The Pharma Innovation Journal 2023; 12(2): 3102-3108

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(2): 3102-3108

www.thepharmajournal.com
Received: 13-12-2022
Accepted: 24-01-2023

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Certain hematological and biochemical changes in blood of Rohu (*Labeo rohita*) in relation to sex, reproductive status and environmental factors

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Abstract
During the present study hematological and biochemical changes in blood of Rohu (*Labeo rohita)* in relation to Sex, Reproductive status and Environmental factors were analysed. Hematological parameters like haemoglobin (Hb), total erythrocyte count (TEC), packed cell volume (PCV), and Mean corpuscular haemoglobin concentration (MCHC) were found higher in males than in females, while the value for total leukocyte count mean corpuscular haemoglobin (MCV) and mean corpuscular haemoglobin (MCH) were found to be more in females than in males. TEC, TLC, Hb, PCV, and MCHC were found in increasing trend with increasing water temperature, while MCV and MCH were found to be in a decreasing trend with water temperature. Total protein content, albumin and globulin content were found to be more in males and were found in inverse correlation with temperature. Analysis of variance showed significant differences between the males and females for hematological and biochemical parameters except for Mean corpuscular volume and Mean corpuscular haemoglobin, the results indicated that the blood parameter levels from March to October were significantly different. The present study shows that that the haematological and biochemical parameters could be affected by sex, reproductive status and environmental conditions.

Keywords: Hematological, haemoglobin, erythrocyte, leukocyte, biochemical

Introduction
Knowledge related to haemotology is an important and effective tool in determining fish health and adaptability of the species to the environment. Hematological parameters of fish when in stress are of great importance in assessing the effects of pollutants in a particular ecosystem, thus serves as an important bioindicator. The hematological parameters are affected by various factors such as sex, reproductive status of fish, environmental conditions, stressors, age, ecological habitat, temperature, fish species and physicochemical parameters (Blaxhall, 1972) [9]. Hematological profile and biochemical indices are related with the energy requirement of the species. Energy changes occurs through the reproductive and physiological process which are mainly because of variations in metabolic activities. Energy changes is also related with age, gender and reproductive status of the fish.
Leucocytes, thrombocytes and Erythrocytes are the important cellular components of blood. Increased WBCs is indicative of damage due to infection of body tissues, physical stress and even leukaemia. Decreased WBCs increases an organisnms vulnerability to stress and infection (Sunomonu and Oyelola, 2008) [31] hence WBC counts are used in fish health studies to validate immune competence. Haematological parameters of fishes have been found to vary with age and development (Syrove, 1970) [33], starvation (Mahajan and Dheer, 1983) [23], Season (Joshi *et al*., 1980) [17], sex (Al-Hassan *et al*., 1993) [6]. Evaluation of the hemogram involves determining the total erythrocyte count (RBC), total white blood cell count (WBC), hematocrit (PCV), hemoglobin concentration (Hb), erythrocyte indices (MCV, MCH, MCHC), white blood cell differential count, and the evaluation of stained peripheral blood film. Variations in hematological parameters of fish depends upon the aquatic biotope, fish species, sexual maturity, sexual maturity age, and health status. The vulnerability of fish to physical and chemical changes in the environment may observed in their blood components, due to their functions in respiration (hemoglobin levels), defense functions (leucocyte), and energetics. Biochemical analyses also provide important information related to the metabolic and reproductive status.

The variation in protein, cholesterol and glucose level is directly related to sex, size and age of the fishes (Khanna and Singh, 1973) [19]. Gonadosomatic index (GSI) is considered as one of the essential parameters of the fish biology, which provides the idea about the fish reproduction and reproductive status of the fishes and assist in ascertaining the breeding period of the fish (Shankar & Kulkarni, 2005) [29]. The cyclic changes in males of rohu (*Labeo rohita)* are noticed mainly due to maturation of testis. The shape, size, colour and length of the testis go through changes during different seasons and reproductive phases of the life cycle. Similar variations have been reported in other teleosts also by other researchers (Ruby and McMillan, 1970). Very little information is available related to hematological and biochemical changes in blood of rohu (*Labeo rohita)* in relation to sex, reproductive status and environmental factors, keeping this in view present study was subjected to the following objectives

1. Hematological changes in relation to sex and reproductive status.
2. Hematological changes in relation to environmental conditions.
3. Biochemical changes in relation to sex, reproductive status and environmental conditions.

Materials and Methods

In the present study the hematological parameters and certain biochemical changes in blood of Rohu (*Labeo rohita*) were estimated to assess their relationship with its sex, season and reproductive status of fish under agro-climatic conditions of tarai region of Uttarakhand. The fish (*Labeo rohita*) were obtained from Instructional fish farm, College of Fisheries, G.B Pant University of Agriculture and Technology, Pantnagar, Udham Singh Nagar, Uttarakhand India. The healthy and disease free male and female Rohu (*Labeo rohita*) were collected from the Instructional Fish Farm, College of Fisheries, Pantnagar from the month of March to October. Every month four male and four female fishes of average weight 600 gms and average length 30 cms were taken for the study. The study included assessment of physicochemical parameters of pond water and analysis of hematological and biochemical parameters of blood of Rohu (*Labeo rohita*).

The parameters such as length, weight, GSI, HSI, hematology and biochemical parameters were also recorded during the study period. The physical and chemical parameters such as Temperature, pH, dissolved oxygen, and free CO_2 of pond water were analysed as per the standard method (APHA, 1998), throughout the experimental period. Blood sampling was done at every month during study period from March to October. Blood was collected by cardiac puncture with a heparin-coated 25 gauge × 0.5 inch needle, attached to an 2 ml syringe in heparinised tubes/ EDTA anticoagulant vials to prevent clotting of blood for hematological analysis and in normal tubes also to allowed to clot for Biochemical analysis. All fishes were considered healthy on the basis of their appearance and the absence of obvious signs of disease.

Hematological parameters estimated were Total Erythrocyte Count (RBC), Total Leucocyte Count (WBC), Packed Cell Volume (PCV), Haemoglobin (Hb), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC) and Mean corpuscular volume (MCV).

Haemoglobin Estimation done by Sahli's hematin method

Serum biochemical parameters

The serum biochemical parameters were studied from March to June. Blood was collected from cardiac puncture, collected in centrifuge tube and allowed to clot. Then it was centrifuged at 2000 rpm for 10 minutes. The clear supernatant serum was collected in the eppendorf tubes and used for further studies. Serum was stored at –20 degree CELSIUS till used for biochemical studies. Biochemical parameters estimated were total serum protein, serum albumin and serum globulin.

Following biochemical parameters were estimated during study period using the serum collected as described above.

Total serum protein

Total protein concentration in serum was estimated by Biuret method using Erba Diagnostic Kit (Transasia Bio-medicals Ltd., Solan (H.P), India) at 546 nm wavelength and result was expressed in g/dl. Total serum protein (g/dl) = Absorbance of test/Absorbance of standard x Concentration of standard (g/dl).

Serum Albumin

Albumin concentration in serum was estimated by Bromocresol green (BCG) end point assay using Erba Diagnostic Kit (Transasia Biomedicals Ltd., Solan (H.P), India) at 630 nm wavelength and result was expressed in g/dl. Total serum protein (g/dl) = Absorbance of test/Absorbance of standard x Concentration of standard(g/dl).

Serum globulin

The serum albumin content was deducted from total protein to calculate serum globulin content and was expressed in g/dl. Globulin (g/dl) = Total serum protein – serum albumin.

Gonadosomatic Index

The weight of the individual fish was noted and the male and female fish gonads were removed carefully and weighed in a electronic balance. The method of June (1953) and Yuen (1955) was employed to calculate the percentage gonad weight or gonado-somatic index by using the formula:

Gonadosomatic index (GSI) = Weight of Gonad/Weight of Fish x100.

Statistical analysis

Hematological and Biochemical parameters were expressed as mean±SD in both male and female fish and compared according to season and sex using one-way analysis of variance (ANOVA). Differences between means was determined by Duncan multiple range test ($p<0.05$). All these statistical analyses were performed using the Microsoft Office Excel 2007.

Results

The present results confirmed that hematological parameters of fish, *Labeo rohita* exhibit mark variations in response to changing levels of water temperature. The hematological parameters have a direct relation with water temperature. The data obtained comprehensively express a significant increase in the RBC dependent parameters *viz*. TEC, Hb, and PCV during spring and summer period and exhibit maximum during summers (June). All the hematological parameters are

positively correlated with temperature except MCV, MCH and MCHC. The rise observed in the RBC dependent parameters *viz*. TEC, Hb and PCV during spring and summer season has been attributed to an adaptation on the part of fish to cope with natural stress caused by an rise in water temperature and low dissolved oxygen content (environmental factor) in aquatic environment and moreover to prepare itself for breeding time which needs greater physical activity and high energy requirement (physiological factor).

RBC dependent parameters *viz*. TEC, Hb and Hct were obtained minimum during month of October. The possible reason for this decline is metabolically inactiveness of fish and low ambient temperature.

1. Gonadosomatic index (GSI)

Maximum GSI was found in the month of June and minimum in October for male and female fish (table 1). The gonadosomatic index measures the cyclic changes in gonad weight in relation to total fish weight, and can be used to determine spawning periods**.** The Hepatosomatic index was found to be higher in males and lower in females, in males it ranged from 0.49 to 1.05% and in females it ranged from 0.52 to 0.96%, as shown in Table.2.

Table 1: Seasonal variation in GSI% of male and female fish

GSI (%)± SD		
Month	**Male**	**Female**
March	0.506 ±0.381	4.14 ±0.327
April	1.205 ±0.586	7.68 ±0.875
May	2.840 ±0.684	12.54 ±1.247
June	3.154 ±0.673	14.90 ±1.860
August	1.400 ±0.472	10.02 ±1.60
September	0.790 ±0.117	2.30 ±1.080
October	0.208 ±0.209	1.87 ±0.362

2. HSI (Hepatosomatic index)

Table 2: Seasonal variation in HSI% of male and female fish

HSI (%)± SD		
Month	**Male**	**Female**
March	0.62 ±0.179	0.82 ±0.147
April	0.94 ±0.280	0.81 ±0.192
May	1.02 ±0.183	0.94 ±0.141
June	1.05 ±0.474	0.96 ±0.185
August	0.98 ±0.314	0.79 ±0.238
September	0.71 ±0.241	0.62 ±0.171
October	0.49 ±0192	0.52 ±0.257

Hematological parameters

3. Haemoglobin

Content of haemoglobin in rohu (*Labeo rohita*) ranged between 5.10 to 7.33 g% during different months with the mean value of 6.48 in males. In the female specimen, value was found in the range of 5.01 to 6.60% g with mean 5.95 g%. The higher concentration of Hb in both sexes was obtained in June and lower value in month of March. Analysis of variance showed significant difference between the sexes at the level of (P<.05). The haemoglobin concentration showed an increasing trend from March to June then Decreasing trend from June to October in both male and female.

4. Total erythrocyte count

The total number of erythrocyte in males were found to be lowest in October i.e. 1.88×10^6 and highest, that is 3.01×10^6 /mm^3 in the month of June with mean value of 2.63×10^6 while in females 1.672×10^6 in the month of October and 2.41×10^6 /mm^3 during the month of June, with mean value of 2.11×10^6. Female possessed lower number of RBC than that of male, While higher value was obtained in June and lower value was obtained in October. Analysis of variance showed significant differences between the sexes at the level of (P<.05) (table 3 and 4)

5. Total leukocyte count (TLC)

The total number of leukocyte count in males were found to be lowest 4.457×10^3 in October and 6.93×10^3/ mm^3 in June with mean value of 6.58×10^3/ mm^3 and in females was 5.99 to 8.65 $\times10^3$/ mm3 with mean value of 7.15×10^3/ mm^3. Female possessed higher number of TLC than male, While lower value was obtained in month of March and higher value was obtained in month of June. Analysis variance showed significant differences between two sex but month wise there is non-significant values at the level of (P<.05) significance.

6. PCV (Haematocrit)

The value of PCV ranged from 25.8% in month of October to 30.02% in the month of June in male fishes, while for female fish it ranged from 20.45% in the month of October to 26.72% in the month of June. On the basis of sex males possesses higher value of haematocrit than females. The mean value of haematocrit for the male was 28.99% and for female fish was 24.22% during the study period, which point out that male fishes have higher value of haematocrit than the female fish. Analysis of Variance showed significant differences between sex at the level of (P<.05).

7. Mean corpuscular volume (MCV)

The value of MCV for male fish ranged between 101.90 fl in the month of June to 147.428 fl highest during the month of October with mean value of 112.43 fl and for female the value of MCV value ranged 107.400 in the month of May to 122.451 fl in the month of October with mean value of 115.235 fl. There was no major difference in male and female fish. Analysis of Variance did not showed significant differences between sex and month wise at the level of (P<.05).

8. Mean corpuscular haemoglobin (MCH)

The value of Mean corpuscular haemoglobin throughout the study period was found to be varied from between 24.59 ρg to 34.02 ρg in male fish with mean value of 27.66 ρg and for female 26.80 ρg to 34.13 ρg with mean value of 29.01 ρg. The MCH value was found higher value in the month of March and lower in June in both sexes. On the basis of sex it was found to be higher in females than in males. Analysis of variance shows non-significant differences between sex and month wise at the level of (P<.05).

9. Mean corpuscular haemoglobin concentration (MCHC)

The value of mean corpuscular haemoglobin concentration, ranged from 20.41% in month of October to 27.22% in month of March in male fish with mean value of 24.89% and in female fish ranged from 24.64% in month of October to 29.21% in the month of March with mean value of 25.84%. The higher value was found in month of March and lower value was found in month of October. The mean value indicated as slightly higher in female fish (25.84%) in comparision to male fish (24.89%). Analysis of variance did not showed any significant differences between the values in relation to sex and months at the level of (P< .05) (table 3, 4).

Table 3: Season wise variation in hematology of Male Fish

Month	TEC ±sd	TLC ±sd	Hb (g%) ±sd	PCV(%)±sd
March	2.22×10^6 ±0.433	5.85×10^3 ±0.451	5.10 ±0.411	26.87 ±1.271
April	2.50×10^6 ±0.342	6.125×10^3 ±0.451	6.27 ±0.588	29.15 ±2.010
May	2.79×10^6 ±0.223	6.935×10^3 ±0.260	7.23 ±.0.471	29.92 ±0.953
June	3.01×10^6 ±0.406	6.585×10^3 ±0.385	7.33 ±.0.386	30.02 ±2.010
August	2.32×10^6 ±1.720	6.280×10^3 ±0.291	6.23 ±0.243	28.41 ±0.914
September	2.18×10^6 ±0.311	5.663×10^3 ±0.363	5.91 ±0.403	27.72 ±1.213
October	1.88×10^6 ±1.091	4.457×10^3 ±0.277	5.22 ±0.209	25.08 ±0.917
Month	**TEC ±sd**	**TLC ±sd**	**Hb (g%) ±sd**	**PCV(%)±sd**
March	2.22×10^6 ±0.433	5.85×10^3 ±0.451	5.10 ±0.411	26.87 ±1.271
April	2.50×10^6 ±0.342	6.125×10^3 ±0.451	6.27 ±0.588	29.15 ±2.010
May	2.79×10^6 ±0.223	6.935×10^3 ±0.260	7.23 ±.0.471	29.92 ±0.953
June	3.01×10^6 ±0.406	6.585×10^3 ±0.385	7.33 ±.0.386	30.02 ±2.010
August	2.32×10^6 ±1.720	6.280×10^3 ±0.291	6.23 ±0.243	28.41 ±0.914
September	2.18×10^6 ±0.311	5.663×10^3 ±0.363	5.91 ±0.403	27.72 ±1.213
October	1.88×10^6 ±1.091	4.457×10^3 ±0.277	5.22 ±0.209	25.08 ±0.917
Month	**MCV(fl) ±sd**	**MCH(µg) ±sd**	**MCHC(%)±sd**	
March	123.325 ±2.246	34.02 ±1.600	27.225 ±1.110	
April	116.825 ±3.816	26.12 ±0.960	24.400 ±2.201	
May	107.680 ±1.159	25.94 ±1.601	24.140 ±1.390	
June	101.900 ±2.880	24.59 ±1.420	23.81 ± 2.091	
August	122.457 ±0.721	26.05 ±1.053	21.92 ±1.323	
September	127.155 ±1.047	27.11 ±0.847	21.32 ± 1.072	
October	147.428 ±0.915	29.25 ±0.739	20.41 ±2.113	

Table 4: Season wise variation hematology of Female Fish

Month	TEC ±sd	TLC ±sd	Hb(%) ±sd	PCV(%) ±sd
March	1.825×10^6±0.430	5.99×10^3±0.297	5.01±1.435	20.62±1.244
April	1.962×10^6±0.160	6.77×10^3±0.145	5.86±0.496	23.10±0.752
May	2.26×10^6±1.103	7.21×10^3±1.258	6.60±0.316	26.47±1.299
June	2.41×10^6±0.246	8.65×10^3±0.141	6.75±1.419	26.72±2.461
August	2.01×10^6±0.533	7.09×10^3±0.987	6.03±2.876	24.31±1.034
September	1.92×10^6±1.091	6.45×10^3±1.456	5.74±0.765	23.04±0.655
October	1.67×10^6±0.814	5.83×10^3±0.544	5.04±2.345	20.45±2.870

Month	MCV (fl) ±sd	MCH (µg) ±sd	MCHC(%) ±sd
March	122.251±4.210	34.13±3.311	29.21±1.711
April	116.070±1.819	26.80±0.650	25.27±2.610
May	107.400±0.462	27.04±2.324	25.07±0.382
June	115.220±3.141	28.09±1.848	25.26±1.772
August	120.940±1.575	30.01±0.659	24.80±1.765
September	120.000±0.439	29.89±0.945	24.91±0.546
October	122.455±1.540	30.17±1.459	24.64±1.945

10. Total protein

Total protein content range of Rohu (*Labeo rohita*) was between 2.256 g/dl to 2.860 g/dl in males with mean value of 2.53 g/dl. In females the value of protein ranged from 2.106 g/dl to 2.552 g/dl with mean value of 2.366 g/dl. The values of protein content was found higher in males than in females. The higher concentration of total protein in males and females were obtained in June and lower value in month of October.

11. Albumin

The values of albumin ranged from 1.230 g/dl to 1.617 g/dl in males with mean value of 1.407 g/dl and in females the value of albumin ranged from 1.043 g/dl to 1.535 g/dl with mean value of 1.379 g/dl. Lowest value was recorded during the month of March in males and highest values in the month of June while in females lowest values are recorded in the month of October and highest values are recorded in month of June. Males possesses higher values of albumin than females. Analysis of Variance showed significant differences in absolute values but did not showed significant differences for mean values between the sex and months at the level of (P<.05).

12. Globulin

The value of globulin ranged from 1.017 g/dl to 1.355g/dl in male fish with mean value of 1.085 g/dl and from 1.162 g/dlto 1.30 g/dl in female fish with mean value of 1.245 g/dl. Female possessed higher globulin content than that of male. While higher value was obtained in month of June and lower value was obtained in month of March (table 5).

Hematological changes on the basis of reproductive status

Hematological parameters are found to vary in relation to reproductive profile also. The development of gonads is reflected by variations in values of GSI in case of male fish was ranged from 0.506% during spring season (Preparatory phase) to 3.154% during summer (Pre spawning phase). The GSI values of female had been increased from 4.14% spring (Preparatory phase) to 14.90%. Summer (Pre spawning phase).

Table 5: Seasonal variation in biochemical composition in Male fish

Month	Protein (g/dl) ±sd	Albumin (g/dl) ±sd	Globulin (g/dl) ±sd
March	2.392±0.173	1.230±0.169	1.162±0.063
April	2.607±0.158	1.330±0.384	1.277±1.265
May	2.752±0.097	1.452±1.163	1.300±0.084
June	2.860±0.306	1.617±0.452	1.243±1.202
August	2.527±1.034	1.434±0.076	1.093±0.548
September	2.316±0.791	1.437±1.194	0.879±0.119
October	2.256±1.331	1.336±0.448	0.920±0.861

Table 6: Seasonal variation in biochemical composition in Female fish

Month	Protein	Albumin	Globulin
March	2.287±0.312	1.102±1.193	1.355±0.262
April	2.345±1.259	1.307±0.252	1.038±0.360
May	2.505±0.369	1.472±1.278	1.032±1.298
June	2.552±0.281	1.535±0.557	1.017±0.389
August	2.424±1.654	1.342±1.783	1.082±0.485
September	2.347±0.345	1.207±0.762	1.140±1.470
October	2.106±1.256	1.043±0.638	1.063±0.564

Discussion

The present results showed that RBC values, Hb, PCV, TEC and TLC values showed significant differences between the male and female fish. The values of Hb, PCV, TEC and TLC were in increasing trend from the month of March to June and after then the values were found to be in a decreasing trend from June to October this states that Haematological parameters are temperature dependent and the values of these parameters shows variation with variation in temperature. During June month, these value were found higher than other months The rise observed in the RBC dependent parameters *viz*. TEC, Hb and PCV during spring and summer season has been attributed to an adaptation on the part of fish to cope with natural stress caused by an rise in water temperature and low dissolved oxygen content (environmental factor) in aquatic environment (Preston, 1960; Joshi *et al.*, 1980; Wells, 1999; Jawad *et al.*, 2004 and De Pedro *et al.*, 2005) [17, 16] and moreover to prepare itself for breeding time which needs greater physical activity and high energy requirement (physiological factor) (Eisler, 1965; Joshi and Tandon, 1977 and Khan,1977) and also because of high body metabolic rate due to high ambient temperature and reproductive activities. These results are supported by findings of Patra *et al*. (2014) [27] in *catla catla*, Pradhan *et al*., (2012) [28] in *Cirrihinus mrigala* in which they observed higher value of TEC, Hb, PCV in summer month. The values of TEC, Hb, PCV were found higher in male fish as compared to female fish throughout study period The higher value in male fish than female fish may be due to physiologically activeness of male fish than female fish. Variation in haematocrit value and other hematological parameters between sexes and the diversity might be due to higher metabolic rates of male fish compared to that of female fish. Higher values of some hematological indices for males than for females of most fish species are caused by varying activity of erythropoietin. In the study period, Total leukocyte count was found higher in female fish than male fish and month wise it showed increasing trend upto month of June. Female fish have higher TLC number throughout the different seasons probably due to the differential genetic makeup of male and female fish. During spring and summer season raised water temperature acts as a natural stressor and creates pathogenic situation in fish. To counter such situations fish react by either increase leucocyte count or their products e.g. lysozymes, peroxides, interferons, lysins etc., an immunostimulatory response and thus grow into immunologically strong enough to face any type of stress in their surroundings. The assumptions are well supported by the findings of Adewoye (2010) who has reported an immunostimulatory response by fish during warm period of the year. MCV, MCH and MCHC values exhibit a negative correlation with temperature therefore as the temperature increases MCV, MCH, MCHC values decreases and vice versa. Decline in the values of MCV, MCH and MCHC with a rise in water temperature finds a relation with the mechanism of erythropioesis through which young erythrocytes (corpuscles) were combined to blood stream of fish. These young erythrocytes being smaller in size have low volume and possess less haemoglobin content (Blaxhell and Daisley, 1973) [9] which result in decrease in the values of MCV and MCH.

Leukocyte levels in the blood may also fluctuate according to environmental quality, nutritional status and presence of infectious agents. Immunosuppression described by previous workers appears that lower water temperature is responsible for the lower count of leucocytes Houston *et al*., (1996). Ibrahim *et al*. (2003) also described a positive correlation between total leucocyte count and feeding status of the fish, as an increase had been observed during the warm period of the year. However low temperature during the winter season usually limits the prevalence of pathogenic events in the aquatic ecosystem and therefore may results in immune depressive state in fish. In winter months total leucocyte counts are observed to be in a declined state.

Total protein and albumin content was found higher in males than in females,it may be due to environmental factors. The serum protein in fishes is dependent on environmental conditions (seasonal temperature) and feeding regime (Ghoroghi *et al*., 2009 a & b) serum protein can be influenced by other factors like age, stress, disease, dietary protein contents and anasthaesia. A variation in serum protein may be a result of gonad maturation and transportation of nutrients from some organs to gonad by blood, mobilization of nutrients to developing oocytes (Chatzifotis *et al*., 2004). Plasma protein is the protein component of the blood and is vulnerable to increase with starvation or any other stress. Plasma protein gives an index of the health status of the brood fish and as indicator of nutritional status. The higher value of total protein in blood was found in June month. (Prespawning phase) and lower value were found in the month of March (Preparatory phase). The results after Statistical analysis showed that the differences in all biochemical parameters were significant at (p<0.05). Protein albumin and globulin are positively correlated with temperature. Significantly higher values (P<0.05) in males were observed for total protein, albumin and globulin. Albumin is a part of total protein and acts in the transportation of steroid hormones. Therefore, their increase with age and gonad maturation is a normal physiological process. A significant direct correlation between Albumin and Total protein is expected.

The reproductive seasonality of breeding fishes is based on the annual cyclicity of several biological phenomenon and Gonadosomatic index is generally used as reliable criteria for expression of gonadal recrudescence and reproductive efforts in fishes. In the month of June higher temperature and low

dissolved oxygen may acts as stimulator for ripening of gonads, hence increase in gonadosomatic index value. The increased metabolic rate associated with the gonadal maturation in the ripening fish necessities gradual increase in the erythrocyte numbers and haemoglobin values as an adjustment to increased oxygen demand. During pre-spawning period teleosts release large amounts of steroids into blood stream, especially steroid C21, a hormone that controls final maturation of oocytes in females and process of spermiation in males.

Summary and Conclusion

The hematological and biochemical parameters of blood of Rohu studied in the present study confirmed that these are influenced by the, sex, reproductive status and environmental factors. The hematological and biochemical parameters of the blood of rohu (*Labeo rohita*) showing variations regarding to sex, reproductive status and environmental factors, and are positively co-related with the temperature and negatively co-related with Dissolved oxygen environmental factors. It is also found that the hematological and biochemical parameters of the fish showed variations during different reproductive phases.

The present study has established a baseline data regarding the selected hematological and biochemical parameters of *Labeo rohita* for certain hematological parameters for further studies. This present study may be helpful as a tool to monitor the health status of fish Rohu (*Labeo rohita*).

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