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Seasonal Variations in Certain Hematological Factors of Catla Catla (Hamilton 1822)

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Abstract: *Hematological parameters are repeatedly used as an essential tool to assess the health condition of fish. The purpose of this study was to assess the reference values of some hematological parameters of Catla, catla catla collected from fresh water pond in a tropical climate of India from July 2011 to 2013. Variation in hematological parameters such as hemoglobin, erythrocyte count, total leucocytes count, hematocrit, mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) of fish were compared according to sex and seasonal differences. Analysis of variance showed that there were significant differences between sexes and the results indicated that the blood parameter levels between the sexes in summer were significantly different than that measure in other seasons except mean corpuscular hemoglobin(MCH) and (MCHC) value ($P < 0.05$). The number of total leukocyte levels was found to be higher in female fish especially in breeding seasons, but the levels of hemoglobin, hematocrit and MCV values were high in male fish in an annual period. However, there was no difference in MCH and MCHC values between the sexes and seasons throughout the study period. These may be related to the seasons of sampling and changing physiological cycles during these months. The correlation between measured hematological parameters was also determined.*
Key words: *Seasonal Variations- Hematology-Catla catla Reference Values*

I. Introduction

Hematological parameters are frequently used as an essential diagnostic tool to assess the health condition of lower vertebrates due to the increasing emphasis on pisciculture and greater awareness of population of natural water resources (Bhaskar and Rao 1985). There are several publications, reports on hematological aspects of the vertebrate species in order to correlate their physiology and evolution, but a break through knowledge of fish physiology is becoming more imperative due to diagnostic evolution, economic importance and comparative study of fish. In recent years, blood parameters have been commonly used to observe and follow the quality of fish (Bhaskar and Rao 1985). One of the difficulties in assessing the state of propagation has been the paucity of reliable references under natural habitat. In pursuant to this objective, many fish physiologists have concentrated to hematological studies probably because it has proved a valuable diagnostic tool in evaluating fish quality. (Hesser1960; Anderson and Klontz 1965; Kori-Siakpre et al. 2005; Oluyemi et al, 2008).

A major part of the world's food is being supplied from fishery sources, thus it is essential to secure the propagation of fishes (Tripathy and Harsh 2002). First of all, the analysis of hematological parameters is one of the most valuable modern diagnostic tools to understand fish health. Recently, Anver(2004) established that the physiological values of hematological parameters are species specific and age dependent. Many publications reported by different workers that qualitative and quantitative variations in hematological parameters including the red blood corpuscles (RBC) and white blood corpuscles (WBC) numbers , cell proportions of leukocyte, the amount of hemoglobin and the size of WBC and RBC are the most significant findings as regards diagnosis. Hematocrit, erythrocytes count and hemoglobin concentrations are the most readily determined hematological parameters under the field and hatchery conditions (Bhaskar and Rao 1990). Among different hematological parameters, hematocrit value is not easily altered as other parameters and should be used in conjunction with erythrocyte and leucocytes count, hemoglobin content osmotic fragility, and differential leucocytes count (Wedemeyer et. al 1983). Most of the several contributions towards a better understanding of fish hematology deal with marine species (Johnson 1968). Variation in hematological parameters of fishes are caused by environmental stress (Hickey 1982), malnutrition (Casillas and Smith 1977), gender (Siddique and Naseem 1979 ; Collazos et al. 1998), fish size (Garcia et al. 1992), seasonal differences and breeding efficiency (Cech and Wohlschlang 1981). The environmental influence on blood parameters of fishes has generally been used as an effective and sensitive index to monitor the physiological and pathological changes in fish (Iwama et al. 1976; Chakrabarti and Banarjee 1988; Orun et al. 2003). Interestingly, the hematology of fish continues to offer valuable diagnostic tool and progress in establishing normal range values for blood parameters of different fish species, but the information regarding Cyprinidae species is incomplete. This study was undertaken to establish reference values for some hematological parameters of a fresh water fish Catla catla living in wild on the basis of sex and seasons.

II. Material and methods

Study site and animal sample collection

C.catla is fresh water teleost belonging to the family Cyprinide and is high-quality flesh together with its good taste made it a high-priced fish in the local market in India. For the present study, fish samples were collected monthly from a fresh pond of Khurda District, Bhubaneswar, India (19^0 40'' N to 20^0 25'' N Latitude and 24^0 55'' E to 36^0 0.5'' E Longitude; area, 2889 km^2) during July 2011 to June 2013. This area and pond was chosen considering tropical climate, average ambit temperature range between 9.5^0C (winter) to 41.4^0C (summer), with annual rain fall of 1,449.1 mm, agricultural, domestic fish farming and human activities. Fifteen to twenty individuals (33.5 ± 3.0 cm in standard length and 1,100 ± 75gm in weight) wre selected in each month from the pond using extension net. Fish samples were put into transport tanks (300L) filled with pond water and transported to the laboratory on the same day. During transport water tank was oxygenated properly.

Blood sample collection

Blood sample were obtained from caudal peduncle and heart by cardiac puncture using disposable plastic syringes fitted with 26-gage needle that was already moistured with heparin and expelled into separate heparinised vials immediately on ice (Orun et al. 2003, Lavanya et al. 2011). For the estimation of hemoglobin, RBCs and WBCs count, we used whole blood.

Hematological analysis

Cynmethaemoblobin method was used for evolution of hemoglobin concentration as described by Lavanya et al. (2011). For counting the number of RBCs and WBCs, we followed standard procedure as published earlier by us (Pal et al. 2008, Parida et al. 2011). The results were expressed as the number of RBC or WBC per 1 mm^3 of blood. We counted in an improved Neubauer hemocytometer using Hayem's diluting fluid the total red blood corpuscle or erythrocyte. The number of cells count was determined as described Svobodova et al. (1991) and Oluyemi et al. (2008). The total RBC count per mm^3=200x50xN= 10,000 N (N=number of RBc counted , dilution factor=200). The total WBC or leucocyte was counted in an improved Neubauer hemocytometer using Tuke's diluting fluid. The total count per cubic mm was obtained as 20x1xL/0.4 cells=50 x L(L= Number of WBC counted dilution factor=50). The determination of packed cell volume(PCV) was performed as published by Adehayo et al. (2007). The PCV was estimated by using micro-hemotocrit reader and expressed in percentage. Erythocyte indices mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated as per formulae of Dacie and Lewis (1975).

MCV=PCV x 100/erythrocyte count
MCH=hemoglobin x 10/ erythrocyte count
MCHC=hemoglobin x 100/PCV

Statistical analysis

Hematological data resulting from the experiment were subjected to one-way analysis of variance (ANOVA) by using the SPSS 17 for windows. Differences between means was determined by Duncan multiple range test ($p<0.05$). The correlation between hematological variables was analyzed by Pearson coefficient for linear correlation (r) at $p<0.05$.

III. Result

Hematological analysis

In order to determine the hematological parameters of each adult fish, sex seasonal variations were considered during the two years of our study period. The average data of the hematological parameters such as RBC, WBC, hemoglobin, hematocrit, MCV, MCH and MCHC were depicted for both the sexes in different seasons (Table-1). As shown in Table-2 there were significant differences (ANOVA) in hematological parameters between sexes and seasons. The correlations between hematological parameters regarding seasons and sexes has been illustrated in Table-3, 4 and 5.

Hemoglobin content

Hemoglobin content of C.catla oscillated between 5.0 and 7.775 g/dl during different seasons (Table-1). In male hemoglobin (Hb), concentration varied from 5.4 to 8.45g/dl with annual mean of 6.97±0.30 g/dl. In the female specimen, value was within the range of 4.6-7.1 g/dl with annual mean of 5.90±0.25 g/dl. The higher concentration of Hb in both the sexes was obtained in summer and lower value in the autumn season (Fig. 1). There are significant differences between sexes.

Total erythrocyte count

The total number of erythrocyte ranged from 1.36 x 10^6 to 1.77 x 10^6/mm^3 blood in male fish and 1.15 x 10^6 to 1.69 x 10^6/mm^3 blood during annual study period (Table-1). Male possessed higher number of RBC than that of female, where as higher value was noted in summer and lower in winter in both the sexes (Fig-2). The annual average of RBC in male and female was 1.644 x 10^6 and 1.44 x 10^6/mm^3 blood, respectively.

Total leukocyte count (TLC)

Season-wise total leukocyte count (TLC) value was higher in summer and lower in the winter in bothj sex C.catla. TLC varied between 2.7 x 10^3 – 5.12 x 10^3/mm^3 of blood and 3.00 x 10^3 – 5.6 x $10^{3g/}$$mm^3$ of blood in male and female fish, respectively (Table-1). The annual mean value of male and female fish was 3.754 x 10^3 and 4.124 x 10^3/mm^3 of blood, respectively. Thus female fish had higher values than that of male fish.

Table-1 Seasonal variations in blood parameters of male and female C.catla

Season	Sex		Hemo	RBC	Leuco	Hemato	MCV	MCHC	MCH
Spring	Male	Mean	7.4100	1.7100	3.9000	31.4900	172.1000	31.6000	55.3100
		SE	0.16743c	0.01155b	0.10392b	0.14434c	3.23316a	0.14434b	0.28868c
	Female	Mean	6.5000	1.5300	4.5000	26.3000	171.4000	31.4000	57.0000
		SE	0.13856b	0.02309c	0.12702b	0.24249d	2.07816a	0.11547a	0.20207d
Summer	Male	Mean	8.4500	1.7700	5.1200	32.9000	179.9000	31.1500	54.0000
		SE	0.21362d	0.02887d	0.30022c	0.17321d	2.51147a	0.24249b	0.20267b
	Female	Mean	7.1000	1.6900	5.6100	27.0000	179.0000	31.5500	55.2000
		SE	0.16166c	0.01732d	0.36188c	0.14434d	1.87639a	0.28868b	0.25981c
Rainy	Male	Mean	7.2000	1.7000	4.6500	31.0000	175.7900	31.8000	53.9000
		SE	0.11547c	0.02021b	0.25981b	0.11547c	3.03109a	0.38682b	0.12702b
	Female	Mean	6.3000	1.5800	4.4100	24.9000	175.5000	32.2000	54.0100
		SE	0.08660b	0.01443c	0.14434b	0.19630c	3.75278a	0.28868b	0.17321b
Autumn	Male	Mean	6.4000	1.7000	3.0000	27.8000	172.9000	31.0000	53.0000
		SE	0.14434b	0.01443b	0.08660a	0.37528b	4.50333a	0.43301b	0.20207a
	Female	Mean	5.0000	1.2500	3.1000	22.1000	171.0000	31.8500	53.0400
		SE	0.08660a	0.03464b	0.11547a	0.34641b	3.46410a	0.28868a,b	0.14434a
Winter	Male	Mean	5.4000	1.3600	2.7000	23.8000	172.2100	30.0000	54.0100
		SE	0.20207a	0.01443a	0.17321a	0.25981a	4.99408a	0.20207a	0.25981b
	Female	Mean	4.6000	1.1500	3.0000	20.7000	169.8000	31.1500	53.0100
		SE	0.23094a	0.01155a	0.28868a	0.20207a	4.33013a	0.34641a	0.20207a

Table-2 Analysis of one-way ANOVA on mean blood parameters by sex and seasons in C.catla

Variables	Source	Sum of squares	ρg	Mean square	g/dl	significance
Hemo	Season	28.588	4	7.147	90.813	0.000
	Sex	8.619	1	8.619	109.516	0.000
	Season* sex	0.472	4	0.118	1.499	0.240
	Error	1.574	20	0.079		
	Corrected total	39.253	29			
RBC	Season	0.827	4	0.207	165.444	0.000
	Sex	0.324	1	0.324	259.584	0.000
	Season* sex	0.125	4	0.031	25.044	0.000
	Error	0.025	20	0.001		
	Corrected total	1.302	29			
Leuco	Season	24.975	4	6.244	37.643	0.000
	Sex	1.027	1	1.027	6.190	0.022
	Season* sex	0.218	4	0.054	0.328	0.856
	Error	3.317	20	0.166		
	Corrected total	29.537	29			
Hemato	Season	238.554	4	56.639	360.463	0.000
	Sex	202.644	1	202.644	1224.805	0.000
	Season* sex	8.940	4	2.235	13.509	0.000
	Error	3.309	20	0.165		0.000
	Corrected total	453.447	29			
MCV	Season	303.813	4	75.953	2.044	0.127
	Sex	11.532	1	11.532	0.310	0.584
	Season* sex	4.671	4	1.168	0.031	0.998
	Error	743.060	20	37.153		

	Corrected total	1063.077	29			
MCHC	Season	6.169	4	1.542	6.114	0.022
	Sex	1.587	1	1.587	6.291	0.021
	Season* sex	2.335	4	0.584	2.314	0.093
	Error	5.046	20	0.252		
	Corrected total	15.138	29			
MCH	Season	35.394	4	8.849	65.744	0.000
	Sex	1.248	1	1.248	9.276	0.006
	Season* sex	6.716	4	1.679	12.475	0.000
	Error	2.692	20	0.135		
	Corrected total	46.051	29			

Hematocrit (PCV)

Season wise PCV value was higher in summer and lower in winter season irrespective of the sexes of C.catla fish. As shown in Table-1, the PCV value ranged from 23.8% to 32.9% in male fish, where as for female fish it varied between 20.70% to 27.0%. The mean value of the male and the female fish was 29.398% and 24.24% respectively, which indicates that male fish was higher than the female fish throughout the study period.

Mean corpuscular volume

MCV value was higher in summer for both sex and lower in the winter. The MCV value of male fish ranged between 172.10 and 179.90 fl and for female fish 169.80 and 179.00fl (Table-1). There was no major difference in male and female fish.

Mean corpuscular hemoglobin

The value of MCH throughout the experimental period was found to be varied between 53.00 and 55.31 ρg in male fish and for female fish it was between 53.01 and 57.00 ρg (Table-1). The MCH value was higher in female fish (54.452 ρg) than that of male fish (54.452 ρg). In both the sexes, higher value was observed in rainy season and lower value in winter season.

Mean corpuscular hemoglobin concentration

The MCHC value ranged from 30.00% in male fish to 31.80% in male fish where as for female fish it was varied between 31.10% and 32.20% (Table-1). Higher value was observed in the rainy season and lower value in winter season in case of male fish, but for female fish it was in spring season. The annual mean value indicated as slightly higher in female fish (31.57%) in comparison with male fish (31.10%).

IV. Discussion

In natural habitat, fish species are pact with different factors such as varied water qualities, pollution, malnutrition, infection and disease and can adapt themselves such environmental conditions by changing their physiological activities. Although all these above factors linked to fish health, it is essential to establish an identity the causes of disease in fish presents as a challenge for the researchers and farmers. Water quality is an important factor, which is an responsible for variation in fish hematology, since fish live in close association with their environment (Casillas and Smith).

Table-3 Corelation analysis of blood parameters by seasons in C.catla

Season	Variable	Hemo	RBC	Leuco	Hemato	MCV	MCHC	MCH
Spring	Hemo	1	0.976**	-0.589	0.941**	0.508	0.982**	-0.667
	RBC	0.976**	1	-0.714	0.985**	0.323	0.922**	-0.794
	Leuco	-0.589	-0.714	1	-0.821*	0.375	-0.426	0.988**
	Hemato	0.941**	0.985**	-0.821*	1	0.187	0.860*	-0.880*
s	MCV	0.508	0.323	0.375	0.187	1	0.662	0.229
	MCHC	0982**	0.922**	0.426	0.860*	0.662	1	-0.514
	MCH	-0.667-	0.794	0.988**	-0.880*	0.299	-0.514	1
Summer	Hemo	1	0.976**	-0.589	0.941**	0.508	0.982**	-0.667
	RBC	0.947**-	1	0.218	0.811	0.743	0.179	-0.552
	Leuco	-0.061	0.218	1	-0.339	0.791	0.992**	0.794
	Hemato	0.955**	0.811	-0.339	1	0.217	-0.401	-0.838
	MCV	0.497	0.743	0.791	0.217	1	0.785	0.334
	MCHC	-0.118	0.179	0.992**-	0.401	0.785	1	0.835
	MCH	-0.644	-0.382	0.794	-0.838*	0.334	0.835*	1
Rainy	Hemo	1	0.997**	-0.234	0.971**	0.325	-0.082	0.047
	RBC	0.997**	1	-0.154	0.947**	0.396	0.000	0.123
	Leuco	-0.234	-0.154	1	-0.462	0.777	0.981**	0.881*
	Hemato	0.971**	0.947**	-0.462	1	0.104	-0.318	-0.176
	MCV	0.325	0.396	0.777	0.104	1	0.883*	0.960**
	MCHC	-0.082	0.000	0.981**	-0.318	0.883*	1	0.951**
	MCH	0.047	0.123	0.881*	-0.176	0.960**	0.951**	1

Autumn	Hemo	1	0.990**	-0.114	0.997**	0.389	-0.434	0.154
	RBC	0.990**	1	-0.172	0.997**	0.303	-0.518	0.060
	Leuco	-0.114	-0.172	1	-0.158	0.844*	0.897*	0.924**
	Hemato	0.997**	0.997**	-0.158	1	0.336	-0.488	0.095
	MCV	0.389	0.303	0.844*	0.336	1	0.658	0.969**
	MCHC	-0.434	-0.518	0.897*	-0.488	0.658	1	0.822*
	MCH	0.154	0.060	0.924**	0.095	0.969**	0.822*	1
Winter	Hemo	1	0.885*	0.224	0.900*	0.735	-0.309	0.991**
	RBC	0.885*	1	-0.252	0.999**	0.347	-0.715	0.918**
	Leuco	0.224	-0.252	1	-0.221	0.781	0.856*	0.128
	Hemato	0.900*	0.999**	-0.221	1	0.379	-0.692	0.931**
	MCV	0.735	0.347	0.781	0.379	1	0.386	0.690
	MCHC	-0.309	-0.715	0.856*	-0.692	0.386	1	-0.393
	MCH	0.991**	0.918**	0.128	0.931**	0.690	-0.393	1

Table-4 Correlation analysis of blood parameters for male C.catla during study period.

	Hemo	RBC	Leuco	Hemato	MCV	MCHC	MCH
Hemo	1	0.861**	0.961**	0.960**	0.558*	0.674**	0.352
RBC	0.861**	1	0.735**	0.905**	0.389	0.773**	0.097
Leuco	0.961**	0.735**	1	0.881**	0.628*	0.615*	0.353
Hemato	0.960**	0.905**	0.881**	1	0.398	0.398	0.350
MCV	0.558*	0.389	0.628*	0.398	1	1	0.259
MCHC	0.674**	0.773**	0.615*	0.749**	0.564*	0.564*	0.405
MCH	0.352	0.097	0.353	0.350	0.259	0.259	1

Table-5

Corelation analysis of blood parameters for female C.catla
*P=0.05, corelation significance
**P=0.01,corelation significance

	Homo	RBC	Leuco	Hemato	MCV	MCHC	MCH
Homo	1	0.861**	0.961**	0.960**	0.558*	0.674**	0.352
RBC	0.861**	1	0.735**	0.905**	0.389	0.773**	0.097
Leuco	0.961**	0.735**	1	0.881**	0.628*	0.615*	0.353
Hemato	0.960**	0.905**	0.881**	1	0.398	0.749**	0.350
MCV	0.558*	0.389	0.628*	0.398	1	0.564*	0.259
MCHC	0.674**	0.773**	0.615*	0.749**	0.564*	1	0.405
MCH	0.352	0.097	0.353	0.350	0.259	0.405	1

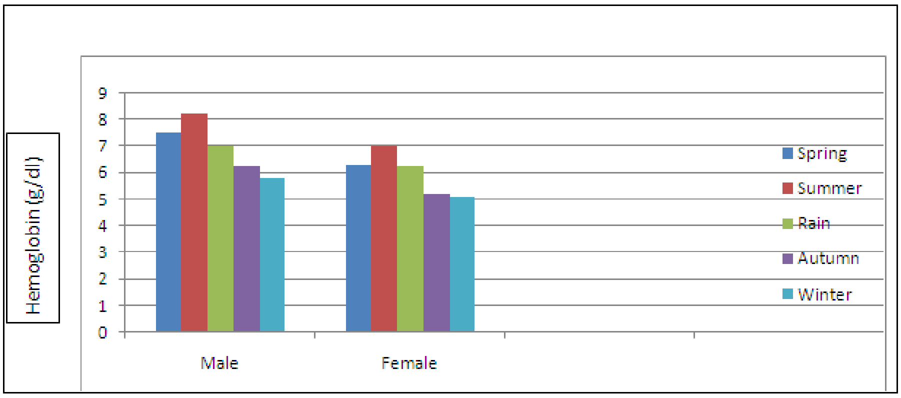

Fig. 1 Average concentration of blood Hb of C. catla a between sexes.

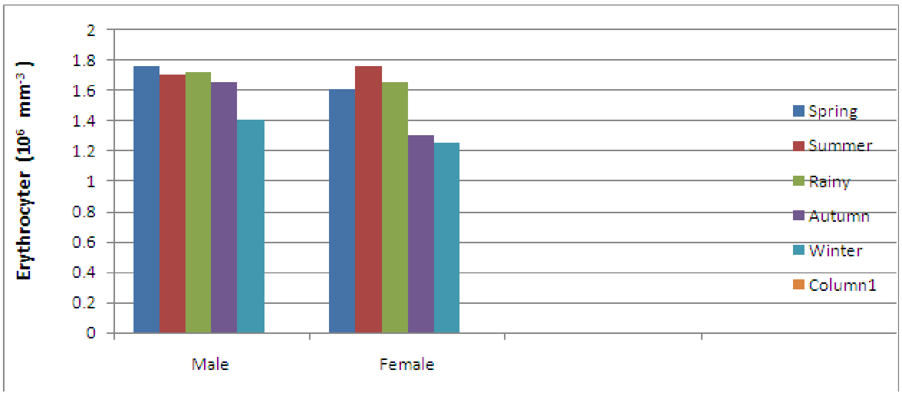

Fig.2 Average number of erythrocyte count of C catla a between sexes.

Recently, Orun et al. (2003) described that fish hematology parameters could be affected by variations in water temperature and oxygen concentration. In this study, water quality was normal range without any interference with other external and environmental factors except rainy season (unpublished data).

There are abundant excellent references used as guides in the unusual fish health and incisive expert needs to use all available diagnostic skills to manage these cases. In this scenarios, the use of clinical pathology greatly enhances this endeavour. Among all the different parameters, hematology offers an easily collected diagnostic gizmo of fish pathology (Lehman and Sturenberg 1975; Hickey 1976). On the other hand, it is important to establish reference ranges for hematological parameters including the RBC and WBC numbers, cell proportions of leukocyte, amount of hemoglobin and the size of RBC and WBC. These are the most significant findings as regards to diagnosis of fish diseases, healtth monitoring and the direction of any ecological and geographical difference between the species. Many hematological reports are available in small and young Cyprinidae fishes by different researchers in different approaches, but the present work basically focused on reference ranges for hematological information in adult species of C.catla. The hematological parameters observed in this study showed marginal differences in comparision withthat of Clarias isheriensis i(Kori-Siakpere 1985) and Clarias buthupogon (Kori-Siakpere and Egor 1997 ; Kori- Siakpere et al. 2005). Preston (1960) observed seasonal fluctuations in hematological parameters of fishes. Many publications reported that there are variations in blood indices values that can be attributed to manmy factors such as age , size of fish, nutrition state, season, spawning, sex and genetic variation. Das in 1965 discovered that the number of blood cells and hemoglobin concentration tends to increase with length and age. Hematocrit value for male fish was increased prior to time of sspawning (Poston 1966). The findings of the present study on blood indices of C.catla, which shows similar seasonal and sex variations, is discussed. Moreover, this study further attributes that during summer time because of high body metabolic rate due to high ambient temperature and reproductive activities, most of the hematological parameters shown higher value than other seasons. The lowest value, on the other hand, that happened during winter might be due to low ambient temperature and low metabolic rate. These results are supported by findings of Adebayo et al. (2007), Joshi (1989) and Orun et al. (2003). Many publications demonstrated that variation in hematocrit value and other hematological parameters between sexes and diversity might be due to higher metabolic rate of male compared to the female (raizada et al. 1983); Fourie and Hatting 1976). Finding of the present work also support this idea, which has been related to an increase in fish activity with increase in size. While considering the sex variations, many workers demonstrated that male fish attributes higher values in almost all hematological parameters except TLC in female fish (Cech and Wohlschlag 1981; Orun et al. 2003). The higher hematological values in favour of male fish may be attributed to physiologically activeness than the female fish. However, MCHC and MCH values did not show any marked difference between seasons and sexes in our results during the 2-year study period.

In conclusion, fish hematology is highly dependent on health, physiological process, habitat and the understanding of different environmental factors that govern reference ranges for fish. Although most of the wild fishes on a pond have been sampled, the small numbers in any specific fish populations limit the interpretation of the results and further validation is required. As the reproductive programs influence the fish physiology, more research will be necessary to determine the effects of microhabitat, environmental conditions, ambient temperature, nutritional status and possible seasonal fluctuations on the fish hematological parameters.

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