mean cell surface area per unit hemoglobin tended to be negatively correlated with weight. 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. Higher blood glucose levels were observed in the more active teleost species.

Atinkson and Judd (1978) stated that various blood parameters reveal physiological adaptations of fishes in their natural habitats and are helpful in determining systematic relationships. Vuren and Hattingh (1978) studied various haematological values of wild yellow fish (*Barbus holubi*), carp (*Cyprinus carpio*) and two species of mud fish (*Labeo umbratus* and *Labeo capesis*) on seasonal basis. Seasonal variations were observed in all of the parameters studied but no sexual differences were found and no significant change in the haematology could be related to breeding season. Wide individual variations were evident in fish of different length groups.

Clark *et al*. (1979) studied physiological stress resulting from environmental influences in largemouth bass, *Micropterus salmonids*, and reported that Haematocrit, hemoglobin and total plasma protein were positively correlated with fish length, Hb, Hct were positively correlated with fish age, while Mean corpuscular hemoglobin negatively correlated with fish age. Both hemoglobin and packed cell volume were related to erythrocyte counts. Siddiqui and Nasim (1979) made haematological observation on *Cirrhina mrigala* and recorded higher hemoglobin and erythrocyte concentration in males than in females.

Mcada *et al*. (1980) studied the distribution of fish in San Rafel river system and found that the native fish dominated in the tributaries but were replaced by introduced fish near the mouth of the river. Smit and Hattingh (1980) made haematological assessment of generally used fresh water fish blood anticoagulants. Heparin injection B.P. containing 0.5% phenol as preservative, EDTA, trisodium citrate (TSC) and ammonium potassium oxalate (APO) were compared as anticoagulants in C*yprinus carpio* and *Sarotherodon mossambicus*. Heparin proved to be the anticoagulant of choice, although unsuitable for some assays.

Mile *et al.* (1983) established normal ranges for diagnostically important haematological and blood chemistry characteristics of rainbow trout *Salmo*

gairdneri. Munkittrick and Leatherland (1983) measured haematocrit values in feral gold fish *Carracius auratus* as indicators of health of the population. They found haematocrit values in males to be larger than in females in all collections of the feral fish except the ones caught in spring. Nair *et al*. (1983) studied the fish fauna of the Ashtamudi, the second largest eustarine system in Kerala. 97 species belonging to 39 families were recorded of which 69 were commercially important. Terashima (1983) described three new species of the cyprinid genus Schizothorax from lake Rara, Northwestern Nepal, *S. macropthalmus* and *S. nepalensis.* The food habits of the three species were different from each other. Reproductive isolation was observed between *S. raraensis* and *S. macropthalmus*. 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) studied the haematological changes in cold water fish, *Schizothorax plagiostomus* (Heckel). They reported that *O. plagiostomus* naturally infected with metacercarae of Diplostomium tertare had decreased total erythrocyte count, PCV, Hb, TLC relative to uninfected controls indicative of pollution. Murray (1984) related haematological characteristics with the sex of fish and season of the year.

Mainwaring and Rowley (1885) studied the effects of ethylenediamine-tetraacetic acid (EDTA), heparin and tri-sodium citrate (TSC) on various haematological parameters in the blenny, *Blennius pholis*. Ralio *et al*. (1985) reported that fish blood parameters of diagnostic importance like erythrocyte and leucocyte count, hemoglobin, haematocrit and leukocyte differential counts readily respond to incidental factors such as physical stress and environmental stress due to water contaminants.

Pickering (1986) studied differential blood cell counts in three different strains of brown trout through three separate spawning seasons. A consistent elevation in the number of circulating erythrocytes was observed in sexually

mature male fish (compared with fish of either sex) during October-December of each season. A marked lymphocytopenia also occurred during October in mature fish of both sexes with lymphocyte numbers still being significantly depressed as late as March of the following year.

Rao, *et al.* (1989) observed 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*). The leukocyte parameters were not related to activity and the habitat of the fish.

Garcia *et al.* (1992) studied the effect of the volume of blood extracted, number of extractions and weight upon the haemocrit value of fresh water teleosts, rainbow trout (*Oncorhynchus mykiss)* and a marine European sea bass (*Dicentrarchus labrax L.*) and found that from trout with the same weight, the increase in the volume of blood extracted resulted in a significant increase in the haemocrit value. Also for the same volume of blood extracted in trout of the same weight the second extraction resulted in the significant increase in the haemocrit value. In sea bass, haemocrit values in relation to the aforementioned parameters were not produced. 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. However a marked increase in the gamma fraction of serum protein 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.

Allen (1994) developed a technique for extracting small quantities of blood up to 16 micro liters from fish of 20-30 mm length from the dorsal aorta of

decapitated fish. Fader *et al*. (1994) studied seasonal variation in heat shock proteins (hsp 70) in stream fish under natural conditions. The study revealed that fish (*Pimephales promelas, Salmo trutta, Ictalurus natalis* and *Ambloplites rupestris)* respond to seasonal changes in their environment by synthesizing heat shock proteins with a molecular weight of approximately 70kd (hsc/hsp-70). Rauthan and Grover (1994) observed 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.

Azizoglu and Cengizler (1996) evaluated some haematological values of fresh water fish *O.niloticus*. Ivanac *et al*. (1996) analyzed haematological values of three species of percidae. The haematological status of three species differed significantly and this was attributed to their ecology and distribution. Roche and Boge (1996) made an investigation on classical stress indicators (cortisol, hemoglobin, and hematocrit) in sea bass (*Dicentrarchus labrax*).

Hrubec *et al*. (1997) investigated the effect of water temperature on haematological and serum biochemical analysis in hybrid stripped brass. Collazos *et al*. (1998) worked on 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*.

Leard *et al*. (1998) measured haematocrit, sodium, glucose and pH in whole blood of 1,522 channel catfish (*Ictalurus punctatus*). The mean monthly haematocrit fluctuated seasonally from a low value of 14.5% in mid winter

to a high of 25.7% in midsummer. Timothy *et al*.(1998) worked out the seasonal values of selected blood parameters of farm-raised channel catfish (*Ictalurus punctatus*) in the Mississippi Delta Hematocrit, sodium, chloride, potassium, calcium, glucose, and pH were measured in whole blood of 1,522 channel catfish collected from 3 commercial food-fish ponds in the Mississippi Delta. The mean monthly hematocrits fluctuated seasonally from a low of 14.5% in midwinter to a high of 25.7% in midsummer. Sodium levels were consistent throughout the year with a mean (SE) of 134 (0.13) mM/liter. Mean chloride values for the year were 120 (0.14) mM/liter but increased to 132 mM/liter in midwinter. 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 were wide variations in haematocrit and MCV of individual healthy fish.

Fagbenro *et al*. (2000) studied haematological profile, food composition and enzyme assay in the gut of the African bony-tongue fish, *Heterotis niloticus.* Aysel *et al*. (2001) carried out study on 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.* Bernet *et al*. (2001) studied the impact of effluent from a sewage treatment works on fish health, serum chemistry variables were investigated in brown trout (*Salmo trutta* L.) held in cages (active monitoring) and wild brown trout (passive monitoring). Means of the measured serum parameters of the different treatment groups were close or within normal ranges. However, fish exposed to effluent from the sewage treatment works had significantly

different blood urea nitrogen and bilirubin values than fish kept in river water. Hrubec *et al*.(2001) worked out age-related changes in hematology and plasma chemistry values of hybrid stripped Bass (*Morone chrysops* & *Morone sexatilis*) The results showed that values for packed cell volume and red blood cell indices were significantly lower, and plasma protein concentration was significantly higher in younger fish. Total white blood cell and lymphocyte counts were significantly higher in fish of six and nine months age. Orun *et al.* (2001) conducted a study to determine and compare blood parameter levels of *Alburnoides bipunctatus* ,*Chalcalburnus mossulensis* and *Cyprinion macrostomus*. Variations in haematological characters of fish were compared according to species, gender and seasonal differences. Effect of water quality on blood parameters was also determined. The 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, erythrocyte were high in male fish in an annual period.

Adham *et al*.(2002) studied the blood chemistry of Nile tilapia, *Oreochromis niloticus* under the impact of water pollution, and environmental factors of ambient water were related to physiology of the cichlid. Homatowska *et al*. (2002) studied haematological indices and cytomorphometry of circulating blood in the sun bleak, *Leucaspius delineates* (Heckel, 1843). 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. Mckenzies, *et al* (2002) studied haematology and serum biochemistry of Tammer wallaby, *Macropus eugenii.* 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. Orun and Erdemil (2002) studied

seasonal effects on blood parameters of *Capoeta trutta* (Heckel, 1843) from Karakaya Dam lake (Malatya, Turkey). Age-Weight-Length values of the fish were determined seasonally during a year and the effect of these independent variables on blood parameters was performed according to sex difference. 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) studied haematology and some blood chemical parameters of young carp till the age of three years, The results suggested that the investigated hematological and biochemical variables could be successfully utilized in monitoring the metabolic balance and health status of fish.

Cullen *et al*.(2003) studied seasonal variation in biochemical indicators of physiological status in *Euphausia superba*. Sowunmi, A.A. (2003) studied the haematology of the African Catfish, *Clarias gariepinus* from Eleyele Reservoir Ibadan.

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*. Hasnain *et al*. (2004) studied biochemical characterization of a protein of albumin multigene family from serum of African cat fish *Clarias gariepinus*. Jawad *et al*. (2004) analyzed the relationship between haematocrit and body length, sex and reproductive state in the Indian *Tenalosa ilisha*, haematocrit value was found to show a quadratic relationship with the fish size. Male fish showed a higher haematocrit value than females. Lerman *et al*. (2004) studied effect of different water temperature on the haematological and metabolic parameters in blood, liver and white muscles of silver cat fish, *Rhamdia quelen*. 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. Tierney *et al*. (2004) studied the differential leucocytes landscape (monocytes, thrombocytes, lymphocytes and diverse forms of granulocytes) encountered in the blood of four teleost species, Coho salmon (*Oncorhynchus kisutch*), pacific herring (*Clupea pallasi*), brook stickle back (*Clupae inconstans*) and feathered minnow (*Pimephales promelas*), and reported that relative leucocyte number responds significantly to changes in water quality.

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 a 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. Caruso *et al*. (2005) performed an investigation to monitor physiological and biochemical parameters in sea bream (*Sparus aurata)* and sea bass (*Dicentrarchus labax*) increase in tissue lactate and plasma cortisol levels and a reduction in haemolytic and haemagglutinating titers were recorded. 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*). 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. In this report thrombocyte was excluded from leucocytes. The existence of gold fish monocytes was demonstrated for the first time in the report.

Asadi *et al*. (2006) studied serum biochemical parameters in endangered species *Huso huso*. Serum samples were analyzed and their serum parameter values were determined as mean ± SD in both sexes. Age, weight, total length and fork length were same between groups. Calcium, total protein, blood urea nitrogen, albumin, glucose, alkaline phosphatase and amylase were studied. Results showed that there was no difference in calcium, total protein, blood urea nitrogen, creatinine, magnesium and amylase between sexes, however male fish have higher glucose, total protein, albumin and globulin than female.

Gbore *et al*. (2006) studied 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, total protein, albumin, and A/G ratio) except for the leukocyte, Hb, albumin and A-G ratio for *Tilapia zilli*, while the blood constants, albumin, A-G ratio values increased for *C. gariepinus*. The changes in the Hb, leukocyte, MCHC and the total protein 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. Tavares-dias and Moraes (2006) worked out the reference intervals for biochemical variables and red blood cell indices of healthy intensively bred channel catfish *Ictalurus punctatus*. The blood variables were determined using standardized clinical methods. The reference intervals (25th and 75^{th} percentiles) were established using a non-parametric method. Reference intervals for plasma glucose, serum total protein, sodium, potassium, calcium, magnesium, chloride concentration, primary and secondary red blood cell indices were established. Trumble *et al*., (2006) studied dietary and seasonal influences on blood chemistry and hematology in seals. Vetansnik *et al*. (2006) carried out haematological analysis 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.

Bayir *et al*. (2007) studied seasonal changes in blood plasma biochemistry of *Siraz capoeta umbla.* The study revealed that blood biochemistry values of siraz, were positively affected by water temperature and pH, except glucose and globulin which were negatively affected by water temperature and pH. Gabriel *et al*. (2007) presented the haematological characteristics of *Sarotherodon melanontheron* from the brackish water creek of Buguma. The highest range of parameters was recorded in thrombocytes, while the lowest was observed in RBC. Significant differences ($p< 0.05$) between males and females were observed in hemoglobin, haematocrit, red blood cells and thrombocytes. Sahan *et al*. (2007) carried out a study in agricultural, industrial, domestic, and slaughter house discharging region of Ceyhan river and found that leukocyte values and neutrophil proportion in fish blood were found increased by means of environmental stressors ($p<0.05$). Vazquez and Guerrero (2007) made a study on the haematology of *Cichlosoma dimerus*. The morphological features of the blood cells were described. Erythrocytes, thrombocytes and four types of leucocytes were distinguished and characterized. This species had similar mean values for packed cell volume and hemoglobin, slightly higher for red blood cells and low lymphocyte counts compared to those found in other fishes. 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. In addition to normal erythrocytes, reticulocytes and division of erythrocytes were observed.

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) presented an investigation to characterize 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, triglycerites, cholesterol, high density lipoprotein. The minimum values were obtained in cold months for red blood cells, haematocrit, MCV, and white blood cells. The highest and lowest value for hemoglobin, MCH, MCHC were found in January and in warm months respectively 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 and Tomova (2008) studied some red cell indices of three freshwater fish –bleak (*Alburnus alburnus L*.), rudd (*Scardinus erythrophtalmusl*) and perch (*Perca fluviatilis L*.) from the studen Kladentsh reservoir. The analysis of all three freshwater fishes inhabiting the Studen Kladenetsh reservoir registered anemic changes in the blood regardless of the season. Bastami *et al*. (2008) carried out a study to obtain a basic knowledge of the haematology and the influence of sex on some blood parameters of wild carp (*Cyprinus carpio*) spawners. The highest haematocrit (PCV), hemoglobin concentration (Hb), RBC, MCH and MCHC were found for males. The highest leucocyte differential counts were found for females. Statistical analysis revealed that differences in haematological parameters between males and female fish were not significant. Latifi *et al*. (2008) studied cytomorphometric evaluation of Koran (*Salmo letnica*) erythrocytes under natural conditions. Nikolov *etal.* (2008) examined 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. Shinde *etal.* (2008) studied the Ichthyofaunal Diversity of Harsool Savangi Dam,District Aurangabad. The results revealed the occurrence of 15 fish species belonging to 3 orders, 4 families and 12 genera. The order cypriniformes found dominant with 11 species, followed by perciformes 3 species and siluriformes with 1 species.

Vijaykumar *et al*. (2008) studied the ichthyofaunal diversity of Kagina River in Gulbarga district, Karnataka. The result of investigation revealed the occurrence of thirteen fish species belonging to five orders. The order Cypriniformes was dominant with ten species followed by order Siluriformes, Osteoglossiformes, Mastacembeliformes, each with one species

Adeyeno *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. Asadi *et al.* (2009) studied serum lipid, free fatty acid, in *Acipenser stellatus*. Serum samples were analyzed and their serum parameter values were determined as mean±SD in both groups.

Jamalzadeh *et al*. (2009) compared the blood indices in healthy and fungal infected Caspian salmon, white blood cells, neutrophile and eosinophile have higher values ($p<0.05$) in fungal infected fishes than healthy Caspian salmon. The other parameters like Hb , Hct, RBC, lymphocyte, monocyte were greater in healthy Caspian salmon. Weight of healthy fishes had positive significant correlation with white blood cell count. However, a negative correlation was seen between length and weight of fungal infected fish and WBC. Olufemi and Owolabi, (2009) studied haematological and serum biochemical parameters of natural population of

Synodontis membranaeca from Jebba lake. The study provided baseline biochemical and haematological data for use in health monitoring and productivity of *S. membranacea*, parameters studied included RBC, Hb, PCV, MCH, WBC, monocyte, albumin, creatinin, uric acid, cholesterol, calcium, potassium, sodium, alanine, only three parameters (i,e, neutrophil, glucose and potassium) differed significantly ($p<0.05$) on gender basis. Patriche *et al*. (2009) made a study on the total blood proteins in cyprinids and found that the level of the total protein in serum indicator of nutritional condition of the organism presenting at the same time ample qualitative and quantitative variations depending on species age, sex, stage of matuarity, water temperature and especially in correlation with the health condition of fish. Shahsavani *et al*. (2009) made a study for determination of some normal serum parameters in starry sturgeon (*Acipenser stellatus pallas*, 1771). Serum biochemical values were determined for sodium, potassium, calcium, glucose, total protein, albumin, cholesterol, creatinine, bilirubin. The serum values for bilirubin, sodium, and creatine were significantly higher in females, whereas blood urea nitrogen and albumin were significantly higher in males. Singh and Tandon (2009) studied the effect of river water pollution on haematological parameters of fish, *wallago attu* (padhan) from river Suheli and river Gomti. The haemoglobin 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. Neutrophils and monocytes were found to be higher in Suheli fish than Gomti fish, however lymphocytes were found higher in Gomti as compared to fish from Suheli River. 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 of 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, glucose, triglyceride and urea nitrogen levels 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.

Akinrotimi *et al.* (2010) investigated the haematological characteristics of *Tilapia guineensis* from Bunguma creek Nigeria. Values of some haematological indices were assessed in 60 adults of *Tilapia guineensis* (mean length 21.14cm ± 4.16; mean weight 386.12g ± 6.12). Andreeva (2010) reported the presence of serum albumins in fish of different classes and orders inhabiting different habitats. A wide spectrum of structural diversity of albumins was reported due to their participation in osmotic, plastic and transport functions under varied conditions of environment and the organism internal media. Dove *et al*. (2010) studied blood cells and serum chemistry in whale shark *Rhincodon typus*. Erythrocyte morphology was similar to other orectolobiforms and major leucocyte types were similar to non-carcharhinid sharks. Lymphocyte population was dominant 46% followed by eosinophilic granulocyte or heterophil (39.5%). The remaining 15% white blood cells were divided among coarse eosinophilic granulocytes or eosinophils, neutrophils, monocytes similar to those of other of most elasmobranchs. Hayes *et al*. (2010) studied Fish distribution patterns and their association with environmental factors in the Mokau River catchment, New Zealand. Nine native diadrornous and 2 exotic fish species were recorded in an intensive survey of tributaries of the Mokau River. At the site level, species diversity was low and much of the fauna had a very restricted distribution. Sites were grouped on the basis of their species composition

using the classification procedure 2-way indicator species analysis. Four groups of sites were identified, characterised by: (1) a longfinned eel-elver assemblage; (2) a longfinned eel-adult redfinned bully assemblage; (3) an inanga-adult redfinned bully assemblage; and (4) a torrentfish-bluegilled bully-redfinned bully-elver assemblage. Relationships between fish assemblage distribution patterns and environmental factors were examined with multiple discriminant analysis. The overriding feature influencing patterns of fish distribution was the prevalence of diadromy in the fauna with species varying in their ability to penetrate upstream. Distance from the sea and gradient from the river mouth were the environmental variables most strongly correlated with distribution patterns. Other, more specific, associated habitat features included water velocity, depth, and substrate coarseness. Ikechukwiu and Obinnaya (2010) carried out haematological studies of African lungfish, *Protopterus annectens* in order to establish a normal range of blood parameters which would serve a baseline 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 thrombocyte counts, hemoglobin content, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular hemoglobin concentration, blood osmolarity, pH, haematocrit, glucose, urea, uricacid, creatinine and ionic concentrations were determined in the various reproductive stages of *p. annectens.*

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) and biochemical such as serum glucose (GLU), protein (PRO), cholesterol and urea (UR) of seven teleost fish species 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, PRO, GLU and UR 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. Satheeshkumar *et al*. (2011) worked out the measurement of haematological and biochemical studies on wild marine carnivorous fishes from Veller estuary south east coast of India. Haematological and serum biochemical parameters were studied and compared between four marine carnivorous fishes. Statistical analysis revealed that the differences in the haematological parameters between marine carnivorous fish were significant ($p<0.05$). The results revealed that hemoglobin, red blood cells / white blood cell ratio, MCV and MCH concentration were significantly correlated at ($p<0.05$) level. RBC/WBC level increased due to the decrease in WBC during the study. Serum biochemistry parameters were used for confirming maturity and monitoring changes in quality of water. 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.

Yousfian *et al*. (2010) studied the serum biochemical parameters of rainbow trout, using an automated blood analyzer. Serum samples of rainbow trout were analyzed and their serum parameter value determined as mean±SD in male and female fish some parameters like total protein, albumin, glucose, phosphorus, magnesium were significantly higher in males than in females, the other biochemical parameters like aspartate aminotransferase, alanine aminotransferase, iron, calcium, blood urea nitrogen were higher in females but the differences were not significant. 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 different temperature. Increasing temperature led to significant increase in haematocrit, calcium and eosonophil, but WBC lymphocyte, cortisol and glucose concentration decreased slightly ($p<0.05$).

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.

Mohamad Reza Imanpoor and Mehdi Abdollahi (2011) studied the **S**erum Biochemical Parameters of Caspian Lamprey, *Caspiomyzon wagneri during* final Spawning Migration. The objective of this survey was to determine some serum ionic and metabolic parameters and those relationships in 22 migratory population of Caspian lamprey in Shirood River. There was no significant ($P<0.05$) difference between level of calcium (8.52±2.9-9.14±0.97 mg/dL), magnesium (2.97±1.04-2.86±0.97 mg/dL), phosphorus (11.23±3.18-13.57±6.61 mg/dL), Iron (0.37±0.19-0.54±0.3 mg/dL), total protein (4.7±2.47-5.81±3.85 g/dL), glucose (93.98±22.89-104.10±32.38 mg/dL) and cholesterol (164.00±59.19-170.99 ±60.77 mg/dL) in male and females. The correlation between magnesium with calcium ($P<0.05$) and cholesterol ($P<0.05$) and glucose ($P<0.01$) was significant. There was a significant correlation between phosphorus and total protein ($P<0.05$). The correlation between cholesterol with glucose ($P<0.01$) was significant. But correlation between total protein with magnesium to phosphorus ratio and calcium to phosphorus ratio ($P<0.05$), was invert.

Work done in Kashmir

Not much work has been done on this aspect in Kashmir except for a few records the notable among them being the work of Qadri (2004).

A critical analysis of the above review of literature indicates that not much literature is available on the local fish species, hence the project was initiated for filling of the gap and understanding of haematological features of *Schizothorax.*

CHAPTER NO –5

RESULTS

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DISCUSSION

The Present study was aimed at studying the ichthyofauna of Lidder stream and haematobiochemistry of *Schizothorax* spp. and impact of environmental factors on haematology and blood biochemistry of *Schizoyhorax* spp. of Lidder stream. For this purpose, three species of fishes namely *Schizothorax plagiostomus, S. labiatus,* and *S. esocinus* were taken for study of haematology and blood biochemistry through various laboratory techniques from August 2010 to May 2011. For a clear understanding, of the observations, this chapter has been divided into following subheadings dealing with various aspects of study.

5.1 Ichthyofauna of Lidder stream

5.2 Distribution pattern of fishes in Lidder stream

5.3 Physico-chemical analysis of water

5.4 Haematology of *Schizothorax* spp.

5.5 Biochemistry of *Schizothorax* spp.

5.1 I chthyofauna of Lidder stream

The ichthyofauna of Lidder stream comprises of nine species of fishes, belonging to four families Cyprinidae, Balitoridae, Sisoridae, and Salmonidae.

Family Cyprinidae includes *Schizothorax plagiostomus, Schizothorax esocinus, Schizothorax labiatus and Crossocheilus diplochilus*.

- ***Schizothorax plagiostomus*** Heckel, 1838 : Local name ***Khont***

Kingdom : Animalia
Phylum : Chordate
Class : Osteichthyes
Order : Cypriniformes
Family : Cyprinidae
Subfamily :Schizothoracinae
Genus : *Schizothorax*
Species : *plagiostomus*

S. plagiostomus, locally known as Khont, is distinguished remarkably by elongate body, with projecting snout. Mouth distinctively inferior, wide and lower jaw very deep, short and with a sharp keratinized antero-ventral cutting edge. Lower lip fold is expanded and papillose. A series of enlarged scales are found along the base of its anal fin. It has minute scales, 89-99 in the lateral line. The fish is typically lotic water species being distributed in fast flowing streams of Kashmir (Kullander etal.,1999)

- ***Schizothorax esocinus*** Heckel, 1838 : Local name ***Churru***

Kingdom : Animalia
Phylum : Chordata
Class : Osteichthyes
Order : Cypriniformes
Family : Cyprinidae
Subfamily : Schizothoracinae
Genus : *Schizothorax*

Species : *esocinus*

S. esocinus, differs from all other Kashmir valley Schizothorax in much longer jaws, without enlarged lips or tuberculate pads. The fish is silvery with numerous dark, small irregular spots on back and flanks of body. The fins are silvery grey with similar dark spots which are more numerous at base. Also the color pattern is distinctive with light ground color and contrasting black spots in most specimens. Its body is streamlined, mouth wide, horse-shoe shaped, cleft is very deep, lips are thick fleshy, lower labial fold is interrupted in middle. Barbells are two pair, rostral pair about 1.5 times longer than eye diameter and maxillary pair slightly shorter. Dorsal fin is inserted slightly nearer to base of caudal fin than to snout tip. Scales are very small, about 104 in lateral line (Kullander etal.,1999).

- ***Schizothorax labiatus*** McClelland & Griffith, 1842 : Local name ***Chush***

  Kingdom : Animalia
  Phylum : Chordata
  Class : Osteichthyes
  Order : Cypriniformes
  Family : Cyprinidae
  Subfamily :Schizothoracinae
  Genus : *Schizothorax*
  Species : *labiatus*

S. labiatus, is distinguished by elongate, fusiform, with a prognathons upper jaw, a lower jaw with wide lip folds usually separated by a distinct raised pad. *S. labiatus* has a rounded lower jaw, with a narrow cornified margin, no keratinized cutting edge, and lips restricted to wide lateral flaps and a more or less well developed median thickening, without enlarged papillae (Kullander etal.,1999).

- ***Crossocheilus diplochilus :*** Heckel, 1838 : local name ***Tetther***

Kingdom : Animalia
Phylum : Chordata
Class : Actinopterygii
Order : Cypriniformes
Family : Cyprinidae
Genus : *Crossocheilus*
Species : *diplochilus*

A small species, reaching approximately 130 mm SL. elongate, fusiform, but with a flatned chest and belly. Eyes dorsolateral. Shallow but distinct ethmoidal furrow; deep rhinal furrow immediately posterior to base of rostral barbell. Barbles two pairs, rostral and maxillary, both short. Tubercles minute, concentrated to infraorbital field. Mouth inferior, broad, upper jaw without lip except in corner of mouth where upper lip and lateral division of lower lip joining in an expanded and tuberculate pad. Colour brownish or grayish, belly and abdomen whitish (Kullander etal.,1999)

Family Balitoridae includes *triplophysa marmorata* and *triplophysa kashmirensis*.

- ***Triplophysa marmorata*** Heckel, 1838**:** Local name ***Ara gurun***

Kingdom : Animalia
Phylum : chordata
Class : Actinopterygii
Order : Cypriniformes
Family : Balitoridae
Genus : *Triplophysa*
Species : *marmorata*

It reaches 112mm, elongate, with eyes high on head, mouth inferior. Scales absent. Barbles two rostral, one maxillary pair. Ground colour pale yellowish or whitish

- ***Triplophysa kashmirensis*** Hora, 1922: Local name ***Ara gurun***

  Kingdom : Animalia
  Phylum : Chordata
  Class : Actinopterygii
  Order : Cypriniformes
  Family : Balitoridae
  Genus : *Triplophysa*
  Species : *kashmirensis*

Elongate, with a long slender caudal penduncle. Body shape notably variable, Eyes high on the head. Scales absent. Mouth inferior, with thick lips. Barbles three pairs, of which two rostral and one maxillary. *Triplophysa kashmirensis* has a bit longer caudal penducle than *Triplophysa marmorata* of same length (Kullander etal.,1999).

Family sisoridae includes *Glyptosternon reticulatum*.

- ***Glyptosternon reticulatum*** McClelland & Griffith, 1842*:* Local name ***Nayid***

  Kingdom : Animalia
  Phylum : Chordata
  Class : Osteichthyes
  Order : Siluriformes
  Family : Sisoridae
  Genus : *Glyptosternon*
  Species : *reticulatum*

Elongate, abdominally, sub cylindrical, Caudally compressed; body depth about uniform from head to caudal fin. Head short, wide and depressed. Eyes minute, dorsal, covered by skin. Barbles four pairs, including nasal, maxillary and two mental pairs. Caudal fin truncate or very slightly convex (Kullander etal.,1999).

Family salmonidae includes *salmo trutta fario*.

- ***Salmo trutta fario*** Linnaeus, 1758: Local name ***Trout***

Kingdom	: Animalia
Phylum	: Chordata
Class	: Actinopterygii
Order	: Salmoniformes
Family	: Salmonidae
Subfamily	: Salmoninae
Genus	: *Salmo*
Species	: *trutta*

The brown trout (*Salmo trutta_ fario)* is distinguished chiefly by the fact that the brown trout is largely a freshwater fish. Freshwater brown trout range in color from largely silver with relatively few spots and a white belly, to the more well known brassy brown cast fading to creamy white on the fish's belly, with medium-sized spots surrounded by lighter haloes. They live in upper rivers. Scales on lateral line, Pored lateral line scales (Kullander etal.,1999).

5.2 Distribution pattern

5.2.1 Distribution pattern at Site-I (upper Lidder Laripora Pahalgam)

The species-wise distribution is as under at Site I:

Schizothorax plagiostomus

It contributed to about 57.51% of total fish catch. This species was present throughout the year at this site.

Schizothorax esocinus

On average it contributed to 15.02% of total catch and ranked 2nd as per the total number.

Salmo trutta fario

It contributed to 13.98% of total catch. This species ranked 3rd at this site as per total catch.

Schizothorax labiatus

It contributed to 10.88% of total catch and thus ranked 4th as per the total catch.

Glyptothorax reticulatum

It contributed to 2.59% of the total catch. *Triplophysa kashmirensis*, *Triplophysa marmorata and* Crossochilus *diplochilus* were not present at this site.

5.2.2 Distribution pattern at Site-II (Lower lidder, Akura Mattan)

The species-wise distribution pattern is as under:

***S. plagiostomus*:**

On an average this fish contributed about 52.94% of total no. of fish. This species ranked 1st as per the no. of fish caught at this site. Here also this species was present throughout the year.

***S. esocinus*:**

On an average this fish contributed to 15.68% of the total no. of fish catch. This species ranked 2nd as per the number of fish caught at this site.

***S. labiatus*:**

On an average this fish contributed to 10.98% of total no. of catch. This species ranked 3rd as per the no. of fish caught.

***Triplophysa kashmirensis*.:**

On an average this fish contributed to 9.41% of total no. of fish. This species ranked 4th as per the number of fish caught.

***Triplophysa marmorata*:**

On an average this fish contributed to 7.05% of total no. of fish. This species ranked 5th as per the number of fish caught.

***Crossocheilus diplochilus*:**

At this site this species ranked 6th per the no. of fish caught. On an average it contributed to 3.52%.*Glyptothorax spp.* contributed to 0.392% *Salmo trutta fario* was completely absent at this site.

5.2.3 Distribution pattern at SiteII-A (Sakhras in between Site-I and Site-II):

The species wise distribution pattern at this place is as:

S. plagiostomus:

The species ranked first as per the number of fish caught. It contributed to 60.20% of total catch.

S. esocinus:

It ranked second as per the no. of fish catch. It contributed to 13.61% of total catch.

***S. labiatus*:**

This species ranked third and contributed to 10.99% of the total catch.

At this site ***Salmo trutta fario*** was present in the months of November, December and May. It contributed to 2.09% of total catch.

Glyptothorax reticulatum ,*triplophysa kashmirensis* and *triplophysa marmorata* were also present with an average contribution of 1.04%,4.71% and7.32% respectively.

5.2.4 Distribution pattern at SiteI-A(Aru in between site-I and origin of lidder)

The species wise distribution pattern at this place is as:

S. plagiostomus:

The species ranked first as per the number of fish caught. It contributed to 54.47% of total catch.

S. esocinus:

It ranked third as per the no. of fish catch. It contributed to 15.67% of total catch.

Salmo trutta fario

It ranked second as per the number of fish catch. It contributed to 17.91% of the total catch.

***S. labiatus*:**

This species ranked forth and contributed to 6.71% of the total catch.

Glyptothorax reticulatum

It contributed to 5.22 % of the total catch *Triplophysa kashmirensis*, *Triplophysa marmorata* and *Crossochilus diplochilus* were not present at this site.

Table 5.1: Showing Distribution of fishes at site-I.

Month	**Total no. of catch**	***S. plagiostomus***	***S. esocinus***	***S. labiatus***	***S. trutta fario***	***Glyptothorax spp.***	***T. kashmirensis***	***T. marmorata***	***Crossochilus diplochilus***
		No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No.(%)	No. (%)
August	14	9 (64.28)	2(14.28)	1(7.14)	2(114.28)	0(0.00)	0(0.00)	0(0.00)	0(0.00)
September	18	10(55.55)	3(16.66)	2(11.11)	3(16.66)	0(0.00)	0(0.00)	0(0.00)	0(0.00)
October	23	14(60.86)	3(13.04)	2(8.69)	4(17.39)	0(0.00)	0(0.00)	0(0.00)	0(0.00)
November	26	14(53.84)	4(15.38)	3(11.53)	4(15.38)	1(3.84)	0(0.00)	0(0.00)	0(0.00)
December	31	16(51.61)	5(16.12)	4(12.90)	4(12.90)	2(6.45)	0(0.00)	0(0.00)	0(0.00)
January	29	14(48.27)	5(17.24)	3(10.34)	6(20.68)	1(3.44)	0(0.00)	0(0.00)	0(0.00)
February	19	12(63.15)	3(15.78)	2(10.52)	1(5.26)	1(5.26)	0(0.00)	0(0.00)	0(0.00)
March	14	9(64.28)	2(14.28)	2(14.28)	1(7.14)	0(0.00)	0(0.00)	0(0.00)	0(0.00)
April	10	7(70.00)	1(10.00)	1(10.00)	1(10.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)
May	9	6(66.66)	1(11.11)	1(11.11)	1(11.11)	0(0.00)	0(0.00)	0(0.00)	0(0.00)
TOTAL	**193**	**111(57.51)**	**29(15.02)**	**21(10.88)**	**27(13.98)**	**5(2.59)**	**0(0.00)**	**0(0.00)**	**0(0.00)**

Table 5.2: Showing Distribution of fishes at site II.

Month	Total no. of catch	*S. plagiostomus*	*S. esocinus*	*S. labiatus*	*S. trutta fario*	*Glyptothora x spp.*	*Triplophysa kashmirensis*	*T.marmorata*	*Crossochilus diplochilus*
		No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
August	20	10(50.00)	5(25.00)	4(20.00)	0(0.00)	0(0.00)	0(0.00)	1(5.00)	0(0.00)
September	24	11(45.83)	6(25.00)	4(16.66)	0(0.00)	0(0.00)	2(8.33)	1(4.16)	0(0.00)
October	22	14(63.63)	4(18.18)	2(9.09)	0(0.00)	0(0.00)	1(4.54)	1(4.54)	0(0.00)
November	28	16(57.14)	5(17.85)	3(10.71)	0(0.00)	0(0.00)	1(3.57)	2(7.14)	1(3.54)
December	37	18(48.64)	4(10.81)	3(8.10)	0(0.00)	0(0.00)	6(16.2)	3(8.10)	3(8.10)
January	30	16(53.33)	2(6.66)	2(6.66)	0(0.00)	1(3.33)	4(13.33)	2(6.66)	3(10.00)
February	28	17(60.71)	3(10.71)	3(10.71)	0(0.00)	0(0.00)	2(7.14)	1(3.57)	2(7.14)
March	21	14(66.66)	2(9.52)	1(4.76)	0(0.00)	0(0.00)	2(9.52)	2(9.52)	0(0.00)
April	22	10(45.45)	4(18.18)	3(13.63)	0(0.00)	0(0.00)	3(13.63)	2(9.09)	0(0.00)
May	23	9(39.13)	5(21.73)	3(13.04)	0(0.00)	0(0.00)	3(13.04)	3(13.04)	0(0.00)
TOTAL	**255**	**135(52.94)**	**40(15.68)**	**28(10.98)**	**0(0.00)**	**1(0.392)**	**24(9.41)**	**18(7.05)**	**9(3.52)**

Table 5.3: Showing Distribution of fishes at site II-A.

Month	Total no. of Catch	*S. plagiostomus*	*S. esocinus*	*S. labiatus*	*S. trutta fario*	*Glyptothorax spp.*	*T. kashmirensis*	*T. marmorata*	*Crossochilus diplochilus*
		No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
August	16	9(56.26)	3 (18.75)	2 (12.50)	0(0.00)	0(0.00)	1 (6.25)	1(6.25)	0(0.00)
September	18	13(72.22)	3 (16.66)	2(11.11)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)
October	18	12(66.66)	2(11.11)	2(11.11)	0(0.00)	0(0.00)	0(0.00)	2(11.11)	0(0.00)
November	20	10(50.00)	2(10.00)	1(10.00)	1(5.00)	1(5.00)	1(5.00)	4(20.00)	0(0.00)
December	18	9(50.00)	3(16.66)	2(11.11)	2 (11.11)	0(0.00)	1(5.55)	1(5.55)	0(0.00)
January	28	20(71.42)	4(14.28)	2(7.14)	0(0.00)	0(0.00)	1(3.57)	1(3.57)	0(0.00)
February	20	12(60.00)	3(15.00)	2(10.00)	0(0.00)	1(5.00)	1(5.00)	1(5.00)	0(0.00)
March	17	11(64.70)	2(11.76)	2(11.76)	0(0.00)	0(0.00)	1(5.88)	1(5.88)	0(0.00)
April	16	9(56.25)	2(12.50)	3(18.75)	0(0.00)	0(0.00)	1(6.25)	1(6.25)	0(0.00)
May	20	10(50.00)	2(10.00)	3(15.00)	1 (5.00)	0(0.00)	2 (10.00)	2(10.00)	0(0.00)
TOTAL	**191**	**115(60.20)**	**26(13.61)**	**21(10.99)**	**4 (2.09)**	**2 (1.04)**	**9(4.71)**	**14(7.32)**	**0(0.00)**

Table 5.4: Showing Distribution pattern at Site-IA

Month	**Total no. of Catch**	***S. plagiostomus***	***S. esocinus***	***S. labiatus***	***S. trutta fario***	***Glyptothorax spp.***	***T. kashmirensis***	***T. marmorata***	***Crossochilus diplochilus***
		No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
August	10	5 (50.00)	2(20.00)	0(0.00)	2(20.00)	1(10.00)	0(0.00)	0(0.00)	0(0.00)
September	11	6(54.54)	1(9.09)	1(9.09)	2(18.18)	1(9.09)	0(0.00)	0(0.00)	0(0.00)
October	14	8(57.14)	2(14.28)	1(7.14)	3(21.42)	0(0.00)	0(0.00)	0(0.00)	0(0.00)
November	17	8(47.05)	3(17.64)	2(11.76)	3(17.64)	1(5.88)	0(0.00)	0(0.00)	0(0.00)
December	22	11(50.00)	4(18.18)	2(9.09)	3(13.63)	2(9.09)	0(0.00)	0(0.00)	0(0.00)
January	20	10(50.00)	3(15.00)	2(10.00)	4(20.00)	1(5.00)	0(0.00)	0(0.00)	0(0.00)
February	16	9(56.25)	2(12.50)	1(6.25)	3(18.75)	1(6.25)	0(0.00)	0(0.00)	0(0.00)
March	10	7(70.00)	2(20.00)	0(0.00)	1(10.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)
April	7	4(57.14)	1(14.28)	0(0.00)	2(28.57)	0(0.00)	0(0.00)	0(0.00)	0(0.00)
May	7	5(71.42)	1(14.28)	0(0.00)	1(14.28)	0(0.00)	0(0.00)	0(0.00)	0(0.00)
TOTAL	**134**	**73(54.47)**	**21(15.67)**	**9(6.71)**	**24(17.91)**	**7(5.22)**	**0(0.00)**	**0(0.00)**	**0(0.00)**

Table 5.5: showing Distribution pattern of fishes at four sites

Fish species	Site-I	Site -II	Site II-A	Site-IA
S. plagiostomus	111	135	115	73
S. esocinus	29	40	26	21
S. labiatus	21	28	21	9
S.T. fario	27	0	4	24
G.reticulatum	5	1	2	7
T.kashmirensis	0	24	9	0
T.marmorata	0	18	14	0
C.diplochilus	0	9	0	0

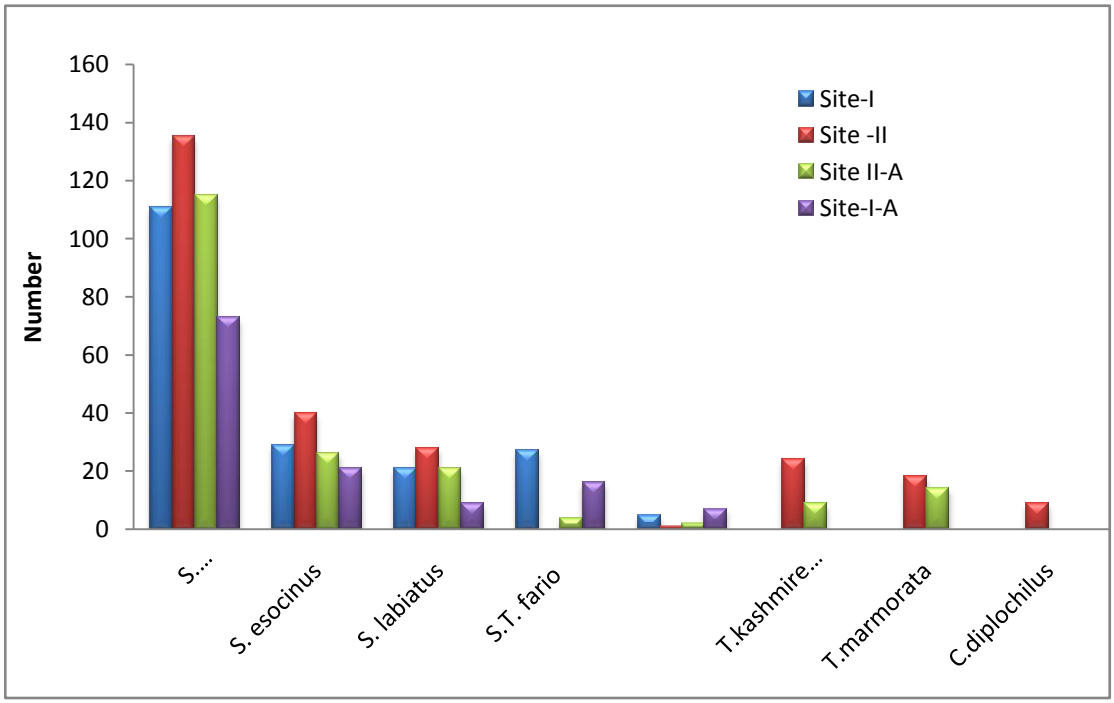

FIG 5.1 Showing the distribution pattern of fishes in lidder stream.

5.3 Physico-chemical analysis of water

5.3.1 Air Temperature

The air temperature of Lidder stream ranged from 2 ^{o}C to 27 ^{o}C. The minimum temperature of 2^{o}C was observed at Site -I during January and the highest of 27 ^{o}C at site -II during the month of August. The air temperature showed an increasing trend from January to May and decreasing trend from August to December.

5.3.2 Water Temperature

The temperature is the parameter most associated with climate. The water temperature ranged from 1 ^{o}C to 20 ^{o}C. The minimum temperature of 1 ^{o}C was observed at Site -I during January and February and the highest of 20 ^{o}C was observed in August at Site - II during the month of August. The water temperature showed an increasing trend from to May and decreasing trend from August to December.

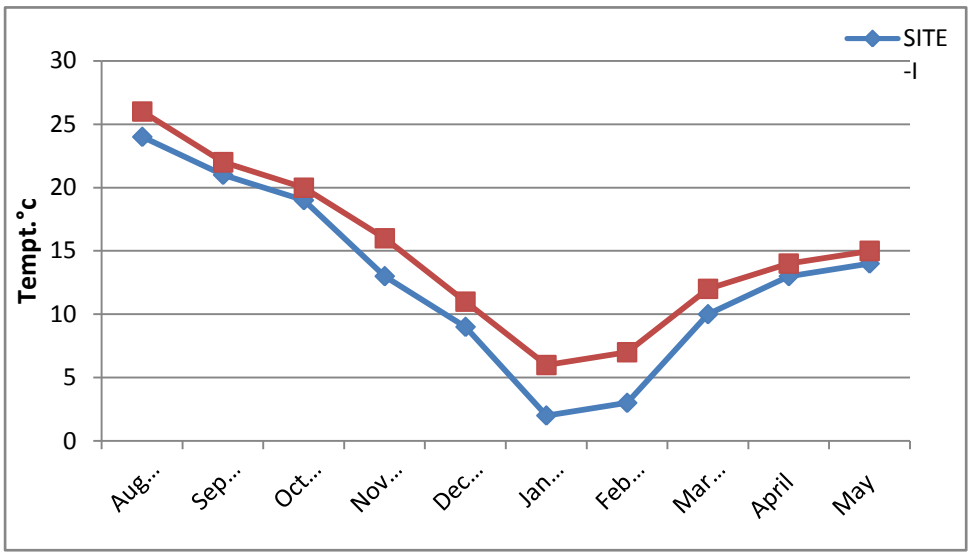

Fig5.2: Monthly variations in the Air Temperature values at the two Sites in Lidder Stream

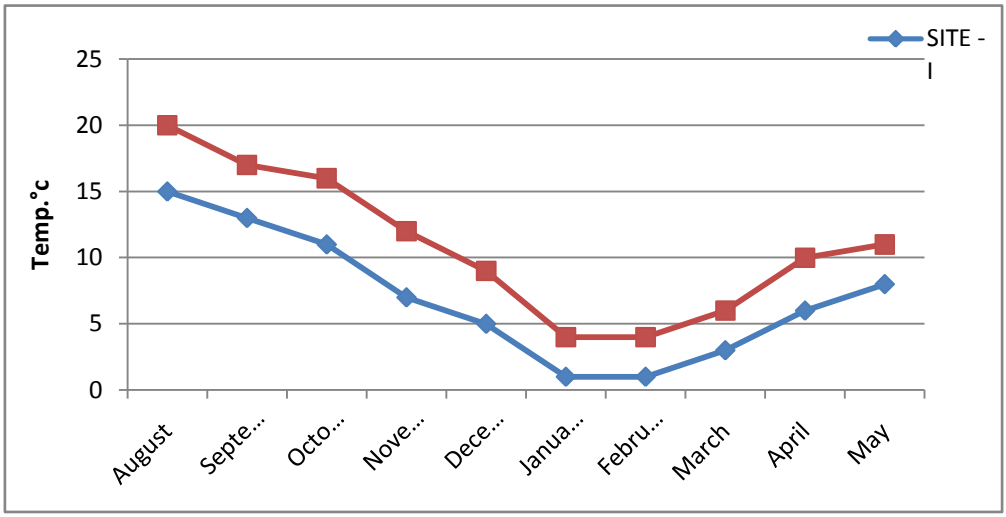

Fig5.3: Monthly variations in the Water Temperature values at the two Sites in Lidder Stream.

5.3.3 pH:

Recordings of pH –values confirm neutral and alkaline conditions. The pH ranged from 7.2 to 8.3 at Site –I. The minimum pH of 7.2 was observed at Site -I during the month of May, and maximum pH of 8.3 was observed in the month of October at the same site. At Site –II, the pH ranged from 7.1 in April to 9 in October.

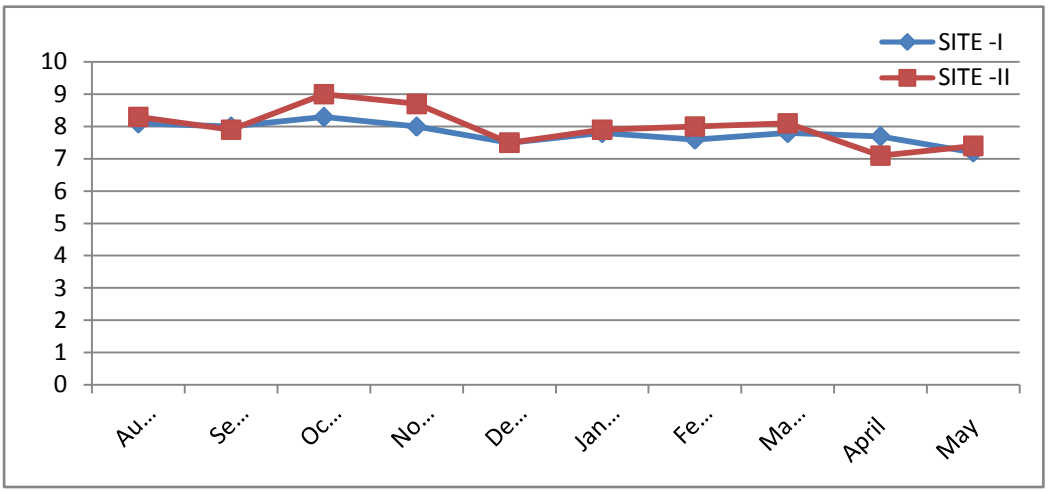

Fig 5.4: Monthly variations in the pH values at the two Sites in Lidder Stream.

5.3.4 Conductivity (µs/cm)

Conductivity ranged from 89 µs/cm in April to 310 µs/cm in August at Site -I and at Site -II it ranged from100 µs/cm in April to 420 µs/cm in August. The conductivity at Site -II were high due to human activities, especially agriculture

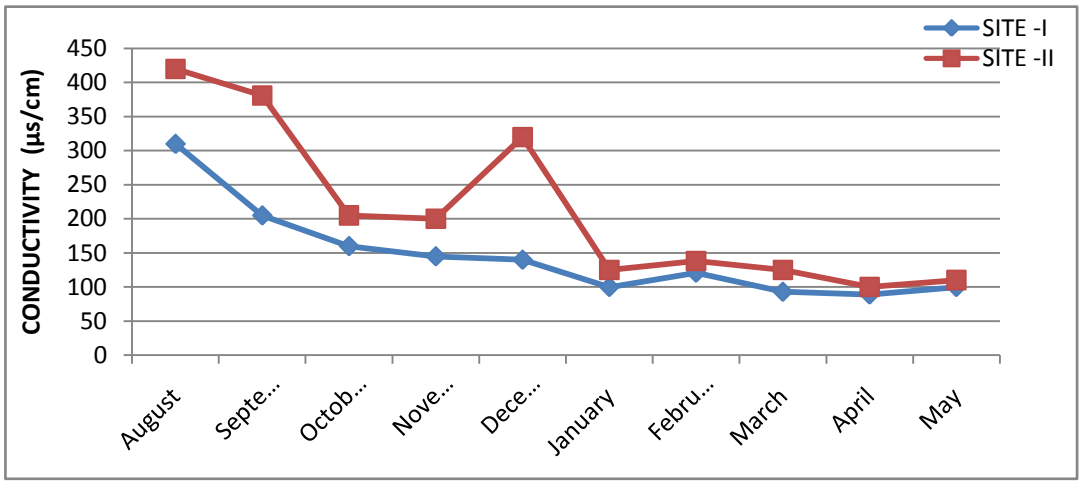

Fig 5.5: Monthly variations in the Conductivity values at the two Sites in Lidder Stream.

5.3.5 DISSOLVED OXYGEN (mg/l)

The lowest concentration of dissolved oxygen was seen at Site –II i.e., 7 mg/l in the months of May and August.At this site it ranged from 7 mg/l to 12 mg/l. The highest concentration was seen at site-I i.e. 14.5mg/l in the month of January. At this site it ranged from 8.5mg/l to14.5mg/l.

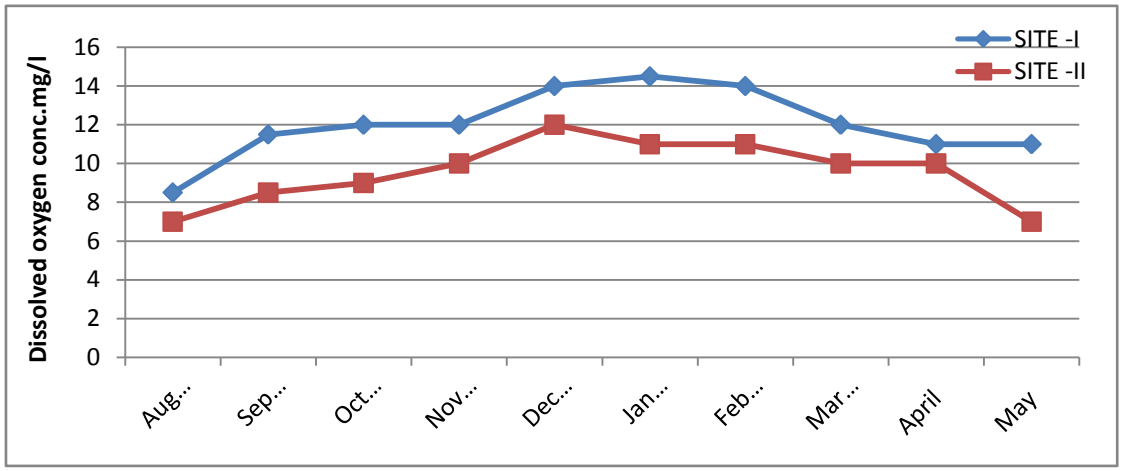

Fig 5.6: Monthly variations in the Dissolved oxygen values at the two Sites in Lidder Stream.

5.3.6 Ammonical Nitrogen (µg/l)

Ammonical nitrogen content was lowest at Site -I in the month of October (2 µg/l) while the highest concentration of (47 µg/l) was recorded at Site -II in the month of May. The concentration of Ammonical nitrogen ranged from 2 µg/l to 20 µg/l at Site –I, while it ranged from 12 µg/l to 47 µg/l at Site -II.

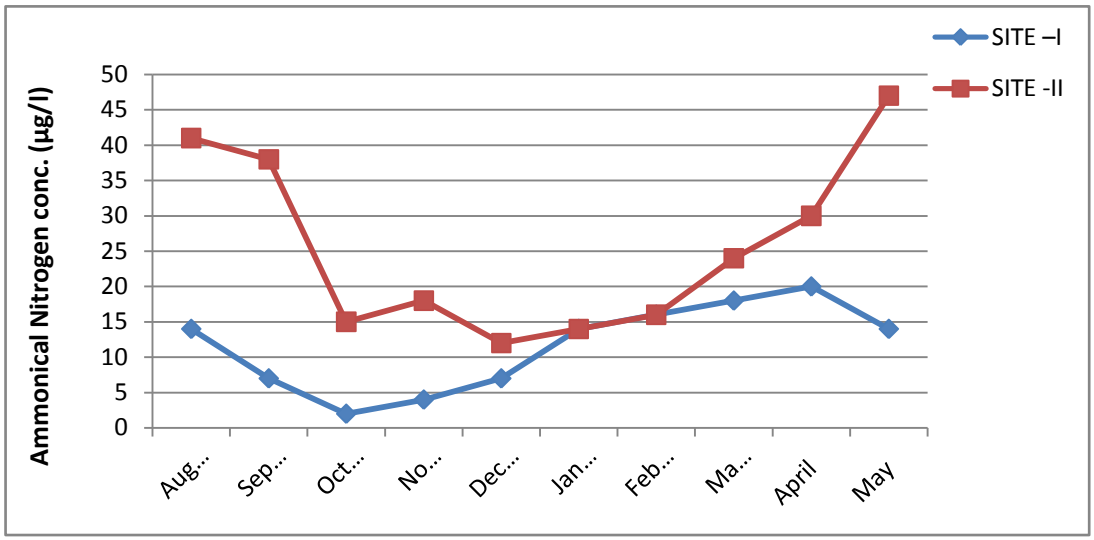

Fig5.7: .Monthly variations in the Ammonical nitrogen values at the two Sites in Lidder Stream.

5.3.7 Nitrate Nitrogen (µg/l)

Nitrate Nitrogen content was lowest (157 µg/l) at Site–I in the month of January, while the highest content (395 µg/l) was recorded at Site - II in the month of October. The concentration of Nitrate nitrogen ranged from 157 µg/l to 318 µg/l at Site – I and from 276 µg/l to 395 µg/l at Site – II.

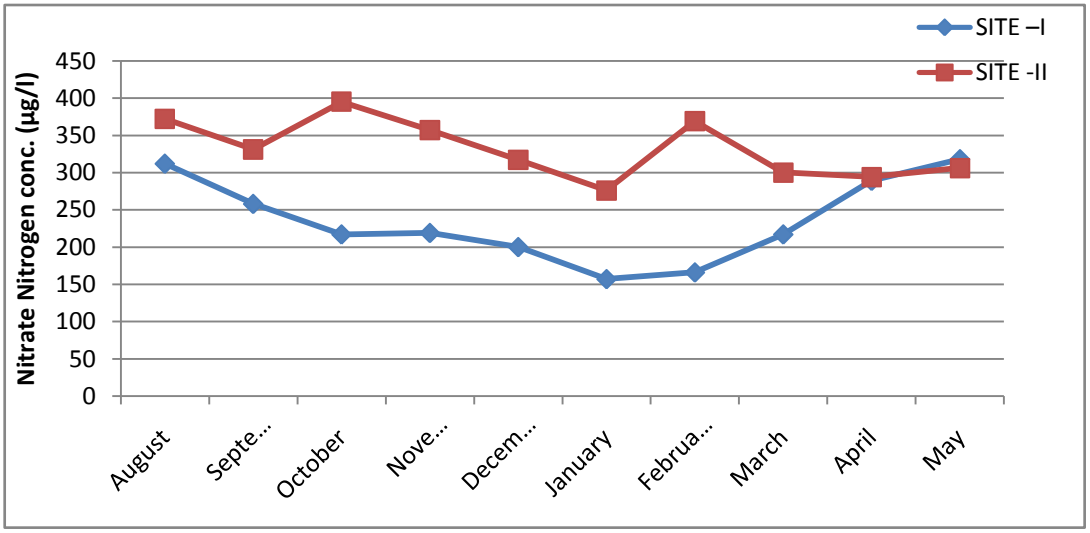

Fig 5.8: Monthly variations in the Nitrate nitrogen values at the two Sites in Lidder Stream.

5.3.8 NITRITE NITROGEN (µg/l)

The lowest concentration of nitrite nitrogen(1 µg/l) was recorded at Site – I during December, January and February and highest concentration (12 µg/l) was recorded at Site – II during August.

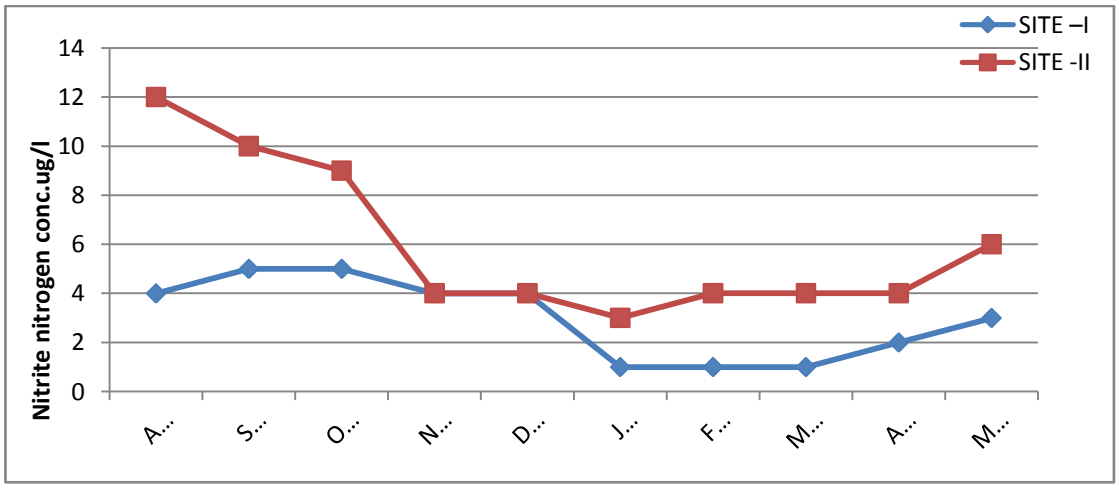

Fig 5.9: Monthly variations in the Nitrite nitrogen values at the two Sites in Lidder Stream.

5.3.9 Ortho phosphorus (µg/l)

Its concentration ranged from minimum value of 1 µg/l in December, January and February at Site – I to a maximum of 14 µg/l in September at Site – II. At Site – I its concentration ranged from 1 µg/l (in December) to 9 µg/l (in September) and at Site – II its concentration ranged from 3 µg/l (in April to 14 (in September).

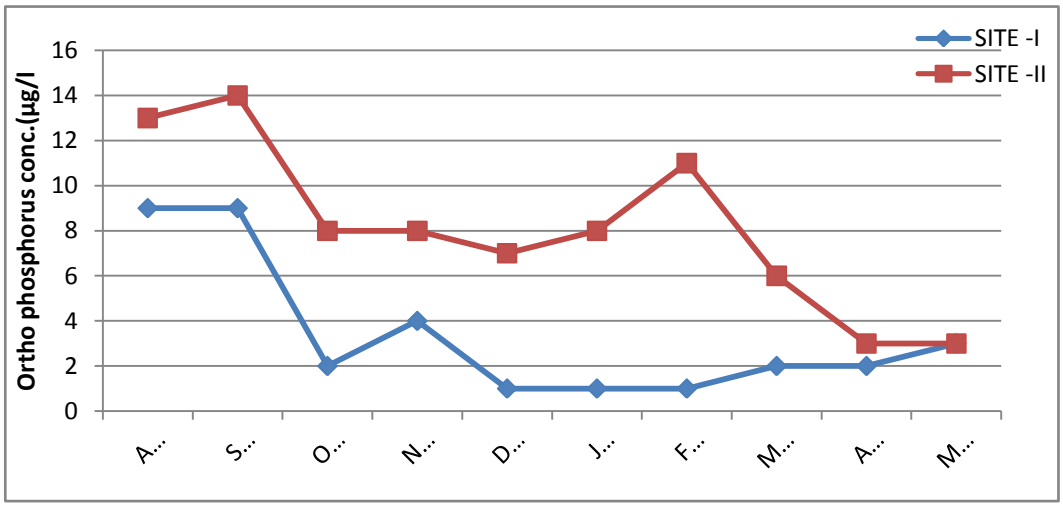

Fig 5.10: Monthly variations in the Ortho phosphorus values at the two Sites in Lidder Stream.

5.3.10 Total phosphorus(µg/l)

The total phosphorus concentration ranged from 4 µg/l in December at Site –I to 23 µg/l in August. At Site –II it ranged from 9 µg/l in April to 72 µg/l in August.

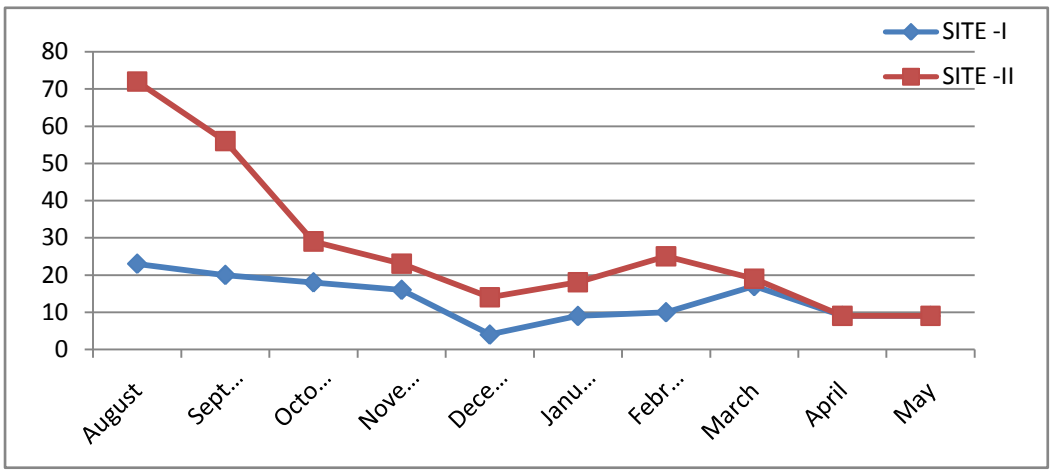

Fig 5.11: Monthly variations in the Total phosphorus values at the two Sites in Lidder Stream.

5.3.11 Velocity (cm/sec)

The highest velocity of 290 cm/sec was recorded in May at Site –I and the lowest of 75 cm/sec in Nov – Dec. at Site –II. Overall velocity decreased from Site –I to Site –II.

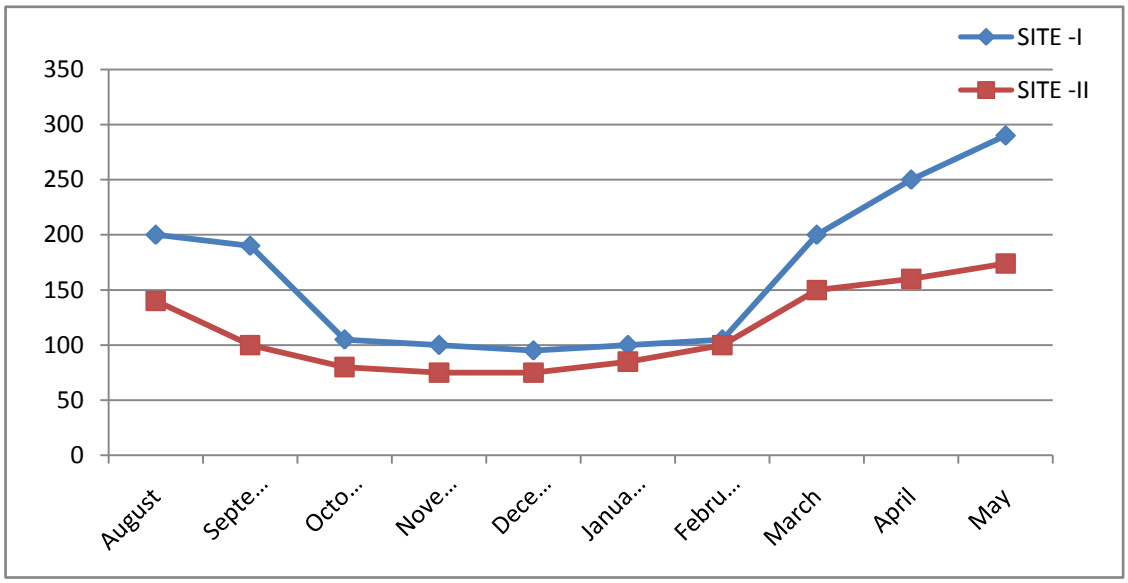

Fig 5.12: Monthly variations in the velocity values at the two Sites in Lidder Stream.

The chemical characteristics of the water are heavily influenced by the particulate matter as well as by the water flow. All the water parameters like pH, dissolved oxygen, conductivity, ammonical nitrogen, nitrate nitrogen, nitrite nitrogen, ortho phosphorus, total phosphorus at site second i.e. lower Lidder were comparatively higher than site first upper lidder which indicates an extra supply to the water while it passes the valley. Sewage effluent and agricultural runoff are the main source of nutrient load. As the Lidder flows down, a number of human settlements lie all along its course, the household wastes from these areas result in increased values of different water parameters.

Table 5.6: Showing Physico chemical parameters at Site-I

Month	Temp. °C		pH	Conductivity(µs/cm)	Dissolved Oxygen(mg/l)	Ammonical Nitrogen(µg/l)	Nitrate Nitrogen(µg/l)	Nitrite Nitrogen(µg/l)	Ortho Phosphorus(µg/l)	Total Phosphorus(µg/l)	Velocity Cm/Sec
	Air	Water									
August	24	15	8.1	310	8.5	14	312	4	9	23	200
September	21	13	8	205	11.5	7	258	5	9	20	190
October	19	11	8.3	160	12	2	217	5	2	18	105
November	13	7	8	145	12	4	219	4	4	16	100
December	9	5	7.5	140	14	7	200	4	1	4	95
January	2	1	7.8	100	14.5	14	157	1	1	9	100
Febuerary	3	1	7.6	121	14	16	166	1	1	10	105
March	10	3	7.8	93	12	18	217	1	2	17	200
April	13	6	7.7	89	11	20	289	2	2	9	250
May	14	8	7.2	100	11	14	318	3	3	9	290

Table 5.7: Showing Physico chemical parameters at Site-II

Month	Temp. ^{O}C		pH	Conductivity(µs/cm)	Dissolved Oxygen(mg/l)	Ammonical Nitrogen(µg/l)	Nitrate Nitrogen(µg/l)	Nitrite Nitrogen(µg/l)	Ortho Phosphorus(µg/l)	Total Phosphorus(µg/l)	Velocity Cm/Sec
	Air	Water									
August	26	20	8.3	420	7	41	372	12	13	72	140
September	22	17	7.9	381	8.5	38	331	10	14	56	100
October	20	16	9.0	205	9	15	395	9	8	29	80
November	16	12	8.7	200	10	18	357	4	8	23	75
December	11	9	7.5	320	12	12	317	4	7	14	75
January	6	4	7.9	125	11	14	276	3	8	18	85
February	7	4	8.0	138	11	16	369	3	11	25	100
March	12	6	8.1	125	10	24	300	4	6	19	150
April	14	10	7.1	100	10	30	294	4	3	9	160
May	15	11	7.4	110	7	47	306	6	3	9	174

5.4 Haematology of Schizothorax spp.

The blood from both upper (site-I) and lower (site-II) fish was screened for various hematological parameters including:

5.4.1 Hemoglobin value (Hb),

5.4.2 Total erythrocyte count (RBC),

5.4.3 Total leukocyte count (TLC),

5.4.4 Packed cell volume (PCV),

5.4.5 Erythrocyte sedimentation rate (ESR)

5.4.6 DLC.

5.4.1 Hemoglobin(g/dl):

The mean hemoglobin value for *Schizothorax plagiostomus* was found to be 9.28±0.52 at Site-I (upper lidder) and 9.16±0.63 at Site-II (lower lidder).The decrease in hemoglobin in case of *S.plagiostomus* was not statistically significant($p>0.05$).

The mean haemoglobin value for *S. esocinus* was 10.0±0.40 at Site-I(upper lidder) and 8.78±0.65 at Site-II (lower lidder). The decrease in haemoglobin content in case of *S. esocinus* was statistically significant($p<0.05$).

The mean haemoglobin value for *S. labiatus* was 10.29± 0.43 at Site I (upper lidder) and 8.98±0.65 at Site-II (lower lidder). The decrease in haemoglobin content in case of *S.labiatus* was statistically significant ($p<0.05$).

Fig. 5.13: Haemoglobin content at site I and site II

5.4.2 Packed cell volume (PCV)(%):

Packed cell volume in *S. plagiostomus* ranged from 29.00±2.35at site-I to 28.2±2.65 at Site II. In case of *S. esocinus* it ranged from 31.3±2.05 at site-I to 27.9±2.55 at Site II and in case of *S. labiatus* it ranged from 31.8±3.3 at site-I to 29.4±2.56 at Site II.

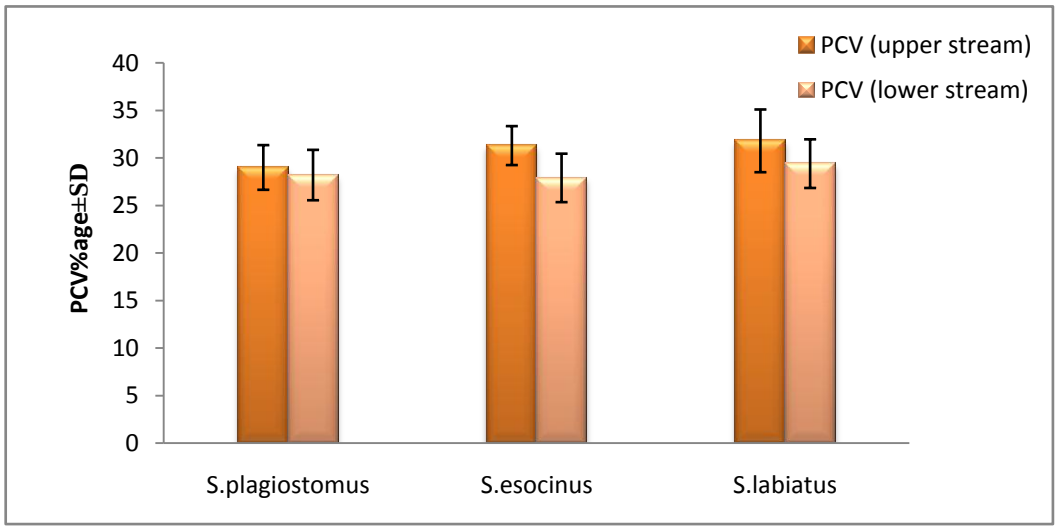

Fig. 5.14: Packed cell volume at site I and site II

5.4.3 ESR(mm1st hr reading):

The normal ESR value expresses the rate of fall of RBC under its own weight in mm Ist hr. reading, when the blood in which anticoagulant was added is allowed to stand undisturbed in a vertical tube for an hour. ESR in *S. plagiostomus* ranged from 1.29±0.21 at site-I to1.69±0.18 at Site II. In case of *S.esocinus* it ranged from 1.47±0.28 at site-I to 1.94±0.22 at Site II and in case of *S. labiatus* it ranged from 1.32±0.21 at site-I to 1.97±0.28 at Site II. The ESR showed positive correlation with pollution load, showing an increase with increase in pollution.

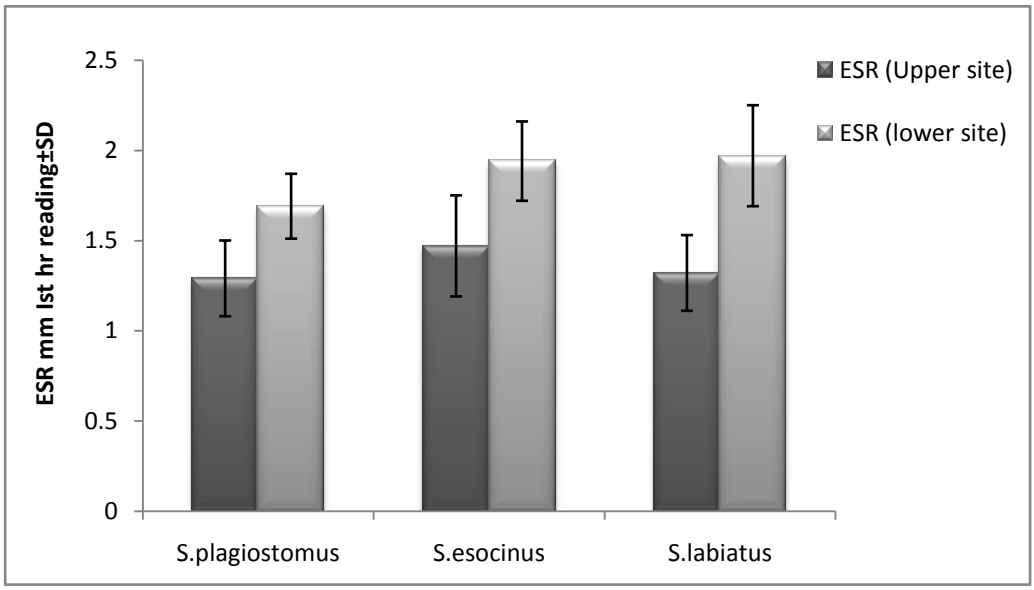

Fig.5.15: Erythrocyte sedimentation rate at site I and site II

5.4.4 WBC : (10^4/mm^3)

The WBC count of fish caught from site-II in all the three species of fish was higher than the fish from site-I. The WBC count of *S. plagiostomus* was 2.395±0.65 at site-I which increased to 4.671±0.36 at Site-II. The WBC count of *S. esocinus* was 2.12±0.59 at site-I and 2.83±0.50 at Site-II. The WBC count of *S. labiatus* was 2.383±0.35 at site-I and 3.501±0.29 at Site-II. The WBC count thus showed positive correlation with flow regime. Increase in WBC count in all the three species of fish was found to be statistically significant ($p<0.05$).

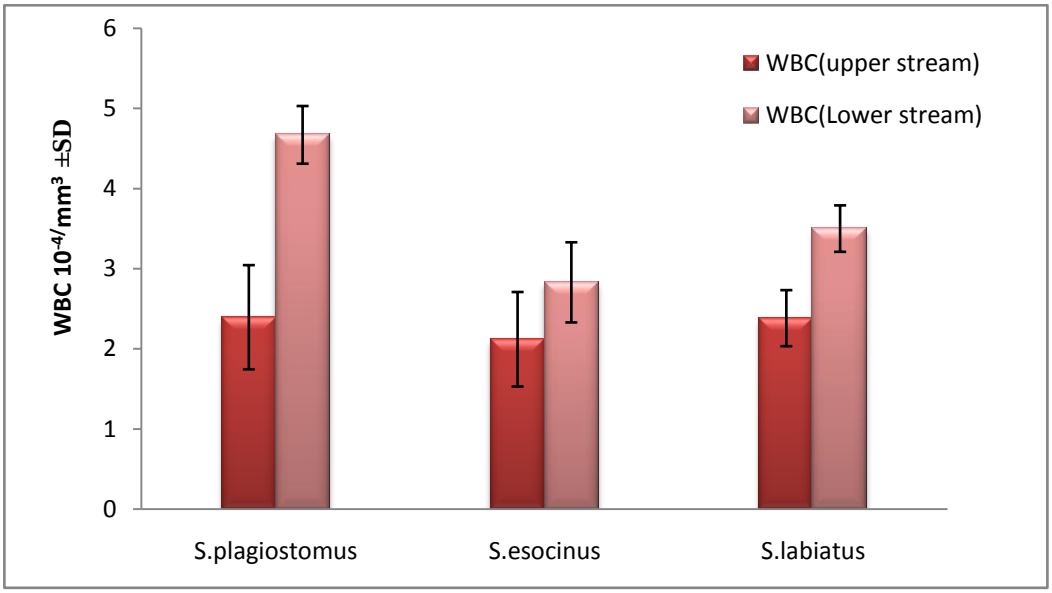

Fig. 5.16: WBC count at site I and site II

5.4.5 RBC COUNT (10^6/mm^3):

The RBC count in all the three species of fish at site II was found to be comparatively lower than the RBC count of fish from site I. The RBC count of *S. plagiostomus* was found to be 2.056±0.17 at site-I which decreased to 2.037±0.21 at Site-II. The RBC count of *S. esocinus* was found to be 2.18±0.12 at site-I which decreased to 1.71±0.26 at Site-II. The RBC count of *S.labiatus* was found to be 2.20±0.19 at site-I which decreased to 1.904±0.23 at Site-II. The decrease in RBC count at site II in all the three species of fish was not stastically significant($p>0.05$).

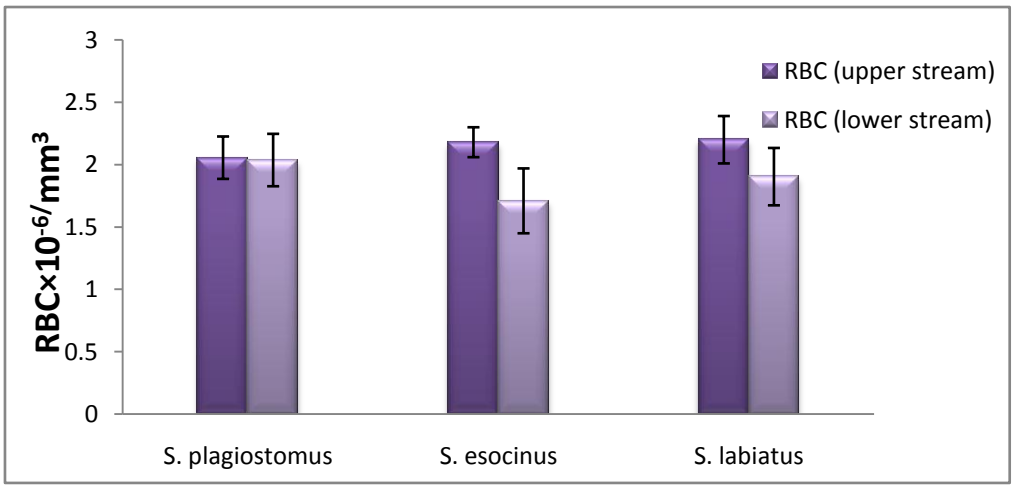

Fig. 5.17: RBC count at site I and site II

5.4.6 DLC:

Overall lymphocytes contributed to 65-70% of the total WBC count. Lymphocytes ranged from 63.4±3.56 at site II to 67.3±2.54 at site I in *S. plagiostomus.* In *S. esocinus* it ranged from 63.2±4.21 at site II to 70.2±2.97 at site I. In case of *S. labiatus* it ranged from 63.2±4.8 at site-II to 67.9±2.76 at site I.

In all the three species of fish neutrophil content at site II was found to be higher than the fish from site I. The neutrophil content in *S. plagiostomus* ranged from 28.6±2.50 at site I to 32.5±2.75 at site II; in *S.esocinus* it ranged from 26.7±2.6 at site I to 30.4±4.35 at site II, while in *S.labiatus* it ranged from 27.4±1.89 at site-I to 31.6±4.8 at site II.

Monocytes, esinophil and basophil contributed to 4 to 6% of the total WBC count.

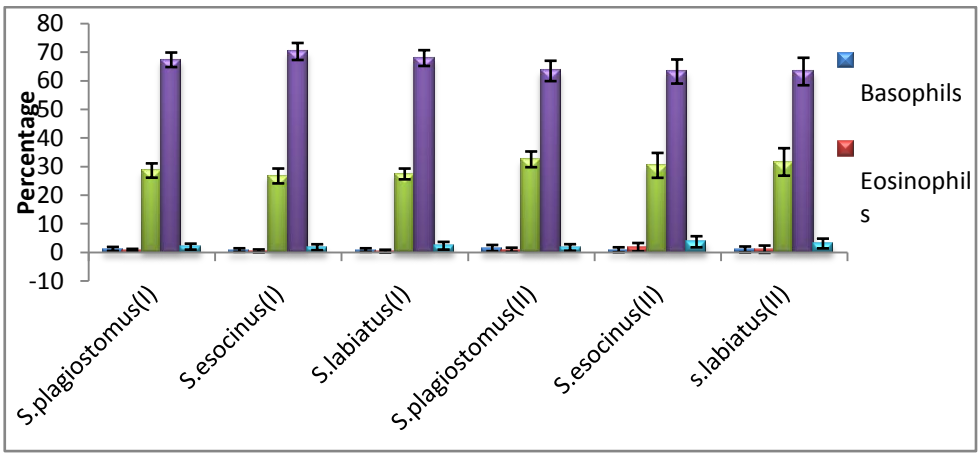

Fig. 5.18: DLC at Site I and Site II

Table 5.8: Haematological parameters of *Schizothorax plagiostomus.*

Parameters	Site-I(Upper Lidder)		Site-II (Lower Lidder)	
	Range	**Mean ±SD**	**Range**	**Mean ±SD**
Body length(inch)	7.2-11.3	8.9±1.21	8.3-10.7	9.42±0.81
breadth(inch)	2.1-4	3.0±0.61	2.4-4	3.34±0.42
Body weight(g)	120-350	221.2±80.92	110-495	293.3±110.66
Hb	8.5-10.2	9.28±0.52	7.8-9.9	9.16±0.63†
RBC	1.77-2.29	2.056±0.17	1.63-2.29	2.037±0.21†
PCV	25-32	29.00±2.35	24-32	28.2±2.65†
ESR	1-1.5	1.29±0.21	1-3	1.69±0.18†
WBC	0.97-5.96	2.395±0.65	2.98-6.97	4.671±0.36*
DLC				
Basophils	0-2	1.2±0.7	0-3	1.5±1.08†
Eosinophils	0-1	0.8±0.42	0-2	0.9±0.73†
Neutrophils	25-33	28.6±2.50	30-37	32.5±2.75*
Lymphocytes	64-72	67.3±2.54	58-67	63.4±3.56†
Monocytes	0-3	2.00±1.05	1-4	1.7±1.15†
MCV	133.02-146.7	140.52±3.55	133.02-147.2	138.65±4.39
MCH	41.9-48.02	45.232±2.07	42.2-48.6	45.126±2.34
MCHC	28-32.8	31.432±1.44	27-34.8	32.06±2.11
Total protein	2.79-5.43	4.0197±0.86	2.75-4.29	3.6748±0.74
Albumin	1.39-4.76	3.0217±1.16	1.286-3.45	2.5336±0.89
Globulin	0.57-1.40	0.998±0.35	0.832-1.47	1.1412±0.21

Data is presented as Mean±SD. Data was analyzed using one way ANOVA, with multiple comparisons using Dunnetts test of significance. Data was considered as significant for $p < 0.05$ vs control group (Site-I) . * $p < 0.05$, † $p > 0.05$.

Table 5.9: Haematological parameters of *Schizothorax esocinus*

Parameters	Site-I (Upper Lidder)		Site-I (Lower Lidder)	
	Range	**Mean ±SD**	**Range**	**Mean ±SD**
Body length(inch)	7.5-13.1	9.3±1.8	7.3-10.8	9.08±1.14
breadth(inch)	2.5-4.1	3.15±0.54	1.9-4.1	2.9±0.73
Body weight(g)	150-495	267.8±119.33	175-240	262.5±53.93
Hb	9.4-10.8	10±0.40	6-9.5	8.78±0.31*
RBC	2.01-2.31	2.18±0.12	1.23-2.01	1.71±0.26*
PCV	28-34	31.3±2.05	24-31	27.9±2.55*
ESR	1-1.9	1.47±0.28	1-2.4	1.94±0.22
WBC	1.48-2.91	2.12±0.59	2.13-3.52	2.83±0.50*
DLC				
Basophils	0-2	0.8±0.63	0-2	0.9±0.87
Eosinophils	0-1	0.5±0.52	0-4	1.8±1.47
Neutrophils	21-31	26.7±2.6	24-38	30.4±4.35*
Lymphocytes	61-77	70.2±2.97	56-69	63.2±4.21
Monocytes	1-4	1.8±1.03	1-7	3.7±1.94
MCV	135.9-149.5	143.183±4.86	134.33-211.38	152.64±21.20
MCH	43.4-48.7	45.79±1.66	40.5-72.35	50.29±8.77
MCHC	30-33.7	31.98±1.40	27.2-36.08	32.78±2.52
Total protein	3.22-5.01	4.118±0.58	3.35-4.11	3.741±0.26
Albumin	2.1-4.1	3.13±0.89	1.77-3.4	2.539±0.62
Globulin	0.51-1.6	0.988±0.43	0.71-1.72	1.202±0.40

Data is presented as Mean±SD. Data was analyzed using one way ANOVA, with multiple comparisons using Dunnetts test of significance. Data was considered as significant for $p < 0.05$ vs control group (Site-I) . * $p < 0.05$, † $p > 0.05$.

Table 5.10: Haematological parameters of *Schizothorax Labiatus*

Parameters	**Site-I (Upper Lidder)**		**Site-I (Lower Lidder)**	
	Range	**Mean ±SD**	**Range**	**Mean ±SD**
Body length(inch)	7.9-11.4	9.88±1.03	7.6-12.5	9.86±1.75
breadth(inch)	2.3-4.8	3.44±0.71	1.8-3.9	2.92±0.60
Body weight(g)	160-500	324.5±110.66	150-450	298.1±115.53
Hb	9-11.3	10.29±0.43	7.5-9.5	8.98±0.65*
RBC	1.87-2.51	2.20±0.19	1.51-2.2	1.904±0.23*
PCV	27-36	31.8±3.3	25-33	29.4±2.56
ESR	1-2.2	1.32±0.21	1-2.8	1.97±0.28
WBC	0.62-3.99	2.383±0.35	2.14-5.32	3.501±0.29*
DLC				
Basophils	0-2	0.8±0.63	0-2	1.0±1.05
Eosinophils	0-1	0.4±0.51	0-4	1.1±1.28
Neutrophils	24-30	27.4±1.89	24-38	31.6±4.8*
Lymphocytes	61-71	67.9±2.76	58-74	63.2±4.8
Monocytes	1-4	2.3±1.35	1-6	3.1±1.7
MCV	133-149.7	144.01±6.52	110.5-158.9	136.29±16.25
MCH	42.2-49.7	46.65±2.04	41.8-56.9	46.52±4.8
MCHC	31-35.5	32.413±1.63	31.48-42.6	34.304±3.2
Total protein	3.82-4.98	4.493±0.41	3.24-3.95	3.664±0.20
Albumin	2.42-4.47	3.728±0.82	1.75-2.59	2.217±0.27
Globulin	0.42-1.47	0.765±0.47	1.29-1.83	1.447±0.22

Data is presented as Mean±SD. Data was analyzed using one way ANOVA, with multiple comparisons using Dunnetts test of significance. Data was considered as significant for $p < 0.05$ vs control group (Site-I) . * $p < 0.05$, † $p > 0.05$.

5.5 Blood biochemistry of fishes:

The blood biochemical parameters included total proteins, albumin and globulin.

Table 5.11: Serum protein analysis of *Schizothorax labiatus*

MONTH	Site-I (Upper Lidder)				Site-II (Lower Lidder)			
	T. Protein	Albumin	Globulin	A/G Ratio	T. Protein	Albumin	Globulin	A/G Ratio
August	4.98	4.47	0.51	8.329	3.95	2.59	1.39	1.748
September	4.65	4.18	0.47	8.893	3.65	2.36	1.29	1.829
October	4.37	3.91	0.46	8.5	3.5	2.21	1.29	1.713
November	4.32	3.9	0.42	9.285	3.52	2.23	1.29	1.728
December	3.82	2.92	0.90	3.244	3.79	1.99	1.80	1.105
January	4.37	2.96	1.41	2.099	3.7	1.87	1.83	1.021
February	3.89	2.42	1.47	1.646	3.24	1.75	1.49	1.174
March	4.79	3.98	0.81	4.913	3.82	2.41	1.41	1.709
April	4.95	4.26	0.69	6.173	3.65	2.33	1.32	1.765
May	4.79	4.28	0.51	8.764	3.82	2.43	1.36	1.904

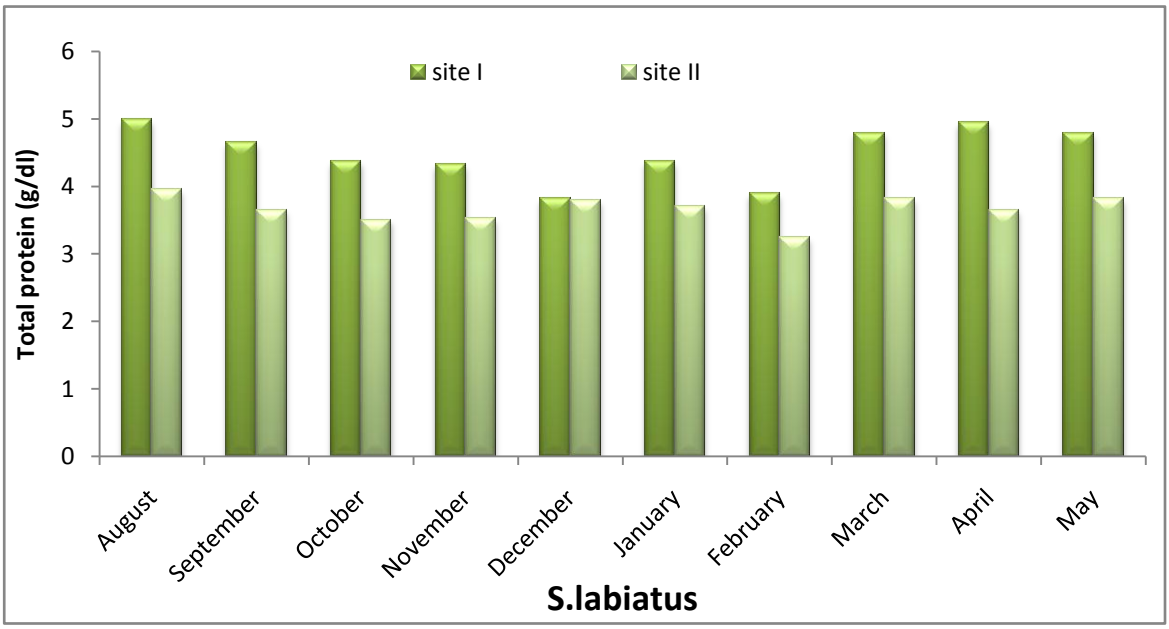

Fig. 5.19: Seasonal and site wise variation in total protein in *S.labiatus*

The mean Total protein in *S.labiatus* at site I was found to be 4.493±0.41 and 3.664±0.20 at site-II. The total protein ranged from 3.82g/dl in December to 4.98g/dl in August at site-I and at site-II it ranged from 3.24g/dl in February

to3.95g /dl in August. Thus highest total protein content was noticed in summer months and lowest in winter months.

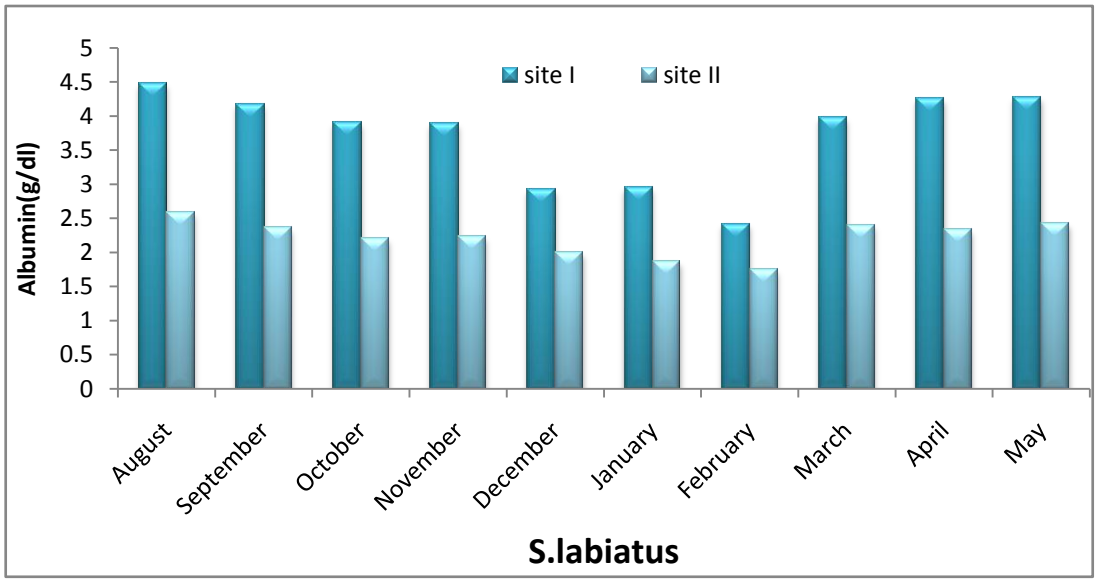

Fig. 5.20: Seasonal and site wise variation in Albumin in *S.labiatus*

The mean Albumin in *S.labiatus* at site-I was found to be 3.728±0.82 and 2.217±0.27 at site-II. The Albumin ranged from 2.42g/dl in February to4.47 g/dl in August at site-I, while at site-II it ranged from1.75g /dl in February to 2.59g/dl in August. Thus highest Albumin content was noticed in summer months and lowest in winter months.

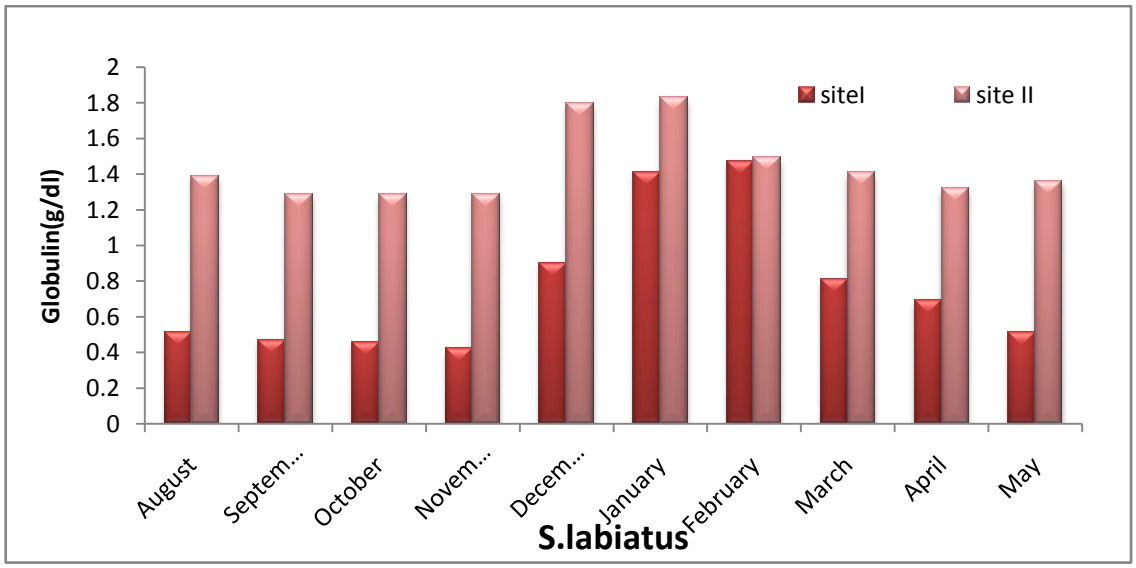

Fig. 5.21: Seasonal and site wise variation in Globulin in *S.labiatus*.

The mean Globulin in *S.labiatus* at site-I was found to be 0.765±0.47 and 1.447±0.22 at site-II. The Globulin ranged from0.51 g/dl in August to1.47 g/dl in February at site-I. At site-II it ranged from1.29 g /dl in September to 1.83g/dl in January. Thus highest Globulin content was noticed in winter months and lowest in summer months.

Table 5.12: Serum protein analysis of *Schizothorax plagiostomus*

MONTH	Site-I (Upper Lidder)				Site-II (Lower Lidder)			
	T. Protein	Albumin	Globulin	A/G Ratio	T. Protein	Albumin	Globulin	A/G Ratio
August	5.43	4.76	0.67	7.104	4.29	3.45	0.84	4.107
September	4.49	3.57	0.92	3.88	4.27	3.29	0.98	3.357
October	4.28	3.31	0.97	3.41	3.97	2.7	1.27	2.125
November	3.64	2.31	1.33	1.736	3.876	2.572	1.304	1.972
December	2.99	1.599	1.391	1.149	2.762	1.286	1.476	0.871
January	2.79	1.39	1.40	0.992	2.75	1.42	1.33	1.067
February	3.1	2.19	0.91	2.406	2.82	1.72	1.1	1.56
March	4.597	3.618	0.979	3.695	3.98	2.79	1.19	2.344
April	4.16	3.32	0.84	3.952	3.91	2.82	1.09	2.587
May	4.72	4.15	0.57	7.28	4.12	3.288	0.832	3.951

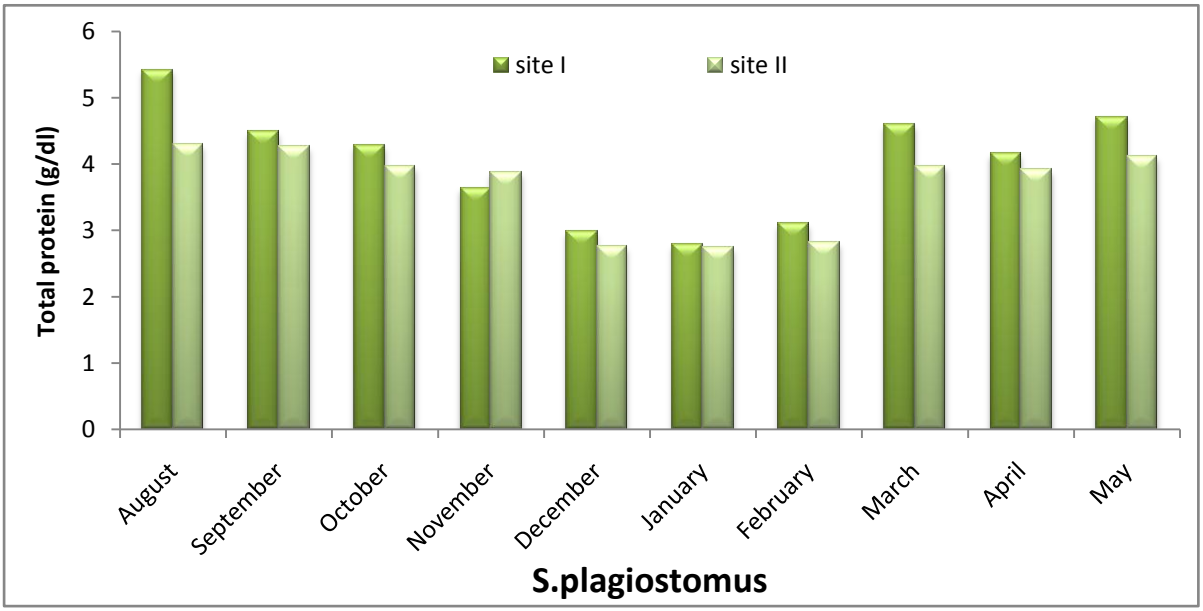

Fig. 5.22: Seasonal and site wise variation in total protein in *S.plagiostomus*

The mean Total protein in *S.plagiostomus* at site-I was found to be 4.0197±0.86 and 3.6748±0.74 at site-II. The total protein ranged from 2.79g/dl in January to 5.43 g/dl in August at site-I and at site-II it ranged from 2.75g/dl in January to 4.29g /dl in August. Thus highest total protein content was noticed in summer months and lowest in winter months.

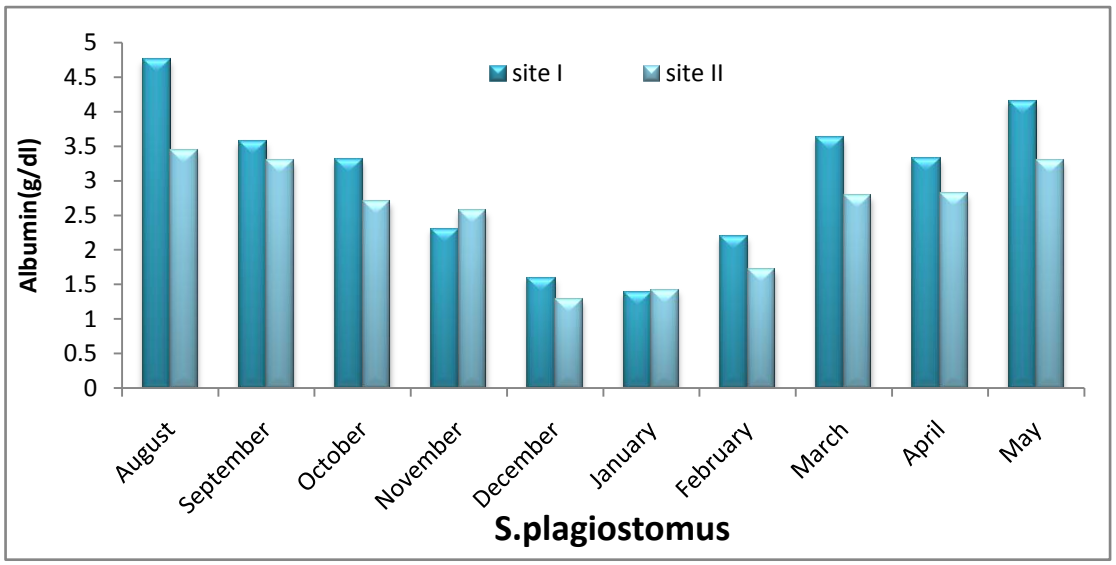

Fig. 5.23: seasonal and site wise variation in Albumin in *S.plagiostomus*

The mean Albumin in *S. plagiostomus* at site-I was found to be3.0217±1.16 and 2.5336±0.89 at site-II. The Albumin ranged from 1.39 g /dl in January to4.76 g/dl in August at site-I and at site-II it ranged from1.286 g/dl in December to 3.45g /dl in August. Thus highest Albumin content was noticed in summer months and lowest in winter months.

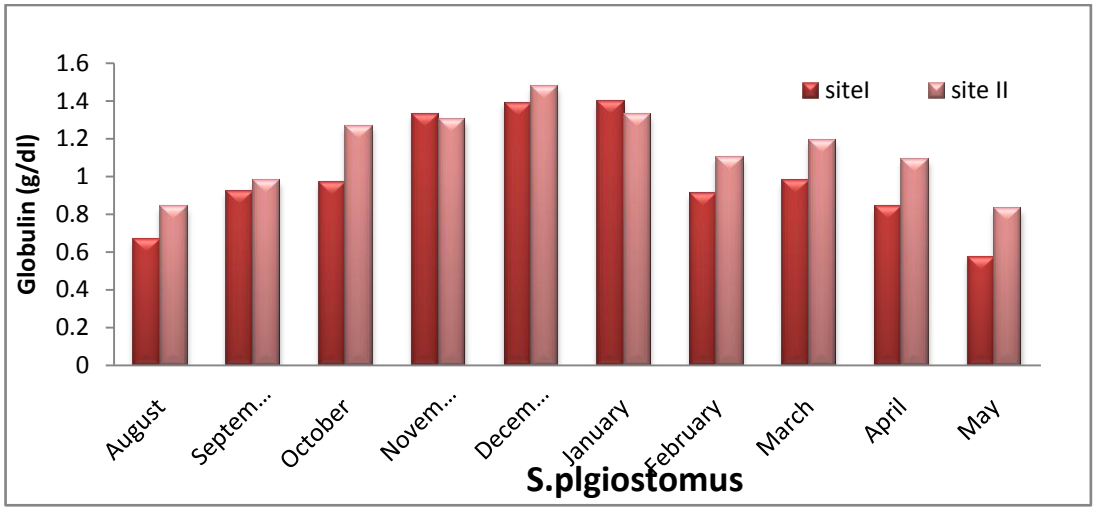

Fig. 5.24: Seasonal and site wise variation in Globulin in *S.plagiostomus*

The mean Globulin in *S.plagiostomus* at site-I was found to be 1.037±0.35and 1.1412±0.21at site-II. The Globulin ranged from0.57 g/dl in May to1.691g/dl in December at site-I and at site-II it ranged from0.832g /dl in May to 1.476g/dl in December. Thus highest Globulin content was noticed in winter months and lowest in summer months.

Table 5.13: Serum protein analysis of *Schizothorax esocinus*

MONTH	Site-I (Upper Lidder)				Site-II (Lower Lidder)			
	T. Protein	Albumin	Globulin	A/G Ratio	T. Protein	Albumin	Globulin	A/G Ratio
August	5.01	4.1	0.91	4.505	4.11	3.4	0.71	4.788
September	4.8	3.9	0.9	4.33	3.81	2.9	0.91	3.186
October	4.6	3.7	0.9	4.11	3.56	2.6	0.96	2.708
November	3.61	2.2	1.41	1.560	3.46	1.82	1.64	1.109
December	3.52	2.12	1.4	1.514	3.48	1.77	1.71	1.035
January	3.8	2.2	1.6	1.375	3.73	2.01	1.72	1.168
February	3.22	2.1	1.12	1.875	3.35	1.79	1.56	1.147
March	4.1	3.49	0.61	5.721	3.96	2.96	1	2.96
April	4.1	3.58	0.52	6.88	3.94	3.04	0.9	3.377
May	4.42	3.91	0.51	7.66	4.01	3.1	0.91	3.406

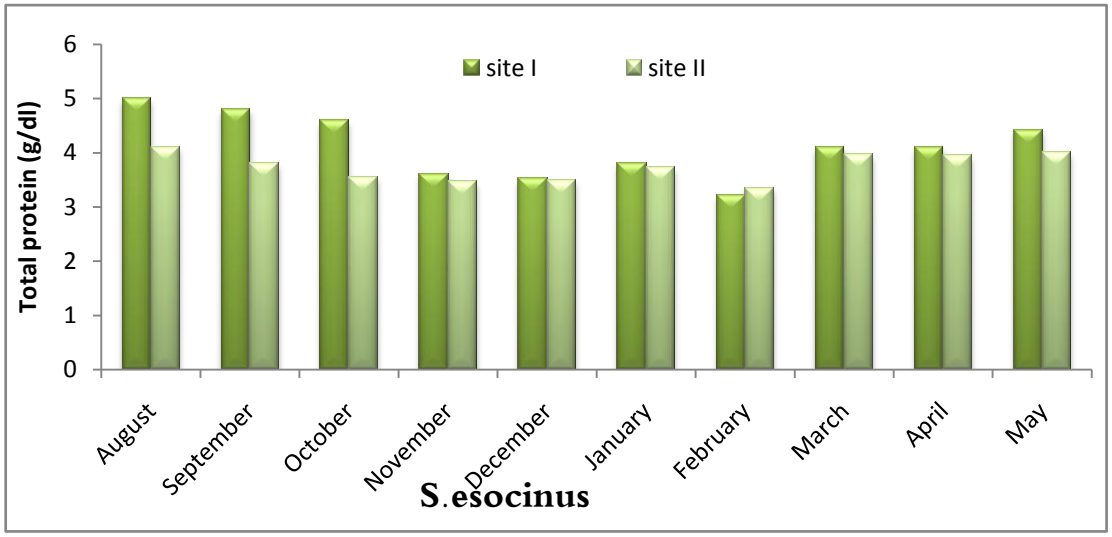

Fig. 5.25: Seasonal and site wise variation in Total protein in *S.esocinus*

The mean Total protein in *S. esocinus* at site-I was found to be 4.118±0.58 and 3.741±0.26 at site-II. The total protein ranged from 3.22g/dl in February to 5.01 g/dl in August at site-I and at site-II it ranged from 3.35g/dl in February to 4.11g /dl in August. Thus highest total protein content was noticed in summer months and lowest in winter months.

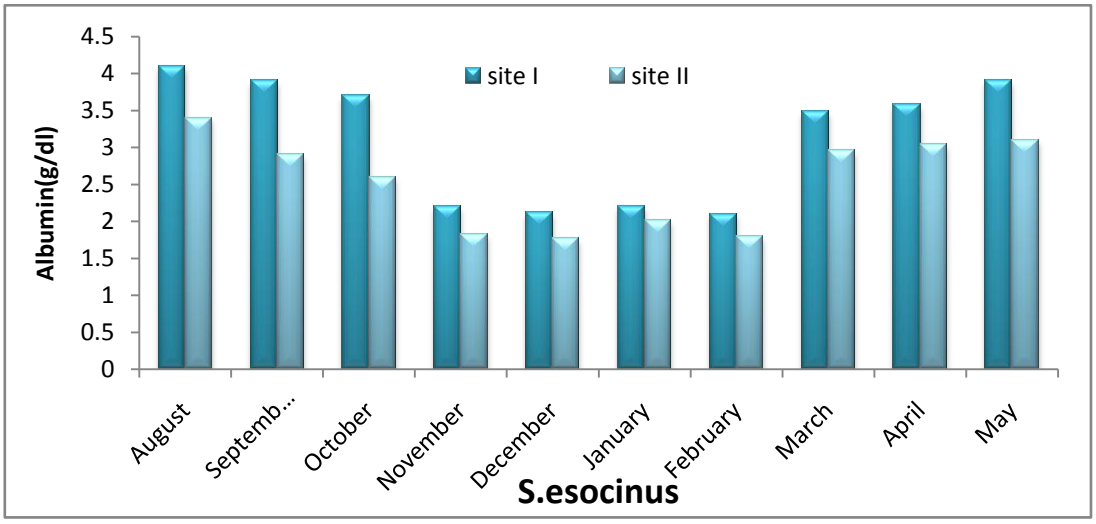

Fig. 5.26: Seasonal and site wise variation in Albumin in *S.esocinus*

The mean Albumin in *S.esocinus* at site-I was found to be 3.13±0.89and 2.539±0.62 at site-II. The Albumin ranged from2.1g /dl in February to 4.1 g/dl in August at site-I and at site-II it ranged from1.77 g/dl in December to 3.4g /dl in August. Thus highest Albumin content was noticed in summer months and lowest in winter months.

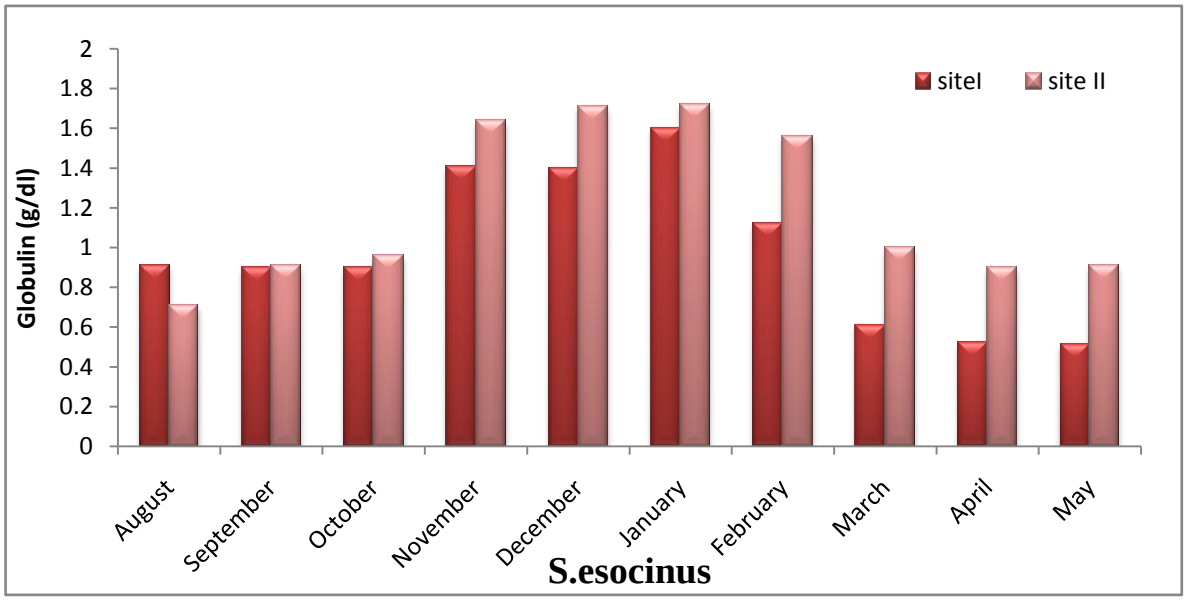

Fig. 5.27: Seasonal and site wise variation in Globulin in *S.esocinus*

The mean Globulin in *S.esocinus* at site-I was found to be 0.988±0.43and 1.202±0.40 at site-II. The Globulin ranged from 0.51 g/dl in May to1.6 g/dl in January at site-I and at site-II it ranged from0.71g /dl in August to 1.72 g/dl in January. Thus highest Globulin content was noticed in winter months and lowest in summer months.

Distribution pattern of fishes in Lidder stream

Fish species abundance and distribution appear to be determined by water quality and food availability factors. *S. plagiostomus, S. esocinus* and *S. labiatus* were the main component of ichthyofauna of Lidder stream at Site-II (Akura Mattan, lower Lidder), while as at Site-I (Pahalgam Loripora, upper Lidder) besides these, *Salmo trutta fario* also contributed a good proportion. The other species i,e *T. kashmirensis, T. marmorata, Glyptosternon reticulatum* and *Crossocheilus diplochilus* were also present. The distribution pattern showed variation at Site-I and SiteII. On the whole *Schizothoracinae* formed 75-80% of total fish catch. Distribution of fishes appeared to be related to physicochemical conditions like conductivity, DO, etc. as the physicochemical condition of Site-I was different from that of site II. *S. trutta fario* was completely absent at Site-II, being present only at Site-I as Site-I, revealed its preference for cold, well oxygenated upland water, especially large streams in mountainous areas. Areas with more pollution load have communities dominated by tolerant species. Similar results on distribution pattern were obtained by Linam *et al.,* (1996) on the fishes in Pecos River, and Hayes *et al.,* (2010) on fish distribution patterns and their association with environmental factors in the Mokau River.

HEMATOLOGY OF FISH (upper and lower):

A major part of the world's food is being supplied from fish source, so it is essential to secure the health of fishes (Tripathi *et al*., 2002). In India as 70% of the chemical formulations employed in agricultural practices are believed to affect non target organisms and to find their way to fresh water bodies, ultimately polluting

them (Bhatnagar *et al*., 1992). Ralio et al., (1985) reported that the blood parameters of diagnostic importance like erythrocyte and leucocytes counts, hemoglobin, haematocrit and leukocyte differential counts would readily respond to incidental factors such as physical stress and environmental stress due to water contaminants. The determination of haematological 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). Variations in blood tissue in fish depends on the stress effects of environmental factors (Orun, 2003; Aldrin *et al*., 1982; Hickey, 1982; Gbore et al., 2006; Arnaudov *et al*.2008);and on biological peculiarities of species as well (Siddiquie and Nasim, 1979; Collazos *et al*., 1998).A comparison of the various haematological data of the fish collected at the two sites in Lidder stream showed significant variations which are clearly related to the ecological set up.

Hemoglobin:

The haemoglobin value decreased at lower lidder compared to upper in all the three species of schizothorax. According to Pamila *et al*., (1991) the reduction in haemoglobin 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 haemoglobin. The pollutant entering into fish system is slowly eliminated (Newman and Mitz., 1988; James and Sampath, 1996; James et al., 1996), and hence the blood parameters get effected on account of pollutant toxicity.

WBCand DLC:

In this study there was a significant increase in WBC quantity and leukocyte cell proportions (neutrophil, monocyte) at site-II, the increase in WBC count can be correlated with an increase in antibody production which helps survival and recovery of fish exposed to pollutants and infections. Similar results were

obtained by Sahan and Cengizler (2002) on carp caught from different regions of seyhan river. 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). The lymphocytes are reported to be responsible for immune response, while monocytes and neutrophils protect the body through their elevated phagocytic activity and eosinophils have been linked to phagocytic activity (Kelenyi and Nemeth, 1969).

In the present study, the increases in WBC and neutrophil quantities in the samples collected from Site II seem to be a response of cellular immune system to pollution. Timur, (1993), Palikova and Navratil, (2001) Şahan and Cengizler, (2002) Saravanan et al., (2003).

RBC,PCV:

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 lower lidder seem to cause stress in the fish and as such seem to be responsible for the changes in the haematological parameters. Decreased RBC, Hb, and PCV in the fish at site-II may be due to anaemic 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).

ESR:

ESR is negatively correlated to RBC (i.e, lower the RBC's higher the ESR). ESR is medically considered as a strong sign of tissue injury (Pandey and Pandey , 2001). Increase in ESR was related to exposure to pesticides by Srivastava and Mishra, 1987;Mishra and Srivastava,1983; Banerjee,1988; Singh and Srivastava, 1994.

Total protein, Albumin and Globulin:

The level of the total protein in serum is first of all an indicator of the nutritional condition of the organism. Modification in value of total protein points out to metabolic perturbances in the fish body. The total protein and albumin were found to be higher in summer months in all the three species and comparatively lower in winter months. The total protein concentrations in the blood of fish are used as a basic index of condition and health status (Svoboda *et al*., 2001). In the present study total protein content and albumin was comparatively higher in summer months than in winter months. The results indicate that warmer conditions have a favorable influence on proteinemia with elevated levels in summer and low levels in winter these results are in agreement with Kaneko *et al*., (1997). Seasonal variations in the blood biochemistry of fish are known to occur and to be significant with regard to their health and well-being (Sandnes *et al.*,1988 Wilkie *et al*.,1996 ; Guijarro *et al*., 2003 ; Kavadias *et al*., 2003 ; de Pedro *et al*., 2005). Significant variations are also known to occur in serum biochemistry throughout the year. Generally the lowest values were determined during winter and the highest during summer. Moreover, these changes are dependent on water temperature and pH (Bayir 2007). Further the alteration in the total protein due to seasonal modification with low levels during winter may be due to intensive utilization of proteins to meet energy needs in the wintering period (Atanasova *et.al*.,2006). Contrary to the present investigation, an increase in plasma protein in cold seasons due to higher synthesis of protein have been reported (Pamparathi,1965;Nielsen *et.al.*, 1977;Siddiqui,1997).However, Helmy *et al.*,(1974) reported essential difference in the amount of total protein in summer months, thus supporting the present findings. A:G ratio was highest in summer corresponding to the levels of protein. Same results were obtained by (Grant *et al*., 1987). There is evidence that variations in fish serum proteins occur as a result of physiological, genetic and environmental factors.

In the present study the decrease in the protein content at lower stream site-II could be attributed to impaired food intake, increased energy cost of homeostasis, tissue repair and detoxification mechanism during stress (Neff, 1985).

CHAPTER NO –6

CONCLUSIONS

CONCLUSION:

- Eight species of fish were recovered from the Lidder stream.
- Schizothoracinae was the dominant group with *S. Plagiostomus* as the dominant species.
- *Salmo trutta fario* was completely absent in the lower waters while it formed the dominant catch in the upper waters, the reason of which can be attributed to the preferable cold and fresh water habitats of the species.
- Hematological parameters of upper and lower stream fish showed varied trends in relation to stress effects of environmental factors.
- WBC count showed significant increase at Site-II which can be attributed to immune response of the organism.
- Biochemical parameters including total protein and albumin showed difference. This difference might be on account of variability in composition of diet.
- There was a decrease in the total protein content at lower stream which can be attributed to either to impaired food intake or increased energy cost of homeostasis.
- Seasonal variation in total protein was observed with increased levels in warm months and decreased levels in cold months.

  Opposite trend was observed in globulin content with increased levels in cold months and decreased levels in warm months.

CHAPTER NO –7

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