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Seasonal variation in some haematological parameters in naturally infected and uninfected roach (*Rutilus rutilus*) with *Cryptobia tincae*

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Abstract

Investigation was carried out to assess and compare the seasonal changes of some haematological parameters in naturally infected and uninfected roach (*Rutilus rutilus*) with *Cryptobia tincae*. Prevalence of *Cryptobia tincae* was 17.8 % in winter, 25 % in spring, 17.9 % in summer and 16.3 % in autumn. On average, between 0.1 and 0.2 parasites per microscopic field were noted at 100× magnification. No statistically significant differences were observed for erythrocyte, leucocyte, haematocrit values between parasitized and unparasitised roach for each season ($p > 0.05$), except for haematocrit in autumn ($p < 0.05$). Seasonally, the significant differences were found for erythrocyte and haematocrit both infected and uninfected roach while it was not found for leucocyte. In the leucocyte profiles, the significant differences were estimated for neutrophiles in spring, for eosinophiles in autumn between infected and uninfected roach ($p < 0.05$).

Key words: Roach, haematological parameters, Sapanca lake, seasonal changes

INTRODUCTION

Fish are poikilotermic animals that are subject to changes in the environment in which they live [1]. Haematological parameters are a sign of these changes. Haematological values of fishes can be affected by environmental and biological factors such as age, weight, sex, food, bacteria, parasites, and water quality parameters including water temperature, oxygen availability, pH etc. [2,3].

Cryptobia is a flagellate parasite belongs to the family Cryptobiidae. In this family, there are 52 species of *Cryptobia* that infect the body surface, gills, bloodstream or the digestive tract of many species of marine and freshwater fishes, but forty species of these parasites are classified as haemoflagellate which are found in the bloodstream. Some of these parasites are known to cause disease and are responsible for killing commercially important fish species such as *Cryptobia salmositica* which causes disease and mortality in all *Onchorhynchus* spp. in North America and Norway [4].. *Cryptobia* have an indirect life cycle and tranmission normally occurs through the bite of a blood sucking leech, *Piscicola* spp., but may also directly between fishes. Infection can result exophtalmia, general oedema, ascites, anemia (microcytic and hypochromic), anorexia. The anemia is a major clinical sign in cryptobiosis and it is correlated with increasing parasitaemia and leech vector [5,6]. Therefore, prevalence and parasitaemia of *Cryptobia* can be changed seasonally. Cryptobiosis may sometimes be result in high mortality in naturally and experimentally infected fish. [4]..The aim of this study was to determine and compare the changes of the haematological values in roach *(Rutilus rutilus*) infected and uninfected with *Cryptobia tincae* during 4 seasons of the year.

MATERIALS AND METHODS

Sapanca Lake is a lake of tectonic origin located between 40^0 41' N – 40^0 44' N and 30^0 09' E – 30^0 20' E in Marmara region (Fig 1). Lake has an oligotropic character and iIts surface area is 46.8 km^2 with mean depth of 29 m. and maximum depth of 55 m.. The lake water is used as a source of drinking water by the city and district of Adapazarı and as a recreational area [7] . Acricultural activity is not so intense but it was determined the total of 25 fish species in this Lake. Roach that has an economical value is one species of these fish species. Fish were caught by gill nets and long-line at a definite area of Sapanca Lake in January- February, May, August and October 2005. The fish were transported to the laboratory in aerated tanks and kept alive until termination. Blood samples were obtained by venous puncture using disposable plastic syringes. A drop blood was examined under the coverslip for the presence of live *Cryptobia* sp. Parasitaemia was estimated by counting the number of *Cryptobia* in field of vision (100× magnification) on wet preparations. Blood samples were also collected in sterile tubes containing EDTA.

Visual counts of erythrocyte and leucocyte were carried out using Thoma haemocytomer. The diluting fluid was Natt-Herrick solution. To estimate the differential leucocyte count, blood smears were prepared, air-dried, fixed in methanol and stained using May Grünwald-Giemsa solution. Leucocytes in a blood smears were categorized into lymphocytes, monocytes, neutrophils and eosinophils [8,9] .

To determine the haemotocrit value, commercially available microhaemotocrit tubes with heparin were used. These tubes were centrifuged in a microhaemotocrit centrifuge for 15 minutes in 12000 rpm. and percentage of the haemotocrit value was calculated.

Haematological values between the two groups (infected and uninfected) were compared by means of non-parametric U Mann- Whitney test. In the seasonal changes the statistical analysis were made using the non – parametric one-way ANOVA [10].

RESULTS

Measured temperature, oxygen and pH values were varied between 9ºC and 26ºC, 8.4 and 10.9 mg/l. and 8.4 and 8.8 respectively in Sapanca lake during the study period.

A total of 173 roach (*Rutilus rutilus*) were examined with 90 in winter, 12 in spring, 28 in summer and 43 in autumn. Prevalence of *Cryptobia tincae* was 17.8 % in winter, 25 % in spring, 17.9 % in summer and 16.3 % in autumn. On average, between 0.1 and 0.2 parasites per microscopic field were noted at 100× magnification. The haematological values in infected and uninfected roach are shown in Table I. No statistically significant differences were observed in erythrocyte, leucocyte, haematocrit values between infected and uninfected roach for any season ($p> 0.05$), except for haematocrit in autumn ($p < 0.05$).

In parasitized roach, the significant differences seasonally were found for erythrocyte ($F_{(2.960)} = 5.245$; P- value = 0.006; $p< 0.05$) and for haematocrit ($F_{(2.960)} = 4.226$; P- value = 0.014; $p< 0.05$), while no significant differences for leucocyte ($F_{(2.960)} = 0.910$; P- value = 0.449; $p> 0.05$).

In unparasitized roach, the significant differences seasonally were found for erythrocyte ($F_{(2.670)} = 36.594$; P- value = 0.0001; $p< 0.05$) and for haematocrit ($F_{(2.670)} = 3.538$; P- value = 0.016; $p< 0.05$), while no significant differences for leucocyte ($F_{(2.670)} = 1.139$; P- value = 0.335; $p> 0.05$).

The statistically significant differences were determined for neutrophiles in spring, for eosinophiles in autumn between infected and uninfected roach ($p < 0.05$) while there was no statistically significant differences for the other parameters in any season ($p > 0.05$).

In the leucocyte profiles, the significant differences seasonally were determined for neutrophiles ($F_{(2.866)} = 6.061$; P- value = 0.002; $p< 0.05$) , for eosinophiles ($F_{(2.866)} = 4.605$; P- value = 0.008; $p< 0.05$) in the infected roach and for neurophiles ($F_{(2.866)} = 6.773$; P- value = 0.001; $p< 0.05$) in the uninfected roach while differences was not found for the other parameters seasonally ($p > 0.05$).

DISCUSSION

Haemoflagellate parasites are common and widespread in both natural and cultured fish populations. Prevalence was reported up to 100 % in carp and tench [11]. In this study, prevalence rose to a maximum value (25 %) in spring. Parasitaemia can be subdivided into three categories, namely low, medium and high infections (cut-off levels of 0 to <2, 2 to <4 and 4 to 12 mean numbers of *Cryptobia* per field of view at ×100 magnification) [11]. The parasitemia for the whole fish used in this study was found in the low category.

It has previously been reported in some studies that experimental haemoflagellate infections of salmonids and cyprinids caused some changes in the haematological parameters and that anaemia was a major clinical sign [2,6]. On the other hand, it has been argued that these changes in the haematological parameters were not seen in the wild fishes and were regarded as evidence of a well balanced host- parasite relationship [12]. While there did not appear to be any difference in the leucocyte count between infected and uninfected fishes [13] , other studies suggest that a decrease in erythrocyte and haematocrit values and an increase in leucocyte count may occur in infected fishes [14]. In this study no differences in these haematological parameters between parasitised and unparasitised roach were noted, except for haemotocrit in autumn , perhaps due to the low levels of infection noted in these fish.

There are a few studies in the literature on the percentage of leucocyte cell type between infected and uninfected fishes. In some studies an increase for eosinophils and neutrophils in infected fish and a decrease for lymphocytes and monocytes has been reported [14,15]. In this study, it was determined an increase for eosinophils in autumn while a decrease for neutrophils in spring in infected roach.

In this study it was also found that there was the differences in the erythrocyte count and percentage of haemotocrit seasonally. Seasonal differences in these parameters count were also determined. But various studies shows us there were so many differantions on values in different seasons. Whereas Van Vuren and Hatting 1978 [16]. determined an increase for erythrocyte and haemotocrit values for *Cyprinus carpio* in winter, Collazos et al 1998 [17]. determined this increase for *Tinca tinca* in spring . In other study, maximum values for these parameters for *Capoeta barroisi* and *Rutilus rutilus* were found in autumn [18]. In this study maximum values for erythrocyte and haemotocrit values in both infected and uninfected roach were determined in autumn.

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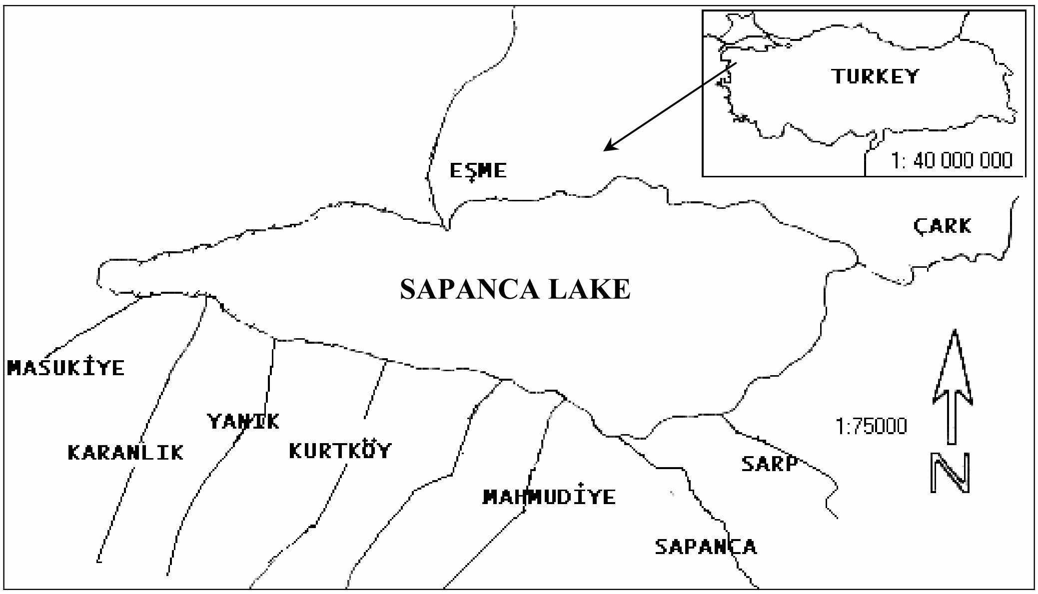

Fig. 1 Map showing the location of Sapanca Lake; Lake extends over an area of 40 km, with an average depth of 29 m., maximum depth of 55 m.

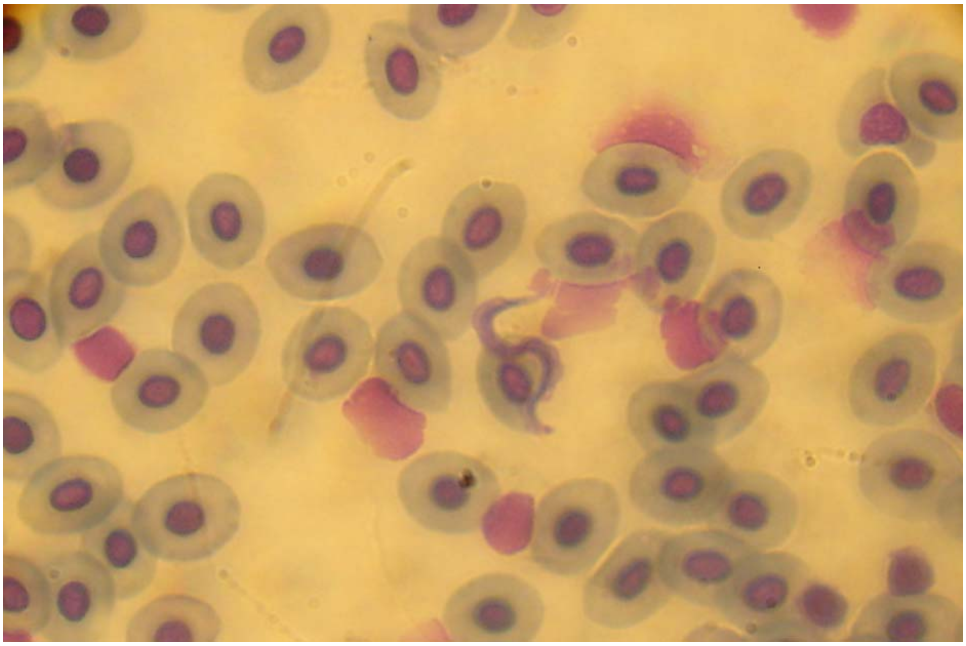

Figure 2 : *Cryptobia tincae* among erythrocytes Giemsa X 1000

Table 1. The seasonal haematological values in infected and uninfected roach

Parameter		Erythrocyte 10^6 / mm^3 Mean ± SE		Leucocyte 10^3/ mm^3 Mean ± SE		Haematocrit % Mean± SE	
Winter	İnfected N= 16	1.082 ±0.314	$t_{(1.987)}=0.284$ P=0.388 p>0.05	32.463 ±15.309	$t_{(1.987)}=0.973$ P=0.166 p>0.05	29.19 ±15.428	$t_{(1.987)}=0.647$ P=0.259 p>0.05
	Uninfected N= 74	1.101 ±0.214		37.799 ±20.486		31.58 ±12.763	
Spring	İnfected N= 3	1.186 ±0.179	$t_{(2.228)}=0.169$ P=0.434 p>0.05	45.88 ± 16.246	$t_{(2.228)}=0.452$ P=0.330 p>0.05	38.33 ± 7.846	$t_{(2.228)}=0.914$ P=0.191 p>0.05
	Uninfected N= 9	1.209 ±0.180		38.23 ± 25.080		30.67 ± 12.463	
Summer	İnfected N= 5	1.706 ±1.098	$t_{(2.055)}=0.390$ P=0.349 p>0.05	36.312 ±9.618	$t_{(2.055)}=0.562$ P=0.289 p>0.05	28.40 ± 3.980	$t_{(2.055)}=1.054$ P=0.151 p>0.05
	Uninfected N=23	1.839 ±0.529		42.980 ±25.037		35.13 ± 13.633	
Autumn	İnfected N=7	2.195 ±0.430	$t_{(2.019)}=0.847$ P=0.201 p>0.05	29.04 ± 14.521	$t_{(2.019)}=1.626$ P=0.056 p>0.05	39.89 ± 12.624	$t_{(2.019)}=1.879$ P=0.03 p<0.05
	Uninfected N=36	1.956 ±0.706		46.17 ± 26.460		49.71 ± 10.885	

Table 2. The seasonal leucocyte profile values in infected and uninfected roach

Season \ Parameter		Lymphocytes % Mean ± SE		Monocytes % Mean ± SE		Neutrophils % Mean ± SE		Eosinophils % Mean ± SE	
Winter	İnfected N= 10	64.40 ±5.480	$t_{(2.101)}=0.984$ P=0.169 p> 0.05	25.60 ±5.122	$t_{(2.101)}=0.151$ P=0.440 p> 0.05	8.10 ±2.426	$t_{(2.101)}=1.706$ P=0.052 p> 0.05	1.90 ±1.758	$t_{(2.101)}=0.455$ P=0.327 p> 0.05
	Uninfected N= 10	61.82 ±5.723		25.20 ±6.063		10.60 ±3.666		2.40 ±2.059	
Spring	İnfected N= 3	63.33 ±8.654	$t_{(2.262)}=0.448$ P=0.332 p> 0.05	32.67 ±10.389	$t_{(2.262)}=0.949$ P=0.183 p> 0.05	2.33 ±1.699	$t_{(2.262)}=2.293$ P=0.023 P< 0.05	1.67 ±2.357	$t_{(2.262)}=0.753$ P=0.233 p> 0.05
	Uninfected N= 8	66.37 ±9.219		24.63 ±11.661		5.38 ±1.798		3.63 ±3.772	
Summer	İnfected N= 5	67.80 ±3.544	$t_{(2.160)}=0.059$ P=0.479 p> 0.05	17.00 ±3.286	$t_{(2.160)}=1.732$ P=0.053 p> 0.05	10.40 ±4.223	$t_{(2.160)}=0.219$ P=0.415 p> 0.05	2.80 ±2.713	$t_{(2.160)}=0.228$ P=0.412 p> 0.05
	Uninfected N= 10	67.60 ±6.560		21.90 ±5.412		9.70 ±5.962		3.30 ±4.148	
Autumn	İnfected N= 7	61.86 ±6.034	$t_{(2.131)}=1.191$ P=0.126 p> 0.05	25.29 ±4.589	$t_{(2.131)}=0.301$ P=0.384 p> 0.05	8.571 ±1.917	$t_{(2.131)}=1.706$ P=0.054 p> 0.05	4.29 ±2.962	$t_{(2.131)}=2.567$ P=0.011 P< 0.05
	Uninfected N= 10	66.20 ±7.521		26.30 ±7.430		6.20 ±3.059		1.30 ±1.487	