RESEARCH ARTICLE

Some Haematological Changes of Zander (Sander lucioperca) In Relation to Age and Its Relationship with Parasitic Infection

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Some Haematological Changes of Zander (*Sander lucioperca*) In Relation to Age and Its Relationship with Parasitic Infection

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Abstract
The blood parameters of *Sander (S.) lucioperca* were investigated among different age classes (2, 3, 4 and 5 year old). Also, alterations of haematological parameters in relation to parasitic infection were tested. The red blood cell (RBC), white blood cell (WBC), hematocrit (Ht), hemoglobin concentration (Hb), mean corpuscular volume (MCV), mean corpuscular hemogbobin (MCH), mean corpuscular hemoglobin concentration (MCHC), lymphocyte, neutrophil, monocyte and myelocyte were measured. No haematological parameters showed significant differences ($P > 0.05$) among age classes. The values of Ht, Hb, neutrophil and monocyte were higher in 4 year compared to other ages. The results showed that MCH, lymphocyte and myelocyte increased with the age. Significant differences were detected for the lymphocytes and neutrophils (infested versus non-infested by *Eustrongylides excises* and *Dactylogyrus* sp) respectively. No differences were found with regard to other haematological parameters of infested fish. Results from this study suggest that age has some degree of influence on the blood parameters of *S. lucioperca.*

Keywords: Haematology; age; blood cell; *Sander lucioperca.*

1. Introduction

Blood is an indicator of physiological condition of an animal. Haematological parameters are used as a diagnostic tool by fish biologists and researchers throughout of the world. This is so because fish are closely associated with the aquatic environment and the blood will reveal conditions within the body of the fish long before there is any outward manifestation of diseases [1]. Several studies have confirmed that haematological parameters are important to the evaluation of fish physiological status [2, 3]. Changes of blood parameters depend on the fish species, age, the cycle of sexual maturity and health condition [4, 5]. It is well recognized that haematological parameters in fish vary with factors such as species, age of fish and season [6]. Blood can be used to study changes in health status revealing lesions in other organs or tissues [7], or to help breeding, given that changes in the hematological profile may indicate infestations and infections or even environmental changes [8]. Hematology is a tool that makes it possible to study organisms' physiological responses to pathogens. It may assist in making diagnoses and prognoses on diseases in fish populations. Red blood cells can be used to identify conditions that cause stress to the fish, and consequently disease [9, 10]. To investigate the fish blood factors and their changes, the normal level of the factors must initially be measured in healthy fish. Studies on haematological alterations of fish have been evaluated in *Cyprinus carpio* [11] and *Piaractus mesopotamicus* [11] infected with *Argulus* sp.; *Mugil platanus* infected with *Trypanosoma*, *Hemogregarina*, *Trichodina*, Monogenoidea, copepods and Hirudinea [8]; *Lepomis macrocephalus* infected with *Trichodina* sp., *Piscinoodinium pillulare* and *Lernaea cyprinacea* [13] and *Oreochromis niloticus* infected with *I. multifiliis* [14]. The pikeperch (*S. lucioperca*) is found in freshwater and brackish water, and it is a semi-anadromous, cool-water species found in the Caspian Sea watershed [15]. Pikeperch is one of the most valuable fishes in freshwater [16]. The most important area for pikeperch spawning in Iran has been the Anzali Lagoon. There is scant knowledge of *S. lucioperca* physiology, especially regarding haematological parameters. Haematology and biochemical parameters of pikeperch were examined in intensive culture by Siwicki *et al.* [17]. Numerous studies have confirmed that age can influence fish blood parameters [17-22]. The aim of the present study was to determine levels of some haematological parameters in the blood of *S.*

lucioperca in relation to age as well as to test the relationship between parasitic infection and haematological alterations.

2. Methods

2.1. Source of fish and parasite inspection

Sixty-four specimens of *S. lucioperca* were captured using seine nets by fishing companies, which are situated in Anzali port, Guilan State, Iran. The fish were divided into different age classes. The age of the zander was determined from scale samples taken between the adipose fin and lateral line. Each fish was measured, the total length to nearest 0.1 cm, the total weight to nearest 1 g. The age classes were 2 year old (n=14), 3 year old (n=16), 4 year old (n=18) and 5 year old (n=16). The mean length and weight of age classes were as follows: 2-year old: mean length 44.61 ± 2.90 cm; mean weight: 656.57 ± 93.24, 3-year old: mean length 49.2 ± 1.2 cm; mean weight: 897.1 ± 171.1g, 4-year old: mean length 68.8 ± 2.2 cm; mean weight: 1.813.8 ± 222.1 g and 5-year old: mean length 71.2 ± 7.9 cm; mean weight: 1336.4 ± 161.2. Parasitic infections (*Eustrongylides excises*, Nematoda and *Diplostomum spathaceum*, Digenea) were recorded from captured fish (infested fish, n=26). Parasitic inspection was performed as follows: The collected parasites separated and placed in a Petri-dish containing physiological saline solution. The parasites were washed in a 0.6% saline solution. Nematodes were compressed between two slides with Glacial Acetic Acid (GAA). This makes the worms transparent and then microscope was used for observation. The Digeneans were washed in a 0.6% saline solution and fixed in 70% ethanol. Haematological parameters were measured according to each parasite.

2.2. Haematological examination

The individual blood samples were taken from caudal vessels of anaesthetized specimens ($MS\text{-}_{222}$; 1g/10 L) using heparinized syringes and then stored in a polystyrene cool bag until analysis. Determination of RBC and WBC counts was performed with Neubauer chambers, using Rees diluting solution (1 g brilliant cresyl blue, 31.3 g sodium citrate, 10 mL formalin (37 %) and 1000 mL distilled water). Differential leukocyte count was determined with blood smears stained with Giemsa solution. The smears were examined by light microscopy (Olympus, Tokyo, Japan) under oil immersion at 100X magnification. Hematocrit (Ht) was determined using micro hematocrit capillaries filled with blood, centrifuged at 3000 rpm for 5 min, and expressed as percentage of total blood volume. Hemoglobin (Hb) was measured with a spectrophotometer at 540 nm absorbance using the cyanmethemoglobin procedure; these results were used to calculate mean red cell volume (MCV), mean red cell hemoglobin (MCH) and mean cellular hemoglobin concentration (MCHC).

2.3. Statistical analysis

Haematological data were analyzed using one-way ANOVA and differences between mean values were considered to be significant by Duncan's multiple range test at $P<0.05$ (SPSS 16). Mann Whitney (U) test was used for normality of data distribution and homogeneity of variance. Data are presented as mean ± SD.

3. Results

Haematological parameters according to age classes are shown in figures 1-11 respectively. According to figures, haematological parameters did not show significant differences among ages. Mean hematological parameters in blood are given in Table 1. The values of Ht, Hb, neutrophils and monocytes were higher in 4 year old fish, but their values were not statistically different ($P>0.05$). In 2 year old fish, values of MCH and MCHC were higher compared with other ages (values were not statistically different). There were significant changes in all parameters expect for lymphocyte and neutrophil in relation to parasitic infection. The change percentages of leucocytes (lymphocyte and neutrophil) in relation to parasitic infestation are presented in Table 2. According to this table, significant differences were observed for the lymphocytes and neutrophils (infested versus non-infested by *Eustrongylides excises* and *Dactylogyrus* sp) respectively.

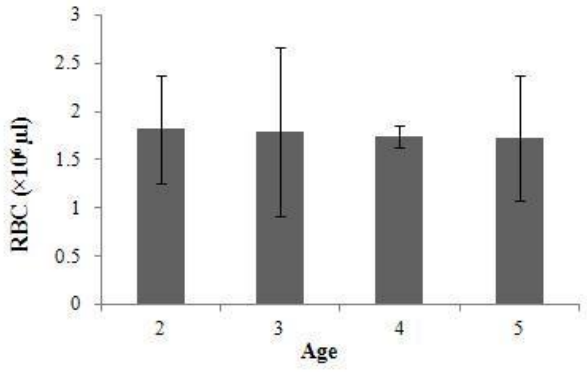

Figure 1: RBC of *S. lucioperca* at four age classes.

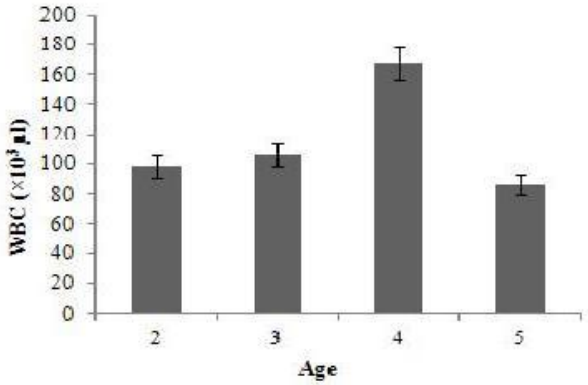

Figure 2: WBC of *S. lucioperca* at four age classes.

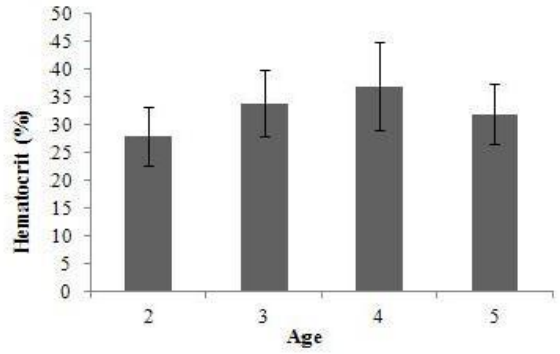

Figure 3: Hematocrit of *S. lucioperca* at four age classes.

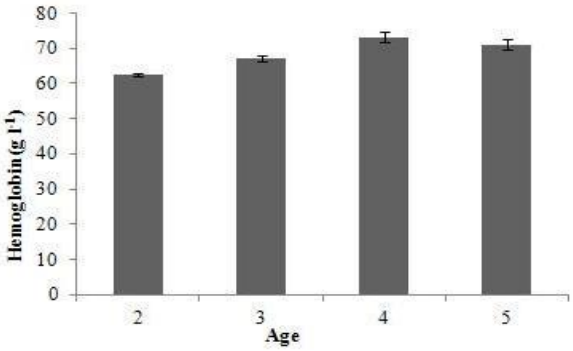

Figure 4: Hemoglobin of *S. lucioperca* at four age classes.

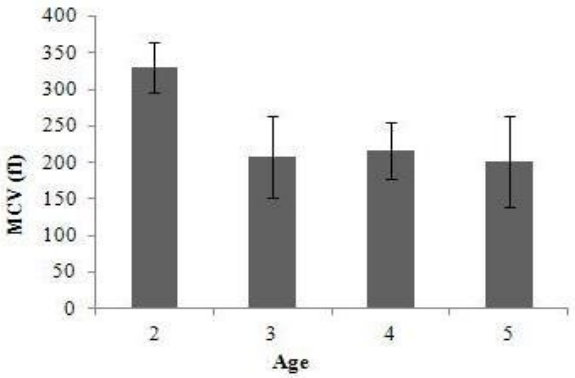

Figure 6: MCH of *S. lucioperca* at four age classes.

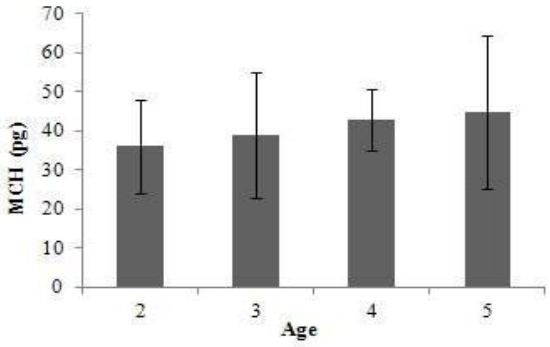

Figure 5: MCV of *S. lucioperca* at four age classes.

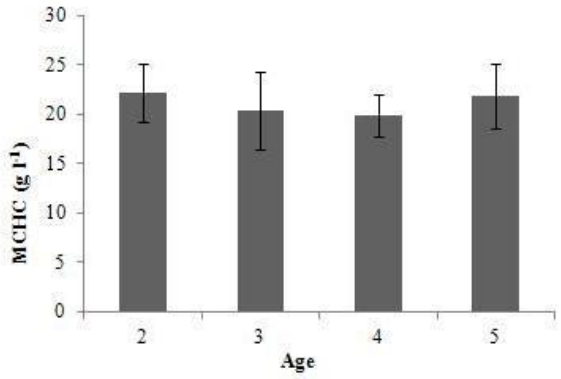

Figure 7: MCHC of *S. lucioperca* at four age classes.

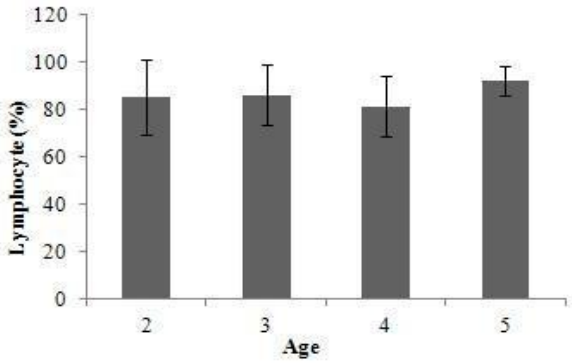

Figure 8: Lymphocyte of *S. lucioperca* at four age classes.

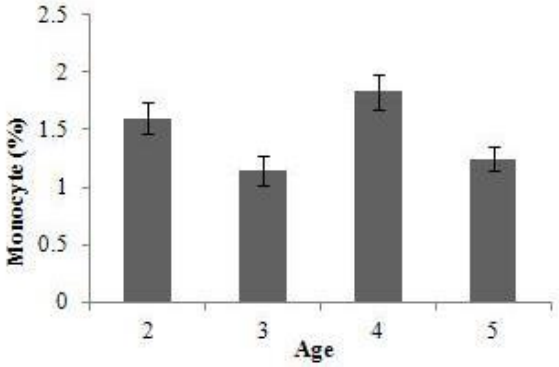

Figure 9: Monocyte of *S. lucioperca* at four age classes.

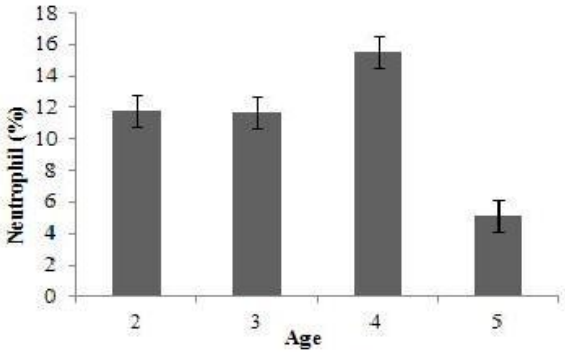

Figure 10: Neutrophil of *S. lucioperca* at four age classes.

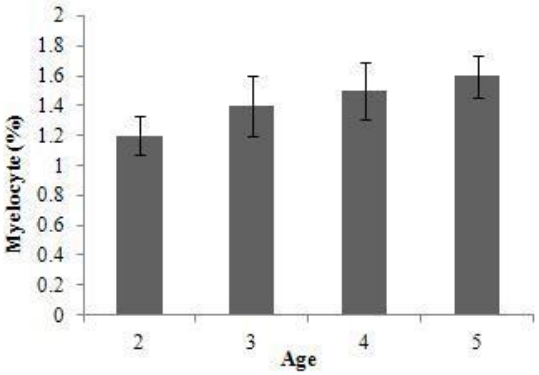

Figure 11: Myelocyte of *S. lucioperca* at four age classes.

Table 1: Mean of hematological parameters in blood of *S. lucioperca* captured from Anzali Lagoon.

Hematological parameters	Mean ± SD
Red blood cell (×10^6 µl)	1.72 ± 0.55
White blood cell (×10^3 µl)	114.4 ± 56.6
Hematocrit (%)	33.05 ± 6.01
Hemoglobin (g l^{-1})	68.5 ± 1.07
Red cell volume (MCV) (fl)	238.9 ± 46.2
Red cell hemoglobin (MCH) (pg)	40.7 ± 1.8
Cellular hemoglobin concentration (MCHC) (g l^{-1})	21.05 ± 1.75
Lymphocytes (%)	86.1 ± 10.6
Monocytes (%)	1.44 ± 0.98
Neutrophils (%)	11.03 ± 0.10
Myelocytes (%)	1.35 ± 0.13

4. Discussion

Present study examined the basic hematological parameters for monitoring fish condition. The data about hematological parameters in this species is rare. Since the literature lacks information regarding some of the hematological parameters of pikeperch, the results of this study were compared with those of another research on pikeperch which are bred intensively in recirculation systems [17]. In our experiment, values of RBC, Ht, Hb and leukocyte were similar to those reported in pikeperch by Siwicki *et al.* [17]. In this study values of Ht, Hb, neutrophils and monocytes were higher in 4 year old fish, but their values were not statistically different (Table 1). Our findings confirm those of Das (1965) and Anthony *et al.* (2010) [23, 24] who reported Hb concentration to be higher when length and age of the fish were increased. The erythrocyte indexes (MCV and MCH) were used to determine physiological changes in fish [25]. In our experiment, MCH and MCHC appeared higher in 2 year old fish compared with other ages although values were not statistically different (Table 1). An increase in erythrocyte index (MCH) was observed in older individuals than in younger ones which are not similar to other studies [22, 26]. In *Cyprinus carpio*, MCHC increases with the age [21]. In Nile tilapia, *Oreochromis niloticus,* reported fish size had no effect on MCV index [27]. In our experiment, lymphocyte increased in larger fish compared to smaller ones. The value of lymphocyte increased in *Capoeta trutta* and Caspian brown trout (*Salmo trutta caspius*) as individuals got larger [20, 22]. Also, Jamalzadeh and Ghomi [22] reported that neutrophil value increased in Caspian brown trout when they became larger.

Table 2: Values of the leukocyte percentages of *S. lucioperca* according to parasitic infestation.

Parasites	Individuals	Lymphocyte (%)	Neutrophil (%)
Eustrongylides excisus	Infested fish	64.5 ± 10.6 [a]	30.5 ± 12.02 [a]
	Non-infested fish	87.03 ± 11.03 [b]	10.2 ± 5.1 [b]
Diplostomum spathaceum	Infested fish	88.04 ± 10.4 [a]	19.43 ± 14.7 [a]
	Non-infested fish	77 ± 14.8 [b]	90.28 ± 6.5 [b]

Rehulka [28] demonstrated that certain infectious skin lesions are accompanied by a number of changes in hematological parameters. There are significant differences in red blood cell parameters and in a whole range of biochemical characteristics between diseased and healthy fish. Hence, research into the clinical haematology of fish may enhance knowledge of pathology, which is needed to improve diagnostic practice and to select the best preventive procedures. There were no significant differences in all haematoloical parameters between infested and non-infested fish expect for percentage of lymphocytes and neutrophils (Table 2). Some haematological parameters (hematocrit, MCV and MCHC) showed reduction trend in *Leporinus macrocephalus* naturally infected

by *Goezia leporini* (Nematoda) [29]. Similar results were described in *Cyprinus carpio*, infected with *Bothriocephalus acheilognathi* [30], whereas no difference in hematocrit was seen in rainbow trout infested with *Lepeophtheirus*.

5. Conclusion

Previous reports by other researchers [31] showed that parasites can act as stress factors which affect hematological parameters such as reduction in RBCs count, Hb value and packed cell volume that may often cause anaemia. Also as a defense mechanism against the parasitic infestation, WBCs count was elevated in the infested fish. The present study provides the first report about haematological parameters of *S. lucioperca* among different age classes and their relationship with parasitic infection.

Competing Interests

This work was financially supported by Islamic Azad University of Agricultural Science and Natural Resource, Islamic Republic of Iran. Otherwise, the authors declare that they do not have any competing interests.

Authors' Contributions

All authors contributed more or less equally to this research work.

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