International Journal of Fauna and Biological Studies
Available online at www.faunajournal.com

IJFBS
International Journal of Fauna And Biological Studies

ISSN 2347-2677
IJFBS 2016; 3(6): 33-38
Received: 07-09-2016
Accepted: 08-10-2016

Zubair Ahmad Sheikh
Fish Nutrition Research Laboratory, Department of Zoology, University of Kashmir, Srinagar, India

Imtiaz Ahmed
Fish Nutrition Research Laboratory, Department of Zoology, University of Kashmir, Srinagar, India

Correspondence
Zubair Ahmad Sheikh
Fish Nutrition Research Laboratory, Department of Zoology, University of Kashmir, Srinagar, India

Seasonal changes in hematological parameters of snow trout *Schizothorax plagiostomus* (Heckel 1838)

Zubair Ahmad Sheikh and Imtiaz Ahmed

Abstract
Hematological parameters are repeatedly used as diagnostic and effective tools to assess the health status of fish and these parameters are not only varied with season but also varied between the sexes with in the species. Therefore, the purpose of the present study was to investigate the variation in hematological parameters of *Schizothorax plagiostomus* with respect to season and sex. This study was carried out in a stretch of time from 2014 to 2015 and the fish were collected from river Jhelum of Kashmir Himalayas. Blood was analyzed using different techniques and difference in hematological parameters including hemoglobin (Hb), total erythrocyte count (RBC), total leucocyte count (TLC), hematocrit (Hct), mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC) from 30 males (Weight, 311.62±25.76gm; length 33.5±3.26cm) and 31 females (Weight 335±32.55gm length 32.99±3.76cm) were reported during the present study. The results indicated that the highest hemoglobin concentration red blood cell count, hematocrit, mean corpuscular hemoglobin, mean corpuscular volume were found in males which were significant ($p<0.05$) different with the hematological values recorded from the female fish, where significantly ($p<0.05$) lower values of hematological parameters were reported except TLC. While it was found to be higher in female fish species as compared to the males fish species. However, no significant difference in mean corpuscular hemoglobin concentration between the sexes was observed. The present findings suggest that hematological parameters varied with sex and season thus, their precise information is necessary to assess the health and physiological conditions of the fish during different months of the year.

Keywords: Seasonal variation, Sex, Hematolgy, *Schizothorax plagiostomus.*

1. Introduction
A major part of the world's food is being supplied from fishery sources, and it is estimated that around 60 % of people in many developing countries including India depend on fish for their protein requirement [1] thus, it is essential to secure the propagation of fishes [2]. Knowledge of hematological parameters of aquatic species are frequently used as an essential diagnostic tool to assess the health condition of lower invertebrates due to increasing emphasis on pisculture and great awareness of the natural resources [3]. Hematological parameters are closely response of the animal to the environment and indication that the environment where fishes live could exert some influence on the hematological characteristics [4, 5]. Several previous reports on hematological aspects of the vertebrates species in order to correlate their physiology and evolution, but thorough knowledge of fish physiology is becoming more imperative due to diagnostic evaluation, economic importance and comparative study of fish. Periodic hematological analysis provide a simple means of evaluating stress, metabolic disorders, reproductive dysfunction, exposure to toxicants and diseases caused by environment husbandry condition in wild and cultured fish [6-8]. Determination of red blood cell count can indicate anemia or stress polycythemia, where white blood cell count may reveal leucopenia or leukocytosis signifies possible immune function alteration. In recent years, hematological parameters have been commonly used to observe and follow the quality of fish [3]. Qualitative and quantitative variations in hemogram including the total erythrocyte count (RBC) and total leucocyte count(WBC) numbers, the amount of hemoglobin, are the most significant findings as regards diagnosis [3, 9] Hematocrit, erythrocytes count, and hemoglobin concentrations are the most readily determined hematological parameters for both the field and hatchery conditions [10]. Variation of hematological parameter caused by physiological and external factors such as species, sex, age, size, nutritional state breeding efficiency, season, environmental stress [11-17, 7]. The influence of environment factors on hematological parameters of fishes has generally been used as effective and sensitive index to monitor the

Physiological and pathological changes in fish health [18, 19]. Interestingly, the hematology of fish continues to offer valuable diagnostic tool and progress in establishing the normal range values for different fish species. This study was undertaken to establish reference values for some hematological parameters of fresh water fish S*chizothorax plagiostomus* living in wild on the basis of season and sex.

2. Material and Methods

Study site, sample collection, blood sample collection

Schizothorax plagiostomus is a freshwater teleost belonging to the family Cyprinidae and its high quality flesh together with its good made it a high-priced fish in the local market in Kashmir. For the present study, fish samples were collected monthly from the river Jhelum in a stretch of time from 2014 to 2015. Fish sample were put into buckets (70l) filled with river water and transported to the laboratory on the same day. After overnight acclimatization, blood sample was collected from the caudal vain using a sterile plastic disposable heparinized syringe (2-3ml) having 0.5mm x16mm microlance needle and transferred into heparinized vial immediately on ice [18, 20]. After collection of blood following hematological parameters were analyzed in the present study, hemoglobin (Hb), total erythrocyte count (RBC), total leucocyte count (WBC), hematocrit (HCT), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular volume (MCV), respectively.

Hematological analysis

Cynomethmoglobin method was used for the evaluation of hemoglobin concentration as described by [20]. Total erythrocyte and leucocyte counts were done with a Neubauer haemocytometer (Marienfeld-Superior, Lauda-Konigshofen, Germany) by using Natt Herrick's [21] diluent with a ratio of 1:200. The number of cells count was determined as described by [22, 23]. The total RBC count per mm^3=200×50×N=10,000 N (N=number of RBC counted, dilution factor=200) and total WBC count per cubic millimeter was obtained as 20×1×L/0.4 cells=50×L (L=number of WBC counted dilution factor=50). The determination of packed cell volume (PCV) was performed as published by [24]. The PCV was estimated by using heparinized micro haematocrit capillaries and centrifuged in a micro centrifuge (REMI RM-12C BL, India) spun in at 12,000 rpm for 5 min to obtain haematocrit value, using micro-hemotocrit reader and expressed in percentage. Erythrocytes indices mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were calculated as per formulae of [25].

MCV=PCV×100/erythrocyte count
MCH=hemoglobin×10/erythrocyte court
MCHC=hemoglobin×100/PCV

3. Results

Difference in hematological parameters of the fresh water *Schizothorax plagiostomus* were statistically analyzed as shown in Table 1**.**

Hemoglobin content

Hemoglobin content of *Schizothorax plagiostomus* oscillated between 7.53 and 11.0 g/dl during different season. In males hemoglobin concentration varied from 8.6 to 11.0 g/dl with annual mean of 9.86±0.37. In the female fish hemoglobin concentration varied from 7.53 to 9.42 g/dl with annual mean of 8.54±0.41. The higher concentration of Hb in both the sexes was obtained in the summer season and lower value in winter season (Fig.1). There are significant differences between the sexes.

Total erythrocyte count

The total number of erythrocyte in the blood of male and female fish of *S. plagiostomus* ranged from 1.37×10^6 to $2.08\times10^6/mm^3$; $1.21\times10^6/mm^3$ to $1.75\times10^6/mm^3$ during annual study period. In males possessed higher number of RBC than that of female, where as higher number of RBC count was obtained in summer season and lower in the winter season in both the sexes (Fig.2). The annual average mean of RBC in the blood of both the sexes was $1.62\times10^6/mm^3$ and $1.42\times10^6/mm^3$ respectively.

Total white blood cell count

The total white blood cell count in the blood of male and female fish varied between the seasons. The higher values of white blood cell count were obtained in summer season and lower in the winter season in both sexes (Fig. 3). White blood cell count varied between 4.05×10^3-$6.15\times10^3/mm^3$; 7.14×10^3 -$9.28\times10^3/mm^3$ of blood in male and female fish, respectively. The annual mean value for male and female fish was 8.05×10^3 and 4.96×10^3 of blood. Thus female fish had higher values than that of male fish.

Hematocrit (PCV)

As shown in Fig.4 PCV value was varied season wise, higher value was obtained in summer and lower in the winter season irrespective of the sexes of fish. The PCV value varied between 32.16% to 41% in male fish. Where as for female fish it varied between 29.24%to 35.60%. The mean value for male and female fish was 36.3% and 32.33% respectively, which indicates that male fish was higher PCV than female fish throughout the study period.

Mean corpuscular volume (MCV)

In Fig.5 MCV values was higher in spring for both the sexes and lower in the summer season. The MCV value for male fish ranged between 204.3-234fl and for female fish 202.0-243.05fl respectively. Thus female fish has higher values than that of male fish.

Mean corpuscular hemoglobin concentration (MCHC)

The value of MCHC of *S. plagiostomus* during the annual study period fluctuates between 25.76-28.45pg. In male and female fish it varied between 26.42- 28.45pg; 25.76-27.43pg. As in Fig. 6 the MCHC value was higher in male fish than that of female fish. In both the sexes the higher value of MCHC was observed in spring and lower values in winter and autumn respectively.

Mean corpuscular hemoglobin

Season wise MCH values was higher in spring season and lower in the summer season in both sexes of *S. plagiostomus* (Fig.7). The MCH values ranged from 53.03- 66.62%in male fish whereas for female fish it was varied between 53.51-66.52%. There was no major difference in male and female fish.

4. Discussion

The results of the present study demonstrated the seasonal variations in haematological parameters of the blood in *Schizothorax plagiostomus*. In recent years there has growing interest in the study of hematological parameters of fish blood, regarded as an important for aquaculture purpose. The study of hematological characters can corroborate the diagnoses and prognoses of morbid condition of fish population [26] and thereby contribute to better comparative physiology, phylogenetic relation, feeding condition and other ecological parameters. Baseline data concerning hematological parameters reference values amenities the fish health condition assessment of diagnostic in fresh water [7]. Several of these studies were attempts to determine if significant variations from normal values of these parameters exist that could be attributable to some internal or external factors, sex, season [27-33] It has been shown that the number of red blood cells and hemoglobin concentration tends to increase with length and age [34]. Many researchers reported that there are variations in blood parameters values that can be attributed to many factors such as age, size of fish, nutrition state, season, spawning, sex, and genetic variation. Although all the above factors linked to fish health, it is essential to establish and identify the cause of diseases in fish which present as a challenge for the researchers and farmers. Water quality is an important factor, which is responsible for variation in fish hematology, since fish live close association with their environment [35]. There are abundant and excellent references used for guiding the unusual fish health, and the incisive expert need to use all available diagnostic cases to manage these cases. Where by the use of clinical pathology greatly enhance this challenge. Once reference values determined in *S. plagiostomus*, they can facilitate monitoring of the fish stress response, their nutritional condition, reproductive state, tissue damage due to frequent handling procedure, detection, and diagnosis of metabolic disturbance or diseases processes in the fish population [36]. Hematocrit value for male fish was increased prior time of spawning [37]. The present study findings on hematological parameters in *S. plagiostomus* show statistical difference in seasonal and sex variations. Moreover this study further attributes that because of high body metabolic rate during summer time, the high ambient temperature and reproductive activities most of the hematological parameters showed higher values than in other seasons. While lowest values was obtained during winter season might be due to low ambient temperature and metabolic rate. These results are supported by our own research and by other researchers in other species [24, 18]. Various authors reported that variation in hematocrit value and other hematological indices among sexes might be due to higher metabolic rate and hormonal activity of male fish as compared to the female fish [38, 39]. While considering the sex variations, various workers revealed that the male fish attributes higher values in almost all hematological indices except TLC in female fish [40, 18]. The higher values of hematological parameters favoring males attributed to higher physiological activeness than the female fishes.

In conclusion, the results of our research provide a contribution to the knowledge of the seasonal changes in hematological parameters of fresh water omnivorous fishes. Establishment of hematological parameters is highly dependent on external and internal factors including season, sex, age, health status, reproductive phase, inadequate feeding regimes and physiological, and genetics of each individual that governs reference values for fish. We suggest that hematological studies on fishes have assumed greater significance due to the increasing emphasis on pisiculture and greater awareness of the pollution of the aquatic ecosystem.

Table 1: Seasonal variation in hematological parameters of male and female *Schizothorax Plagiostomus*

Season	Sex		HB	RBC	TLC	PCV	MCV	MCHC	MCH
Spring	Male		9.63^{a}	1.44^{a}	4.52^{a}	33.83^{a}	234.10^{a}	28.46^{a}	66.62^{a}
		SE	0.18	0.56	0.09	0.26	2.41	0.53	1.43
	Female		8.41^{a}	1.26^{a}	7.47^{a}	30.68^{a,d}	243.05^{a}	27.44^{a}	66.53^{a}
		SE	0.22	1.15	0.12	0.52	2.46	0.70	1.45
Summer	Male		11.06^{b}	2.08^{b}	6.16^{b}	41.78^{b}	204.34^{b}	26.52^{a,c}	53.04^{b}
		SE	0.10	3.00	0.33	0.91	6.23	0.36	0.79
	Female		9.43^{b,c}	1.76^{a}	9.28^{b,c}	35.61^{b,c}	202.04^{b}	26.63a	53.52b
		SE	0.06	1.15	0.63	0.87	4.82	0.78	0.52
Autumn	Male		9.93^{a}	1.61^{c}	5.15^{a}	37.72^{a}	233.57^{a}	26.43^{b,c}	61.87^{c}
		SE	0.06	2.73	0.31	0.87	3.00	0.52	0.92
	Female		8.82^{a,c}	1.46^{c}	8.33^{a,c}	33.84^{a,c}	231.19^{a}	26.20^{a}	57.98^{b,c}
		SE	0.23	3.62	0.57	1.06	5.77	0.76	2.17
Winter	Male		8.62^{c}	1.37	4.05^{a}	32.06^{a}	233.95^{a}	27.47^{a,b}	64.06^{a}
		SE	0.19	1.81	0.08	0.61	6.36	0.60	1.40
	Female		7.53^{d}	1.21^{a}	7.14^{d}	29.24^{d}	240.39^{a}	25.76^{a}	62.00^{a,c}
		SE	0.21	2.78	0.28	0.76	6.53	0.33	2.09

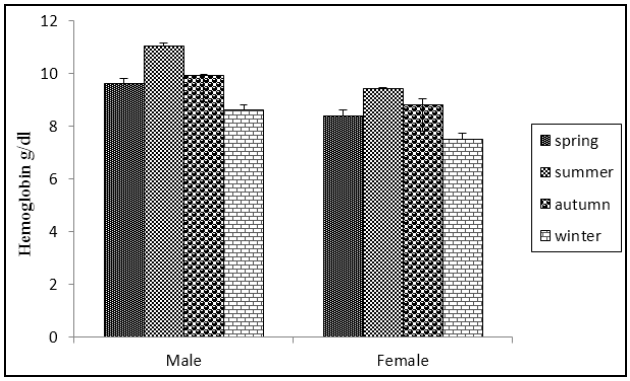

Fig 1: shows average hemoglobin value of *S. plagiostomus* between sexes and season.

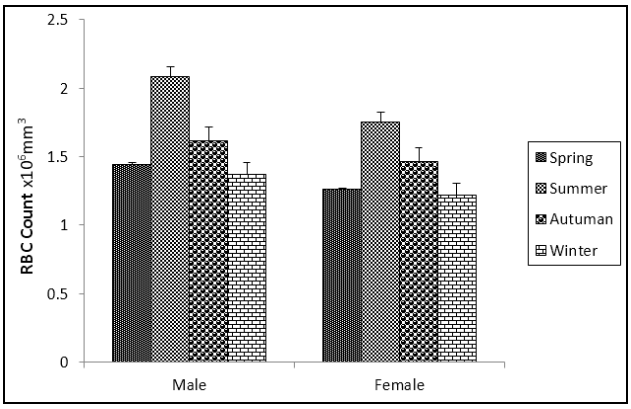

Fig 2: shows average number of red blood cells (RBC) of *S. plagiostomus* between sexes and season

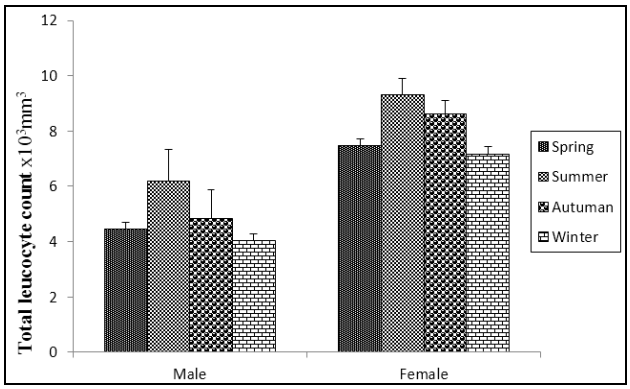

Fig 3: Average number of leucocyte count of *S. plagiostomus* between sexes and season

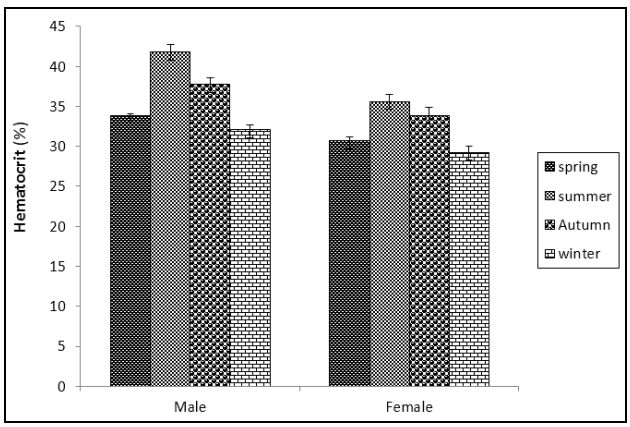

Fig 4: Average Hematocrit value of *S. plagiostomus* between Sexes and season.

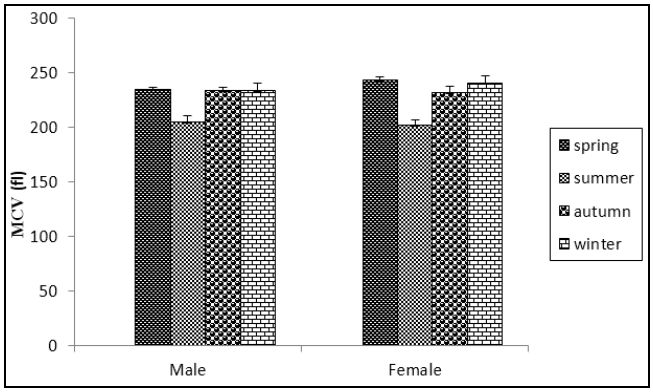

Fig 5: Average mean corpuscular volume (MCV) of *S. plagiostomus* between sexes and season.

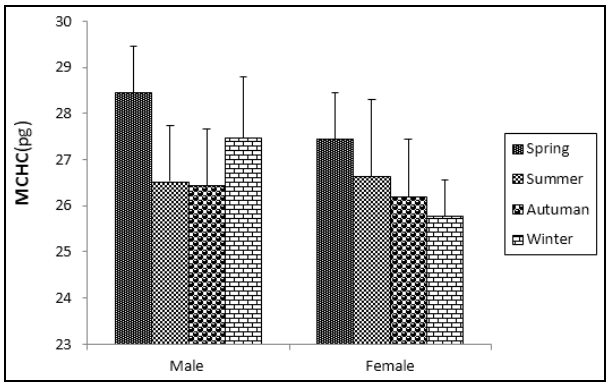

Fig 6: Average mean corpuscular hemoglobin concentration (MCHC) of *S. plagiostomus* between sexes and season

Fig 7: shows average mean corpuscular hemoglobin value of *S. plagiostomus* between sexes and season

5. References

1. Osibona AO, Kusemiju K, Akande GR. Proximate composition and fatty acids profile of the African catfish, *Clarias gariepinus*. Journal of Life and Physical Sciences Acta Satech. 2009; 3:85-89.
2. Tripathi G, Harsh S. Fenvelerate-induced macromolecular changes in the catfish, *Clarias batrachus.* Journal of Environmental Biology. 2002; 23:143-146.
3. Bhaskar BR, Rao KS. Some haematological Parameters of tarpon, *Megalops cyprinoids* (Broussonet) from Visakhapatham harbor. Matsy 1985; 11:63-69.
4. Kori-Siakpere O, Ake JEG, Idoge E. Haematological characteristics of the African snakehead, *Parachanna obscura*. African Journal of Biotechnology. 2005; 4(6):527-530

5. Gabriel UU, Ezeri GNO, Opabunmi OO. Influence of sex,source, health status and acclimation on the haematology of Clarias gariepinus(Burch, 1822). African Journal of Biotechnology 2004; 3:463-467.
6. Filiciotto F, Fazio F, Marafioti S, Buscaino G, Maccarrone V, Faggio C. Assessment of hematological parameter range values using an automatic method in European sea bass (*Dicentrarcbus labrax* L.). Natura Rerum 2012; 1:29-36
7. Pradhan SC, Patra AK, Sarkar B, Pal A. Seasonal changes in hematological parameters of *Catla catla* (Hamilton 1822). Comparative Clinical Pathology A 2012; 21:1473-1487.
8. Handy RD, Depledge MH. Physiological responses: their mea-surement and use as biomarkers in ecotoxicology. Ecotoxicology 1999; 8:329-349.
9. Luskovav V. Annual cycle and normal values of hematological parameters in fishes. Acta Sci Nat Brn. 1997; 31(5):70
10. Bhaskar BR, Rao KS. Use of hematological parameters as diagnostic tools in determining health of milk fish, *Chanos chanos* (Forskal), in brakish water culture. Aquaculture and Fisheries Management, 1990; 21:125-129
11. Hlavova V. References values of the haematological indices in grayling (*Thymallus thymallus* linnaeus). Comparative Biochemistry and Physiology Part A: Physiology. 1993; 105: 525-532.
12. Joshi PC. Seasonal changes in the blood parameters of a hill stream teleost, *Channa gochua*. Comperative Physiology Ecology. 1989; 14:71-73.
13. Nespolo RF, Rosenmann M. Intraspecific allometry of hematological parameters in *Basilichthys australis*. Journal of Fish Biology. 2002; 60:1358-1362.
14. Ranzani-Paiva MJT, Godinho HM. Estudos hematologicos em curimbatá, Prochilodus scrofa Steindachner, 1881 (Osteichthyes, Cypriniformes, Prochilodontidae). Série Vermelha. Boletim do Instituto de Pesca. 1985; 12:25-35.
15. Svetina A, Matasin Z, Tofant A, Vucemilo M, Fijan N. Hematology and some blood chemical parameters of young carp till the age of three years. Acta Veterinaria Hungarica 2002; 50:459-467.
16. Lim C, Klesius PH. Influence of feed deprivation on hematology, macrophage chemotaxis, and resistance to Edward-siella ictaluri challenge of channel catfish. Journal of Aquatic Animal Health. 2003; 15:13-20.
17. Singh BP, Tandon PK. Effect of river water pollution on hematological parameters of fish, *Wallago attu*. Reserach Environmental Life Science 2009; 2:211-214.
18. Orun I, Dorucu M, Yazlak H. Hematological parameters of three Cyprinid fish species from Karakaya dam lake, Turkey. Online Journal of Biological Sciences. 2003; 3:320-328.
19. Chakrabarthy P, Banerjee V. Effect of sublethal toxicity of three organo phospheres pesticide on the peripheral haemogram of the fish, (Channa punctatus). Environment Ecology 1988; 6:151-158
20. Lavanya S, Ramesh M, Kavitha C, Malarvizhi A. Hematological, biochemical and iono regulatory responses of Indian major carp *Catla catla* during chronic sublethal exposure to inorganic arsenic. Chemosphere 2011; 82:977-985
21. Natt MP, Herrick CA. New blood diluent for counting the erythrocytes and leucocytes of the chicken. Poultry Science 1952; 31(4):735-738.
22. Svoboda M, Kouril J, Hamackova J, Kalab P, Savina L, Svobodova Z *et al*. Biochemical profile of blood plasma of tench (*Tinca tinca* L.) during pre and post spawning period. Acta Veterinaria Brno, 2001; 70:259-268.
23. Oluyemi KG, Adecparusi EA, Olanrewage J. Basic hematological parameters in African catfish, *Clarias gariepinus*, (Burchell, 1822) fed ascorbic acid supplemented diets. Research Journal of Animal Sciences 2008; 2:17-21.
24. Adebayo OT, Fagbenro OA, Ajayi CB, Popoola OM. Normal haematological profile of *Parachanna obscura* as diagnostic tool in aquaculture. International Journal of Zoological Research. 2007; 3:193-199.
25. Tavares-Dias M, Tenani RA, Gioli LD, Faustino CD. Características hematológicas de teleósteos brasileiros. II. Parâmetros sangüíneos do Piaractus mesopotamicus Holmberg (Osteichthyes, Characidae) em policultivo intensivo. Revista Brasileira de Zoologia 1999; 16:423-431.
26. Munkittrick KR, Leatherland JF. Hematocrit values in feral gold fish, *Carassius auratus*, as indicators of the health of the population. Journal Fish Biology. 1983; 23:153-162
27. Gabriel UU, Alagoa JK, Allison ME. Effects of dispersed crude oil water dispersion on the hemoglobin and haematocrit of *Clarias gariepinus*. Journal Applied Science and Environment Management. 2001; 2:9-11.
28. Jamalzadeh HR, Ghomi MR. Hematological parameters of Caspian salmon *Salmo trutta caspius* associated with age and season. *Marine Fresh Behavior and Physiology*. 2009; 42:81-87.
29. Orun I, Erdemil AU. A study on blood parameters of *Capoeta trutta* (Heckel, 1843). *Online* Journal of Biological Sciences. 2002; 2:508-511.
30. Jamalzadeh HR, Ghomi MR. Hematological parameters of Caspian salmon *Salmo trutta caspius* associated with age and season. Marine Fresh Behavior and Physiology. 2009; 42:81-87.
31. Dawson MA. Blood chemistry of the windowpane flounder *Scophthalmus aquosusin* Long Island Sound: geographical, seasonal and experimental variations. Fishery Bulletin. 1990; 88:429-437.
32. as BC. Age related trends in the blood chemistry and hematology of the Indian carp (*Catla catla*). Gerontologia, 1965; 10:47-64.
33. Casillas E, Smith LS. Effect of stress on blood coagulation and hematology in rainbow trout, *Salmo gairdneri*. Journal of Fish Biology. 1977; 10:481-491.
34. Poston HA. Effects of sex and reproductive stage on hemoglobin levels in brown trout. Fish Res Bull 1966; 29:28-29.
35. Joshi BD. Cricannual fluctuation in some blood components of the fish Rita rita, in relation to certain eco-physiological conditions. Uttar Pradesh J Zool. 1982; 2:62-66
36. Raizada MN, Jain K, Raizada S. Monthly variations in the hematocrit values (PCV) in a teleost, *Cirrhinus mrigala* (Ham.). Comperative Physiology 1983; 8:196-198
37. Poston HA. Effects of sex and reproductive stage on

haemoglobin levels in brown trout. Fish Res Bull 1966; 29:28-29.
38. Raizada MN, Jain KK, Raizada S. Monthly variations in the hematocrit values (PCV) in a teleost, *Cirrhinus mrigala* (Ham.).Comp Physiol 1983; 8:196-198.
39. Fourie FLR, Hatting J. A seasonal study of hematology of carp, *Cyprinus carpio* from a locality in the Transvaal, South Africa. Zool Afri 1976; 2:75-8.
40. Cech JJ, Wohlschlag DE. Seasonal patterns of respiration, gill ventilation and hematological characteristic in the striped mullet, *Mugal cephalus*. Bull. Mar. Sci. 1981; 31:112-119.