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Frequency of black spot disease in *Cobitis* cf. *turcica* from Pınarbaşı Springs (Haymana, Turkey)

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Abstract. During a survey on the biology of *Cobitis* cf. *turcica* living in Pınarbaşı Springs (Haymana district, Turkey), symptoms of black spot disease were frequently observed on the specimens. Of the 1295 loach collected, 240 (19 %) specimens were found to be infected with metacercaria of *Posthodiplostomum cuticola*, a common digenean parasite causing black spot disease in freshwater fish in Eurasia. During spring and autumn, the infection rate was significantly higher than during winter and summer, most probably due to the increasing density of migration of birds in this area. No statistically significant difference was found in condition, weight and length between infected and non-infected specimens; indicating low physiological effects of the encysted metacercaria on the host specimens. However, the prevalence of black spot had a tendency to decrease with age, remarking a higher mortality rate of infected specimens. This increased mortality rate seems to be the most important impact of an infection with metacercaria for a *Cobitis* population.

Key words: *Posthodiplostomum cuticola*, prevalence, Cobitidae, Pınarbaşı Springs

Introduction

Parasites may have considerable impacts on many fish populations and parasitic infections are among the significant factors that may affect host natural populations (Barber & Poulin 2002). However, studies on parasite fauna of fish are more often focused on commercially important species. Spined loaches are widespread through Eurasia and known to be host for numerous parasite species and important secondary hosts for many other parasites (Zrnčić et al. 2009). Parasitological examinations of loach populations in Europe revealed infections caused by trematode species *Diplostomum* sp., *Tylodelphys clavata*, *Allocreadium transversale*, *Posthodiplostomum cuticola*, *Metorchis xanthosomus*, Echinostomatidae gen. sp., (Robotham & Thomas 1982, Halačka et al. 2000, Popiołek & Kotusz 2003, 2004); nematode species *Rhabdochona ergensi* (Popiołek & Kotusz 2003); cestodes *Ligula colymbi* and *Neogryporhynchus cheilancristrosus* (Halačka et al. 2000, Scholz et al. 2004, Kessler et al. 2009).

Black spot is a common fish disease easily recognisable by the presence of encysted metacercariae surrounded by an accumulation of blackspots of melanin in the skin, muscle and fin (Lasee 1995). Many species of digenean trematodes cause blackspot disease in most freshwater fish species. The life cycle of these trematodes is complex and involves a series of hosts; fish serve as an intermediate host in the development of parasites. Infection occurs when a fish encounters free swimming cercariae that penetrate its integument and await the opportunity to pass to the final host (bird) when the fish is eaten (Laase 1995). In response to the metacercarial cyst, the fish forms a capsule of connective tissue that contains melanophores. In Europe, the most common agent of blackspot disease is *Posthodiplostomum cuticola* (Ondračková et al. 2002). Blackspot disease caused by metacercaria of *P. cuticola* in loach populations were reported by Halačka et al. (2000) and Popiołek & Kotusz (2003). Studies on the parasites of *Cobitis* are very rare in Turkey. Kartal & Öztürk (2009) reported the occurrence of *Gyrodactylus cobitis* from *C. simplicispina*, and Keskin & Özeren (2002) has presented that blackspot was frequently found on *C. simplicispina* from

Sakarya River Basin. But in this study *P. cuticola* was found on *Cobitis* cf. *turcica* from the closed basin of Central Anatolia.

Cobitis cf. *turcica* is an endemic loach distributed throughout the Tuz Lake Basin in Central Anatolia. Although this species is listed as endangered in the IUCN Red List since 2005, there is no detailed information about its ecology and biology. We frequently observed black spots on the fish body, and the main goal of this study is to point out high prevalence of black spot disease, which could be regarded as a potential future threat for this endangered loach species.

Study Area, Material and Methods

Fish samples were collected from a semiarid area where continental climate prevails. The species is dwelling in a small brook fed by Pınarbaşı Springs (39°14′21″ N 32°44′59″ E) and flowing into Kozanlı-Gök (Saz) Lake which is an important bird area (Magnin & Yarar 1997).

Sampling was performed along 100 m of the watercourse, where depth ranged from 50 to 80 cm, width was about 2 to 3 m and the current was low. The bottom was mainly sandy, muddy and covered with aquatic vegetation and willows. The water temperature ranged between 8.2 °C (March) and 19.3 °C (June) (Fig. 1). The minimum and maximum dissolved oxygen contents were 4.35 mg/l and 11.49 mg/l respectively.

Samples of *Cobitis* cf. *turcica* were collected by a dip net with a mesh size of 1 mm. A total of 1295 specimens were caught on a monthly basis between March 2003 and August 2004. Total and standard lengths of anaesthetized fish were measured in mm and weighed in mg. Scales were used for age determination. The condition factor was determined from the length and weight of each specimen using Fulton's condition factor (*KF*) given as $KF = W10^5/TL^3$ (*W*: body weight in mg and *TL*: total length in mm). Uninfected specimens were released back into the water after all measurements were completed, the infected specimens were preserved in 4 % buffered formaldehyde for laboratory examination.

The number of cysts on fish was counted and recorded. To identify the parasite species, some parasites were preserved in 70 % ethyl alcohol. Fixed parasite specimens were stained with aceto-carmine and they were dehydrated into alcohol series and mounted in slides with Canada balsam. The determination of parasites was performed according to Bykhovskaya-Pavlovskaya et al. (1962) and Markevich (1951).

The level of parasite infection was estimated according to Bush et al. (1997). The parasitological indices prevalence (percentage of infected hosts), mean intensity (average intensity of the parasite species among the infected members of the host species) and mean abundance (number of parasite in a sample of host divided by the total number of hosts) were calculated. Total length, weight and condition of infected and non-infected specimens in each age class were compared by the Mann-Whitney *U*-test. Statistical analyses were carried out by SPSS™ 16 package program. The $p < 0.05$ was considered as significant.

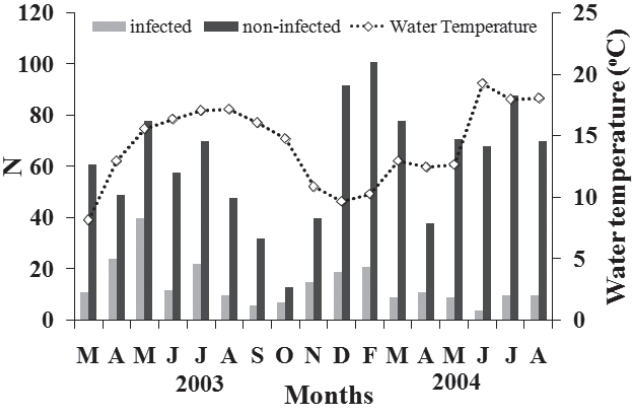

Fig. 1. *Monthly variations in water temperature, number of infected and non-infected specimens.*

Results and Discussion

Diagnosis of blackspot disease is usually by presence of dark areas on the surface of the fish. In the present case, cysts from the skin of *Cobitis* cf. *turcica* were elliptical with about 1 mm in diameter. The metacercaria body consisted of a wide anterior segment and a small, narrow posterior segment. The oral sucker measured 0.065 × 0.060 mm and the ventral sucker measured 0.080 × 0.080 mm. The ventral sucker is located at the middle of the body. The Brandes' organ was round and bigger than the ventral sucker. There were two intestinal trunks ending in the posterior part of body. The excretory system opening is located at the posterior body tip. On the base of these characters, we identified the parasite as *Posthodiplostomum cuticola*.

In this study, metacercaria of *P. cuticola* in black cysts were observed primarily on the skin, fins and gills of fish. A total of 240 infected individuals by *P. cuticola* were found in 1295 examined specimens of *Cobitis* cf. *turcica*. Monthly variation of water temperature and both the number of infected and non-infected specimens were depicted on Fig. 1. Although cold

continental climatic conditions prevail in the study area, due to the nature of spring water the temperature of stream never decreases under 8 °C during the winter and even in very hot days of summer the water temperature did not reach up to 20 °C.

Environmental conditions of the study area were convenient for the occurrence of blackspot disease. The sampling area is a small stream with slow water flow, soft muddy bottom and abundant vegetation, a suitable microenvironment for both snails and *Cobitis* cf. *turcica*, since the snails are the intermediate hosts for *P. cuticola*, cercariae that leave the snail may easily infect this benthic fish. Moreover, Pınarbaşı Springs is close to Kozanlı-Gök (Saz) Lake, which is considered as an important bird area and the piscivorous birds (definitive hosts of *P. cuticola*) may be one of the major causes for spreading the parasitological infections in this area.

The prevalence of *P. cuticola* was about 19 % for all specimens. It was determined that the prevalence of black spot had a general tendency to decrease with age (Fig. 2). However, this may be due to the fact that massively infected individuals may have died and not be represented in samples as noted by Berra & Au (1978). On the other hand, the prevalence was 27 % at age of 0, this figure reached to 7 % at V age group (Fig. 2). Although age VI seemed to have the highest prevalence, it should be taken into consideration that the number of samples at this age is only four and half of those specimens that were infected. As the number of samples at age VI is not sufficient, this group is not taken into account during evaluation. It is still a matter of debate on whether the intensity of strigeoid trematode infection increases in older fish (Steedman 1991).

Juvenile fish are more susceptible to blackspot disease. Severe infections of young fish may cause spinal curvature, abnormal muscle development or death and infected adults may be susceptible to secondary infections (Steedman 1991). However, we observed a single sample with spinal abnormality among 1295 specimens.

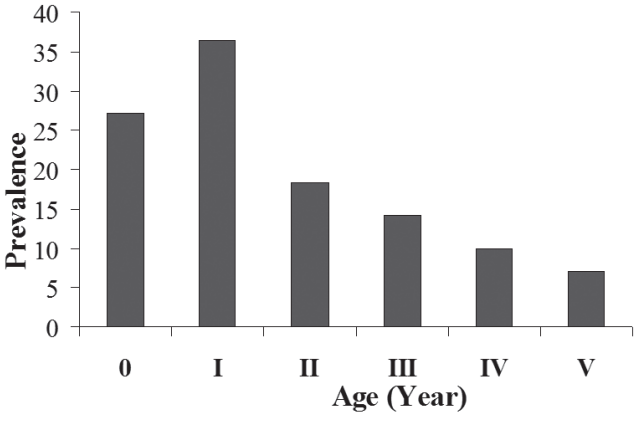

Fig. 2. *Variations in prevalence of blackspot in different age groups of* Cobitis *cf.* turcica.

We observed seasonal variation for prevalence such as an increase during spring and autumn (Fig. 3). Lowest prevalence was observed in summer with 14.47 %. There is little evidence to suggest strong seasonality in the abundance of black spot disease in fish populations (Steedman 1991). There may be a relation with the bird migrations and the seasonality in abundance, as many migratory birds use both Gök Lake and remains of Samsam Lake adjacent to the study area intensively during migration in spring and autumn, and may act as a vector of this parasite.

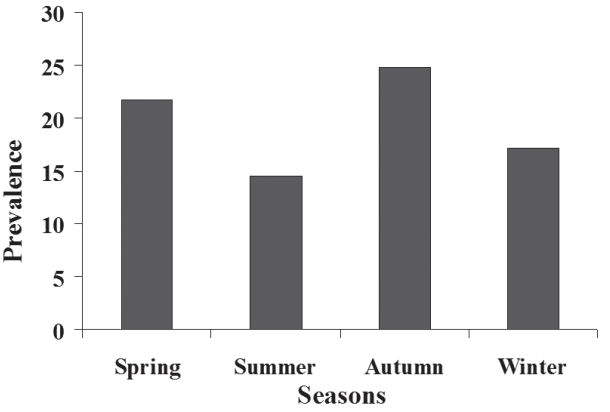

Fig. 3. *Seasonal variations in prevalence of blackspot in* Cobitis *cf.* turcica.

The condition factor, weight and length of infected and non-infected individuals in the same age groups were compared and statistically no significant difference was found (Mann-Whitney *U*-Test, $p > 0.05$). Total length, weight and condition factor of the infected and non-infected specimens are given in Table 1. Ondračková et al. (2006) claimed that condition factor was more poor in fish with higher prevalence of *P. cuticola*. However, in contrary to our expectations, the result of this study revealed that the condition factor was not significantly different between infected and non-infected individuals. Despite of the high prevalence, insignificance of correlation coefficient (*TL*, *W* and *KF*) may be explained by low mean intensity and mean abundance.

The length-weight relationship for infected specimens ($W = 0.1 \times 10^{-4} TL^{2.83}$) was not statistically different that of non-infected fish ($W = 0.1 \times 10^{-4} TL^{2.77}$) (Student's *t*-test, $p > 0.05$). Both data are presented in Table 1 and length-weight relationship equations

Table 1. *Total length (TL), weight (W) and condition factor (KF) with mean values, standard deviation (SD) for infected and non-infected* Cobitis *cf.* turcica *specimens and mean intensity (MI) and mean abundance (MA) values in different age groups (N: number of specimens).*

Ages	N		*TL* (mm) Mean (± SD)		*W* (g) Mean (± SD)		*KF* Mean (± SD)		MI (± SD)	MA (± SD)
	Infected	Non-infected	Infected	Non-infected	Infected	Non-infected	Infected	Non-infected		
0	7	15	51.60 (±5.78)	47.40 (±7.53)	1.02 (±0.82)	0.56 (±0.27)	0.52 (±0.08)	0.48 (±0.08)	5.14 (±1.77)	1.64 (±2.63)
I	50	87	65.98 (±9.08)	66.45 (±13.25)	1.50 (±0.67)	1.61 (±0.83)	0.50 (±0.08)	0.52 (±0.08)	5.80 (±3.87)	2.12 (±4.05)
II	111	497	79.24 (±9.23)	79.86 (±7.89)	2.67 (±0.96)	2.74 (±0.82)	0.52 (±0.09)	0.53 (±0.08)	4.28 (±2.26)	0.77 (±2.15)
III	62	378	88.51 (±9.75)	88.84 (±9.95)	3.50 (±1.07)	3.57 (±1.14)	0.50 (±0.07)	0.50 (±0.07)	4.31 (±6.02)	0.61 (±6.02)
IV	7	63	104.14 (±10.06)	104.78 (±10.31)	5.69 (±1.63)	5.41 (±1.63)	0.49 (±0.05)	0.46 (±0.08)	4.00 (±3.21)	0.40 (±2.76)
V	1	13	115.0	116.82 (±4.45)	6.442	7.74 (±1.22)	0.424	0.48 (±0.05)	4.00 (±1.77)	0.29 (±1.77)
VI	2	2	124.18 (±3.64)	118.90 (±3.25)	8.17 (±2.34)	8.28 (±0.88)	0.42 (±0.09)	0.49 (±0.01)	8.00 (±7.49)	4.0 (±6.73)
TOTAL	240	1055	-	-	-	-	-	-	4.68 (±4.51)	0.86 (±2.65)

indicated that growth rate of infected fish were similar to non-infected individuals.

Mean intensity and mean abundance were found as 4.68 and 0.86 respectively for all specimens (Table 1). It was determined that both mean intensity and mean abundance had the tendency to decrease with age. Due to the high number of infected specimens, more intense parasite infection may be expected in the studied population. But our results revealed that the infection intensity was relatively low, this may be due to the lost of black spots caused by the friction between the fish skin and substratum.

Males were predominant with ratio of 1 : 0.59 (822 male and 483 female). Although males were more abundant in the population, the prevalence of black spot in males was lower (16 %) than in females (30%). The presence of parasites has great influence on the longevity of the organism so the prevalence of blackspot should be considered carefully. In his review, Steedman (1991) reported that several studies have shown that black spot infections were most severe in stream fishes that inhabited pool areas, as we have observed in the presented case. Pollution may have effects on the abundance of snails, which are the intermediate host of *P. cuticola*. Although we do not have any quantitative data on the pollution, we observed organic material discharging from the farms that may cause to the pollution in the creek. Presence of blackspot disease not only reduces the aesthetic value of the fish, it may also cause mortality especially when infections are heavy and water temperature declines (Lasee 1995) and changes in fish behaviour (Mikheev & Pasternak 2006). On the other hand, fish infected by *P. cuticola* appear to be easier prey for predators due to the formation of clearly visible black spots (Ondračková et al. 2006). But this claim does not seem to be acceptable for the case of *Cobitis* cf. *turcica* as this is a bottom dwelling species.

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