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±0.89 cm and total weight 20.6±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 1.41×10±5.07 per mm, white blood cells (WBCs) were 3860±554.17 per mm, hemoglobin concentration was 8.09±2.34 g/dL and hematocrit concentration was 30.9±6.20%. Differential count of WBCs showed that 87.6±7.24% belonged to lymphocyte, 18.8±7.00% to neutrophil, 1.9±0.73% to monocyte and 0.7±0.48% to eosinophil. Also blood indicators were included of MCV 236.44±66.21µ, MCH 60.92±15.64Pg, MCHC 2.5±0.17%. No significant correlation was obtained between blood parameters of *Chalcalburnus chalcoides* and total length in range of 13.94±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 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 affect 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⁻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 microhematocrit 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)

\[
MCV = \frac{\text{Hematorcrit} \times 10}{\text{RBC} \left( \frac{\text{micron}}{\text{mm}^3} \right)}
\]

Mean corpuscular haemoglobin (MCH)

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

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

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Mean±SD</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total length (cm)</td>
<td>13.94±0.89</td>
<td>12.8</td>
<td>15</td>
</tr>
<tr>
<td>Weight (g)</td>
<td>2.98±20.6</td>
<td>16</td>
<td>24</td>
</tr>
<tr>
<td>RBC (mm³)</td>
<td>5.07±1.41 × 10⁶</td>
<td>6.9</td>
<td>2.34</td>
</tr>
<tr>
<td>WBC (mm³)</td>
<td>554.17±3860</td>
<td>32</td>
<td>48</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>2.34±8.09</td>
<td>6.3</td>
<td>14.3</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>6.20±30.9</td>
<td>23</td>
<td>43</td>
</tr>
<tr>
<td>Neutrophil (%)</td>
<td>7.00±18.8</td>
<td>10</td>
<td>28</td>
</tr>
<tr>
<td>Lymphocyt (%)</td>
<td>7.24±78.6</td>
<td>69</td>
<td>88</td>
</tr>
<tr>
<td>Monocyt (%)</td>
<td>0.73±1.9</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Eosinophil (%)</td>
<td>0.48±0.7</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>MCV (fL)</td>
<td>66.21±236.44</td>
<td>183.7</td>
<td>358.02</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>15.64±60.92</td>
<td>46.9</td>
<td>92.75</td>
</tr>
<tr>
<td>MCHC (%)</td>
<td>0.17±2.5</td>
<td>2.3</td>
<td>2.74</td>
</tr>
</tbody>
</table>

Table 2: Reciprocal correlation between hematological parameters

<table>
<thead>
<tr>
<th>Variable</th>
<th>RBC</th>
<th>WBC</th>
<th>Hb</th>
<th>Hct</th>
<th>Neutrophil</th>
<th>Lymphocyte</th>
<th>Monocyt</th>
<th>Eosinophil</th>
<th>MCV</th>
<th>MCH</th>
<th>MCHC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total length</td>
<td>-0.28</td>
<td>-0.35</td>
<td>-0.48</td>
<td>-0.36</td>
<td>0.15</td>
<td>-0.13</td>
<td>0.17</td>
<td>-0.37</td>
<td>-0.05</td>
<td>-0.25</td>
<td>0.022</td>
</tr>
<tr>
<td>Total weight</td>
<td>-0.69*</td>
<td>-0.36</td>
<td>-0.713*</td>
<td>-0.715*</td>
<td>-0.20</td>
<td>0.17</td>
<td>0.53</td>
<td>-0.47</td>
<td>0.3</td>
<td>0.15</td>
<td>0.31</td>
</tr>
</tbody>
</table>

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

In the present study, total length of *Chalcalburnus chalcoides* was 13.94±0.89 cm and total weight was 20.6±2.98 g. Number of RBCs were 1.41±10⁶±5.07 per mm³, number of WBCs was 3860±554.17 per mm³, 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±66.21 fL. Reciprocal correlation between hematological parameters with length and weight of *Chalcalburnus chalcoides* are shown in 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 10³ to 282×10⁶ is variable. The number of white blood cell in some teleosts reaches over 100×10⁶ 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±10⁶/mm³, number of WBCs was 3860±554.17/mm³, 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 1.67±0.000, hematocrit concentration was 9.4%, hemoglobin concentration was 9.1 g/dL and total number of WBCs was 10000/mm³ [25]. In the Indian carp, hemoglobin concentration was reported 7.4 g/dL and number of WBCs was reported 6000/mm³ [26]. Also, in silver carp, number of RBCs was reported 1.01×10⁶, number of WBCs was 37000/mm³, 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±66.21 fL, MCH: 15.64±60.92 pg 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 1.41×10⁶±0.035, 12.5×10⁶±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 1.23×10⁶±40400, 7281±463, 8.33±4.85g/dL, 41.86±1.3 %,
383.81±92.8 fL, 70.14±32.17 pg and 12.41±823% that. 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.

REFERENCES


