

## Determination of Some Hematological Parameters of Male Gypsy King Fish (*Chalcalburnus chalcoides* Güldenstadt, 1772) During Reproductive Migration to the Khoshkrud River

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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 with total length of  $13.94 \pm 0.89$  cm and total weight  $20.6 \pm 2.98$  g were captured from the Khoshkrud River. After biometry by cutting tail stem, blood sampling was taken from fish. Results showed that the numbers of red blood cells (RBCs) were  $1.41 \times 10^6 \pm 5.07$  per  $\text{mm}^3$ , white blood cells (WBCs) were  $3860 \pm 554.17$  per  $\text{mm}^3$ , hemoglobin concentration was  $8.09 \pm 2.34$  g/dL and hematocrit concentration was  $30.9 \pm 6.20\%$ . Differential count of WBCs showed that  $87.6 \pm 7.24\%$  belonged to lymphocyte,  $18.8 \pm 7.004\%$  to neutrophil,  $1.9 \pm 0.737\%$  to monocyte and  $0.7 \pm 0.48\%$  to eosinophil. Also blood indicators were included of MCV  $236.44 \pm 66.21 \mu^3$ , MCH  $60.92 \pm 15.64$  Pg, MCHC  $2.5 \pm 0.171\%$ . No significant correlation was obtained between blood parameters of *Chalcalburnus chalcoides* and total length in rang of  $13.94 \pm 0.89$  cm ( $P > 0.05$ ). But there was a significant negative correlation between total weight and RBC ( $r = -0.691$ ,  $P < 0.05$ ) and hemoglobin ( $r = -0.713$ ,  $P < 0.05$ ) and hematocrit ( $r = -0.715$ ,  $P < 0.05$ ).

**Key words:** *Chalcalburnus chalcoides* • Blood Parameters • Reproductive Migration

### INTRODUCTION

Gypsy king fish (*Chalcalburnus chalcoides*) is one benthopelagic species that live in Caspian Sea, Black Sea and Baltik 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 *Chalcalburnus chalcoides* 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 *Chalcalburnus chalcoides* 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 *Chalcalburnus chalcoides* [8-10].

Blood is a liquid vital fluid and important index for health, environmental effect, growth and reproduction cycle. Cells of blood consist of RBCs (erythrocytes), WBCs (leucocyte) 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. Therefore, understanding of normal range of blood parameters can be used as biomarker [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]. At fish, hematology parameters might be affected by physiological factors such as gender, reproduction stages, age, size and their 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 hematology 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 season 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 perform artificial reproduction of these fishes and successfully introduced a wild species to the aquaculture is understanding the reproductive cycle and also 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 (*Chalcalburnus chalcoides*) during reproductive migration.

## MATERIALS AND METHODS

**Broods and Sampling:** Sampling was taken at Khoshkrud River at May 2011. Sex determination was taken with pressure to genital area and macroscopic observation. Males were anaesthetized by immersion in Tricaine Methane sulphonate (MS-222) at 100 mg L<sup>-1</sup>. 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:

Mean corpuscular volume (MCV)

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

Mean corpuscular haemoglobin (MCH)

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

Mean corpuscular haemoglobin concentration (MCHC)

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

**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 *Chalcalburnus chalcoides* are summarized in Table 1.

Table 1: Mean±SD of Hematological parameters of *Chalcalburnus chalcoides* during reproductive migration to the Khoshkrud River

Parameters	Mean±SD	Minimum	Maximum
Total length (cm)	13.94±0.89	12.8	15
Weight (g)	2.98±20.6	16	24
RBC (mm <sup>3</sup> )	5.07±1.41 × 10 <sup>6</sup>	6.9	2.34
WBC (mm <sup>3</sup> )	554.17±3860	32	48
Hemoglobin (g/dL)	2.34±8.09	6.3	14.3
Hematocrit (%)	6.20±30.9	23	43
Neutrophil (%)	7.004±18.8	10	28
Lymphocyte (%)	7.24±78.6	69	88
Monocyte (%)	0.73±1.9	1	3
Eosinophil (%)	0.48±0.7	0	1
MCV (fL)	66.21±236.44	183.7	358.02
MCH (pg)	15.64±60.92	46.9	92.75
MCHC (%)	0.17±2.5	2.3	2.74

Table 2: Reciprocal correlation between hematological parameters

Variable	RBC	WBC	Hb	Hct	Neutrophil	Lymphocyte	Monocyte	Eosinophil	MCV	MCH	MCHC
Total length	-0.28	-0.35	-0.48	-0.36	0.15	-0.13	0.17	-0.37	-0.05	-0.25	0.022
Total weight	-0.69*	-0.36	-0.713*	-0.715*	-0.20	0.17	0.53	-0.47	0.3	0.15	0.31

\*P<0.05. Hb: Hemoglobin, Hct: Hematocrit

In the present study, total length of *Chalcalburnus chalcoides* was 13.94±0.89cm and total weight was 20.6±2.98 g. Number of RBCs were 1.41±10<sup>6</sup>±5.07per mm<sup>3</sup>, number of WBCs was 3860±554.17per mm<sup>3</sup>, hemoglobin concentration was 8.09±2.34 g/dL and hematocrit concentration was 30.9±6.20%. Differential count of WBCs showed that 78.6±7.24% belonged to lymphocyte, 18.8±7.004% to neutrophil, 1.9±0.737 to monocyte and 0.7±0.48 to eosinophil. Also blood indicators were included of MCH: 60.92±15.64 pg, MCHC: 2.5±0.171 %, MCV: 236.44±66.21fL. Reciprocal correlation between hematological parameters with length and weight of *Chalcalburnus chalcoides* are shown in the Table 2. As shown in Table 2, there was negative significant correlation between total weight with RBCs (r= -0.691, P<0.05), hemoglobin (r= -0.713, P<0.05) and hematocrit (r= -0.715, P<0.05). Total weight was negatively correlated with WBCs, neutrophil and eosinophil, but these correlates were not significant. Also relationship between total length with RBCs, WBCs, hemoglobin, hematocrit, lymphocyte, eosinophil, MCV and MCH was reverse but these correlates were not significant (P<0.05).

## DISCUSSION AND CONCLUSION

According to studies at other species, blood cells was consist of erythrocytes, lymphocytes, monocytes, neutrophils, trombocytes, heterocyte, basophil, 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. Based on other studies, range of white blood cells are much different and based on species from 10 ×10<sup>3</sup> to 282×10<sup>3</sup> is variable. The number of white blood cell in some teleosts reaches over 100×10<sup>3</sup> 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 *Chalcalburnus chalcoides* was 1.41±10<sup>6</sup>/mm<sup>3</sup>, number of WBCs was 3860±554.17/mm<sup>3</sup>, hemoglobin concentration was 8.09±2.34 g/dL and hematocrit concentration was 30.9±6.20 %. To comparing between blood parameters at other family of Cyprinidae with *Chalcalburnus chalcoides* shown that number of RBC of gold fish was 1.670000/mm<sup>3</sup>, hematocrit concentration was 9.4%, hemoglobin concentration was 9.1 g/dL and total number of WBCs was 10000/mm<sup>3</sup> [25]. In the Indian carp, hemoglobin concentration was reported 7.4 g/dL and number of WBCs were reported 6000/mm<sup>3</sup> [26]. Also, in silver carp, number of RBCs was reported 1.01×10<sup>6</sup>, number of WBCs was 37000/mm<sup>3</sup>, hematocrit concentration was 32 % and hemoglobin concentration was 8.9 g/dL [16]. At the present study, results of blood indicators were, MCV: 236.44±66.21fL, MCH: 15.64±60.92pg and MCHC 2.5±0.171 % respectively. Khaje *et al.* [27] studied on hematology parameters of *Barbus grypus* that level of RBCs, WBCs, hemoglobin, hematocrit, neutrophil, lymphocyte, monocyte, eosinophil, MCV, MCH, MCHC were 1.41×10<sup>6</sup>±0.035, 12.5×10<sup>3</sup>±0.57, 6.5×0.1 g/dL, 36.9±0.7%, 40±0.52%, 56.2±0.59%, 2.8±0.22%, 1.1±0.15%, 261±4.87 femeto L, 45.7±0.88 pg and 17.6±0.27% respectively. Rahimibashar *et al.* [28] these values reported for *Rutilus frissi kutum* respectively 1.23×10<sup>6</sup>±40400, 7281±463, 8.33±4.85g/dL, 41.86±1.3 %, 304

383.81±92.8 fL, 70.14±32.17 pg and 12.41±823% that. This 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<sup>6</sup>, 135.7fL, 34.9 pg and 25.7% respectively. Feldman *et al.* [24] in a review 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 0.77-4.2×10<sup>9</sup> respectively. Comparison the values of studied parameters with values of hematological parameters in other species showed that *Chalcalburnus chalcoides* have values of MCHC less than the *Rutilus frisii kutum* and *Barbus grypus* and also number of WBCs in *Chalcalburnus chalcoides* was lower than the other species. Pickering [2] with 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. In this study, there was significantly correlation between length body and hematological parameters of *Chalcalburnus chalcoides* (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 hematology parameters in different seasons in all four species and the lack of significant difference in hematology 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*. This study examined only hematological parameters in the males at spawning season. 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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