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Community Structure of the Parasites of the Endemic Chocolate Chub *Squalius carinus* Özuluğ & Freyhof, 2011 (Cyprinidae) from Işıklı Lake, Çivril, Turkey

Erhan Soylu*[1], Selçuk Uzmanoğlu[1], Sibel Özesen Çolak[2] & Meral P. Soylu[1]

[1]Vocational School of Technical Sciences, Fisheries Department, Marmara University, Göztepe, 34722 Kadıköy-Istanbul, Turkey
[2]Fisheries Faculty, Istanbul University, Ordu Cad. No. 200, 34470 Laleli, Fatih-Istanbul, Turkey

Abstract: This study presents the first data on parasites of the chocolate chub, *Squalius carinus* Özuluğ & Freyhof, 2011. The composition and the structure of the parasite community infecting this endemic fish from the Işıklı Lake are described. A total of 54 specimens of *S. carinus* are examined; 51 (94.4%) infected by at least one parasite species. In total, 1192 parasite individuals are collected, with mean intensity of 23.4 and mean abundance of 22.1 parasites/fish. The parasite community of *S. carinus* consists of 14 species: *Trichodina* sp., *Ichthyophthirius multifiliis*, *Chilodonella cyprini* (Protozoa); cysts of *Myxobolus* sp. (Myxozoa); *Dactylogyrus vistulae*, *Gyrodactylus* sp. (Monogenea); *Bothriocephalus acheilognathi* (Cestoda), metacercariae of *Diplostomum* sp. and *Thylodelphys clavata* (Digenea); larvae of Nematoda indet. (Nematoda), *Pomphorhynchus* sp. (Acanthocephala); *Lamproglena pulchella* and *Lernaea cyprinacea* (Crustacea); and glochidia of Mollusca indet. (Mollusca). The highest dominance index among parasites is recorded for *Pomphorhynchus* sp. (47.9%), and the lowest for Nematoda indet. (0.3%). The mean species richness of parasite infracommunities is 2.3 species per individual fish. The parasite community of *S. carinus* is dominated by *Pomphorhynchus* sp. The remaining parasite species are found with low prevalence and abundance, except for *Diplostomum* sp. and *Dactylogyrus vistulae*, which are moderately abundant.

Key Words: Fish, endemic *Squalius carinus*, parasite communities, Işıklı Lake

Introduction

The freshwater fish fauna of Turkey is highly diverse and consists of about 248 species (plus 13 introduced species) in 14 major freshwater fish regions (Fricke et al. 2007). Our study area, Işıklı Lake, is located in the Büyük Menderes drainage in the Anatolian Mediterranean Sea watershed. Işıklı Lake, Işıklı Spring and Kufi Stream, which run into the lake, contain 12 endemic, five non-native and one translocated fish species (Güçlü et al. 2013). Under the pressure of population growth, extensive agricultural activities, pollution and eutrophication, as well as the diversion of water for irrigation, the native Turkish fish fauna is now highly threatened and additional measures are required to ensure its conservation (Fricke et al. 2007). The chocolate chub, *Squalius carinus*, is one of the endemic fish of Işıklı Lake and has been included in the IUCN (International Union for Conservation of Nature) Red List of Threatened Species. The populations of *S. carinus* are mostly impacted by water regime change due to irrigation for farming and weir construction. There may be detrimental competitive or predatory effects of rainbow trout (*Oncorhynchus mykiss*), which escapes from fish farms on a large scale, on native fish species in Işıklı Lake and Işıklı Spring. The occurrence of other non-native fish, such as *Carassius gibelio* can also have negative impacts on the survival of native fish species in the

*Corresponding author: esoylu@marmara.edu.tr

lake (Gaygusuz et al. 2013). Introduced non-native fish can affect species richness, composition and size distribution of native communities (Turek et al. 2013) and have been demonstrated to cause declines in native freshwater fish species in Turkey (Gaygusuz et al. 2007, Aydin et al. 2011, Tarkan et al. 2012). Alien fish species compete with native fish through predation and competition for food, space and breeding opportunities (Jonsson & Jonsson 2006). Tarkan et al. (2015) recorded 30 introduced freshwater fish species in Turkey, including 11 that have been translocated. Nineteen non-indigenous fish species have established self-sustaining populations in the areas in Turkey to which they were introduced. Five species (*Cyprinus carpio*, *Gambusia holbrooki, Atherina boyeri, Carassius gibelio* and *Pseudorasbora parva*) have established extensive populations and are qualified as problematically invasive. In the present study, the composition and the community structure of *S. carinus* parasites from the Işıklı Lake were examined at the infracommunity and component community levels.

Materials and Methods

A total of 54 chocolate chubs (*Squalius carinus* Özuluğ & Freyhof, 2011) were examined between July 2014 and June 2015: 32 female and 22 male specimens. They had a mean (± SD) total length of 21.4 ± 2.4 cm (range 15.2–25.7 cm) and a mean (± SD) weight of 129.0 ± 48.2 g (range 43.2–254.1 g). The fish specimens were collected from the Işıklı Dam Lake (38º13′42.94″N and 29º53′56.68″E), Işıklı Spring (38º18′57.57″N and 29º51′37.29″E) and Kufi Stream (38º19′29.13″N and 29º48′24.44″E) during the spawning migration in April. Fish were caught using gillnets with a knot to knot mesh size of 10, 20 and 30 mm. Nets were 50 m long with a 1.5 m hanging depth and cast net. The fish were transported to the laboratory alive, where they were weighed and measured. The sex of each individual was determined by visual and microscopic examination of the gonads. The fish were necropsied as soon as possible; the external surfaces of the body, gills, eyes and internal organs (oesophagus, stomach, intestine, pyloric caeca, liver, heart, spleen, gall bladder and gonads) were examined separately.

Wet smears of skin and gills were prepared and examined in order to detect the presence of protozoan parasites. Monogenean parasites were removed and placed on a slide with ammonium picrate-glycerine or lactophenol and covered with a cover-glass to prepare flat mounts. Trematode and cestode specimens were identified alive or fixed in 70% alcohol. Parasites were stained with acetocarmine, dehydrated through an alcohol series, cleared in dimethyl phthalate and examined as permanent mounts in Canada balsam. Most of these parasite specimens were slightly flattened before fixation. Nematode and crustacean parasites were cleared in lactophenol and mounted in Canada balsam. Slides were studied with a microscope at 40x and 100x magnifications.

The prevalence, mean intensity and abundance of species were determined as defined by Bush et al. (1997). The following indices were used to characterise the parasite communities: Berger-Parker Dominance Index, $d=n_{max}/N$; Shannon-Wiener Species Diversity Index, $H'=-\Sigma(pi\times ln\ pi)$, Simpson Index, $D=\Sigma ni(ni-1)/N(N-1)$ and Index of Evenness = H/H_{max}. The condition factors of the fish were calculated using Fulton's formula: $K=Wx100/L^3$. A Kruskal-Wallis test was used to analyse the differences between size classes in terms of the number of individuals of *Pomphorhynchus* sp. present and in terms of the condition factor of the fish. Parasites recovered were fixed and preserved according to Bylund et al. (1980). Acanthocephalan specimens were identified as described by *Spakulova et al. (2011) and Emde et al. (2012*) using unfixed fresh material. The other parasite species were identified according to Lom & Dykova (1992), Niewiadomska (2003), Pugachev et al. (2010) and Bykhovskaya-Pavlovskaya et al. (1962).

Results

Totally, 54 *Squalius carinus* specimens were examined; 51 (94.4%) were infected by at least one parasite species. Overall, 31.5% were infected by one species, 25.9% by two, 22.2% by three, 3.7% by four, 5.6% by six, 3.7% by seven and 1.8% by eight parasite species. A total of 14 parasite species were identified from 1192 individuals: *Trichodina* sp., *Ichthyophthirius multifiliis*, *Chilodonella cyprini* (Protozoa); *Myxobolus* sp. (Myxozoa); *Dactylogyrus vistulae*, *Gyrodactylus* sp. (Monogenea); *Bothriocephalus acheilognathi* (Cestoda); metacercariae of *Diplostomum* sp. and *Thylodelphys clavata* (Digenea); Nematoda indet. (Nematoda); *Pomphorhynchus* sp. (Acanthocephala); *Lamproglena pulchella*, *Lernaea cyprinacea* (Crustacea); glochidia of Mollusca indet. (Mollusca). No intestinal digeneans were found.

The epidemiological parameters of parasite infection of *S. carinus* are given in Table 1. Parasite infracommunities of *S. carinus* were dominated by *Pomphorhynchus* sp. (85.2%), with a mean intensity of 12.4 and a mean abundance of 10.6 parasites/

Table 1. Epidemiological parameters of infection by parasites of *Squalius carinus* from Işıklı Lake. Abbreviations: IFN: Infected Fish Number; TPN: Total Parasite Number; P: Prevalence; MI: Mean Intensity; MA: Mean Abundance

Parasite	IFN	TPN	P (%)	MI	MA	Site of infection
Myxobolus sp.	6	-	11.1	-	-	Gill
Trichodina sp.	1	-	1.9	-	-	Gill
Ichthyophthirius multifiliis	5	-	9.3	-	-	Gill, skin
Chilodonella cyprini	1	-	1.9	-	-	Gill, skin
Dactylogyrus vistulae	15	105	27.7	7.0	1.9	Gill
Gyrodactylus sp.	5	65	9.3	13.0	1.2	Skin
Diplostomum sp.	20	247	37.0	12.4	4.6	Lens of eyes
Thylodelphys clavata	7	127	13.0	18.1	2.4	Vitreous humor
Bothriocephalus acheilognathi	3	5	5.5	1.7	0.1	Intestine
Nematoda sp.	1	4	1.9	4.0	0.1	Intestine
Pomphorhynchus sp.	46	571	85.2	12.4	10.6	Intestine
Lernaea cyprinacea	4	25	7.4	6.3	0.5	Skin
Lamproglena pulchella	5	8	9.3	1.6	0.1	Gill
Mollusca sp. (glochidia)	8	35	14.8	4.4	0.6	Gill, fins

Table 2. Dominance index of the metazoan parasite community of *Squalius carinus* from Işıklı Lake

Parasite	Dominance index D (%)
Dactylogyrus vistulae	8.8
Gyrodactylus sp.	5.5
Diplostomum sp.	20.7
Thylodelphys clavata	10.7
Bothriocephalus acheilognathi	0.4
Nematoda indet.	0.3
Pomphorhynchus sp.	47.9
Lernaea cyprinacea	2.1
Lamproglena pulchella	0.7
Mollusca indet. (glochidia)	2.9

Table 3. Diversity indices of the parasite community of *Squalius carinus* from Işıklı Lake

Index	Value
Number of chub	54
Number of metazoan taxa	10
Species richness	14
Shannon-Wiener Index	1.55
Shannon-Wiener Evenness	0.67
Simpson's Diversity	0.30
Dominant taxon	*Pomphorhynchus* sp.

fish. The parasite community was characterised by the presence of species with a low prevalence and abundance, except for *Pomphorhynchus* sp., *Diplostomum* sp. and *D. vistulae*, and by high values of parasite species richness and diversity. Parasite communities of *S. carinus* were composed of six (42.9%) endoparasite and eight (57.1%) ectoparasite species. The allogenic community was comprised of five taxa, three of which were present as larval stages. The remaining nine parasites belonged to the autogenic community.

There were broad differences in composition of the parasite species between Işıklı Spring and Kufi Stream that corresponded to different environmental conditions. *Pomphorhynchus* sp. was abundant in the spring and *Lamproglena pulchella* was also found only in the spring, whereas protozoans and *Lernaea cyprinacea* were recorded in Kufi Stream. The overall mean parasite species richness at the infra-community level, for all species combined, was 2.3. The mean species richness was significantly higher in samples from Kufi Stream (5.8) than from Işıklı Spring (1.8). The mean parasite species richness was 2.3 in female and 2.5 in male fish hosts. The highest dominance index of the parasites was recorded for *Pomphorhynchus* sp. (47.9%) and the lowest for Nematoda (0.3%; Table 2). The mean parasite diversity (Shannon's H index) was 1.55 and the Shannon-Wiener Evenness (E) had a mean value of 0.67 (Table 3).

There were significant differences in the number of *Pomphorhynchus* sp. individuals between the size classes of *S. carinus* (Kruskal-Wallis $p < 0.05$). The mean intensity of *Pomphorhynchus* sp. increased with increasing the total length of the host from 11.0 in the 17.0–18.9 cm length class and to 34.8 in the > 25.0 cm length class, which was the class with the highest mean intensity and abundance of *Pomphorhynchus* sp. The condition factor of each size group was calculated to analyse the influence of *Pomphorhynchus* sp. on fish condition; no significant differences were found (Kruskal-Wallis $p > 0.05$).

The identification of acanthocephalan specimens remained problematic. The fresh and unfixed

worms had morphology of the proboscis hooks similar to that described for *P. tereticollis* (first four to five hooks longest, fifth or sixth hooks stoutest, posterior hooks of fifth or sixth rows significantly shorter, hooks at the posterior half of the proboscis with proximal projections on the base, and the last hook row stands at the posterior end of the proboscis). However, PCR studies showed that these specimens were genetically distinct from *P. tereticollis* (Špakulová & Perrot-Minnot, personal communication). In addition, we identified as "*Pomphorhynchus* sp. acanthellae" or "cystacanths of *Pomphorhynchus* sp." acanthocephalans that were found to infect 36.9% of 2201 *Gammarus obnixus* individuals examined in March, which was the prevalent amphipod in the spring, with a mean intensity of 2.6 and a mean abundance of 0.9.

Discussion

The composition and structure of parasite communities of *Squalius carinus* in Işıklı Lake was studied at the infracommunity and component community levels. The present study also aimed to evaluate how parasite infra-communities were related to the condition factor of the fish host. It has been demonstrated that fish condition factors are affected by environmental quality and food sources (Bolger & Connolly 1989) as well as by the presence or abundance of certain species of parasites (Tavares-Dias et al. 2000). Gaygusuz et al. (2013) compared relative body condition of native fish species in the absence (allopatry) and presence (sympatry) of non-native fish species from three different regions in western Anatolia and found differences in body condition between allopatric and sympatric populations. The *S. carinus* population in the Işıklı Lake is affected by the presence of both parasites and non-native fish species.

The present study provides the first record of parasites from this endemic fish in the Işıklı Lake. The high recorded ectoparasite species richness and the lack of digeneans could be related to the ecological characteristics of the area and the feeding habits of the fish host. The diet of chub includes both plant material and invertebrates, confirming their generalist and opportunistic feeding behaviour (Balestrieri et al. 2006) but plant material predominates (Hellawell 1971). Older *S. cephalus* in age groups IV, V and VI from the Tödürge Lake, Turkey, behaved mostly as carnivores but also consumed some macrophytes (Ünver & Erk'akan 2011). In the Işıklı Lake, *Gammarus obnixus*, an intermediate host of *Pomphorhynchus* sp., is likely to be common prey of *S. carinus*, therefore resulting in the dominance of this acanthocephalan in *S. carinus* parasite communities. *Diplostomum* sp. was found to be the second most common parasite species and this may be related to the feeding and resting behaviour of *S. carinus*. Marcogliese & Compagna (1999) suggested that benthic fish are more vulnerable to *Diplostomum* infection. Additionally, lake eutrophication may increase the abundance of digeneans, especially diplostomids (Hartmann & Numann 1977).

In conclusion, *S. carinus* presented high parasite richness at the component community level but one species, *Pomphorhynchus* sp., dominated the infracommunities. The mean abundance of all other parasite species was very low. The mean infracommunity richness was higher in male fish hosts than in females. Although the mean intensity and abundance of *Pomphorhynchus* sp. increased with increasing host size class, there were no significant differences in the condition factor between these size classes.

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