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, Hematology, 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 leukopenia or leukocytosis signifies possible immune function alteration. In recent years, hematological parameters have been commonly used to observe and follow the quality of fish [9]. 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]. 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 *Schizothorax 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 vein 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**

Cynomethglobin method was used for the evaluation of hemoglobin concentration as described by \[20\]. Total erythrocyte and leucocytes were done with a Neubauer haemocytometer (Marienfeld-Superior, Lauda-Königshofen, 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\(^3\)=200x50xN=10,000 N (N=number of RBC counted, dilution factor=200) and total WBC count per cubic millimeter was obtained as 20x1xL/0.4 cells=50xL. (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-hematocrit 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 \[25\].

\[
\begin{align*}
MCV &= \text{PCV} \times 100/\text{erythrocyte count} \\
MCH &= \text{hemoglobin} \times 10/\text{erythrocyte count} \\
MCHC &= \text{hemoglobin} \times 100/\text{PCV}
\end{align*}
\]

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 1.37x10\(^6\) to 2.08x10\(^6\)/mm\(^3\); 2.10x10\(^6\)/mm\(^3\) to 1.75x10\(^6\)/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 average annual mean of RBC in the blood of both the sexes was 1.62x10\(^6\)/mm\(^3\) and 1.42x10\(^6\)/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 4.05x10\(^3\)-6.15x10\(^3\)/mm\(^3\); 7.14x10\(^3\)-9.28x10\(^3\)/mm\(^3\) of blood in male and female fish, respectively. The annual mean value for male and female fish was 8.05x10\(^3\) and 4.96x10\(^3\) 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 pisciculture and greater awareness of the pollution of the aquatic ecosystem.

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

<table>
<thead>
<tr>
<th>Season</th>
<th>Sex</th>
<th>HB (x10^6)</th>
<th>RBC (x10^6/l)</th>
<th>TLC (x10^9/l)</th>
<th>PCV</th>
<th>MCV</th>
<th>MCHC</th>
<th>MCH</th>
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<tbody>
<tr>
<td>Spring</td>
<td>Male</td>
<td>9.63±</td>
<td>1.44±</td>
<td>4.52±</td>
<td>33.83±</td>
<td>234.10±</td>
<td>28.46±</td>
<td>66.62±</td>
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<td></td>
<td>SE</td>
<td>0.18</td>
<td>0.56</td>
<td>0.09</td>
<td>0.26</td>
<td>2.41</td>
<td>0.53</td>
<td>1.43</td>
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<td>Female</td>
<td>8.41±</td>
<td>1.26±</td>
<td>7.47±</td>
<td>30.68±</td>
<td>243.05±</td>
<td>27.44±</td>
<td>66.53±</td>
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<tr>
<td></td>
<td>SE</td>
<td>0.22</td>
<td>1.15</td>
<td>0.12</td>
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<tr>
<td>Summer</td>
<td>Male</td>
<td>11.06±</td>
<td>2.08±</td>
<td>6.16±</td>
<td>41.78±</td>
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<td>26.52±</td>
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<tr>
<td></td>
<td>SE</td>
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<td>3.00</td>
<td>0.33</td>
<td>0.91</td>
<td>6.23</td>
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<td>1.76±</td>
<td>9.28±</td>
<td>35.61±</td>
<td>202.04±</td>
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<td>0.87</td>
<td>4.82</td>
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<tr>
<td>Autumn</td>
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<td>1.61±</td>
<td>5.15±</td>
<td>37.72±</td>
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<tr>
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<td>2.73</td>
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<td>Female</td>
<td>8.82±</td>
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<td>5.77</td>
<td>0.76</td>
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<tr>
<td>Winter</td>
<td>Male</td>
<td>8.62±</td>
<td>1.37</td>
<td>4.05±</td>
<td>32.06±</td>
<td>233.95±</td>
<td>27.47±</td>
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<tr>
<td></td>
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<td>1.21±</td>
<td>7.14±</td>
<td>29.24±</td>
<td>240.39±</td>
<td>25.76±</td>
<td>62.00±</td>
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<tr>
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<td>2.78</td>
<td>0.28</td>
<td>0.76</td>
<td>6.53</td>
<td>0.33</td>
<td>2.09</td>
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</table>
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


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