World Journal of Fish and Marine Sciences 5 (2): 121-126, 2013
ISSN 2078-4589

DOI: 10.5829/idosi.wjfms.2013.05.02.71110

Seasonal Variations in Hematological Parameters of *Alburnoides eichwaldii* in Zaringol Stream-Golestan Province, Iran

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Abstract: The aims of the present study were to studying seasonal variation of hematological parameters in Spirlin (*Alburnoides eichwaldii*) and compare them between male and female. For this goal, 90 specimens (including 56 female and 34 male) were collected seasonally by electrofishing (150 V, 10 A)during October 2010 to September 2011 from Zaringol Stream of Golestan Province. After biometry, blood samples were taken from caudal vein for hematological measurements. Also, water quality criteria were assessed. Mean total length and weight were 8.42±1.38 cm and 6.19±2.38 respectively.No significantly effect of sex was noted except in hematocrit. Hematocrit, hemoglobin, MCHC, red and white blood cells counts showed significant increase in warm seasons comparing with colds. Results of leucocytesdifferential count showed higher frequency in monocyte in summer, too. It seems that hematological parameters of Spirlin reflect environmental conditions and physiological status of fish.

Key words: *Alburnoides eichwaldii* · Blood Parameters · Season · Sex · Water Quality · Zaringol Stream

INTRODUCTION

Alburnoides eichwaldii belongs to Cyprinidae family which is living in the barbell zone of the stream [1] and distributed widelyin Europe, North and West Asia including the Turkey and Iran [2]. It was reported that it distributed in many rivers and streams of Iran like Caspian, Uromieh, Karaj and Jajrud, Zayandeh-rud and Shorrud, Ghare-Ghom basin's rivers [3]. Since its habitats requirements for living and spawning are in very narrow range, it is sensitive to habitat change and human activities. It is considered as a biological indicator of the environment quality because of its low tolerance to water quality and pollutants, too [4].

Unfortunately, human activities disturb many of its suitable habitats and Spirlin is reported as a "nearly threatened" species [5]. In addition to, most of the previous studies focused on growth, natural diet [6-8], reproduction features [9], distribution [10] and morphology features [11] and there is limited data on its hematological parameters. So it is necessary to determine its natural range of hematological parameters and studythe effect of biological and environment factors on them [12]. It will help us to protect its population in the future.

Hematological parameters of fish are closely related to the response of fish to environmental and biological factors [13-15]. Many factors can seasonally affect blood parameters changes in fish such as the reproduction cycle [16,17], diet [18], temperature [19], pH [20] and photoperiod [21].

Orun *et al.* [22] investigated hematological parameters of three Cyprinid fish species in Karakaya Dam Lake, Turkey and suggested seasonal changes like water temperature and dissolved oxygen effect on some of hematological parameters significantly. As the hematocrit, hemoglobin, red and white blood cell counts higher in warm seasons that cold ones. Bayir *et al.* [23] reported that maximum value of globulin and glucose of biochemical profile of blood serum of *Capoeta capoetaumbla*was measured in winter and have positive correlation with temperature.

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Although Interpretation of fish hematological data is quite difficult due to internal and external variations, analysis of blood indices is a valuable guide in assessing the condition of fish. So the aim of this study was to obtain reference values for hematological parameters of Spirlin (*A. eichwaldii*) and comparison its values in different sexes and seasons.

MATERIAL AND METHODS

This study was carried on Zaringol stream, Golestan province, Iran (Geographical location; longitude of 54°, 57′ and latitude of 36°, 52′) during a year from December 2009 to September 2010.

Ten water quality parameters of Zaringol Stream including temperature, salinity, turbidity, pH, electrical conductivity, dissolved oxygen, nitrite, nitrate, phosphate and sulfate ions were measured by using Water Checker Water checker u-10 and Spectrophotometer, seasonally.

A total number of 90 *A. eichwaldii*(56 females and 34 males)wascaught by electro fishing(150V, 10 A), simultaneity with water sampling. Sampling time was in second week of third month of each season on every occasion.

They transferred to fishery laboratory of Gorgan University of agricultural sciences and natural resources and kept on 5Lglass tank (filled with stream water) to remove handling stress for 6 hours.Fish length and weight were measured by digital caliper [±1mm]and scales (±0.01g) respectively. Average length and weigh for female were 8.7±1.5 cm and 6.8±2.37 g, they were 8±1.9 cm and 5.2±2.1 g for males. Sex was determined by gonad observation microscopically.

No anesthetic was applied to fish before blood sampling as it may affect blood parameters and hemolised tissues.

Blood samples were taken from the caudal vein using an insulin syringe and Heparin was used as an anticoagulant atconcentration of 5,000 IU heparin sodium salt in 1 ml [24].

Hematological examination was carried out immediately after sampling to assess indices of erythrocytes and leukocytes profiles. Number of red blood cells (RBCs$\times 10^6$/mm^3) and number of leukocytes (WBCs$\times 10^3$/mm^3) were determined in blood diluted by Daice diluting fluid solution using a Neubauer hemocytometer. Number of RBCs and WBCs was determined on each blood smear and calculated.

Hematocrit (HCT) was determined by transferring blood samples in heparinized microcapillary tubes, centrifugingat 3000 rpm for 5 min. and expressing as percentage of the total volume.

Hemoglobin (Hb, g/dl) was measured with spectrophotometer at 540 nm absorbance using cyanmethemoglobin method [25].

Blood smears were prepared immediately and air-dried, fixed in 95% methanol for 5 min. then stained with Giemsa. One to two blood smears were prepared per individual and read 100 numbers of leucocytes. Cells were identified on the basis of morphology and cell ultrastructure as documented in previous fish leucocytes studies [26].

The erythrocyte indices including mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) was calculated according to Haneym *et al.* [27].

Data checked for normality distribution with the Kolmogorov-Smirnov test and then all were analyzed using independent sample t-test, one-way ANOVA and Duncan's post-hoc test, assuming a significance level of α=0.05 by SPSS 17 software package.

RESULT AND DISCUSSION

Seasonally water quality parameters are given in Table 1. Most of the water quality parameters show significantly variation in different seasons, especially between warm and cold seasons like water temperature, dissolved oxygen, turbidity and electrical conductivity.

Summaries and comparison of hematological values in male and female *A. eichwaldii*are shown in Table 2. The results showed that there is no significant difference in relation with gender except in hematocrit. As it can be seen it has the higher value in females. It should be mentioned that basophil is notobserved in blood smear. So the average value is equal to zero and is not shown in table 2.

Because there were no significant differences in most of the hematological parameters of males and femalesbased on results of Table 2, the sex effect do not consider in seasonal comparison of hematological parameters of *A. eichwaldii* in Table 3except in hematocrit value.

Table 1: Water quality parameters of Zaringol stream (2009-2010)

Parameter/season	Autumn	Winter	Spring	Summer
Temperature (°C)	18.25±0.63[b]	14.6±0.14[a]	22.3±0.42[c]	23±0.28[c]
Dissolved Oxygen (mg/l)	9.02±0.02[b]	9.32±0.02[b]	8.64±0.05[a]	8.9±0.01[a]
Salinity (g/l)	0.075[b]	0.035[a]	0.035[a]	0.06[b]
Electrical conductivity (µm/cm)	1.71±0.02[c]	0.96±0.05[a]	1.06±0.01[b]	1.67±0.03[c]
Turbidity (FUT)	31±1.41[a]	261±1.41[d]	191.5±2.12[c]	86±1.41[b]
pH	8.29±0.01[b]	8.32±0.02[b]	8.13±0.04[a]	8.33±0.05[b]
Phosphate (mg/l)	0.01±0[a]	0.055±0.007[b]	0.055±0.007[b]	0.29±0.014[c]
Sulfate (mg/l)	63±1.41[b]	29±1.41[a]	94±1.41[d]	76.5±2.12[c]
Nitrite (mg/l)	0.01±0.004[b]	0.008±0.002[b]	0.001±0[a]	0.011±0.001[b]
Nitrate (mg/l)	3.31±0.01[c]	0.79±0.01[a]	2.29±0.01[b]	2.64±0.5[bc]

*Different letters shows significant difference (P< 0.05).

Table 2: Hematological values of male and female *A. eichwaldii*

Parameter/sex	Female (n=56)	Male (n=34)	P value
Hematocrit (%)	39.1±9.49	33.39±6.13	0.024*
Hemoglobin (g/dl)	6.94±1.46	6.96±1.02	0.965
RBC ($\times 10^6$/mm^3)	2.32±0.77	2.35±0.66	0.879
WBC ($\times 10^3$/mm^3)	9.13±2.29	9.78±2.26	0.442
Lymphocytes (%)	77.8±6.84	78.75±13.29	0.99
Monocytes (%)	4±1.81	3.75±1.7	0.718
Neutrophils (%)	16.33±6.12	15.83±5.62	0.829
Eosinophil (%)	1.78± 0.35	1.67± 0.42	0.118
MCV (fl)	181.08±61.14	158.66±41.79	0.275
MCH (Pg)	35.45±9.46	34.29±9.5	0.756
MCHC (%)	20.64±5.83	21.77±3.94	0.561

*Significant difference (P<0.05)

Data are presented as mean± standard division.

Table 3: Seasonal variation in Hematological values of *A. eichwaldii*

Parameter/sex	Autumn (n=15)	Winter (n=30)	Spring (n=20)	Summer (n=25)
Male hematocrit (%)	23.75±3.18[a]	30.75±3.82[b]	38±4.25[c]	38.75±3.47[c]
Female hematocrit (%)	35.06±8.92[ab]	32.66±4.12[a]	44.42±8.28[b]	42.61±10.1[b]
Hemoglobin (g/dl)	6.55±0.34[a]	6.02±0.41[a]	7.61±0.64[b]	9.1±0.94[c]
RBC ($\times 10^6$/mm^3)	1.94±0.11[a]	1.53±0.36[a]	2.6±0.36[b]	2.78±0.55[b]
WBC ($\times 10^3$/mm^3)	8.09±0.78[b]	6.28±0.8[a]	9.16±1.32[b]	11.78±1.13[c]
Lymphocytes (%)	80.5±4.9	81±1.7	83±10.3	77.05±10.5
Monocytes (%)	3±1.2[a]	3±0.95[a]	4±0.81[ab]	5.61±1.64[b]
Neutrophils (%)	14.5±0.71	14.66±0.57	11.5±2.64	16.05±6.13
Eosinophil (%)	2±1.3	1.34±0.25	1.5±0.41	1.29±0.35
MCV (fl)	144.16±50.62	231.45±61.63	168.44±41.57	149.28±42
MCH (Pg)	39.76±9.07	40.65±12.75	28.18±4.34	33.21±6.52
MCHC (%)	27.71±7.27[c]	19.06±2.13[a]	17.11±2.19[a]	23.02±5.17[ab]

*Different letters shows significant difference (P< 0.05).

Data are presented as mean± standard division.

According to results of Table 3, some of hematological values of *A. eichwaldii* show significantly difference between seasons like hematocrit, hemoglobin, number of red and white blood cells. As they increase in warm seasons (summer and spring).Results of differential counts of white blood cell show that frequency of monocytes increase in summer significantly, Mean corpuscular hemoglobin concentration(MCHC)increased in autumn though.

Knowledge of the hematological characteristics is an important tool that can be used as an effective and sensitive index to monitor physiological and pathological changes in fish [28]. Because environmental factor can effect on blood parameters, water quality parameters of Zaringol stream were determined in this study.

Results of analyzing water quality parameters of Zaringol stream show that values of some ions like phosphate, nitrite and nitrate increase in summer.

Since the aquaculture and agricultural activitystart or rise in warm seasons (spring and summer) in this region and they release their effluents into the stream, the water quality parameter changed. In addition to, climate (increasing in water temperature) and hydraulic variations (like decreasing in discharge) change some water quality parameters like concentration of dissolved oxygen in this season.

Based on types of blood cells, erythrocytes are the dominant cell type in the blood of the vast majority of fish species [29]. It is accepted that fishes have common leucocytes pattern consisting of granulocytes, monocytes, lymphocytes and thrombocytes [30]. Lymphocytes are usually the most commonly present leucocytes type in some fish, accounting for as much as 85% of the total leucocytes population [31]. In the present study lymphocytes are the dominate type (table 2 and 3; more than 75% of leucocytes). They produce fish's immune response [32]. Neutrophils have the second place after them. Also there is no basophil is observed in blood smear. Hetrophils (neutrophil) and eosinophils are the most common, whilst basophils are much rare types of granulocytes in fishes.

Results of hematological parameters in Spirlin show that there were no significant differences between males and females except of hematocrit. Some previous studies reported no significant differences in blood parameters in*Prochilodus lineatus*[33], *Clarias gariepinus*[34], *Cyprinus carpio*[30] and *Barbus grypus* [12]. Although Al Hasan *et al.* [35] reported that value of hematocrit in males of *Acanthopagrus latus* was higher than females. Similar result was reported by Jawad *et al.* [36] in *Tenuolosa ilisha*and [22] in three Cyprinidae species, while in present study it is higher in females.

Previous studies on fish hematology revealed that blood parameter levels could be affected by variation in water temperature and oxygen concentration [37-41]. In present study, values of hematocrit, hemoglobin and number of red blood cell are higher in warm seasons(spring and summer). It is related to hematological response of fish to water temperature and dissolved oxygen variation for more gas exchanging. Orun *et al*. [22] reported that some of hematological parameters of *Chalcalburnusmossulensts, Cyprinion macrostomus* and *Alburnoides bipunctatus* increased in summer significantly like total hematocrit, hemoglobin, erythrocytes, total leucocytes and lymphocyte. Theysuggested that these changes due to seasonal water temperature and dissolved oxygen variations. Also results of Mohammadi Zaragabadi *et al.* [42] Who studied effect of rearing temperature on hematological parameters of *Husohuso*juvenile and confirmed that some hematological parameters like hematocrit and frequency of eosinophil increase and some others like white blood cell count and lymphocyte decrease regarding to increasing temperature.

Warm seasons (spring and summer) are the periods of year which are the reproduction seasons for fish too. Joshi [43], Ezzat *et al.* [44] and Cech and Wohlschlag [45] described that some of the blood parameters levels may increase in related to high energy demand of fish in this period. Orun *et al*. [22] suggested that high erythrocyte number, hemoglobin and hematocrit level in male individuals of three Cyprinidae fishes may be caused by high metabolic activity in male fish. Increasing of total leucocyte with developing of gonadic stages of *Rutilus frissiikutum* was reported by Rhimi-Bashar*et al*. [46], too.In this study higher abundant of neutrophil and monocyte in this period andalso their higher level in females of *Alburnoides eichwaldii*, may be due to increasing of its reproduction activity, metabolism and defense mechanism. Although more study will be needed for describing the relationship between developing of gonadic stages and hematological parameters of *Alburnoides eichwaldii*.

In summary, the results of our research provide a contribution to the knowledge of the characteristics of blood cells and hematological parameters of the *Alburnoides eichwaldii* and its seasonal variation.

ACKNOWLEDGEMENTS

This work was carried out in the Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran. We express our thanks to this center's experts and fisheries group.

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