

Hematological characteristics associated with parasitism in Pikeperch *Sander lucioperca* (Percidae) from Anzali Wetland

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Abstract A total of 120 *Sander lucioperca* (Percidae) were captured and investigated for parasites and also haematological parameters were analysed and compared between infected and uninfected fish. The haematological analysis showed reductions in haematocrit, haemoglobin, red blood cell, whereas with blood cell (WBC) and lymphocyte significantly increased ($P < 0.05$) in infected fish, whereas with blood cell (WBC) and lymphocyte significantly increased ($P < 0.05$) in infected fish. Parasitological inspections revealed the following infestations: *Eustrongylides excisus* (Nematoda), *Dactylogyrus* sp. (Platyhelminthes) and *Achtheres percarum*, *Diplostomum spathaceum* (Platyhelminthes) and *Trichodina* sp. (Ciliophora). The prevalence and intensity of the infection with parasites were varied at age groups of host. Significant differences were found for the white blood cell and lymphocyte in relation to parasitism. Parasitism had no influence on studied blood parameters of *S. lucioperca* in natural conditions.

Keywords Parasitic fauna · Hematology · Anzali wetland · Pikeperch

Introduction

Fish disease defines as an abnormal condition characterized by a gradual degeneration of a fish ability to maintain normal physiologic functions. Diseases are the most serious limiting factors in aquaculture because of increased density of fish in restricted water where the fish pathogens can easily transmit from one fish to another (Brassard et al. 1982). Much of economic loss is however, preventable with proper fish health management (Woo 1998). In recent years, knowledge about parasites of fish has been progressed due to their impact on growth and behaviour of fish and economic losses (Barassa et al. 2003). Haematological parameters are commonly used as a tool for testing health status in fish (Blaxhall 1972). Moreover, qualitative and quantitative variations in haematological parameters are the most significant findings as regard diagnosis (Martins et al. 2004). According to Rolbiecki (2006) fish belonging to different length classes differ in their degree of exposure to parasites, hence abundance and composition of parasitic faunas change with the age. Parasites may often cause anemia, which is characterized by reduced haemoglobin concentration, hematocrit and erythrocyte number (Martins et al. 2004). Therefore, it is necessary to evaluate changes in haematological parameters with various parasites to establish a database to allow precise diagnoses and as a reliable tool for interpretation of treatment or preventive measures, which are vital in fish farming. The objectives of our study were to identify parasites of *Sander lucioperca* and determine changes that occur in the blood in association with parasitism.

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Materials and methods

A total of 120 *S. lucioperca* were captured using seine nets by fishing companies in Anzali wetland (37°28'16"N 49°27'44"E), Guilan Province, Iran. After capture, they were delivered alive to the laboratory of teleost fish researches institute (TFRI) in Anzali port, Iran. The fish were divided into age classes. The age classes were: 2 years old ($n = 29$), 3 years old ($n = 35$), 4 years old ($n = 32$) and 5 years old ($n = 36$). The mean weight and length were 890 ± 4.26 g and 45.4 ± 4.7 cm respectively. Fish were initially examined for the presence of any parasites visible to naked eye. After that, wet mounts of scrapings (body surface mucus from behind the pectoral fin adjacent to the dorsal fin) for parasites using a compound light microscope at $\times 10$ magnification. The methods and techniques used for collection, relaxation, fixation, staining and mounting of parasites are followed by Roberts (2012). The collected parasites separated and placed in a petri-dish containing physiological saline solution (0.6 %). They 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 to observation. The monogeneans were washed in a 0.6 % saline solution and fixed in 70 % ethanol. They were stained with alum carmine, dehydrated and then cleaned in xylene and mounted in Canada balsam. Identification was carried out according to keys suggested by Gussev (1985) and Moravec (1994). Prevalence and mean intensity were calculated according to Bush et al. (1997). The individual blood samples were taken from caudal vessels of anaesthetized specimens using syringes containing EDTA (10 %) and then stored until analysis. Determination of red blood cells (RBC) and white blood cells (WBC) counts were 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 performed with blood smears stained with Giemsa solution. The smears were examined under light microscopy (Olympus, Tokyo) at $100\times$ magnification. Haematocrit was determined using micro-haematocrit capillaries filled with blood, centrifuged at 5000 rpm for 5 min, and expressed as percentage of total blood volume. Hemoglobin content was measured with a spectrophotometer at 540 nm absorbance using the cyanmethemoglobin procedure. The haematological indices including mean cell volume (MCV), mean cell hemoglobin (MCH) and mean cell hemoglobin concentration (MCHC) were calculated according to Haney et al. (1992). Mann–Whitney (U) test was used for normality of data distribution and homogeneity of variance, as well as Kruskal–Wallis (K) analysis were applied to the

data for determine the existence of any meaningful difference in mean intensity of the parasite species. The correlation between prevalence of infection and age of fish were checked using Chi square test. All statistical analyses were performed using the statistical program SPSS 10.0. Data are presented as mean \pm SD.

Results

The parasites *S. lucioperca* are given in Table 1. As seen in Table 1, parasites were found in the following part of fish: Tissue- *Eustrongylides excisus* (Nematoda), Gill- *Dactylogyrus* sp (Platyhelminthes) and *Achtheres percarum* (Arthropoda), Eye- *Diplostomum spathaceum* (Platyhelminthes) and Skin- *Trichodina* sp. (Ciliophora). The values of prevalence, intensity and abundance of parasites, by age, are given in Table 2. The highest prevalence of total parasite was 63.8 % in 2 years old fish (length: 37 cm and weight: 451 g) and the lowest 31 % in 4 old fish (length: 52 cm and weight: 1199 g). Abundance value ranged from 5 to 300. The lowest abundance value (11) was recorded from 3 years old (length: 39 cm and weight: 583 g) fish and the highest value (260) was in 5 years old group (Table 2). Haematological characteristics in infected and uninfected fish of *S. lucioperca* were presented in Table 3. The data represent that haematocrit, haemoglobin, RBC, and MCV were reduced in infected fishes (only MCV had significant differences and other values were not significantly different), whereas WBC and lymphocyte significantly increased ($P < 0.05$) in infected fish.

Discussion

The prevalence of infection and intensity were positively correlated with host age and size (Oniye et al. 2004). This means that as fish grows, chances of infection increase, because a long period of exposition to infective stages, and the amount of food it consumes, which including the larval stages of parasite (Polyanski 1961). According to Luque and Alves (2001), correlation between the host size and parasite prevalence and intensity is a pattern widely recorded in marine fish and documented with numerous cases in freshwater fishes. Barzegar and Jalali (2009) reported *S. lucioperca* and *Perca fluviatilis* (European perch) were infected with *A. percarum* in Sefid-rud River and in Anzali Wetland. Polyanski (1961) reported that the main factors determining the variety of parasite fauna as well as the intensity and prevalence of infection could be related to following reasons; diet of the host, lifespan of the host, mobility of the host throughout its life including the variety

Table 1 Parasitic fauna of *S. lucioperca* in Anzali wetland

Parasites	No. of infected fish	No. of not infected fish	Prv	Mi	Abu
<i>Eustrongylides excisus</i>	85	29	60.25	5.1	3–11
<i>Achtheres percarum</i>	67	48	55.25	3.8	3–9
<i>Trichodina</i> sp.	41	23	85.2	6.7	7–16
<i>Dactylogyrus</i> sp.	88	26	21.8	2.3	2–7
<i>Diplostomum spathaceum</i>	56	24	40.12	3.4	5–8

Io infected organ, *Prv* prevalence (%), *Mi* mean intensity, *Abu* abundance

Table 2 Comparative prevalence, mean intensity and abundance of parasites at different age groups of *S. lucioperca*

Parameters	2	3	4	5
<i>Eustrongylides excisus</i>	0.2a	0	0.08b	0
	3.0 ± 0a	0	1 ± 0b	0
	0–3	0	0.1	0
<i>Achtheres percarum</i>	0	0.14	0	0.12
	0	1.0 ± 0	0	2.0 ± 0
	0	0–1	0	0–2
<i>Trichodina</i> sp.	0.2a	0.14b	0.41c	0.12a
	3.0 ± 0	1.0 ± 0	4.8 ± 1.48	2.0 ± 0
	0–3	0–6	3–7	0–2
<i>Dactylogyrus</i> sp.	0.4a	0.14b	0.25c	0.12d
	4.0 ± 2.12a	2.0 ± 0b	3.33 ± 2.05c	5.0 ± 0c
	3–5	0–2	1–7	0–5
<i>Diplostomum spathaceum</i>	0	0	0.8a	0
	0	0	1 ± 0	0
	0	0	0–1	0
<i>Eustrongylides excisus</i>	0.2	0	0.08b	0
	3.0 ± 0a	0	1 ± 0	0
	0–3	0	0–1	0

Values with alphabetic letters are significantly different ($P < 0.05$)

Table 3 Hematological characteristics in infected and non-infected of *S. lucioperca*

Parameters	Infected fish	Un infected fish
Haematocrit (%)	35.6 ± 7.2a	32.5 ± 6.4b
Haemoglobin (gr/dL)	7.1 ± 1.2a	6.7 ± 1.3b
Red blood cell (mm ³)	185.12 ± 64.5a	167.5 ± 49.6b
White blood cell (mm ³)	159.06 ± 106.3a	856.6 ± 55.9
MCV (%)	256.9 ± 18.8a	200.1 ± 13.6
MCH (%)	40.2 ± 12.4a	42.8 ± 15.1a
MCHC (%)	20.4 ± 3a	21.2 ± 3.1a
Lymphocyte (%)	77.8 ± 12.3a	93.5 ± 4.8b
Neutrophil (%)	78.8 ± 11.4a	95.3 ± 8.4b
Monocyte (%)	1.75 ± 1.3a	1.25 ± 1.2a
Myelocyte (%)	1.38 ± 1.8a	1.38 ± 1.6a

Values with alphabetic letters are significantly different ($P < 0.05$)

of habitats it encounters, its population density and the size attained. The subject of organ specificity among fish parasites has been reported by various researches for example, Williams and Jones (1994) demonstrated that host and organ specificity is determined by ecological requirements of the hosts and the parasites. An increase in the intensity of infection with the size of the host was observed in the *Siganus sutor* (Rabbit fish Valenciennes, 1835) and *Selar crumenophthalmus* (Martens and Moens 1995). As shown in previous investigations, parasitic fauna undergo a number of qualitative and quantitative changes accompanying fish growth (Starovojtov 1995). Many parasites actively attack a host. In that case, the host size is the most important factor facilitating infection. Larger fish, with a large body surface, are a target that is easy to attack by parasites especially free-living ones. For example, occurrence of *Ancyrocephalus paradoxus* and *Dactylogyrus amphibothrium* (Monogenea) increased when fish became larger (Rolbiecki 2006). Some authors have analyzed relationships between infection and fish age, for example, Gonzalez-Lanza and Alvarez-Pellitero (1982) found that the prevalence of a *Dactylogyrus* species on the host fish aged 2–7 years was higher than other ages. Koskivaara et al. (1991) found no parasites on roach under 3 years old. Also, a relationship between existence of *Dactylogyrus* sp and host fish size was found by Koskivaara et al. (1991) and Ozturk and Altunel, (2002). An increase in infection of *S. lucioperca*, with *Achtheres paradoxus* accompanying by increase in fish size was reported in Gdansk Gulf (Rolbiecki and Rokicki 1996). In addition, an *A. paradoxus* infection that increased with fish length was also observed in Lake Jamno (Kozikowska et al. 1956) in Gdansk Gulf (Rolbiecki and Rokicki 2000) and in Lake Peurunka (Valtonen et al. 1993). There were significant differences in some haematological parameters between infested and uninfested fish (Table 3). The reduction in RBC count, Hb value and haematocrit in the infested catfish occurred as a result of the parasitic infestation that often leads to anemia (Martins et al. 2004). Furthermore, the parasites simply act as a stressor; and during primary stages of stress the haematocrit changes due to the release of catecholamine, which can mobilize RBCs from spleen (Wells and Webber 1990) or induce RBC swelling as a result of fluid shift into the intracellular compartment (Chiocchia and Motias 1989). Some haematological parameters (hematocrit and MCHC) showed reduce trend in *Leporinus macrocephalus* naturally infected by *Goezia leporini* by the nematod (Martins et al. 2004). On the contrary, Lebelo et al. (2001) reported significant increases in Hb value and haematocrit and non significant increases in RBC counts in striped bass infested with Henneguyosis. It is known that WBCs are normally lower in non-infested fishes and can be used as a significant indicator for infectious diseases. In the present

study, the increase in WBC and lymphocyte quantities in infected fishes was accepted as a response of cellular immune system to parasitic infection. That increase in WBCs counts occurred as a pathological response since these WBCs play a great role during infestation by stimulating the haemopoietic tissues and the immune system by producing antibodies and chemical substances working as defense against infection (Lebelo et al. 2001). In this experiment lymphocyte and neutrophil count with the degree of the parasitic infestation were changed. Other researchers found significant differences for the Ht and MCHC in relation to infested or uninfested by *Trichodinidae*; MCHC in relation to infestation by Copepoda. On the contrary, there were no changes in blood characteristics for *Mugil platanus* (Tainha Günther, 1880, estuarine region of Cananeia, São Paulo, Brazil) infested by trypanosomes (Ranzani-Paiva et al. 1997). Khan (1977) experimentally infested *Gadus morhua* (Atlantic Cod) with *Trypanosoma murmanensis* and then found alterations in blood characteristics.

The current study provides valuable information about parasitic fauna of *S. lucioperca* in Anzali wetland. The entire study reveals that the intensity of infection was responsible for altering the haematology of pikeperch, *S. lucioperca*. Increased number of WBC and lymphocytes values may be associated with the defense mechanism and immunological responses against infectious diseases.

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