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Assessment of some seasonal variables on some blood parameters of a local fish '*Schizothorax plagiostomus*' in 'River lidder' in Kashmir valley (India)

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Abstract
Present work was carried out in order to find out some seasonal variables on some blood parameters of *Schizothorax plagiostomus* caught from the world-famous River lidder (Pahalgam Kashmir, India). Effects of the independent variables e.g., sex, weight and length values were determined seasonally. Analysis revealed that highest number of leukocytes was found in spring and lowest number was found in winter. Hemoglobin and Haematocrit values were highest during the months of summer and lowest during winter. It was also seen that Males were having greater values for hemoglobin and Haematocrit than females, whereas total leukocyte count in females was higher than males. It was also found that there was a positive correlation between Length/weight and Hemoglobin and Haematocrit values where as a negative correlation between length or weight and Total leukocyte counts.

Keywords: Schizothorax, leukocytes, haematocrit values, correlation

Introduction
Due to great importance of blood in the diagnosis of diseases in medicine, hematological and serological investigations are now employed on an increasing scale for diagnostic purposes in fish pathology. Hematology concerns with the cells present in the blood and serology deals with the constituents in the fluid part of the blood, such as proteins, minerals, carbohydrates, pigments, enzymes, hormones, immunoglobulin's etc. Hematological investigations on fish have confirmed that the variations in the blood give indications of health conditions (Soivio, A, and A Oikari. 1976) [33].

Marked variations in hematological data, caused by the environment would necessitate an exact establishment of normal values of various environmental conditions and in different seasons of the year. In fisheries it is important to diagnose the illness of a fish by evaluating hematological data, particularly blood parameters (Ramesh M, *et al*, 2008) [32].

To picturize the actual hematological data is quite different because of marked internal and external variations sampling techniques, laboratory techniques, seasonal variations, habitat size, sex, population density, oxygen content, pH, water, ammonia, feeding etc. (Wilhelm Filho *et al*, 2001) [16]. Research on blood of Cyprinus Carpio had shown that blood parameters were affected by seasonal variations (Denton and Yousuf, 1975) [14]. In addition to this age, sex, weight and length of fish also affect the blood parameters of fish (Ezzat A *et al*, 1982) [20].

Materials and Methods.
In the present study 10 male and female specimens of *S. plagiostomus* were collected from River lidder with the help of local fisherman. Sex, weight and length of the specimens was male and females, 180-3100gms and 22-33 cms respectively. Many techniques were utilized for the collection of blood e.g., Heart punctures, severing of caudal peduncle (Blaxhall and Daisley, 1973; Hatting 1975) [8].

Blow was given on the head of the fish and the needle was inserted at an angle of 45° in to the heart and the blood was collected in to the glass tubes already containing EDTA (Ethylene diamine tetra acetic acid). Blood analysis was carried out very quickly after sampling. Different leukocyte counting was performed by transporting blood samples diluted with turck's solution with a leukocyte pipette on to a neubar's counting chamber and examined for leukocytes (Blaxhall and Daisley, 1973) [8].

Leishman staining was used to determine the percent leukocyte (Goel *et al*, 2011) [21]. The hemoglobin was determined according to the method of Haldane. Haematocrit values were determined by the microhaematocrit (Jewet *et al*, 1991) [22].

The blood without EDTA was transferred in to Haematocrit pipettes and centrifuged at 10,000×g rpm for 10 minutes and the ratio of the blood components in plasma was determined. The slides were examined under a binocular microscope with 100x objectives to see the percentage of neutrophils, eosinophils, lymphocytes and monocyte.

Results

Water quality plays a vital role in determining any hematological parameter of the fish. Water quality criteria like Temperature of River lidder between January 2018 and December 2019 was different in different seasons. Total leukocyte count, eosinophil, neutrophil and monocyte percentages were significantly (p<0.05) higher for both sexes in spring -summer seasons than those of other seasons. Lymphocyte ratio also increased (p<0.05) in autumn-winter season. The amount of hemoglobin and Haematocrit value increased significantly (p<o.o5) in spring-summer season.

Table 1: Seasonal variations of total leukocytes and its types in males of *S. plagiostomus*.

Season/Temp.	No. of fishes	Total leukocyte count(x10/mm3)	Neutrophil%	Eosinophil%	Lymphocyte%	Monocyte%
Spring/16ºc	10	13.00+0.20	26.10+0.50	1.45+0.1	61.40+1.50	12.20+0.30
Summer/28 ºc	10	12.60+0.50	25.40+0.40	1.02+0.2	64.70+1.20	9.50+0.50
Autumn/20 ºc	10	11.40+0.50	24.40+0.20	0.950+0.5	68.50+0.50	6.50+0.20
Winter/5 ºc	10	9.50+0.50	21.32+0.70	0.92+0.3	63.20+0.30	8.60+0.70

Table 2: Seasonal variations of total leukocytes and its types in females of *S. plagiostomus*.

Season/Temp.	No. of fishes	Total leukocyte count(×10/mm³)	Neutrophils%	Eosinophils%	Lymphocyte%	Monocyte%
Spring/16ºc	10	18.00±0.50	33.00±0.70	1.05±0.02	53.05±0.30	11.50±0.17
Summer/28ºc	10	16.50±0.30	29.50±0.50	0.90±0.50	61.02±1.20	12.70±1.09
Autumn/20ºc	10	13.70±0.70	23.50±0.30	0.40±0.30	67.00±0.30	10.05±0.50
Winter/5ºc	10	10.50±0.20	21.30±0.70	0.48±0.50	73.05±0.10	10.50±1.51

Table 3: Male dependent seasonal variations in Hemoglobin and Haematocrit values.

Season/Temp.	No. of fishes	Hemoglobin Density(g/dl)	Haematocrit values (%)
Spring/16ºc	10	9.50± 0.05	33.80± 0.30
Summer/28ºc	10	11.20± 0.20	37.65± 0.45
Autumn/20ºc	10	7.30± 0.70	31.50 ±0.37
Winter/5ºc	10	7.09 ± 0.30	25.30 ± 0.70

Table 4: Female dependent seasonal variations in Hemoglobin and Haematocrit values.

Season/Temp.	No. of fishes	Hemoglobin Density(g/dl)	Haematocrit values (%)
Spring/16ºc	10	6.50± 0.08	28 ± 0.50
Summer/28ºc	10	8.20± 0.05	28± 0.20
Autumn/20ºc	10	5.90 ± 0.09	22± 0.10
Winter/5ºc	10	5.50 ± 0.08	17.80± 0.20

Table 5: Comparison of Blood parameters between male and female *S. plagiostomus* between March 2018 to Mar.2019.

Blood parameters	Males	Females
Total leukocyte count	13.52± 1.50	11.50 ± 0.50
Leukocyte types (%)		
Neutrophils	25.20± 0.50	29.50± 0.30
Eosinophils	0.70 ± 0.01	0.40 ± 0.01
Lymphocytes	0.50± 0.80	55.20± 1.50
Monocyte	8.50 ± 1.00	10.50± 0.80
Hemoglobin Density(g/dl)	7.20 ± 0.50	6.02 ± 0.50
Haematocrit values (%)	28.50± 0.70	22.50± 0.23

Table 6: Length-weight group dependent frequencies of hematological parameters of *S. plagiostomus*.

Parameter				
No. of fishes	10	10	10	10
Length(cm)	17-23	24-30	31-33	29-33
Weight(gm)	180-210	180-280	282-320	300-320
Hemoglobin Density(g/dl)	6.07 ± 0.12	6.18± 1.50	7.12 ± 0.32	9.50 ± 0.3
Haematocrit values (%)	19.55± 0.50	20.32± 1.62	23.55± 1.32	25.80± 1.32
TLC(×10/mm³)	25.42± 1.30	22.60± 0.80	22.70± 1.30	21.80± 0.50
Neutrophils (%)	10.60± 1.50	11.50± 0.20	14.50± 1.30	11.30± 0.20
Eosinophils (%)	0.60 ± 1.31	0.49 ± 0.05	0.55 ± 0.30	0.55 ± 0.70
Lymphocytes (%)	83.80± 0.50	78.50± 1.32	82.30 ± 1.32	77.30± 0.30
Monocyte (%)	09.50± 0.50	11.30± 0.50	11.20± 0.70	12.50± 0.40

The leukocyte count, neutrophils and monocyte percentages were compared regarding sex difference, they were found to be higher in females than males but the level of hemoglobin and Haematocrit values and lymphocyte ratios were found to be higher in males than females. The amount of hemoglobin, Haematocrit value, neutrophil and monocyte ratio also increased in length and weight but lymphocyte ratio decreased significantly.

Discussion

Although less work has been done on the hematology of *S. plagiostomus* there are few reports of normal blood values and published values are severely limited by low fish numbers. Annual changes in blood parameters showed a clear increase in spring-summer period and a decrease in autumn-winter period. The leukocyte numbers and Neutrophil ratios for female specimen were found to be higher compared to male specimen during the reproduction period (March-August). The same trend occurred with the values of hemoglobin, haematocrit, neutrophil and monocyte percentages. Our results are in accordance with other results of (Denton and yousuf, 1975; Ezzat, A. A, *et al*. 1974; Beelen, R. *et al*, 1998; Azizoglu and Cengizler, 1996) [14, 18, 4]. When sex was taken into consideration, the amount of hemoglobin, Haematocrit and lymphocyte values for male fish were significantly higher than for female fish. Monocyte ratio, neutrophil and leukocyte values were higher in females at reproduction periods than in males. So, our results are in accordance with the results of Ezzat *et al*. 1974; Murray 1984; Terao *et al*. 1984; Lumsden 2011 [18, 28, 34, 27]. Length to weight independent variables were determined to cause an increase in hemoglobin, hematocrit, neutrophil and Monocyte values but the ratio of lymphocytes decreased to a moderate level. The length weight variables on blood parameters (lymphocyte formation) are an essential component of immune system in the early stages of growth period.

To conclude, the large variation in hematological parameters obtained emphasizes the need of more extensive study. The changes in micro-environment and the macro-environment have a direct effect on the blood components of fish which makes the comparison difficult to be made. Evaluation of hematological analyses will enhance the culture of fish by facilitating early detection of infectious diseases and identification of sub lethal conditions affecting production performance this will contribute to more specific, timely and effective disease treatment in the future. Therefore, we are of this opinion that there should be separate data collection and comparison from healthy and unhealthy fish to obtain reliable hematological data. These results of our study may be used as reference values of blood parameters for other fishes.

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