Hematological parameters associated with parasitism in pike, *Esox lucius* caught from Anzali wetland

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Abstract This study involved 120 pike, *Esox lucius*, captured from Anzali wetland. Parasite fauna were identified in captured fish. Also, changes of haematological parameters were compared both infected and uninfected fish. Parasitological inspections revealed the following infestations: Skin: *Lerne cyprinacea*, *Argulus foliaceus* (Crustacean) and *Tricodina* sp. (Ciliata). Gill: *Dactylogyrus* sp. (Digenea) and *Tetraonchus monenteron* (Monogenea). Eye: *Diplostomum spathaceum* (Digenea). Gut: *Eustrongylides exises*, *Rhipdocotyle illense*, *Raphidascaris acus* (Nematode), *Corynosoma Strumosum* (Acanthocephala). Most prevalence and intensity were related to *Eustrongylides exises* and *Rhipdocotyle illense*. Following haematological parameters were evaluated: haematocrit, haemoglobin concentration, erythrocyte and leukocyte counts, mean cell volume (MCV), mean cell haemoglobin, mean cell haemoglobin concentration lymphocytes, monocytes and eosinophils. Significant difference was found for MCV between infected and uninfected fish.

Keywords *Esox lucius* · Hematology · Parasitism · Pike · Estuarine region

Introduction

Keeping in view the increasing importance of fish as a cheap source of protein rich diet, parasitic infections in fresh water fish has drawn attention of the fish biologists, ichthyologists and parasitologists under fish pathology. Some haematological indices such as erythrocyte count, hematocrit (HT), and hemoglobin (HB) concentration have been used as markers for evaluating fish health (Houston 1997). The determination of haematological parameters of freshwater fish gives an idea of their physiological status and the influence of various environmental factors (Ranzani-Paiva et al. 1997). Measurement of blood parameters has been used for many years as a tool for monitoring the health of fish. 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 (Sebastiao et al. 2011). Pathological conditions in fish are manifested in the form of changes in the behavior (symptoms) and/or in the integrity of the tissues (lesions), leading to a decrease in weight gain and often, death (Roberts 1981). Parasites, causing little apparent damage in feral fish populations, may become causative agents of diseases of great importance in farmed fish, leading to pathological changes, decrease of fitness or reduction of the market value of fish. A success in aquaculture is keeping health fishes, resistant, to diseases and handling. Fish health maintenance is arising in economic importance. This requires diagnoses and fight against sickness, important step for good quality in research and to fish culture. Ectoparasites are the most common ones infecting Iranian fish both cultured and wild fishes (Barzegar and Jalali 2009). The main objective of the present study was to identify parasites of faunas as well as determine changes that occur in the blood in association with parasitism in pike.

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Materials and methods

One hundred twenty specimens of *E. lucius* were captured using hoop net by fishing companies in Anzali port, Guilan State, Iran. Then each fish was measured in total length (49.2 ± 14.4) and total weight (897.15 ± 75.9). Fish were initially examined for the presence of any parasites visible to the naked eye. Next, 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 $10\times$ magnification. The methods used for collecting, fixing, staining, and mounting of parasite specimens were reported as follows: Regarding Monogenea, fish gills were cut out and examined under a microscope at $40\times$ magnification. Vigorously moving worms were separated from those organs with a pipette and fixed under a cover slip according to Fernando et al. (1972) and Gussev (1983) in ammonium picrate and glycerol-gelatine, respectively. Digenea were collected in a 0.6 % saline solution. The sample was placed with a little saline on a glass slide and appropriate pressure was applied. It was fixed with 90 % alcohol and washed in 70 % alcohol and then stained with alum haematoxylin according to Fernando et al. (1972). Nematodes were compressed between two slides with glacial acetic acid (GAA). This makes the worms transparent and then microscope was used to observation. Fishes were also examined for internal parasites, the abdominal cavity of each fish was cut open and the gut was separated and placed in a Petri-dish containing physiological saline (0.06 NaCl) and the acanthocephalans were found with loop. Identification of parasite specimens was carried out in accordance with the keys presented by Gussev (1985), Moravec (1994) and Yamaguti (1971). Prevalence and mean intensity were calculated according to the definitions given by Bush et al. (1997). The individual blood samples were taken from caudal vessels of infected and uninfected fish respectively using heparinized syringes. Then blood samples were stored in a polystyrene cool bag 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 1,000 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 $100\times$ magnification. Ht was determined using micro HT capillaries filled with blood, centrifuged at 3,000 rpm for 5 min, and expressed as percentage of total blood volume. 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). Mann–Whitney (*U*) test was used for normality of data distribution and homogeneity of variance. Average most prevalence and intensity of parasites were analyzed by Chi square test. All statistical analyses were performed using the statistical program SPSS 10.0. Data are presented as mean $\pm$ SD.

Results

As can be shown in Table 1, ten species were found in *E. lucius* in Anzali wetland region. Three types of the parasites belong to Nematodes; two other types of them belong to Crustacean and Digenea parasites respectively. Also, one genus was *Tricodina* sp. (Ciliate), *Tetraonchus monenteron* (Monogenea) and *Corynosoma Strumosum* (Acanthocephala) respectively. Most prevalence and mean intensity were related to *Eustrongylides exises* and *Rhipdocotyle illense* respectively. Largest numbers of parasites were observed for *Rhipdocotyle illense* which separated from gut. Comparison haematological values between infected fish and uninfected ones revealed a change in MCV index (Table 2).

Discussion

The pike is host to a vast assemblage of parasites. Hoffman (1967) listed the following parasites for this species in North America: fungi, Protozoa, Trematodes, Cestoda, Nematoda, Acanthocephala, leeches, Mollusca, and Crustacea. In this study we observed that pike hosted 10 species either Ectoparasites or endoparasites. Similar to our findings, (Khara et al. 2007) identified ten parasites from pike which caught from Amir Kalaieh lagoon. Number parasites of pike are different in many places, for example; Watson and Dick (2006) were reported pike hosted 18 species of parasites. In another study was found seven parasites in pike of Chamkhaleh River (north-east region, Guilan state, Iran) (Khara et al. 2004). Also, some endoparasites including *Bathybothrium rectangulum* (Cestoda), *Raphidascaris acus*, *Camallanus truncatus* (Nematoda) and *Neoechinorhynchus rutili* (Acanthocephala) were detected in pike inhabiting the Işikli Dam Lake, Turkey (Kir and Ozan 2005). In the present study most prevalent parasite was belonged to *E. exises* (26.66 %), but most prevalence in pike of Chamkhaleh River and Amir Kalaieh lagoon were related to *Raphidascaris acus* (26.9 and 37.39 % respectively). Higher mean intensity in our research was observed for *R. illense*, whereas in Pike captured from Chamkhaleh River was *Raphidascaris acus* (Khara et al. 2004). In our experiment highest range in parasite infection was seen for *R. illense* (3–300). The results of this study

Table 1 Parasitic fauna of pike, *Esox lucius*, captured in Anzali lagoon

Parasites	Locality	Prevalence (%)	Intensity (mean $\pm$ SE)	Range
<i>Lernea cyprinacea</i>	Skin	6.66	1.75 $\pm$ 0.89	1–3
<i>Argulus foliaceus</i>	Skin	6.66	6.12 $\pm$ 3.75	1–3
<i>Trichodina</i> sp.	Skin	3.33	4.25 $\pm$ 2.50	1–7
<i>Dactylogyrus</i> sp.	Gill	6.66	2 $\pm$ 1.41	1–5
<i>Tetraonchus monenteron</i>	Gill	13.33	35.5 $\pm$ 22.88	2–60
<i>Eustrongylides exises</i>	Gut	26.66	18.56 $\pm$ 14.12	1–60
<i>Rhipidocotyle illense</i>	Gut	13.33	88.5 $\pm$ 26.91	3–300
<i>Raphidascaris acus</i>	Gut	6.66	2.875 $\pm$ 2.10	1–7
<i>Corynosoma strumosum</i>	Gut	6.66	7.375 $\pm$ 6.12	1–18
<i>Diplostomum spathaceum</i>	Eye	13.33	2.25 $\pm$ 1.34	1–4

Table 2 Haematological parameters of infected and uninfected in pike

Parameters	Infected fish	Uninfected fish
White blood cells ($\times 10^6$ μ l)	7.3 $\pm$ 1.5	7.09 $\pm$ 1.7
RBC ($\times 10^3$ μ l)	11.3 $\pm$ 3.03	9.8 $\pm$ 1.8
Hemoglobin (g/dL)	6.27 $\pm$ 1.16	5.42 $\pm$ 0.85
Haematocrit (%)	26.8 $\pm$ 5.53	23.02 $\pm$ 2.57
MCV (fl)	264.13 $\pm$ 35.43 ^a	245.02 $\pm$ 23.70 ^b
MCH (pg)	56.50 $\pm$ 8.59	56.46 $\pm$ 5.17
(MCHC) (g/dL)	22.7 $\pm$ 1.31	22.3 $\pm$ 1.22
Neutrophil (%)	35.7 $\pm$ 6.7	36.31 $\pm$ 4.80
Lymphocyte (%)	60.5 $\pm$ 6.8	58.92 $\pm$ 22.4
Monocyte (%)	1.75 $\pm$ 0.76	1.83 $\pm$ 0.78
Eosinophile (%)	1.9 $\pm$ 0.85	1.73 $\pm$ 0.87

Columns with the different alphabetic letters are significantly different $p < 0.05$

confirmed other findings (Khara et al. 2004; Kir and Ozan 2005) which demonstrated that parasite community of pike had much diversity due to piscivorous diet. Blood parameters can be useful for the measurement of physiological disturbances in parasitized fish and thus provide information about the level of damage in the host and the prognosis for the diseases. Also, haematological parameters are widely used indicators of environmental stress in fish. Indices such as HB, HT, red and white cell counts, erythrocyte sedimentation rates, and differential blood smears have all been used as indicators of disease and stress (Blaxhall and Daisley 1973; Wedemeyer and Yasutake 1997). To investigate the fish blood parameters and their changes, the normal rate of these factors must be initially measured in healthy fish. In the present study, no changes were observed for haematological parameters in infected fish compared to uninfected ones. In our experiment no change was observed in differential counts between healthy and infected fish. Changes in WBC and differential counts have been reported to play important roles in the assessment of the state of health of fish (Gabriel et al. 2004). It is known that leukocyte cells are normally lower in healthy fish and could be used as a significant indicator for

infectious diseases. The other parameters including Hb and Ht were greater in healthy pike, but values were not significantly different. The present study revealed that MCV index is more prominently altered by parasite infestations. Shah et al. (2009) reported that change in haematological indices of some carps which infected with helminth parasites. Some haematological parameters (HT, MCV and MCHC) showed reduce trend in *Leporinus macrocephalus* naturally infected by *Goezia leporini* (Nematoda) (Martins et al. 2004). Natarajan and Felix (1987) observed in *Myslus glilio*, contaminated by various parasites, including *Ergasilus* sp. a drastic reduction of RBC, Hb and Ht, with an elevation in MCV, MCH and MCHC as compared to the not infested fish. In contrast to our findings Van Der Heijden et al. (1996) determined an increase in the numbers of lymphocyte and granulocyte cells of fish infected with *A. crassus*.

Conclusion

The current research provides important information about parasitic fauna of pike, in Anzali wetland. This work is the

first report about parasitic fauna and its relationship with haematological parameters of pike in Anzali wetland.

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