CHAPTER 1
INTRODUCTION

Recent years have witnessed considerable attention in fish hematology. The reason for this surge of interest is that several blood parameters serve as sensitive tools of physiological well-being of fishes and their responses to the environmental variables. Besides the chemical and physical factors of the aquatic environment which at certain levels affect the fishes adversely, the normal life processes of these animals in certain seasons create stress conditions in the body. Blood is quick to respond to these factors. A seasonally based study of the blood profile thus throws light on the variations in the internal environment of fishes as part of their normal living as different from those caused by dynamics of external environment. Idea of the hematological syndrome is useful inasmuch as it provides normal values of blood characteristics which are unreservedly an important aspect of hematology in research and practice.

Despite considerable work on ichthyohematology, no satisfactory information on major carps, namely Catla catla, Labeo rohita and Cirrhina mrigala is available, although these
Teleosts form substantial part of capture and culture fishery in many Asian countries. This study provides a comprehensive account of seasonal variations in blood parameters including total erythrocyte count, percentage of immature erythrocytes, size of erythrocytes and nuclear/cytoplasmic ratio, hematocrit or packed cell volume, hemoglobin concentration, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), leucocrit, total and differential white blood cell–thrombocyte (WBC-T) count and erythrocyte sedimentation rate (ESR). Effort has been made to identify, as far as possible, the factors responsible for these fluctuations.

MATERIALS AND METHODS

Live specimens of both the sexes of three species of major carps, namely *C. catla*, *M. rohita* and *S. auripinnis* were captured from a stocking pond (total area 0.3 hectare), situated in the university campus. This pond is perennial and fishes subsist on the food produced naturally. Samples of fishes were obtained on monthly basis from October 1981 to July 1982. Commercial fishery depleted the fish stock of the pond to the extent that the catch per unit effort was so hopelessly poor that further sampling had to be discontinued after July. Sampling was carried out in the third week of
every month and at fixed hours in the forenoon (9-10 am). Data pertaining to atmospheric temperature and rainfall were obtained from the university's meteorological laboratory. Together with these the values of dissolved oxygen content and temperature of pond water are presented in Table I and Fig. 1. Fishes were caught by the help of cast nets.

Immediately upon capture, fishes were immobilized by giving a blow on the head, and were measured for their length and weight. Blood was drawn by severing the caudal peduncle to allow the blood from dorsal aorta to flow out, since cardiac puncture method was quite problematical and without any advantage. The procedure adopted is being used increasingly in recent years for small and medium sized fishes. Blood was collected in heparinized microhematocrit tubes and processed for the determination of hematocrit and leucocrit. For analysis of other parameters samples were stored in ordinary heparinized vials. A minimum of 6-7 specimens of each species were analysed every month to provide the maximum number of samples that could be processed promptly in one day. Sixty specimens of each species were examined. Blood samples were analysed in duplicate or triplicate. Sex was identified by examination of gonads. Liver and gonad were weighed on a sensitive electric balance. Hepato-somatic index was calculated as:

\[ \text{Hepato-somatic Index} = \frac{\text{Liver weight (g)}}{\text{Body weight (g)}} \times 100 \]
Fig. 1 Monthly variations in air, water temperature and dissolved oxygen content of medium.
TEMPERATURE(°C)

Dissolved Oxygen (PPM)

- Maximum Atmospheric Temperature
- Minimum Atmospheric Temperature
- Water Temperature
- Dissolved Oxygen

OCT NOV DEC JAN FEB MAR APR MAY JUN JUL
The gonado-somatic index was determined as:

\[
\text{Gonado-somatic Index} = \frac{\text{Gonad weight (g)}}{\text{Body weight (g)}} \times 100
\]

Hematocrit and leucocrit values were evaluated according to the methods described in detail by Hecay and Gordon (1977). Separinized microhematocrit tubes containing blood samples were centrifuged, hematocrit and leucocrit values were measured within 30 minutes. This time interval was standardized. Hematocrit, which represented the proportion of blood occupied by erythrocytes was expressed on percentage basis. Leucocrit was determined by measuring the height of the buffy layer (greyish-white layer of cells separating the erythrocytes from the plasma) to the nearest of 0.02 mm, using an ocular micrometer. Each tube was rotated during measurement, and the average height of the buffy layer was found out. The leucocrit value, defined as the volume of packed leucocytes and thrombocytes as a percentage of the blood volume, was calculated as follows:

\[
\text{Leucocrit (c)} = \frac{\text{Height of buffy layer (mm)}}{\text{Weight of total blood volume (mm)}} \times 100
\]

Hemoglobin was determined by cyanmet-hemoglobin method described by Blaxhall and Baisley (1973). All forms of circulating hemoglobin (hemoglobin, oxyhemoglobin,
carboxyhemoglobin and methemoglobin) are readily converted to cyanmethemoglobin except sulfhemoglobin, which is rarely present in appreciable amount. After thoroughly shaking the blood, 0.02 ml was placed in 4.0 ml of Drabkin's reagent. The solution was gently mixed by inversion and allowed to stand for about 10 minutes for full conversion of hemoglobin to cyanmethemoglobin. Small coagulum which formed was removed by the help of glass rod. Color intensity of the solution was read on Dencod and color Spectronic 20 spectrophotometer at a wavelength of 540 μm. Concentration of hemoglobin was read out by the help of calibration curve, using commercially available cyanmethemoglobin as the standard. Values were expressed as grams of hemoglobin per 100 ml of whole blood.

For enumeration of total number of erythrocytes and that of white blood cells plus thrombocytes (WBC) the procedure suggested by Messer (1960) involving Shaw's solution was followed. This solution enabled an easy differentiation of erythrocytes, leucocytes and thrombocytes. Blood was diluted 1:200 in a standard blood diluting pipet. Different types of cells were identified and counted under a microscope by the aid of Neubauer hemocytometer. Each value reported here represents mean of four separate counts.

The erythrocyte volume, the amount of hemoglobin in each erythrocyte, and the concentration of hemoglobin in the
erythrocytes were calculated from the data on hemoglobin content of whole blood, erythrocyte number and packed cell volume.

The mean corpuscular volume (MCV) was derived by the help of the formula:

$$\text{MCV} (\mu m^3/\text{red cell}) = \frac{\text{Volume of packed erythrocytes (µl) per 1000 ml blood}}{\text{Erythrocyte number (millions per µm}^3)}$$

The amount of hemoglobin per cell (in picograms, pg) was evaluated by calculating the mean corpuscular hemoglobin (MCH):

$$\text{MCH} (\text{pg/ red cell}) = \frac{\text{Hemoglobin (g) per 1000 ml blood}}{\text{Erythrocyte number (millions per µm}^3)}$$

Mean corpuscular hemoglobin concentration (MCHC) which represented the concentration of hemoglobin in the erythrocytes was determined by dividing the amount of hemoglobin per 100 ml blood by the packed cell volume expressed in percentage. Multiplication of the value by 100 gave the MCHC in terms of percentage. More precisely it measures the concentration of hemoglobin in grams per 100 ml of erythrocytes. The equation is:

$$\text{MCHC (g)} = \frac{\text{Hemoglobin (g) per 100 ml of blood} \times 100}{\text{Packed cell volume (µl)}}$$

Erythrocyte sedimentation rate (ESR) was determined by using Micro-wintrobe method described by Blaxhall and Daisley (1973). Known volume of heparinized blood was drawn into microhematocrit
tubes (75 cm long and with 1.1 - 1.2 mm internal diameter) and one end of the tube was sealed. The tubes were placed in vertical position for one hour at room temperature. The reading was recorded using a finely calibrated millimeter scale, the measurement was made from the top of the column of sedimented erythrocytes to the surface of the plasma. ESR value was expressed as mm per hour.

Blood films were prepared and stained by the technique of Williams (1977). Wright's stain was used. Differential leucocyte-thrombocyte count was based on the first 200 cell types observed. The cells identified and enumerated include small and large lymphocytes, monocytes, neutrophils, eosinophils and thrombocytes. Two hundred cells were counted separately from the best two of smears under oil immersion lens and their mean percentage tabulated. Counting was carried out from the same areas in each slide, away from the extreme edges. For determination of the ratio of small lymphocytes (w) / large lymphocytes (W), 200 cells of both types were counted separately from the above exercise.

The immature red blood cells (polychromic cells) were counted in two different blood slides of each fish out of a total of 1000 cells, and recorded as percentage of the total number of red cells.
Erythrocyte morphometry with reference to possible seasonal cycle in erythrocyte dimensions was also worked out. Ten cells from each slide in duplicate selected for excellence of staining and internal cytology were examined with an ocularometer standardized with micrometer scale as a parallel magnification used in conjunction with an oil immersion objective. Measurements of maximum cell width (minor axis) and length (major axis) of the erythrocytes as well as the nuclear width and length were made. Because of the ellipsoidal shape of erythrocyte and its nucleus the surface area was calculated using the standard mathematical formula applied for calculating the areas of elliptical objects:

\[
\text{Surface area (\(\mu m^2\)) = } \frac{\pi}{4} \times a \times b
\]

where, 'a' and 'b' represent the width, \(\mu m\) (minor axis) and length (major axis) of erythrocyte or its nucleus (as the case may be). The cytoplasmic area was calculated by subtracting the nuclear area from the total erythrocyte area. By the help of nucleus and cytoplasmic areas, the nuclear and cytoplasmic ratio (N/C ratio) was computed. This N/C ratio was multiplied by 100 to express on percentage basis (index).
RESULTS

Seasonally based data on hematological characteristics of the three teleostean species, Catla catla, Labeo rohita and Cirrhina mrigala have been embodied in Tables II/III and represented graphically (Figs. 3-4). The results reveal identity in the pattern of seasonal fluctuations but interspecific differences in the magnitude of change in the various blood parameters.

MATUROCYTES

Erythrocytes of the fishes were ellipsoid, with lightly stained cytoplasm which was clear and devoid of vacuoles. Nucleus was purple colored and centrally placed with its axis lying parallel to longitudinal plane of the cell. Immature erythrocytes (polychromatic cells) were, however, round and with bluish tinge; their nucleus which acquired pink color, was spherical, larger and had loosely packed chromatin granules. Both medium and large-sized erythroblasts were clearly recognizable and distinct from the mature erythrocytes. Chromatin granules become progressively compact as the erythroblasts tend to mature. Signs of such a change become evident in late erythroblasts or early erythrocytes. Other structural changes accompanying maturation are reduction in size of the nucleus as well as modification in its shape from
Fig. 3 Monthly variations in erythrocyte number, hematocrit and hemoglobin concentration in
Catla catla (male, O—O; female, O—O),
Labeo rohita (male, O—O; female, O—O),
Cirrhina mrigala (male, A—A; female, A—A).
Vertical lines indicate one standard error.
Fig. 4 Monthly variations in blood parameters of *Catla catla*, *Labeo rohita* and *Cirrhina mrigala* (male: unbroken lines; female: broken lines).
Fig. 5  Monthly variations in abundance of immature erythrocytes ( ), and surface area and nuclear/cytoplasmic ratio of mature cells in *Catla catla* (O—O), *Labeo rohita* (O—O) and *Sirrhina microlepis* (Δ—Δ). Vertical lines indicate one standard error.
Fig. 6 Monthly variations in WBC, leucocrit and ESR in *Catla catla* (●●●), *Labeo rohita* (○○○), and *Cirrhina arизма* (▲▲▲). Vertical lines indicate one standard error.
Fig. 7 Differential leucocyte analysis (percentage basis) in different months, *Catla catla* : white bars; *Labeo rohita* : black bars; *Cirrhina mrigala* : dotted bars. Vertical lines indicate one standard error.
Fig. 3 Monthly variations in small lymphocyte/large lymphocyte ratio in *Catla catla* (○—○), *Habeo rohita* (■—■) and *Cirrhina arsiela* (▲—▲). Vertical lines indicate one standard error.
spherical to ellipsoid. The mature erythrocytes ranged 9.0-12.0 μm in length and 6.0-12.25 μm in width (C. catla), 9.0-12.0 μm in length and 7.5-9.75 μm in width (C. rohita), and 10.5-13.5 μm in length and 7.5-9.0 μm in width (C. mrigala). Interspecific differences were also observed in nuclear dimensions: (C. catla: length, 3.0-4.5 μm and width, 1.5-3.0 μm; C. rohita: length, 3.0-4.5 μm and width, 1.5-3.0 μm; C. mrigala: length, 3.75-5.25 μm and width, 1.5-2.25 μm). Erythrocytes of C. mrigala were more ellipsoidal and with elongated nucleus whose ends taper sharply. Mean value of the total erythrocytic area was also found to be slightly greater in C. mrigala (Table V, Fig. 5). Seasonal change in the surface area and n/c ratio in the three species were observed (Table V, Fig. 5). Surface area of the erythrocytes was higher in winter and at the onset of gonad maturation. Since nuclear area remained unaffected, a reduction in n/c ratio was obvious. There was no sex-linked difference in these parameters.

Erythrocyte count exhibited interspecific variation. The erythrocytes were 2.31-3.31 million/mm³ in male and 2.47-3.78 million/mm³ in female (C. catla); 1.53-2.74 million/mm³ in male and 1.56-2.71 million/mm³ in female (C. rohita); 2.17-3.0 million/mm³, male and 1.91-3.03 million/mm³, female (C. mrigala). The number of erythrocytes varied with the
season (Fig. 3); it declined sharply during winter and increased steadily with temperature from March, attaining peak in April-May. It was interesting to note that erythrocyte counts decreased with increase in size of erythrocytes.

The proportion of immature erythrocytes in the total blood cell population dropped abruptly during winter, registering lowest value in January. Thereafter, the cells become more preponderant and the maximum numbers were recorded in April-May. Decline was clearly evident in successive months (June-July).

Hematocrit and Hemoglobin

Hematocrit and hemoglobin concentration showed a similar pattern of seasonal change as did the total erythrocyte count. The interspecific differences in these parameters have been indicated in Fig. 3, Tables II-IV. Lowest values were encountered in L. rohita. Except June-July, the sex-linked differences were not discernible. This change was coincident with gonadosomatic indices (Fig. 2). Hematocrit and hemoglobin declined with the gonadal development. These changes were more marked in the females.
Fig. 2 Monthly variations in gonado-somatic index (male, O--O; female, O---O) of *Gadus* gadus, *Labeo* rohita and *Cirrhina* mrigala. Vertical lines indicate one standard error.
VOLUME AND HEMOGLOBIN CONTENT OF ERYTHROCYTES

Seasonal variations in MCV, MCH and MCHC were noticed in the three species of carps (Fig. 4). The mean corpuscular volume was generally high during winter and low during warmer months. An increase in MCV was also observed during gonad maturation. The mean corpuscular volume in _A. rohita_ was higher (male: 137.34-148.16 μm³/red cell; female: 137.92-144.14 μm³/red cell) in comparison with the other two species: _L. catla_ (male: 125.12-135.10 μm³/red cell; female: 125.0-131.66 μm³/red cell), and _P. norma_ (male: 133.73-143.15 μm³/red cell; female: 131.78-143.09 μm³/red cell). The MCH and MCHC, however, registered higher values during October-November and April-May, when the volume of the erythrocyte was low. An inverse relationship was found between MCV and MCHC. This has been formulated:

\[
\text{MCV(μm}^3/\text{red cell) = 149.20 - 0.74 (MCHC)}\quad \text{for } L. \text{ catla,}
\]
\[
\text{MCV(μm}^3/\text{red cell) = 211.10 - 2.55 (MCHC)}\quad \text{for } A. \text{ rohita,}
\]
\[
\text{MCV(μm}^3/\text{red cell) = 166.75 - 1.07 (MCHC)}\quad \text{for } P. \text{ norma.}
\]

The correlation coefficients 'r' were -0.714, -0.757 and -0.755 for _L. catla_, _A. rohita_ and _P. norma_, respectively, significant at \( p < 0.01 \). The MCV was also negatively correlated with the number of erythrocytes present in the blood as indicated by the equation:
\( C. \text{ satla} \): MCV (\( \mu m^3/\text{red cell} \)) = 144.58 \pm 4.69 \text{ RBC numbers (million/mm}^3\),
\[ r = -0.805, \ P < 0.01; \]
\( L. \text{ rohita} \): MCV (\( \mu m^3/\text{red cell} \)) = 161.09 \pm 5.29 \text{ RBC numbers (million/mm}^3\),
\[ r = -0.784, \ P < 0.01. \]

The correlation coefficient was as low as \(-0.203\) in case of
\( C. \text{ brigala} \) and thus regression formula was of no importance. There
was no significant sex-related difference in MCV, MCH and MCHC.

**ERYTHROCYTE SEDIMENTATION RATE**

ESR was higher in \( L. \text{ rohita} \) compared to the other two species
of carps (Fig. 6, Tables II-IV). The mean value ranged from
1.73 to 5.57 mm/hr in \( L. \text{ rohita} \), 1.05 to 4.17 mm/hr in \( C. \text{ satla} \) and
0.95 to 3.97 mm/hr in \( C. \text{ brigala} \). ESR varied seasonally. It was
negatively correlated with the number of erythrocytes. Value of
ESR was slightly higher during winter and peaked in June, the
period of maximum gonad development as indicated by gonadosomatic
index.

**LEUCOCYTES**

No structural difference was observed in the leucocytes and
thrombocytes of the three carps. Neutrophils and eosinophiles
(granulocytic series), lymphocytes: large and small, monocytes
(agranulocytic series) and thrombocytes were present in the blood
but eosophilic granulocytes were absent.

Small lymphocytes were the most preponderant of the leucocytes. They are round, and measure about 6.0-7.5 μm in diameter. The nucleus which has compact mass of chromatin and stains dark purple occupied most of the cell. The small rim of cytoplasm is blue with lighter areas and irregular cell line. Large lymphocytes measure 9.0-10.5 μm in size. Their shapes varied from circular to amoeboid with pseudopodial formation. The nucleus occupies about 3/4 of the cytoplasm and stains lightly compared to the nucleus of small lymphocytes.

The thrombocytes were numerically inferior to lymphocytes and variable in shape. Usually they were elongated, dumb-bell shaped or with a long 'tail' at one or both ends. Nucleus resembled the shape of the cells and stained in the same way as that of the erythrocytes. The cytoplasm was clear, and acquired light blue color.

Monocytes were larger, with a diameter of 10.5-12.0 μm. The nucleus occupied almost half of the cell. The cytoplasm was agranular and was found to take little stain.

Amongst the cells of granulocytic series, neutrophils are most commonly encountered. They are generally round and the diameter ranged from 11.25 to 12.75 μm. The nucleus has a variable form, usually with two to four lobes and stains
purple. On staining cytoplasm acquired a pale blue-grey to light pink color and seemed to be studded with fine granules. The eosinophils on the other hand are relatively less numerous, oval and smaller in size (diameter: 9.75-10.5 μm), with nonfilamentous nucleus lying on the periphery of the granulated cytoplasm. The granules were larger than those observed in the neutrophils and were more uniform in size and distribution.

Besides the cells described above some other forms of cells which could not be identified with certainty were also encountered rarely. These have been recorded as unidentified cells (Table VI).

**TOTAL WBC-T COUNTS AND LEUCOCRIT**

Total white blood cell-thrombocyte count and leucocrit of all the three species of carps clearly exhibited seasonal changes (Tables II-IV, Fig. 6). There was, however, no observable sex-linked difference. The data revealed a significant decrease in WBC-T count during winter (December-January-February), and at the time of peak gonadal maturation (June-July). This decline was much more pronounced than the one that occurred in June-July. Significant interspecific differences were noticeable. Values of leucocrit and WBC-T count of *P. catla* and *P. wroblewskii* were closer and these were
higher in comparison with those observed in *A. rohita*. The pattern of seasonal variation was indeed similar.

**DIFFERENTIAL COUNTS**

The three species differed in the differential leucocyte-thrombocyte counts. These parameters showed significant seasonal changes (Fig. 7, Table VI).

The observations revealed that lowering of WBC-T count and leucocrit during peak winter and summer months were mainly due to the reduction in numbers of small lymphocytes and thrombocytes. The number of large lymphocytes varied reciprocally but the changes were less marked. The ratio of small lymphocytes (SL)/large lymphocytes (LL) was computed (Table VII, Fig. 3). It was interesting to note that this SL/LL ratio was lower during peak winter and summer months. The higher values coincided with moderate temperature and fast growth.

Percentage of granulocytes (neutrophils and eosinophils) increased during winter; the peak was observed in January - February. Subsequently, the values declined gradually, registering the lowest in May. Thereafter, the granulocytes increased in June - July. The mean percentage of neutrophils ranged 3.75-13.75% (*A. catla*), 3.25-11.5% (*A. rohita*) and 3.6-11.25% (*A. mrigala*). Monocytes which
followed the same pattern of seasonal change as the granulocytes, however, registered higher values in summer months.

No consistent linear relationship between any of the hematological parameters and length, weight, 'condition factor' and 'hepatosomatic index' of fish seemed to exist. During non-breeding season it indeed appeared that hematocrit, hemoglobin concentration and erythrocyte count increased with gain in body weight and 'condition factor'. But by the commencement of gonadal maturation the picture was altered and variations caused by a multitude of factors were so profoundly different in random samples that establishment of any regression model based on hard and fast basis was considered futile.

**Discussion**

The present data reveals seasonal variation in the hematological profile of the three species of major carps studied. Decline in erythrocyte number, hematocrit, hemoglobin, HbR, HbO and in the proportion of immature erythrocytes during winter months (January - February), a common feature observed in *C. catla*, *L. rohita* and *C. mrigala*, may be correlated with reduced metabolic rate and lower oxygen demand in this season. Reduction in the rate of
erythropoiesis at lower temperature is well established. Investigations on hematological response to temperature carried out by several workers (Spoor, 1951; Devilde and Houston, 1967; Houston and Devilde, 1968; Denton and Housef, 1975; Bridges et al., 1976; Houston and Rupert, 1976; Houston, 1980 and Koos and Houston, 1981) substantiate the results of this study. Reciprocal relation noted in the mean corpuscular volume and the mean corpuscular hemoglobin concentration points to the inclemency of winter vis-a-vis hemoglobin biosynthesis despite enlargement of erythrocytes. Decrease in the N/C ratio despite constancy of nuclear size signifies inflammatory tendency of the extranuclear part of the cells. While Fourie and Van Vuren (1976) have reported seasonal change in erythrocyte dimensions in *Cyprinus carpio* and *Barbus holubi*, Nikinmaa (1982) considered such a variation as a physiological adjustment in response to prevailing oxygen tension. Johansson-Johneck et al. (1975) observed the cellular swelling of erythrocytes in European eels during starvation and linked this phenomenon to hydration of erythrocytes resulting from a chloride shift and iron deficiency. The author views that this erythrocyte swelling in major carps may be an adaptation to expose a larger surface area of hemoglobin inside the red blood cells for more efficient exchange of gases at a time when environmental conditions do not favour endogenous synthesis of hemoglobin.
Reduction in erythrocyte numbers, hematocrit and hemoglobin concentration during breeding season was perhaps a response to gonadal development rather than a seasonal environmental phenomenon. Iwao (1969) also observed decrease in hematocrit value with gonad buildup in both sexes of rainbow trout *Salmo gairdneri*. In a very elaborate article Mustafa (1977) traced the sequence of events in the internal environment during sexual maturation. He attributed the 'physiological emergency' of the body at this step of life to two factors, viz. decline/cessation of food intake, and diversion of biochemical constituents from somatic tissues, especially liver and musculature towards developing gonads. These conditions, particularly the poor nutritional status, may account for decline of erythropoiesis, and lower value of hematocrit, erythrocyte count and hemoglobin concentration. Interestingly, ESR increased during this period. This may be a manifestation of increase in the relative volume of plasma and more so its water component, because of reduction in the erythrocytes. Coupled with this, mobilization of plasma proteins decrease the specific gravity and viscosity of plasma, allowing the cells to sediment more quickly (Love, 1970).

Sex-linked differences noticed in some of the hematological parameters (erythrocyte count, hematocrit and hemoglobin concentration), with males having higher values of
these parameters can be attributed to difference in the endocrine activity and hormone titers as suggested by Rickey (1976). Ochiai et al. (1975) while working on the Japanese eel, Anguilla japonica, found reduction in hematocrit, hemoglobin content and erythrocyte count with increase in the gonadotrophin and estrogens. He also observed that induced sexual maturation of the female fish with gonadotrophin and estrogen caused gradual reduction in the plasma protein. Erelev (1977b) has elaborated the erythropoietic effect of physiological and pharmacological dosages of androgen in case of mammals. The pharmacological doses were found effective in generating red cell population (Shahidi, 1973) by stimulating the production of 'erythropoietin' (Alexanian, 1969). The work of Zanjani (1969) and Erelev (1977a) leaves no doubt that this humoral substance is capable of adjusting the number of red cells to the oxygen demands of the body. On the other hand, studies carried out by Jukes and Goldwasser (1961) suggested suppression of erythropoiesis but throws no light if the effect is due to disruption in the pathway of erythropoietin biosynthesis.

Winter decline in the numerical strengths of leucocytes and thrombocytes in the major carps seems to be a stress-mediated response of low water temperature. However, it is difficult to attribute differences to one factor in an uncontrolled natural environment, since both extrinsic and intrinsic environmental factors varied simultaneously.
Love (1980) is right in his assessment that the term 'seasonal variation' is used quite loosely just to describe phenomenon of unknown origin. The differential leucocyte-thrombocyte count revealed that reduction in AMC-T and leucocrit is mainly due to significant decrease in small lymphocytes and thrombocytes, since percentage of other type of cells including neutrophils, eosinophils and monocytes was found to rise. Decrease in the number of circulating small lymphocytes and thrombocytes during winter has been reported by Gardner and Fevich (1969) and McLeay (1975b). If low environmental temperature can be considered as an stress, either by way of its direct effect, or through decreased feeding intensity or both, decline in AMC-T count of fishes in the present study can be correlated with increased activity of pituitary-interrenal axis. Data published by Mazaoud et al. (1977) and Strange et al. (1977) explicitly indicates that stress caused enhanced output of ACTH and corticosteroids. That these effect reduction in some white blood cells such as lymphocytes and thrombocytes has been documented by McLeay (1973b, 1975a,b) and Ellis (1977). AMC-T value showed marked recovery in the summer months of April and May but declined during the period of peak maturation (June - July). Presumably, this may be related to turnover of larger quantities of corticosteroids as has been authenticated by Love (1980).
Reduction in the S/L ratio during the peak winter and summer months was mainly due to decline in the output of small lymphocytes and their substitution by the large ones. A critical examination of the dynamics of these cells in the light of life processes of fishes points to the existence of some sort of a 'feedback mechanism' in the production of small and large lymphocytes. There is no scepticism that large lymphocytes generate the small lymphocytes (Blazhkov and Daisley, 1973) which are involved in the formation of antibodies and hence in the disease resistance (Ellis, 1977; Mossal and Ada, 1977). It is quite likely that changes in the internal environment of the fishes and variation in their susceptibility might in some way be linked to differential preponderance of these types of cells at different times of the year.
<table>
<thead>
<tr>
<th>Month</th>
<th>Atmospheric temperature (°C)</th>
<th>Water temperature (°C)</th>
<th>Dissolved oxygen (ppm)</th>
<th>Rainfall (mm/month)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Minimum</td>
<td>Maximum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OCT</td>
<td>14.5</td>
<td>26.1</td>
<td>47.0</td>
<td>2.0</td>
</tr>
<tr>
<td>NOV</td>
<td>10.5</td>
<td>29.0</td>
<td>18.0</td>
<td>2.9</td>
</tr>
<tr>
<td>DEC</td>
<td>6.3</td>
<td>23.7</td>
<td>16.0</td>
<td>2.5</td>
</tr>
<tr>
<td>JAN</td>
<td>6.4</td>
<td>19.8</td>
<td>16.0</td>
<td>2.0</td>
</tr>
<tr>
<td>FEB</td>
<td>6.0</td>
<td>23.0</td>
<td>17.5</td>
<td>3.7</td>
</tr>
<tr>
<td>MAR</td>
<td>3.2</td>
<td>31.3</td>
<td>17.5</td>
<td>2.9</td>
</tr>
<tr>
<td>APR</td>
<td>13.0</td>
<td>38.7</td>
<td>20.0</td>
<td>2.9</td>
</tr>
<tr>
<td>MAY</td>
<td>17.5</td>
<td>41.8</td>
<td>32.0</td>
<td>3.0</td>
</tr>
<tr>
<td>JUN</td>
<td>19.3</td>
<td>42.8</td>
<td>29.0</td>
<td>3.2</td>
</tr>
<tr>
<td>JUL</td>
<td>25.3</td>
<td>44.1</td>
<td>28.5</td>
<td>2.0</td>
</tr>
</tbody>
</table>
**TABLE VII**

Small lymphocyte/large lymphocyte (S/L/L) ratio in monthly samples of *Catla catla*, *Labeo rohita* and *Cirrhina mrigala*. Values are mean ± standard error.

<table>
<thead>
<tr>
<th>Months</th>
<th><em>Catla catla</em></th>
<th><em>Labeo rohita</em></th>
<th><em>Cirrhina mrigala</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>OCT</td>
<td>8.61 ± 0.93</td>
<td>11.25 ± 1.50</td>
<td>7.86 ± 0.89</td>
</tr>
<tr>
<td>NOV</td>
<td>9.30 ± 1.36</td>
<td>15.31 ± 1.17</td>
<td>14.07 ± 1.75</td>
</tr>
<tr>
<td>DEC</td>
<td>8.29 ± 0.26</td>
<td>10.57 ± 0.54</td>
<td>18.30 ± 1.23</td>
</tr>
<tr>
<td>JAN</td>
<td>7.41 ± 0.63</td>
<td>6.32 ± 0.50</td>
<td>3.52 ± 0.31</td>
</tr>
<tr>
<td>FEB</td>
<td>6.68 ± 0.18</td>
<td>6.68 ± 1.08</td>
<td>6.02 ± 0.58</td>
</tr>
<tr>
<td>MAR</td>
<td>5.09 ± 0.30</td>
<td>0.79 ± 1.01</td>
<td>0.13 ± 0.68</td>
</tr>
<tr>
<td>APR</td>
<td>0.82 ± 0.83</td>
<td>0.40 ± 1.09</td>
<td>5.41 ± 1.32</td>
</tr>
<tr>
<td>MAY</td>
<td>3.93 ± 1.95</td>
<td>1.37 ± 1.30</td>
<td>3.80 ± 1.73</td>
</tr>
<tr>
<td>JUN</td>
<td>3.81 ± 1.63</td>
<td>4.89 ± 0.52</td>
<td>4.66 ± 0.78</td>
</tr>
<tr>
<td>JUL</td>
<td>3.44 ± 0.35</td>
<td>6.18 ± 0.51</td>
<td>3.99 ± 0.39</td>
</tr>
</tbody>
</table>