www.trjfas.org
ISSN 1303-2712
Turkish Journal of Fisheries and Aquatic Sciences 16: 585-596 (2016) DOI: 10.4194/1303-2712-v16_3_28

RESEARCH PAPER

Histopathology and Blood Parameters of Bogue Fish (*Boops boops*, Linnaeus 1758) Parasitized by *Ceratothoa oestroides* (Isopoda:Cymothoidae)

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Received 26 April 2016
Accepted 01 June 2016

Abstract

Bogue fish (*B. boops*, Linnaeus 1758) were captured using a seine net in the Lagoon of Lapseki, located in the Dardanelles, Turkey. We examined a total of 200 fish and assessed the hematological, biochemical, immunological parameters and histopathology of the buccal cavity of them (40 of them not parasitized, 40 of them parasitized). No significant difference was found between parasitized and not parasitized fish groups in terms of the average biometric indices, body weight and length values. Blood leucocytes counts, haemoglobin and hematocrit values, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, glucose, triglyceride, total protein, globulin, aspartate aminotransferase, NBT and lysozyme activity significantly reduced and bilirubin, alkaline phosphatase, creatine kinase, lactate dehydrogenase and myeloperoxidase activity significantly increased in *C. oestroides* parasitized fish compared to not parasitized ones. Infestation by this parasite resulted in histopathological manifestations such as hemorrhage, edema, necrosis, deformation in striated muscle cells, hypertrophy in chondrocytes and epithelial cells, mononuclear cell infiltration. According to the results obtained in the present study, it can be suggested that blood and histopathological variations influenced by parasites in the bogue fish can cause the fish to be more susceptible to pollutants, predators and diseases."

Keywords: Bogue fish, *Ceratothoa oestroides*, blood parameters, histopathology, Lapseki Lagoon (Turkey).

Kupez Balığı (*Boops boops*, Linnaeus, 1758)' nın Bazı Kan Parametreleri ve Histopatolojisine *Ceratothoa oestroides* (Risso, 1826) Parazitinin Etkisi

Özet

Bu çalışmada kullanılan kupez balığı (*Boops boops*) Çanakkale Lapseki Dalyanı'ndan (Türkiye) uzatma ağı ile avlanmıştır. Toplamda 200 adet balık örneklenmiş ve bu balıkların hematolojik, biyokimyasal ve immünolojik parametreleri ile ağız boşluğu histolojisi değerlendirilmiştir (40 adet parazitli, 40 adet parazitsiz). Çalışmada parazitli ve parazitsiz balıkların ortalama biyometrik indeksileri, vücut ağırlıkları ve boy verileri arasında istatistiksel açıdan önemli bir farklılık olmadığı bulunmuştur. Çalışmada parazitli balıkların beyaz kan hücre sayısı, hemoglobin ve hematokrit değerleri, eritrosit başına düşen ortalama hemoglobin, eritrosit başına düşen ortalama hemoglobin konsantrasyonu, glikoz, trigliserit, toplam protein, globulin, aspartat aminotransferaz, NBT ve lizozim aktiviteleri önemli oranda azalırken, bilirubin, alkalen fosfataz, kreatin kinaz, laktat dehidrogenaz ve myeloperoksidaz aktivitesi parazitli olmayanlara göre önemli derecede artış göstermiştir. Parazitli balıklarda hemoraji, ödem, nekroz, çizgili kas hücrelerinde deformasyon, mononuklear hücre infiltrasyonu, ve dokunun total yapısında defektler ve deformasyonlar gibi histolojik bulgular tespit edilmiştir.

Çalışma sonucunda elde edilen bulgulara göre parazitin kupez balığının kan ve histopatolojik parametrelerinde neden olduğu değişimler balığın doğal ortamındaki diğer hastalıklara, kirleticilere ve predatörlere karşı daha duyarlı olmasına neden olabilir.

Anahtar Kelimeler: Kupez, *Ceratothoa oestroides*, kan parametreleri, histoloji, Lapseki Dalyanı (Türkiye).

Introduction

Cymothoids are ectoparasite isopods of the sea, freshwater and brackish water teleost fish. Cymothoids individuals generally live on various commercially important fish species and their families are spread mainly in tropical and subtropical regions (Brusca, 1981). It is known that *C. oestroides*, which is one of the most commonly seen parasites of Cymothoidae family (Vagianou *et al*., 2006), live on

various fish families such as Sparidae, Carangidae, Clupeidae, Maenidae, Scorpaenidae and Mugilidae (Charfi-Cheikhrouha *et al*., 2000). *C. oestroides*, which bears prostandric hermaphrodite quality, spends all its life on the fish (Mladineo, 2003). This parasite, which is male at the first developmental stage, transitions into female later on. Male and female *C. oestroides* generally coexist on the same host. It is known male *C. oestroides* locates into the buccal cavity of the hosts since their swimming ability is decreased and accordingly lose their quality to pass to a different host at the puberty (adolescence) period (Mladineo, 2003; Gökpınar *et al*., 2009). Particularly in the fish on which *C. oestroides* is detected, weight loss, inertia, hemorrhage on the operculum, respiratory difficulty, burning in the gills and focal necrosis are reported (Horton and Okamura, 2001; Varvarigos, 2003; Korun and Akaylı, 2004). Besides, it is known that this parasite might result in growth retardation in the fish mature enough to be put into the market (Sarusic, 1999) and even might cause death in small fish which are infected by a vast number of parasites (Vagianou *et al*., 2006).

Fish blood has recently been a topic underlined by the researchers. Blood parameters are an important tool in monitoring the diseases and health conditions and physiology of the fish in natural and culture environment and assessing their immune system (Aldrin *et al*., 1982; Viljoen and Van vuren, 1991; Ballarin *et al*., 2004; Tavares-Dias and Moraes, 2004; 2007; Fazio *et al.*, 2015a; 2015b).

Infestation by *Argulus sp.* (Shimura *et al*., 1983; Ranzani-Paiva *et al.*, 1987; Ruane *et al*., 1999; Tavares-Dias *et al*., 1999; Haond *et al*., 2003*), Ichthyophthirius multifiliis* (Tavares-Dias *et al*., 2002), *Argulus sp., I. multifilis* and *Dactylogyrus vastator* (Küçükgül Güleç and Şahan, 2010), *Henneguya branchialis* (Sabri *et al*., 2009), *Caligus rogercresseyi* (Peña-Rehbein *et al*., 2013), *Dolops carvalhoi* (Tavares-Dias *et al*., 2007), *Dactylogyridae, Urocleidoides ermitus, Anacanthorus sp*., (Correa *et al*., 2013), *Clinostomum complanatum* (Kaur *et al*., 2012), *Trachelobdella lubrica* (Çelik and Aydın, 2006), *Monogenea, Protozoa, Crustacea* (El-Seify *et al*., 2011), *Anacanthorus penilabitus, Piscinoodinium pillulare, I. multifiliis, P. pillulare* (Tavares-Dias *et al*., 2008) can influence blood parameters in different host fish. Only one study which investigates the impact of *C. oestroides* on the blood parameters of the fish is available. This study reported that this parasite caused posthemorrhagic anemia in *Dicentrarchus labrax* fish (Horton and Okamura, 2003).

In addition to affecting the blood parameters of the fish, ectoparasites adversely affect the health of the fish by causing histopathologic damage in the fish tissues where they locate (Timur *et al*., 2005; Korun, 2006; Koyuncu, 2006; Bamidele, 2007; Adeyemo and Agbede, 2008; Raissy and Ansari, 2011). Fish ectoparasites commonly lead to acute or chronic inflammation, degenerative changes, necrosis arising out of vascular pathology (hemorrhage) and hyperplasia (Feist and Longshaw, 2008).

Bogue is a delicious and abundant fish species in Turkish waters that belonging to the family Sparidae and has a significant role in the economy of the Country. Its natural infestation with *C. oestroides* was reported previously in a study performed in the North-East Atlantic, Western Mediterranean and Eastern Mediterranean (Pérez-del Olmo et al., 2007; Ramdane *et al*., 2013). Only one histopathological study on the impact of *Meinertia (=Ceratothoa) oestroides*, located into the buccal cavity of the fish was found in the literature (Romestand and Trilles, 1977a). Romestand and Trilles (1977b) also studied the haematological blood values of wild bogue fish parasitized by *C. oestroides*. However, no study on the impact of *C. oestroides* on the serum biochemical and immunological parameters of bogue fish naturally parasitized by *C. oestroides*. Therefore the aim of this study is to evaluate the haematological, biochemical, immune parameters and histopatology of bogue fish naturally parasitized by *C. oestroides*.

Material and Methods

Bogue fish (*B. boops*, Linnaeus 1758) were captured using a seine net in the Lagoon of Lapseki, located in the Dardanelles, Turkey in 2013 (June). We examined a total of 200 fish (mean weight=50.18±0.96) and assessed the hematological, biochemical, immunological parameters and histopathology of the buccal cavity of them (40 of them not parasitized, 40 of them parasitized). The body, liver, spleen and bile of each fish were weighed and the total length of each fish was measured after removal of the parasites. During the study, some water quality parameters were measured by YSI MPS 556 probe. Values were found as follows temperature, 23.4±0.45ºC; salinity, 27.9±0.31 ‰; pH, 8.4±0.15; dissolved oxygen, 8.03±0.66 mg L^{-1}.

Prevalence, mean intensity and parasite abundance values were calculated according to Bush *et al*. (1997). The condition factor (CF), hepatosomatic index (HSI), spleensomatic index (SSI), bile somatic index (BSI) and gonadosomatic index (GSI)were calculated for each fish as outlined by White and Fletcher (1985).

Parasitological Examination

Parasitological examination was carried out for the identification of external parasites (*C. oestroides*) on the buccal cavity of the samples. Following blood sampling, all isopods parasites were removed from hosts. Isolated parasites were stored in 70 % ethanol and brought to laboratory for identification based on literature data (Trilles, 1964; 1969).

Blood Sampling

For blood sampling, fish were anesthetized with 20 mg/L clove oil (Harman Business, Istanbul) (Mylonas *et al*., 2005). Fish were well wiped and cleaned in order to avoid mucus mixing into the blood, and then, blood was taken from the fish through the caudal vein by a 5 ml plastic syringe, avoiding any harm to the fish (Kaya *et al*., 2014). Then, a part of blood was transferred to EDTA tubes (MiniCollect® Tube, Austria) for haematological analysis. Another part of blood was harvested in Z serum sep. tubes (MiniCollect® Tube, Austria). Blood tubes were centrifuged at 4000x *g* for 10 min, and blood serum was separated (Kaya *et al*., 2016). Serum samples were stored at -20 °C and below until analysis (30 days).

Haematological Analysis

Red blood cells (RBC, 10^6 mm^3), hematocrit (Hct, %) and haemoglobin (Hb, g/dL) were determined by the methods of Blaxhall and Daisley (1973). RBC was counted with a Thoma hemocytometer after dilution of the blood sample with a Dacie's diluting fluid. Hct was determined through hematocrit centrifuge. Hb concentration was determined by spectrophotometry (540 nm) via cyanmethemoglobin method. Differential leukocytes were examined with May-Grunwald–Giemsa stained peripheral blood smears. Each slide was examined under oil-immersion at 100 X. For each slide, 100 leukocytes were identified as lymphocytes (LYM), neutrophils (NEU) and monocytes (MON) (Yılmaz *et al*., 2014).

Biochemical Analysis

The biochemical parameters that were detected during the test included glucose (GLC), cholesterol (COL), triglyceride (TG), albumin (ALB), globulin (GLO), total bilirubin (TBIL), total protein (TP), urea, uric acid (UA), creatinine (CRE), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), creatine kinase (CK), lactate dehydrogenase (LDH), phosphorus (P), magnesium (Mg), calcium (Ca), iron (Fe) and chlorine (Cl). Serum biochemical indices were determined through bioanalytic test kits (Bioanalytic Diagnostic Industry, Co) by a shimadzu spectrophotometer (PG Instruments, UK) (Yılmaz *et al*., 2014).

Immunological Analysis

The respiratory burst of the neutrophils and monocytes was quantified by the reduction nitroblue tetrazolium (NBT) to formazan as a measure of the production of oxygen radicals (Siwicki and Anderson, 1993). Serum lysozyme was assessed using the turbidometric assay (Ellis, 1990). Total myeloperoxidase content in the blood serum was measured according to Quade and Roth (1997).

Histopathological Examinations

The tissue complex surrounding the buccal cavity was removed by dissecting the 40 parasitized and 40 not parasitized bogue fish, blood samples of which were taken for histopathological examination. Upon 12-hour fixation of the tissue samples within Bruin's solution, they were kept within 70% ethyl alcohol. 5-8 µm sections were stained with haematoxylin & eosin preparing paraffine blocks following routine histological procedures (Mills *et al*., 1992). Finally, histological imaging of the preparations was carried out using a camera mounted on an Olympus BX51 light microscope and the findings were analyzed through DP2-BSW software.

Statistical Analysis

Each value was expressed as mean ±standard error of mean (SEM) for each measured parameter. Body weight, body length and Blood values of control and parasitized fish were analyzed by student's *t test*. The others were analyzed by Mann–Whitney U tests. All statistical analyses were performed using SPSS 17.0 packaged software (Logan, 2010). Differences were considered to be significant at $P < 0.05$.

Results

During the present investigation, 200 specimens of *B. boops* were examined. Out of 200 specimens, 110 (prevalence of 55%) were found infected by *C. oestroides* (Isopods: Cymothoidae). In June, 150 *C. oestroides* specimens were obtained from the buccal cavity of *B. boops*. The mean intensity and abundance of isopods in the fish were recorded as 1.36 and 0.75, respectively. However, no significant difference was found between the parasitized and unparasitized fish groups in terms of the average hepatosomatic index (HSI), spleensomatic index (SSI), bile somatic index (BSI), gonadosomatic index (GSI), condition factor (CF), body weight and length (Table 1).

The haematological parameters of not parasitized and *C. oestroides* infected *B.boops* were presented in Table 2. The RBC, Hb, Hct, MCH and MCHC significantly reduced ($P < 0.05$) in *C. oestroides* infected fishes compared to healthy ones. We found insignificant differences ($P > 0.05$) between parasitized and not parasitized fish in terms of MCV, LYM, NEU, MON and EOS (Table 2).

Biochemical blood parameters of not parasitized and naturally parasitized bogue fish were shown in Table 3. The GLC, TG, TP, GLOB and AST significantly decreased ($P < 0.05$) and TBIL, ALP, CK and LDH significantly increased ($P < 0.05$) in parasitized *B. boops* in comparison to not parasitized individuals. COL, ALB, Urea, UA, ALT, P, Mg, Ca, Fe and Cl did not differ compared to unparasitized fish (Table 3).

The changes of innate immune parameters for parasitized and not parasitized bogue fish are presented in Figure 1. The NBT, LA and MPO activities were observed as 1.81±0.09 mg NBT formazan/ml, 432.00±29.73 U/ml and 31.28±3.83 U/L in not parasitized fish and 0.99±0.03 mg NBT formazan/ml, 266.00±38.68 U/mL and 69.13±3.30 (U/L) in infected fish, respectively. The decrease in

Table 1. The weight and length, condition factor (CF), hepatosomatic index (HSI), spleensomatic index (SSI), bile somatic index (BSI), gonadosomatic index (GSI) in the studied Bogue fish (*B. boops*)

Characteristic	Not parasitized mean±SE (n=40)	Parasitized fish mean ±SE (n=40)
[a]Body weight (g)	49.16±2.68 [a]	50.92±3.81 [a]
[a]Body length (cm)	16.87±0.35 [a]	17.32±0.43 [a]
[b]CF	1.01±0.04 [a]	0.95±0.02 [a]
[b]HSI	0.94±0.05 [a]	0.96±0.05 [a]
[b]SSI	0.06±0.01 [a]	0.07±0.02 [a]
[b]BSI	0.15±0.01 [a]	0.12±0.01 [a]
[b]GSI	0.54±0.06 [a]	0.58±0.06 [a]

a student's *t test*, b Mann–Whitney U tests were used for comparative analysis, the criterion for significance was set at P<0.05.

Table 2. Hematological parameters of not parasitized and parasitized bogue fish with *C. oestroides*

Parameters	Not parasitized fish (n=40)	Parasitized fish (n=40)
RBC ($\times 10^6$ mm^3)	2.15±0.06[a]	1.26±0.07[b]
Hb (g/dl)	6.96±0.36[a]	2.63±0.09[b]
Hct (%)	42.50±1.36[a]	25.38±1.10[b]
MCV (fl)	197.56±2.17	202.69±5.93
MCH (pg)	32.43±1.47[a]	21.40±1.51[b]
MCHC (%)	16.45±0.84[a]	10.51±0.52[b]
LYM (%)	64.50±7.09 [a]	68.00±3.69 [a]
NEU (%)	5.00±1.32 [a]	7.33±1.94 [a]
MON (%)	2.50±1.15 [a]	2.17±0.98 [a]
EOS (%)	28.00±5.93 [a]	22.5±2.14 [a]

RBC, red blood cells; Ht, haematocrit; Hb, haemoglobin; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; LYM, lymphocytes; NEU, neutrophils; MON, monocytes; EOS, eosinophils.
All values are means ± the standard error. Student's *t test* was used for comparative analysis, the criterion for significance was set at P<0.05.

Table 3. Biochemical blood parameters of not parasitized and parasitized bogue fish with *C. oestroides*

Parameters	Not parasitized fish (n=40)	Parasitized fish (n=40)
GLC (mg/dl)	58.38±5.24[a]	26.35±6.80[b]
COL (mg/dl)	80.53±10.68 [a]	65.87±0.96 [a]
TG (mg/dl)	126.95±2.90[a]	91.13±7.92 [b]
TP (g/dl)	12.76±0.80[a]	8.77±0.91 [b]
ALB (g/dl)	1.73±0.11 [a]	1.69±0.14 [a]
GLOB (g/dl)	11.04±0.91[a]	7.08±0.84 [b]
TBIL (g/dl)	0.11±0.02 [b]	0.18±0.02 [a]
UREA (mg/dl)	0.06±0.04 [a]	0.10±0.01 [a]
UA (mg/dl)	0.37±0.18 [a]	0.36±0.07 [a]
CRE (mg/dl)	1.83±0.44 [a]	1.50±0.25 [a]
AST (U/L)	270.46±56.92 [a]	69.48±11.81 [b]
ALT (U/L)	68.10±13.97	59.21±1.40
ALP (U/L)	32.17±5.12 [b]	77.81±5.84 [a]
CK (U/L)	52.31±9.65 [b]	363.63±68.33 [a]
LDH (U/L)	285.81±50.06 [b]	539.00±21.38 [a]
P (mmol/L)	1.60±0.33 [a]	1.29±0.38 [a]
Mg (mmol/L)	1.21±0.01 [a]	1.44±0.21 [a]
Ca (mmol/L)	4.68±0.46 [a]	7.29±0.99 [a]
Fe (µg/dl)	105.55±8.65 [a]	99.86±5.03 [a]
Cl (mmol/L)	235.33±10.19 [a]	191.42±34.58 [a]

GLC, glucose; COL, cholesterol; TG, triglyceride; TP, total protein; ALB, albumin; GLOB, globulin; TBIL, total bilirubin; UA, uric acid; CRE, creatinine; AST, aspartate aminotransferase, ALT, alanine aminotransferase; ALP, alkaline phosphatase; CK, creatine kinase; LDH, lactate dehydrogenase; Mg, magnesium; P, phosphorus; Ca, calcium; Fe, iron; Cl, chloride.
All values are means ± the standard error. Student's *t test* was used for comparative analysis, the criterion for significance was set at P<0.05.

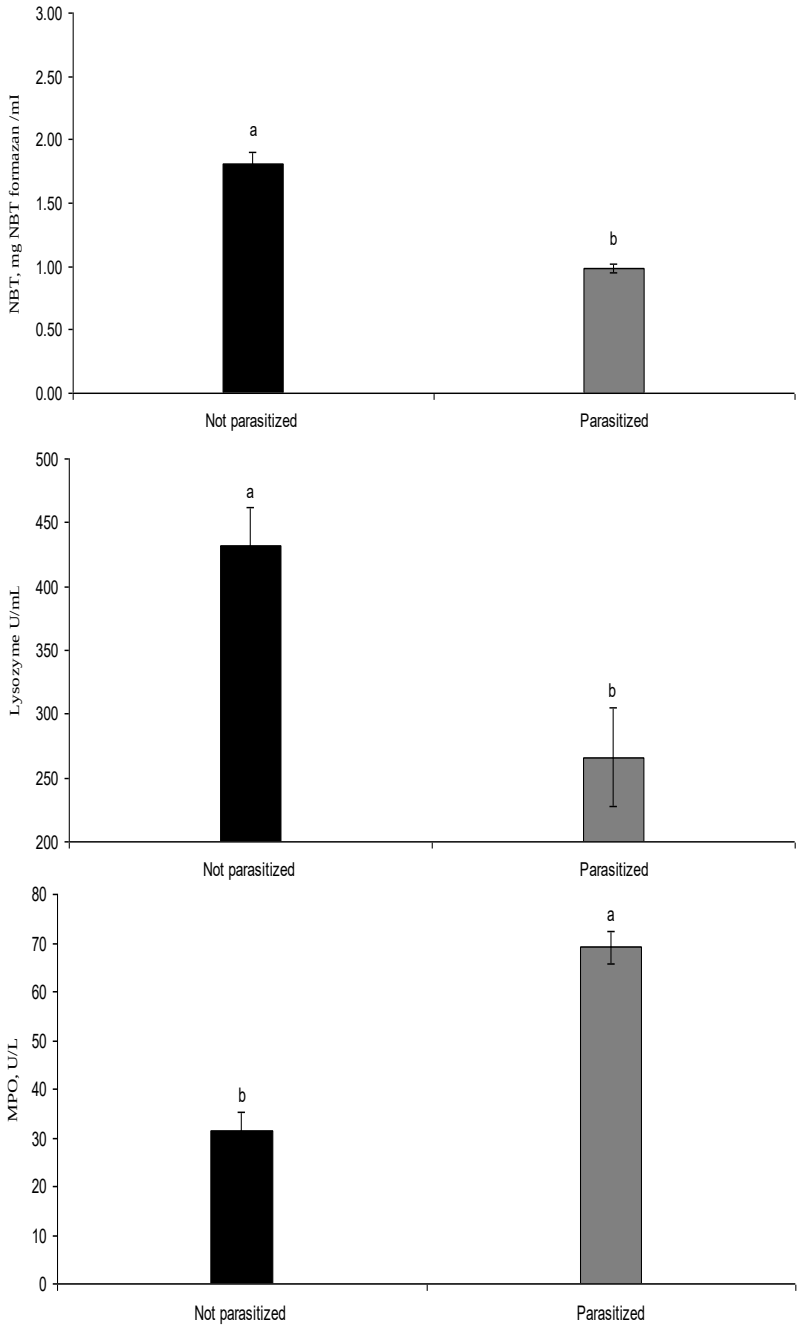

Figure 1. Innate immune parameters of not parasitized and parasitized bogue fish with *C. oestroides*. All values are means ± the standard error. Student's *t test* was used for comparative analysis, the criterion for significance was set at P<0.05.

the NBT and LA activities and the increase in MPO activity of not parasitized fish were found significant compared to those in parasitized fish (P <0.05).

The macroscopical observations in naturally parasitized bogues with *C. oestroides* showed: hemorrhages, inflammation and necrosis of the buccal cavity.

Histopathological examinations did not indicate a histopathological anomaly in the tissue sections that surround the buccal cavity of the not parasitized fish (Figure 2a). However, an increase was observed in the hemorrhagic activity in the sections of the tissue samples parasitized with *C. oestroides* that pass the same area (Figure 2b). It was found out that the increased activity was local. Multi focal hemorrhages were detected in some sections and their intensity was found to be changing. Necrosis and edema were monitored according to the intensity of the infestation (Figure 2c). These histopathological findings demonstrated that the samples were affected by massive infection. Cellular deformations and local hemorrhage were detected in striated muscle cells around buccal cavity (Figure 2d). It was found out that in these sections striated muscle cells and plasma membranes were deformed, cellular borders were lost and partial fusions emerged. Histopathologic changes

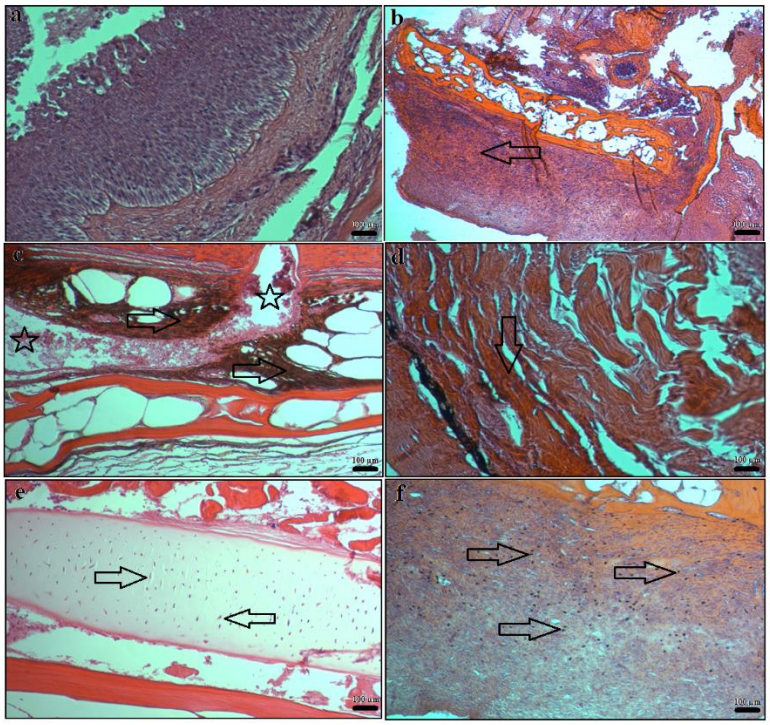

Figure 2. Histological sections regarding control group and the surrounding area of buccal cavity in *B. boops* samples infested with *C. oestroides.* a. Control group, b. Increase in hemorrhagic activity (marked with an arrow), c. Necrosis (marked with an arrow), edema (marked with a star), d. Striated muscle deformations (marked with an arrow), e. hypertrophic chondrocytes (marked with arrows), f. mononuclear cell infiltration (marked with arrows), H&E.

that emerge in the cartilaginous tissue that are commonly seen in the palatal structure are quite important. Particularly, hypertrophic changes observed in the chondrocytes reveal the severity of the infestation (Figure 2e). Severe cartilaginous deformations and hypertrophic chondrocytes were identified in all the parasitized fish in our study. Also the presence of the mononuclear cell infiltrations observed in the sections indicate that there is inflammatory reaction (Figure 2f) in the samples. That eosinophiles and mononuclear leukocytes are intensely seen in the sections of the buccal cavity where the parasite locates show that inflammatory reactions are at serious levels.

Discussion

Blood parameters can be useful in measuring the physiological disorders in the parasitized fish and provide information to us make inferences regarding the diseases and the level of damage in the host (Tavares-Dias *et al*., 2007).

In this study, from among the hematologic parameters of the parasitized bogue fish, a significant decrease was observed in the number of RBC, Hb, Hct, MCH and MCHC values in comparison with the not parasitized fish. A similar decrease was also observed in the number of RBC, Hb and Hct values of the bogue fish infested with the *M. oestroides* parasite (Romestand and Trilles, 1977b); the number of RBC, Hct and Hb values of the *D. labrax* fish parasitized by the *C. oestroides* (Horton and Okamura, 2003), the number of RBC and Hct values of the *Scorpaena porcus* fish infested with the *T. lubrica* (Çelik and Aydın, 2006), the number of RBC and Hb values of the *Nandus nandus* fish parasitized by *C. complanatum* (Kaur *et al*., 2012); and Hct value of the hybrid tambacu fish parasitized by the *Dolops carvalhoi* (Tavares-Dias *et al*., 2007). In this study, the decline in the Hb value of the parasitized fish can be attributed to the weak mobilization of Hb from the palate to other hematopoietic organs (Scott and Rogers, 1981). Additionally, in line with the decline observed in the RBC, Hb and Hct values of the fish, decrease in Hb synthesis was associated with hypochromic microcytic anemia, a type of anemia (Sachar and Raina, 2014). Similar to our study, the decline is believed to be caused by *C. oestroides*. The reason for the decline in MCH and MCHC values can be shown as the decrease in Hb synthesis in RBC and this might be attributed to anemia (Soivio and Nikinmaa, 1981).

A significant decrease was observed in the GLC value of the parasitized fish in comparison with the not parasitized ones. Similarly, the decreased GLC value was also observed in the *S. porcus* fish infested with the *T. lubrica* (Çelik and Aydın, 2006). The decrease in GLC can result in hypoglycemia depending on the distress caused by the parasite (Çelik and Aydın, 2006). The significant decrease

observed in GLC concentration in our study can be associated with liver dysfunction or malnourishment caused by the parasite (Jacobson-Kram and Keller, 2001). Besides, the decrease in GLC level (with the damage that the parasite made in the buccal cavity it located) can be explained by muscle tissue degeneration (Çelik and Aydın, 2006).

A significant decrease was observed in the TG value of the *B. boops* fish with *C. oestroides* infection. A similar decrease was also observed in *S. porcus* fish parasitized with the *Trachelobdella lubrica* (Çelik and Aydın, 2006). While it is indicated that TG value can be decreased by infectious diseases, this parameter was also reported to be associated with the kidney and liver functions and lipid metabolism (Yang and Chen, 2003; Yıldız and Aydın, 2006; Çelik and Aydın, 2006). Besides, it is thought that GLC and TG values are decreased due to decline in nutrition as a result of the infestation of the buccal cavity of the bogue fish by the parasite and the necessary energy need is met by the liver storage.

In this study, the decrease observed in the total protein and globulin values of the parasitized fish was also seen in the TP value of the *S. porcus* fish infested by the *Traclelobdella lubrica* (Çelik and Aydın, 2006). It was reported that a decrease in TB value is seen as a result of long fasting and various distress factors (McDonald and Millican, 1992). Additionally, the decrease in TP can be attributed to the consumption of the nutrient materials by the parasites and the inhibition of the protein and nutrient absorption in the nutrient materials (Eissa *et al*., 2012). Protein loss arising out of cell destruction, malabsorption and fasting might be reflecting the common impact of the decreased TP. The parasites investigated on the fish can have detrimental impact on the immunological response and the decrease in the serum GLO value of the parasitized fish can be considered as a result of this (Çelik and Aydın, 2006).

The increase in the TBIL levels of the parasitized fish can be attributed to a liver dysfunction and damage in liver tissues, increase in destruction of the red blood cells and a potential increase that might be seen in conjugated bilirubin associated with dysfunction of the gall bladder (obstruction) (Arthur and John, 1986). The increase seen in the total bilirubin level was also seen in the dolphins (*Delphinus sp*.) infested with the trematode parasites grounded (Ridgway and Dailey, 1972).

AST and ALT aminotransferases symbolize the protein metabolism and they catalyze the intermolecular transfer of the amino groups between α-ketoacidosis and amino acids (Jee *et al*., 2006). In this study, a significant decrease was found in the AST value of the parasitized fish in comparison with the unparasites ones. Low AST activity indicate that oxaloacetate and glutamate are not suitable for krebs cycle via this routine transamination (Çelik *et al*., 2012). Besides, decreased AST and ALT activities might be signs of liver cell insufficiency. The decrease observed in the AST activities seen in the fish as a result of fasting caused by the parasitized fish can be attributed to the fasting. This can be attributed to the decreased protein regeneration in the malnourished fish under the influence of the decline in gluconeogenesis in the amino acids and the aminoacid deficiency in the digestive system (Tlak *et al*., 2008).

A significant increase was observed in the LDH value of the parasitized fish in comparison with the not parasitized ones. A similar increase was also recorded in the Tilapia sp infested with the *Pygidiopsis geneta* and *P. summa* parasites (Elnemaki, 2003). Increase in LDH activity can be attributed to the damage in muscle tissue and liver (Rui and Zuzuki, 1997). Also the increase in LDH level indicates metabolic changes like glycogen catabolism and glucose changes direction towards lactate form in distressed fish, mainly in muscle tissues (Simon *et al*., 1983).

ALP is an enzyme produced in body tissues, liver and gall bladder cells (Agrahari *et al*., 2007). ALP is a good stress indicator in biological systems and has an important role in cell transport and phosphate hydrolysis as well. ALP measurement is valuable in controlling the cellular membrane health and liver dysfunctions (Banaee *et al*., 2011; Banaee, 2013). The increase in ALP obtained in this study might be related to the tissue damage in the buccal cavity or liver damage caused by the parasite. Also the increase can be attributed to the in transphosphorylation activity (Sharma, 1990).

CK is an enzyme which is chiefly seen in the muscles, heart, gills and the brain (Banaee *et al*., 2011) and has an important function in the cell energy homeostasis and thus is a good indicator that indicates the damage in these tissues. Physiological stress increases plasma CK levels and release of the cytological CK in the blood and the damage in the muscle may result in a potential increase at CK levels (Řehulka and Minařik, 2007; Shahsavani *et al*., 2010). In this study, the increase in CK level might be associated with the damage that the parasite created in the buccal cavity where it located. Further studies are required to confirm this claim.

In addition to respiratory destruction, the particles engulfed by the immune cells are destroyed with lysosomal digestive enzymes in the cell (Diker, 2005). Fish lysozyme is an exceptionally widespread defence molecule of the innate immune system, which is important for protection against fish pathogen (Saurabh and Sahoo, 2008). Myeloperoxidase is contained in the polymorphonuclear neutrophils, monocytes, and macrophages (Klebanoff, 1992). It is known to participate in microbicidal activity and is released into phagolysosomes following the junction of phagosome and lysosome (Siwicki and Anderson, 1993). In this study while NBT and LY activities were decreased, MPO activities were increased in the parasitized fish. Similarly, in a study carried out on *D.*

labrax, lysozyme activity was decreased in the parasitized (*Lernanthropus kroyeri*) fish at the end of 6 weeks (Henry *et al*., 2009). The decreased lysozyme activity in parasitized fish might be related to that the fish have high levels of sensitivity against the parasite they are infested with (Alvarez-Pellitero, 2008). Lysozyme activity, nitric oxide and phagocytic index in the gilthead sea bream (*Sparus aurata*) infested with *Polysporoplasma sparis* were reduced significantly and this was attributed to the immunosuppression impact caused by the parasite (Karagouni *et al*., 2005). Also the increase serum peroxidases in the fish can be attributed to the severe damage during immunopathological impacts (Muñoz *et al*., 2007). Various studies reported changes in innate immune parameters in parasitized fish. For example, serum peroxidase (more specifically MPO) amount of Sharpsnout sea bream (*Diplodus puntazzo*) fish infected with *Enteromyxum leei* showed increase (Muñoz *et al*., 2007). While lysozyme activity showed decrease in *Labeo rohita* fish infected with a low intensity *Argulus siamensis* 1-10 lice fish^{-1}), it did not show a statistically significant difference in the fish infected with medium level (10-25 lice fish^{-1}) and intense (>25 lice fish^{-1}) level parasite (Saurabh *et al*., 2010). In the same study, serum MPO activity was found to be statistically similar in all the fish infected with *A. siamensis*. A similar study indicated that serum MPO activity remained unchanged in *L. rohita* fish infected with *A. siamensis*, lysozyme activity increased on the 3rd day and decreased on the 21st day and NBT activity increased on the 14th day and no change was observed in the immunological parameters on the other days (Kar *et al*., 2015). In the turbot (*Scopthalmus maximus*) fish infected with *Enteromyxum scophthalmi* parasite, an increase was observed in the number of the NBT positive cells and a decrease was observed in the lysozyme activity on the 29th day (Sitjà-Bobadilla *et al*., 2006). Looking at the referred studies, it was seen that parasites caused changes in the innate immune parameters of the fish. These changes showed variances depending on the fish and parasite species, parasite intensity and the time of infestation with the parasite.

Hemorrhages, inflammation and necrosis were seen in the buccal cavity of the parasitized fish in the morphological observations. In the Bogue infected with *M. oestroides* parasite, showed histopathological changes such as epidermal deformations, disorganization of the connective tissue, total deformation of the cartilage tissues, partial deformation of the bone tissue (Romestand and Trilles, 1977a). Additionally the same result was obtained in *Dicentrarchus labrax* fish cultured and parasitized with *C. oestroides* (Korun and Akaylı, 2004). Moreover, histopathological examination indicated that the tissue surrounding the buccal cavity in the fish had a complex structure and contained hyaline cartilage, striated muscle, connective tissue elements and bone tissue structures that shows endochondral development in some areas. *C. oestroides* which is addressed within the scope of this study locates into the buccal cavity of the fish and shows parasitic effect (Mladineo, 2003). There is a vast number of studies conducted on the histopathological changes that different parasite types create in some tissues and organs and impacts similar to our study results were found (Çiltaş *et al*., 2000; Timur *et al*., 2005; Koyuncu, 2006; Korun, 2006; Bamidele, 2007; Adeyemo and Agbede, 2008; Raissy and Ansari, 2011; Mohammadi *et al*., 2012). The sections surrounding the buccal cavity of the fish were investigated histopathologically in this study. The most remarkable finding obtained as a result of the *C. oestroides* infestation is the increase in the hemorrhagic activity. Also, it was observed that the deformations in the striated muscle tissue and hemorrhage are at serious levels. Since hypertrophy observed in the epithelial cells are commonly seen in similar parasitic infestations, it was also observed in the sections of our study. A different study reported hypertrophy in the tissues of the *Xiphophorus maenlatus* fish infested with the parasite (Timur *et al*., 2005). Moreover, the increase in the mucosa secretion in the epithelial cells can be considered as the attempt by the tissue to protect itself from the infestation. However, the serious amounts of mucosa secretion may result in the death of the cells and primarily the tissue and the secondly the life of the organism can be put at death risk (Mc Donald and Wood, 1992). Hyperplasia and obstruction were detected in the tissues of the fish infested with the *Capoeta aculeata*, damage is reported in the primary and secondary lamellas in the gill epithelium with the mucous cell increase (Raissy and Ansari, 2011). Histopathological changes existing in the cartilaginous tissue, which is abound in the palatal complex, are quite important. Particularly hypertrophy in the chondrocytes reveals the intensity of the infestation. In our study, deformations and hypertrophic chondrocytes were observed in the cartilaginous tissue surrounding the buccal cavity of all the infested fish. Mononuclear cell infiltration observed in the sections was attributed to an inflammatory case.

In conclusion, this paper reports the *C. oestroides* infestation of bogue fish in Lapseki Lagoon, located in the Dardanelles, Turkey. Infestation by this parasite resulted in histopathological manifestations such as hemorrhage, edema, necrosis, deformation in striated muscle cells, hypertrophy in chondrocytes and epithelial cells, mononuclear cell infiltration. Moreover, significant changes emerged in the hematological (RBC, Hb, Ht, MCH, MCHC), serum biochemical (GLC, TG, TP, GLOB, TBIL, AST, ALP, CK, LDH) and innate immune (NBT, lysozyme and myeloperoxidase) parameters of the Bogue fish parasitized with *C. oestroides*. These parameters in Bogue fish could provide useful information for the evaluation of

physiological effects of *C. oestroides* infestation, however, prior to implementing the finding of this study, further comprehensive laboratory works are encouraged for the use of a biomarker.

Acknowledgment

This study was supported by the Çanakkale Onsekiz Mart University Scientific Foundation (Project 2012/021).

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