

Determination of some Hematological Parameters of *Vimba vimba persa* during Reproductive Migration

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Abstract: In this study the effect of reproductive migration on some hematological parameters such as white blood cells differential and total count, the total count of red blood cells, hemoglobin, hematocrit, MCV (mean corpuscular volume), MCH (mean corpuscular hemoglobin) and MCHC (mean corpuscular hemoglobin concentration) was investigated. Ten adult fish *Vimba vimba persa* with total length of 16.11 ± 1.31 cm and total weight 39.5 ± 5.01 g were captured from the Khoshkrud River. After biometry by cutting tail stem, blood sampling was taken from fish. Results showed that the number of red blood cells (RBCs) was $3.62 \times 10^6 \pm 1.42$ per mm^3 , white blood cells (WBCs) were 2312.9 ± 5510 per mm^3 , hemoglobin concentration was 8.16 ± 1.21 g/dL and hematocrit concentration was $32.6 \pm 5.54\%$. Differential count of WBCs showed that $77.4 \pm 12.06\%$ belonged to lymphocyte, $19 \pm 10.70\%$ to neutrophil, $1.9 \pm 0.875\%$ to monocyte and $1.7 \pm 1.49\%$ to eosinophil. Also blood indicators were included of MCV 239.19 ± 55.19 fL, MCH 60.35 ± 15.24 Pg and MCHC 2.5 ± 0.119 g/dL. No significant correlation was obtained between blood parameters *Vimba vimba persa* fish to total length and total weight ($P > 0.05$).

Key words: *Vimba vimba persa* • Blood Parameters • Reproductive Migration

INTRODUCTION

Gypsy king fish (*Vimba vimba persa*) is one benthopelagic species that live in Caspian Sea, Black Sea and Baltic Sea [1]. Populations of this species lives in the freshwater and brackish water and in the Caspian Sea basin entered to the rivers of south basin (especially rivers of western half) for reproduction [2, 3]. In recent years, reproductive migration of anadromous fish such as *Vimba vimba* reduced due to destruction of rivers, building the dam in the migration path and pollution of spawning areas and also illegal fishing strongly reduced their stocks [4]. Kiabi *et al.* [5] based on classification of International Union for Conservation of Nature reported that *Vimba vimba persa* is considered as vulnerable species exposed to threat. Also, in the case of biological studies of different species of domestic water of Iran has been less studied [6, 7] and this type of studies is limited about *Vimba vimba persa* [8-10].

Blood is a liquid vital fluid and important index for health, environmental effect and growth and reproduction

cycle. Blood cells consist of RBCs (erythrocytes), WBCs (leucocytes) and thrombocytes. WBCs consist of granocyte, lymphocyte and monocytes that granocytes consist of neutrophil, eosinophil and basophil [11]. One of the ways, studies of physiological characteristics of fishes is determination of blood parameters that to other ways is simply and cheaper. By attention that each fish have especial pattern of blood, investigation solely can determine exact information about physiological characters of the species. Another aspect of this research is that these parameters can be changed with environmental changes [12]. Hematological features of fishes are one of the most important evidence of the physiological stages and reflecting the relationship characteristic of water ecosystem and their health [13]. Changes in blood characteristics of fishes in response to environmental conditions are response to environmental stress and can be used as a biomarker [14]. In fish, hematological parameters might affected by physiologic factors such as gender, reproduction stages, age, size and health [13, 15-18]. Many studies carried out in related with

hematological characteristics of fishes have focused on cultured species [17, 19, 20] and information about non-farmed and marine species is limited [21]. In general application of hematological science is consists of determination the physiological status of blood cells and comparison with natural conditions and also as a clinical tool in the diagnosis of diseases. In Iran, hematological studies is performed on fishes especially sturgeon fish, such as investigation and compare blood cells and white cells differential count of *Acipenser persicus* and *Acipenser stellatus* [22], determination of some the blood parameters of *Acipenser stellatus* at part of southeast Caspian Sea [23]. Also in the other countries such as Norway, Canada and Germany studies have been conducted on blood parameters and physiological changes in related with seasonal changes, temperature and effect of chemical material have been investigated [22]. As regards this fish is one economical fish and the population of this species is declining and in order that Iranian fisheries intends to perform artificial reproduction of these fishes and successfully introduced a wild species to the aquaculture is evaluation of hematological conditions that is created by osmotic changes. The purpose of this study is determination of some the hematological parameters of male Gypsy king fish (*Vimba vimba persa*) during reproductive migration.

MATERIALS AND METHODS

Broods and Sampling: Sampling was taken from Khoshkrod River in May 2011. Sex determination was determined with pressure to genital area and macroscopic observation. Males were anaesthetized by immersion in Tricaine Methane sulphonate (MS-222) at 100 mg L⁻¹. Weight and total length are determined and recorded. Blood samples were taken from the caudal vein using heparinized capillary tubes and samples were transferred to Aquaculture Research Center of Gorgan University of Agricultural Sciences and Natural Resources.

Measurement of Hematological Parameters: Hematological parameters including RBCs count, WBCs count, Hb, hematocrit, MCV, MCH and MCHC concentration, white cells differential count such as neutrophils, lymphocyte, basophil and eosinophil were determined.

An aliquot of blood was diluted 1:200 with 0.4% formaldehyde and 3% trisodium citrate, to determine the number of RBCs in a Neubauer counting chamber (hemocytometer). The hematocrit value was determined by centrifuging the blood in a capillary or micro-hematocrit tube at 12,000 rpm for 5 min. The hemoglobin concentration was obtained using the cyanmethaemoglobin method.

MCV, MCH and MCHC concentration were calculated:

$$MCV(fl) = \frac{\text{Hematocrit} \times 10}{RBC \left(\frac{\text{micron}}{mm^3} \right)}$$

Mean corpuscular volume (MCV)

$$MCV(pg) = \frac{\text{Hemoglobin} \times 10}{RBC \left(\frac{\text{micron}}{cum} \right)}$$

Mean corpuscular hemoglobin (MCH)

$$MCHC(\%) = \frac{\text{Hemoglobin concentration} \times 10}{\text{Hematocrit}}$$

Mean corpuscular hemoglobin concentration (MCHC)

Statistical Analysis: The correlation between blood parameters and length and weight of brood were analyzed using the bivariate correlation coefficients of Pearson (SPSS, ver. 16).

RESULTS

The mean values and standard deviation of the hematological parameters of *Vimba vimba persa* are summarized in table 1.

In the present study, Total length of *Vimba vimba persa* was 16.11±1.31 and total weight was 39.5±5.01, Number of RBCs was 3.62×10⁶±1.42 per mm³, number of WBCs was 2312.9±5510 per mm³, hemoglobin concentration was 8.16±1.21 g/dL and hematocrit concentration was 32.6±5.54. Differential count of WBCs showed that (12.6±77.4%) belonged to lymphocyte, (19±10.70%) to neutrophil, (1.9±0.875) to monocyte and (1.7±1.49 %) to eosinophil. Also blood indicators were included of MCH: 6.35±15.24 pg, MCHC: 2.5±0.119 % and MCV: 239.19±55.19 fL. Reciprocal correlation between hematological parameters with length and weight of *Vimba vimba persa* are shown in the table 2.

Table 1: Hematological parameters of *Vimba vimba persa* during reproductive migration to the Khoshkrud River

Maximum	Minimum	Mean± SD	Parameters
18.1	3.13	1.31 ± 16.11	Total length (cm)
48	32	39.5 ± 5.01	Weight (g)
87.1	4.7	3.62 ± 1.42	RBCs (mm ³)
89	25	2312.9 ± 5.51	WBCs (mm ³)
10	5.6	21 ± 8.16.1	Hemoglobin (g/dL)
40	24	32.6 ± 5.54	Hematocrit (%)
39	9	10.70 ± 19	Neutrophils (%)
89	56	77.4 ± 12.06	Lymphocyte (%)
4	1	0.875 ± 1.9	Monocyt (%)
5	0	1.49 ± 1.7	Eosinophil (%)
85.342	200	55.19 ± 2.39	MCV (fL)
19.89	6.48	60.35 ± 15.24	MCH (pg)
75.2	39.2	0.119 ± 2.5	MCHC (%)

Table 2: Reciprocal correlation between hematological parameters

MCHC	MCH	MCV	Eosinophil	monocyte	Lymphocyte	Neutrophil	Hct	Hb	WBCs	RBCs	Variable
114.0	371.0	404.0	151.0-	415.0-	068.0	114.0	178.0-	186.0-	067.0	403.0-	Total length
257.0-	048.0	144.0	111.0	089.0	490.0-	257.0-	060.0	020.0-	621.0	063.0-	total weight

DISCUSSION AND CONCLUSION

According to studies at other species, blood cells was consist of erythrocytes, lymphocytes, monocytes, neutrophils, trombocytes, heterocyte, bazophil, eosinophils and mature cells that for these cells are listed similar activity with mammalian cells. The red blood cells of fish unlike mammals is nucleate and with the advancement of cell development raised Mean corpuscular volume, mean corpuscular hemoglobin and Mean corpuscular hemoglobin concentration. Basis on other studies, range of white blood cells are much different and based on species from 10×10^3 to 282×10^3 is variable. The number of white blood cell in some teleosts reaches over 100×10^3 and even in one species is variable based on age, season and sexual maturity [24]. According to results of this study, number of RBCs of *Vimba vimba persa* was $3.62 \times 10^6 \pm 1.42/\text{mm}^3$, number of WBCs was $2312.9 \pm 5510/\text{mm}^3$, hemoglobin concentration was 8.16 ± 1.21 g/dL and hematocrit concentration was 32.6 ± 5.54 (%). To comparing between blood parameters at other family of cyprinid with *Vimba vimba persa* shown that number of RBCs of gold fish was $1.670000/\text{mm}^3$, hematocrit concentration was 9.4%, hemoglobin concentration was 9.1 g/dL and total number of WBCs was $10000/\text{mm}^3$ [25]. In the Indian carp, hemoglobin concentration was reported 7.4 g/dL and number of WBCs were reported $6000/\text{mm}^3$ [26]. Also, in silver carp, number of RBCs was reported 1.01×10^6 , number of WBCs was

$37000/\text{mm}^3$, hematocrit concentration was 32 (%) and hemoglobin concentration was 8.9 g/dL [16]. At the present study, result of blood indicators were, MCV: 239.19 ± 55.19 fL, MCH: 15.24 ± 6.35 pg and MCHC: 2.5 ± 0.119 % respectively. Khaje *et al.* [27] studied on hematology parameters of *Barbus grypus* that level of RBC, WBC, hemoglobin, hematocrit, neutrophil, lymphocyte, monocyte, eosinophil, MCV, MCH, MCHC were $1.41 \times 10^6 \pm 0.035$, $12.5 \times 10^3 \pm 0.57$, 6.5×0.1 g/dL, $36.9 \pm 0.7\%$, $40 \pm 0.52\%$, $56.2 \pm 0.59\%$, $2.8 \pm 0.22\%$, $1.1 \pm 0.15\%$, 261 ± 4.87 femeto L, 45.7 ± 0.88 pg and 17.6 ± 0.27 respectively. Rahimibashar *et al.* [28] reported the following values for *Rutilus frisii kutum* for the previous mentioned parameters respectively $1.23 \times 10^6 \pm 40400$, 7281 ± 463 , 8.33 ± 4.85 g/dL, 41.86 ± 1.3 %, 383.81 ± 92.8 fL, 70.14 ± 32.17 pg and $12.41 \pm 823\%$. These finding is in agreement with our results. Hrubec *et al.* [29] reported hematocrit concentration, hemoglobin concentration, number of RBCs, MCV, MCH and MCHC at tilapia (*Oreochromis hybrid*) 23%, 8.2 g/dL, 2.31×10^6 , 135.7 fL, 34.9 pg and 25.7% respectively. Feldman *et al.* [24] reported extent of variation of hematocrit, hemoglobin, MCV, MCH, RBCs at different species 0.17-55%, 1.5-15 g/dL, 81-553 fL, 14-106 pg and $0.77-4.2 \times 10^9$ respectively. Comparison the values of studied parameters with values of hematological parameters in other species showed that *Vimba vimba persa* have values of MCHC less than the *Rutilus frisii kutum* and *Barbus grypus* and also number of WBCs in *Vimba vimba persa* was lower than the other species.

Pickering [2] reported in a study on brown trout in the spawning season proved that in the males reduced the values of lymphocytes in the breeding season. The important point in the study of hematological parameters is that hematological parameters significantly influenced environmental and biological factors; therefore in the interpreting the results of study of hematological parameters must be aware from influence mentioned factors on the hematological parameters [30]. Of course seasonal differences in the hematologic characteristics of fishes can relate to natural cycle of physiological and environmental conditions or both. No significant correlation was obtained between blood parameters *Vimba vimba persa* fish to total length and total weight ($P>0.05$). that Hatingh and Van vuren [31] studied the effect of season on hematological parameters in four species, *Barbus holubi*, *Cyprinus carpio*, *Labeo umbratus* and *Labeo capensis* and differences in values of hematological parameters in different seasons in all four species and the lack of significant difference in hematological parameters in both male and female and also absence of communication between length and weight with hematological parameters was reported, but there was significant correlation between number of RBCs and body weight, that Parma [32] reported significant correlation between number of RBCs and body weight in the *Prochilodus lineatus*. Hrubec *et al.* [33] expressed age of factors effective on hematocrit concentration and number of RBCs and reported that levels of hematocrit and hemoglobin increased with increasing age. Siddiqui and Nasim [34] reported that level of hemoglobin and RBCs in male of *Cirrhinus mrigala* is higher than female. Barnhart [35] reported age and sex are effective on hematological parameters in the *Oncorhynchus mykiss*. In the present study, it is observed that differences and similarities between values of each parameters with similar parameters, although this comparison according to the effect of environmental and biological conditions in hematological parameters is not correct, but indicating that hematological parameters in different species of fishes is different.

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