Haematological characteristics associated with parasitism in bream, *Abramis brama orientalis*

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Abstract A parasitological investigation was done on 175 specimens. Infections of *A. brama orientalis* were analyzed according to the age and sex. The fish also were examined for evaluation changes of haematological parameters in relation to parasitic infection. Four parasites were found, including—*Caryophyllaeus laticeps* and *Ligula intestinalis* (Cestoda), *Diplostomum spathaceum* (Platyhelminthes) and *Trichodina* sp. (Ciliophora). Among identified parasites maximum prevalence and mean intensity were related to *Ligula intestinalis* and *Caryophyllaeus laticeps* respectively. The values of prevalence and mean intensity showed significant differences among ages. Our results revealed prevalence, mean intensity and abundance had not significant difference between males and females. Parasite infection provoked reduction ($P < 0.05$) in haematocrit, mean cell volume and lymphocyte. On the other hand, significant increase ($P < 0.05$) in white blood cell (WBC), mean cell haemoglobin concentration and neutrophil in blood of infected fish was observed. Significant differences were detected for the WBC, lymphocyte and neutrophil (infected versus uninfected by *Trichodina* sp., *Diplostomum spathaceum* and *Caryophyllaeus laticeps*). In addition to WBC and lymphocytes, significant change was

observed for the haemoglobin (Hb) (infected versus uninfected by *Ligula intestinalis*).

Keywords Parasites · Haematological parameters · Infection · Bream

Introduction

The parasites generally attack fishes largely and destroy them by making wounds or diseases on their flesh and thus making them inedible. The damage caused on the host depends on parasite species, type of spoliation of host tissue, number of parasites and the health status of the host (Tavares-Dias et al. 1999). 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). According to Rolbiecki (2006) fish belonging to different length classes differ in their degree of exposure to parasites, hence abundance and composition of parasitic fauna change with the age. Changes of the parasite fauna of a host with age have been studied by some workers, with length and weight of fish being used as indication of age (Sinha and Srivastava 1958). Generally, reports about the susceptible host sex to infection are varied and conflicting. Such studies have generally been used as an effective and sensitive index to monitor physiological and pathological changes in fishes (Chekrabarty and Benerjee 1988). Changes associated with haematological parameters due to various parasites establish a data base and allow precise diagnosis guiding the implementations of treatment or preventive measures which are indispensable in fish farming and fish industry (Roberts 1981). Indices such as haemoglobin, Ht, red and white cell counts and differential

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blood smears have all been used as indicators of disease and stress (Wedemeyer and Yasutake 1977). Parasites may often cause anemia, which is characterized by reduced haemoglobin and hematocrit concentrations and erythrocyte number. Studies on haematological alterations fish has been performed on some species such as *Cyprinus carpio* (Ranzani-Paiva et al. 1987) and *Piaractus mesopotamicus* (Tavares-Dias et al. 1999) infected with *Argulus* sp.; *Mugil platanus* infected with *Trypanosoma*, *Hemogregarina*, *Trichodina*, Monogeneoidea, copepods and Hirudinea (Ranzani-Paiva et al. 1997) and *Oreochromis niloticus* infected with *I. multifiliis* (Tavares-Dias et al. 2002). Despite the importance of bream as a highly valued commercial species, little information is available about parasitic infections of *A. brama orientalis* at Anzali lagoon. The objectives of our study were to identify parasites of *A. brama orientalis* and determine changes that occur in the blood in association with parasitism.

Materials and methods

A total of 175 *A. brama orientalis* were collected from October 2010 to August 2011 using hoop net at Anzali port, Guilan State, Iran. Then each fish was measured in total length (49.2 ± 14.4) and total weight (897.15 ± 75.9). The fishes were delivered alive to the laboratory of teleost fish researches institute (TFRI) at Anzali port, Iran. The fish were divided into four age classes as follow: 2-year old: mean length 24.3 ± 2.90 cm; mean weight: 178.2 ± 56.6 g, 3-year old: mean length 27.5 ± 3.4 cm; mean weight: 254.3 ± 76.9 g, 4-years old: mean length 29.7 ± 2.8 cm; mean weight: 321.6 ± 61.9 g, 5-years old: mean length 32.9 ± 2.5 cm; mean weight: 438.4 ± 55.4 g. Also sex was determined internally. 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 (Olympus, Tokyo, Japan) at $10\times$ magnification. The methods used for collecting, fixing, staining, and mounting of parasite specimens were reported as follows: The digeneans were washed in a 0.6 % saline solution and fixed in 70 % ethanol alcohol. They were stained with alum carmine, dehydrated and then cleaned in xylene and mounted in Canada balsam. 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 cestodes were found with loop. Identification was carried out in accordance with the keys Gussev (1985) and Moravec (1994). Prevalence and mean intensity were calculated according to Bush et al. (1997).

Blood examination

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 RBC and 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. Hematocrit (Ht) was determined using micro hematocrit capillaries filled with blood, centrifuged at 3,000 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).

Data analysis

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 χ^2 test. All statistical analyses were performed using the statistical program SPSS 10.0. Data are presented as mean $\pm$ SD.

Results

The parasites of *A. brama orientalis* are given in Table 1. As seen in Table 1, parasites species were found in the respective organs: Intestine—*Caryophyllaeus laticeps* and *Ligula intestinalis*, Skin—*Trichodina* sp., Eye—*Diplostomum spathaceum*. The values of prevalence, intensity and abundance of parasites, by age, are given in Table 2. The highest prevalence of total parasite was 41 % in 4 years old fish and the lowest was 29 % in 2 year individuals. The highest mean intensity of parasites was 24.4 in 5 years old fish (length: 32.9 ± 2.5 cm and weight: 438 g) and the lowest was 15.8 in 2 year individuals (length: 24 cm and weight: 178 g). Abundance value ranged from 4 to 6. The lowest abundance value (4) was recorded from 4 year old group (length: 29 cm and weight: 321 g) and the highest value (6) was in 5 years old group (length: 32.9 ± 2.5 cm and weight: 438 g). Prevalence, mean intensity and

Table 1 Intensity and prevalence of parasite of *A. brama orientalis*

Parasites	Infected organ	Prevalence (%)	Mean intensity
<i>Caryophyllaeus laticeps</i>	Intestine	56	1.75 ± 0.89
<i>Ligula intestinalis</i>	Intestine	73	3.9 ± 0.41
<i>Trichodina</i> sp.	Skin	22	2.6 ± 0.35
<i>Diplostomum spathaceum</i>	Eye	23	2.25 ± 1.34

Table 2 Comparative prevalence, mean intensity and abundance of parasites in different age groups of *A. brama orientalis*

Age of fish (year)	No. of hosts		Prevalence (%)	Mean intensity	Abundance (range)
	Examined	Infected			
2	38	29	29	15.8	5
3	70	57	35	19.4	6
4	42	33	41	18.9	4
5	25	19	39	24.4	6

Fig. 1 Comparative prevalence, abundance and mean intensity of parasites between males and females

abundance of parasites by host sex are shown on Fig. 1. There was no variation in prevalence, mean intensity and abundance with sex of the host; females had slightly higher intensity, but values were not statistically different. Also a similar pattern was observed for male's fish in values of abundance. Haematological characteristics in infected and uninfected of *A. brama orientalis* are presented in Table 3. The values of Ht, MCV and lymphocyte are influenced by parasitic infection and a reduction was observed in infected fish. On the other hand, significant increase in WBC, MCHC and neutrophil in blood of infected fish was observed. Significant differences were found for the following variables: WBC, lymphocytes and neutrophil in relation to infested or not infested by all detected parasites (Tables 4, 5). Also, significant differences were found for Hb in relation to infection or uninfected by *Ligula intestinalis*.

Discussion

A few parasites species have been found in a wide range of fresh or brackish water fish species throughout the world.

In this study various parasite species were found in *A. brama orientalis* including *Caryophyllaeus laticeps* and *Ligula intestinalis*, *Diplostomum spathaceum* and *Trichodina* sp. Among them, *Diplostomum spathaceum* is a very common parasite in the freshwater fishes of Iran. Moreover, it has been frequently reported from fishes in different regions of Iran (Barzegar et al. 2008; Azadikhah et al. 2009). Karatoy and Soylu (2006) found ten metazoan parasites in bream, *A. brama* in the Lake Durusu. Also, Li et al. 2010 investigated parasites of *A. brama orientalis* in Ergis River, China and eight parasites were found. Host size serves as a factor in determining the burden of parasitic communities in a host. An increase in size is a reflection of increase in length which is considered a measure of age (Torres et al. 1977). The prevalence and intensity positively correlated with host age and size (Oniye et al. 2004). This study showed that prevalence, mean intensity and abundance were higher in 5 years old of *A. brama orientalis* have got higher than other age groups. As reported by Roberts (1978), bigger fishes provide larger surface area for infection than smaller ones. It is therefore a plausible explanation that the big fish provides a good ground for the parasites to multiply over time. Vankara et al. 2011 found that parasite richness and diversity are poorly correlated with the host size. Blackburn and Lawton (1994) and Blackburn and Gatson (1997) documented relationship between parasite abundance and body size. Machado et al. (1994) found that increase in size and age of fish led to significant increase in the levels of parasitism. Szalai and Dick (1990) mentioned that the *Contracaecum* spp. larvae were absent in age 0 and age 1 bass (*Micropterus salmoides*), but prevalence and mean intensity increased with age, for bass age 2 or older. Host sex is one of the biotic factors which are important in studying host-parasite relationships. Effects of host sex on the levels of parasitism was studied by Thomas (1964) which might

Table 3 Haematological characteristics in infected and uninfected of *A. brama orientalis*

Parameters	Infested fish	Not infested fish
Haematocrit (%)	24.4 ± 3.1 ^b	31.4 ± 5.1 ^a
Haemoglobin (gr/dL)	7.7 ± 1.01	7.1 ± 1.3
Red blood cell (mm ³)	244.4 ± 29.1	243.9 ± 29.6
White blood cell (mm ³)	173.09 ± 219 ^a	130.5 ± 24.1 ^b
MCV (%)	117.4 ± 12.8 ^b	128.2 ± 16.6 ^a
MCH (%)	32.1 ± 5.4	29.4 ± 5.1
MCHC (%)	27.5 ± 4.7 ^a	22.7 ± 4.6 ^b
Lymphocyte (%)	75.7 ± 6.3 ^b	86.5 ± 4.2 ^a
Neutrophil (%)	23.5 ± 6 ^a	11.4 ± 3.8 ^b
Monocyte (%)	1.12 ± 1.3	1.21 ± 0.95

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

Table 4 Values of the white blood cell and lymphocytes of *A. brama orientalis* according to the degree of the parasitic infestation

Parasites	WBC ($\times 10^3$ mm)	Lymphocyte (%)
<i>Caryophyllaeus laticeps</i>		
Infected	155.5 ± 21.6	89.9 ± 4.8
Uninfected	172.6 ± 24.5	75.8 ± 7.02
<i>Ligula intestinalis</i>		
Infected	177.70 ± 18.7	74.5 ± 6.1
Uninfected	147.8 ± 26.6	82.1 ± 5.6
<i>Trichodina</i> sp.		
Infected	167.27 ± 24.5	63.7 ± 6.5
Uninfected	176.67 ± 25.03	77.5 ± 7.1
<i>Diplostomum spathaceum</i>		
Infected	166.22 ± 25.3	63.3 ± 5.4
Uninfected	181.40 ± 19.6	76.5 ± 6.9

Table 5 Values of the neutrophil and haemoglobin of *A. brama orientalis* according to the degree of the parasitic infestation

Parasites	Neutrophil (%)	Hb (g/dL)
<i>Caryophyllaeus laticeps</i>		
Infected	15.7 ± 4.5	27.6 ± 7.2
Uninfected	23.3 ± 6.8	21.7 ± 4.7
<i>Ligula intestinalis</i>		
Infected	24.8 ± 5.6	6.8 ± 1.01
Uninfected	16.2 ± 5.5	7.4 ± 1.1
<i>Trichodina</i> sp.		
Infected	25.8 ± 6.4	7.8 ± 0.9
Uninfected	21.3 ± 6.5	7.6 ± 1.1
<i>Diplostomum spathaceum</i>		
Infected	29.3 ± 5.6	26.8 ± 4.7
Uninfected	21.7 ± 6.8	27.3 ± 4.9

be due to the physiological and biological factors and behavioral difference between males and females which produce a small albeit consistent sexual trend in infection levels. There was slightly significant difference between the infection of males and females. It can also be noted that even though the difference is not statistically significant.

Males fish were more infected than females ones. Females are more likely to be heavily infected when compared to males which might be due to the stress caused during reproductive periods and lead into behavioral changes, thus making them more vulnerable to heavy parasitization. In contrast, Folstad and Karter (1992) found more

parasitization in males which might be due to high levels of testosterone which causes immunosuppression making them more susceptible to parasites than females. In the present study, host sex had no influence on parasitization. Ranzani-Paiva et al. (1997) found males of *Mugil platanus* had higher infestation percentage than the females. Studies should be developed to better establish whether the higher percentage of infestation is really due to the sex of the fish, or to the environment they live in. In the present study, infection *A. brama orientalis* was altered Ht, MCV and lymphocyte. Similar results were described in carp infected with *Bothriocephalus acheilognathi* (Sopinska 1985), while no difference in hematocrit values were seen in rainbow trout infested with *Lepeophtheirus salmonis*. Anemia was also associated with *Heteropneustes fossilis* infected with metacercariae (Murad and Mustafa 1988) and carp infected with *Myxobolus artus* (Yokoyama et al. 1996). An increase in WBC, MCHC and neutrophil of infested fish was detected. It is well recognized that leukocyte cells are normally lower in healthy fish and could be used as a significant indicator for infectious diseases. Natarajan and Felix (1987) observed in *MySlus glilio* contaminated by various parasites, including *Ergasilus* sp., a drastic reduction of, Hb and Ht, with an elevation in MCV, MCH and MCHC as compared to the uninfected fish. No significant alteration was shown as to erythrocyte, leukocyte count, haemoglobin concentration and monocyte percentage of *Leporinus macrocephalus* naturally infected by *Goezia leporini*. The entire study reveals that the intensity of infection was responsible for altering the hematology of bream, *A. brama orientalis*.

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