Endohelminth communities of the perch, *Perca fluviatilis* (Perciformes, Percidae) from Srebarna Biosphere Reserve, Bulgaria

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Summary

Altogether 180 specimens of *Perca fluviatilis* (L.) from Srebarna Lake, North-eastern Bulgaria, were examined for helminths during spring, summer and autumn of 2005 – 2006. Of them, 143 (79.4 %) perch were infected with helminths. Thirteen helminth species were found: metacercariae of *Bolbophorus confusus*, *Diplostomum pseudospathaceum*, *D. spathaceum*, *Ichthyocotylurus pileatus*, *Posthodiplostomum cuticola* and *Tylodelphys clavata*; cestode *Proteocephalus percae*; acantocephalans *Acanthocephalus anguillae* and *A. lucii* and nematode larvae *Contracaecum microcephalum*, *Eustrongilides excisus*, *E. tubifex* and *Rhaphidascaris acus*. The digenean *T. clavata* was dominant species of the perch component community. Little seasonal differences were found in the prevalence, abundance and mean infection of helminths. *Eustrongilides tubifex* reported from the territory of Bulgaria for the first time, represent the new geographical records and new species of Bulgarian helminth fauna.

P. fluviatilis was reported as a new host record for two digeneans, namely *Diplostomum pseudospathaceum* and *Posthodiplostomum cuticola*, the cestode *Proteocephalus percae*, the acantocephalan *Acanthocephalus anguillae* and the nematodes *Contracaecum microcephalum* and *E. tubifex* from the territory of Bulgaria. *P. fluviatilis* was reported as a new host record for *D. pseudospathaceum*, *P. cuticola*, *C. microcephalum* and *E. tubifex* from Balkan peninsula.

Keywords: Helminths; helminth communities; biodiversity; *Perca fluviatilis*; Srebarna Biosphere Reserve; Bulgaria

Introduction

The Srebarna Lake has been declared as a biosphere reserve under the Programme on Man and the Biosphere

(UNESCO). As a site of the World Natural Heritage, it was also included in the List of Wetlands of International Importance (Ramsar Convention) and Important Bird Areas (BirdLife International). The Srebarna Biosphere Reserve is situated in North-eastern Bulgaria (44°7'N, 27°5'E) and it includes the Srebarna Lake (a Danubian oxbow lake) and adjacent wet grasslands and inundated forests. The Srebarna Lake is freshwater eutrophic lake connected via an artificial canal with the Danube (Michev *et al.*, 1998; Uzunov *et al.*, 2001; Pehlivanov *et al.*, 2006) and characterized by a significant diversity of highly protected species, including globally threatened fish-eating birds such as Dalmatian pelican, *Pelecanus crispus* Bruch, 1832, Pygmy Cormorant, *Phalacrocorax pygmeus* (Pallas, 1773) and Ferruginous duck, *Aythya nyroca* (Güldenstädt, 1770) (Michev *et al.*, 1998). The total number of registered invertebrate taxa in the macrozoobenthos of the Srebarna lake was estimated to be 236 (Varadinova *et al.*, 2009, etc.). The list of fishes from Srebarna lake include twenty six species – 18 species of Cyprinidae, 4 species of Percidae, 3 of Gobiidae and by one species of Esocidae, Umbridae, Siluridae, Gobiidae, Centrarchidae and Gasterosteidae (Pehlivanov, 2000; Pehlivanov & Pavlova, 2009). The fish fauna is composed mainly of common Danubian species with perch, *Perca fluviatilis* (L.) representing one of the most abundant fish species of the lake (Pehlivanov, 2000; Pehlivanov *et al.*, 2005; Pehlivanov & Pavlova, 2009).

Helminth parasites of fish from Srebarna Lake have never been studied in detail (Michev *et al.*, 1998). The only ichthyoparasitological data are those by Margaritov (1959), who reported only three helminth species, among them *Bolbophorus confusus* (Krause, 1914) parasitizing perch. The present study on helminth parasites of perch represents a part of comprehensive investigations carried out in the Srebarna Lake during the years 2004 – 2006 with the aim

to characterize perch helminths as an integral part of the biodiversity inventory. Since data on fish helminth communities from the Lower Danube area are scarce, the other aim of this study was to analyze the structure of helminth communities of perch from the Srebarna Lake as well.

Materials and Methods

During spring, summer and autumn of 2005 – 2006, a total of 180 perch (the body length from 103 to 305 mm, average 154 ± 4.5) were examined for helminth parasites. Thirty host individuals were studied per season during every year and on sixty perch per season for both years (2005 and 2006, N = 60). Fish were captured by local fishermen using various methods (netting, angling or electro-fishing). They were transported alive in the water from the lake to the laboratory of Field Station - Srebarna (Laboratory of Bulgarian Academy of Sciences). After fish were dissected

and immediately examined. A small part of samples was either fixed in 4 % formaldehyde or frozen for subsequent studies. A total number of 2,122 helminths was obtained and examined during the whole period of investigation. Helminths were separated from internal organs and tissues and also from fins, skin, gills, eyes and brain.

All parasites were identified and counted. Cestodes and trematodes were stained in iron acetocarmine, dehydrated in ethanol series with increasing concentrations, cleared in eugenol and mounted in Canada balsam (Bykhovskaya-Pavlovskaya, 1985; Georgiev *et al.*, 1986; Scholz & Hanzelová, 1998). Larval developmental stages of digeneans (metacercariae) of genus *Diplostomum* were cleared in dimethylphthalate and mounted in Canada balsam (Niewiadomska & Niewiadomska-Bugaj, 1995; Shigin, 1986). Nematodes and acanthocephalans were studied as temporary glycerol mounts (Bykhovskaya-Pavlovskaya, 1985; Moravec, 1994). The ecological and epidemiological

Table 1. Species diversity of helminth parasites of the perch, *Perca fluviatilis*, from the Srebarna Lake (p - number of parasites; P - prevalence; MA - mean abundance; MI - mean intensity)

Helminth species	p	P (%)	MA ± SD	Intensity		Site
				MI ± SD	Range	
Trematoda						
<i>Bolboforus confusus</i> (Krause, 1914), metacercariae	8	4.4	0.04 ± 0.21	1.00	1	mesentery, gills (encysted)
<i>Diplostomum pseudospathaceum</i> , (Newiadomska, 1984), metacercariae	5	1.1	0.03 ± 0.31	2.50 ± 2.12	1 – 4	lens
<i>Diplostomum spathaceum</i> (Rudolphi, 1819), metacercariae	5	1.7	0.03 ± 0.25	1.67 ± 1.15	1 – 3	lens
<i>Ichthyocotylurus pileatus</i> (Rudolphi, 1802), metacercariae	20	1.1	0.11 ± 1.05	10	10	stomach and pyloric caeca
<i>Posthodiplostomum cuticola</i> (Nordmann, 1832), metacercariae	2	0.6	0.01 ± 0.15	2.00	2	skin, gills
<i>Tylodelphys clavata</i> (Nordmann, 1832), metacercariae	>2000	75.0	11.11 ± 22.25	14.81 ± 24.61	1 – 118	vitreous humour
Cestoda						
<i>Proteocephalus percae</i> (Müller, 1780)	9	1.7	0.05 ± 0.39	3.00	3	stomach, intestine
Acanthocephala						
<i>Acanthocephalus anguillae</i> (Müller, 1776)	1	0.6	0.01 ± 0,07	1.00	1	intestine
<i>Acanthocephalus lucii</i> (Müller, 1776)	1	0.6	0.01 ± 0.07	1.00	1	intestine
Nematoda						
<i>Contracaecum microcephalum</i> (Rudolphi, 1809), larvae	2	1.1	0.01 ± 0.11	1.00	1	intestine
<i>Eustrongilides excisus</i> Jägerskiöld, 1909, larvae	44	13.9	0.24 ± 0.88	1.76 ± 1.74	1 – 9	mesentery, skin, muscles, surface of internal organs, (encysted)
<i>Eustrongilides tubifex</i> (Nitzsch, 1819), larvae	1	0.6	0.01 ± 0.07	1.00	1	mesentery (encysted)
<i>Rhaphidascaris acus</i> (Bloch, 1799), larvae	24	3.3	0.13 ± 0.77	4.00 ± 1.55	2 – 5	intestine, abdominal cavity

terminology followed Bush *et al.* (1997). Brillouin's Diversity Index and Berger-Parker Dominance Index were calculated as described by Maguran (1988). The dominance was determined according to the criteria proposed by Kennedy (1993) on the basis of the prevalence (P): satellite species ($P < 10$), secondary species ($10 < P < 20$) and core species ($P > 20$). Mean abundance (MA) and mean intensity of infection (MI) were calculated using Microsoft Excel and STATISTICA 6.0 program. Seasonal differences were evaluated by applying Chi-square test for prevalence, Bootstrap 2-sample t-test for mean intensity of infection (Quantitative Parasitology - version 3.0 available at website <http://www.behav.org/qp/qp.htm>; Rozsa *et al.*, 2000) and Kruskal-Wallis test H for the abundance (STATISTICA 6.0). The species of parasites were determined by Bykhovskaya-Pavlovskaya, 1982; Scholz & Hanzelová, 1998; Shigin, 1986; Moravec, 1994).

Results

Species diversity of helminthes

Thirteen endohelminth species were identified: 6 digeneans - *Bolboforus confusus*, *Diplostomum spathaceum*, *D. pseudospathaceum*, *Ichthyocotylurus pileatus*, *Posthodiplostomum cuticola* and *Tylodelphys clavata*; 1 cestode species - *Proteocephalus percae*; 2 acanthocephalans - *Acanthocephalus anguillae* and *A. lucii* and 4 nematodes - *Contracaecum microcephalum*, *Eustrongilides excisus*, *E. tubifex* and *Rhaphidascaris acus*. No monogeneans were found. Three species, the tapeworm *P. percae* and both acanthocephalans (23.1 %) occurred as adults and remaining 10 species (76.9 %) were found at the developmental stage of the larvae (Table 1). *P. percae*, *A. lucii*, *A. anguillae* and *R. acus* were autogenic species and allogenic species group comprised of other nine helminth species parasitizing fish-eating birds at their mature stage.

Table 2. Seasonal variation of helminth infection indices in perch from the Srebarna Lake (P - prevalence; MI - mean intensity, N-number of examined host)

Helminth species	Spring (N = 60)		Summer (N=60)		Autumn (N=60)	
	P (%)	MI $\pm$ SD	P (%)	MI $\pm$ SD	P (%)	MI $\pm$ SD
		Range		Range		Range
<i>Bolboforus confusus</i>	3.3	1.00 1	10.0	0.83 $\pm$ 0.41 1	1.7	1.00 1
<i>Diplostomum pseudospathaceum</i>					3.3	2.50 $\pm$ 2.12 1 – 4
<i>Diplostomum spathaceum</i>	3.3	1.00 1	1.7	3.00 3		
<i>Ichthyocotylurus pileatus</i>			3.3	10 10		
<i>Posthodiplostomum cuticola</i>			1.7	2.00 2		
<i>Tylodelphys clavata</i>	56.7	15.29 $\pm$ 25.78 1 – 109	81.7	8.51 $\pm$ 10.20 1 – 44	86.7	20.44 $\pm$ 31.51 1 – 118
<i>Proteocephalus percae</i>	1.7	3.00 3			3.3	3.00 3
<i>Acanthocephalus anguillae</i>	1.7	1.00 1				
<i>Acanthocephalus lucii</i>	1.7	1.00 1				
<i>Contracaecum microcephalum</i>				-	3.3	1.00 1
<i>Eustrongilides excisus</i>	8.3	1.40 $\pm$ 0.55 1 – 2	10.0	2.50 $\pm$ 3.21 1 – 9	23.3	1.57 $\pm$ 1.09 1 – 4
<i>Eustrongilides tubifex</i>					1.7	1.00 1
<i>Rhaphidascaris acus</i>			10.0	4.00 $\pm$ 1.55 2 – 5		

Component community

Helminths were found in 143 (79.44 %) of 180 fish hosts examined during two years. The metacercariae of *T. clavata* showed the highest epidemiological indices ($P = 75.0$ %; $MA = 11.1$; $MI = 14.8$) over the entire period of our study. This species also represented the only core species parasitizing perch in the Srebarna Lake. On the other hand, the lowest parameters of infestation were found in *P. cuticola*, *A. anguillae*, *A. lucii* and *E. tubifex* (Table 1). *E. excisus*, with the prevalence ranging seasonally (between 8.3 – 23.3%) was the secondary species and all other helminths were ranked among satellite parasites of perch (Table 1 and 2).

Significant seasonal differences in the prevalence of perch helminths were recorded in metacercariae of *T. clavata* and nematode larvae of *E. excisus* (Chi-square test, $df = 2$, $p < 0.05$). Both species occurred less frequently in spring in comparison with summer and autumn seasons. Variation of the mean abundance of perch helminths was slightly more expressive. Besides former two species, significant differences among three seasons were evidenced also in *R. acus* nematode larvae (Kruskal-Wallis test: *T. clavata* - H (2, N = 180) = 16.02137, $p = 0.0003$; *E. excisus* - H (2, N = 180) = 12.34168 $p = 0.0021$; *R. acus* - H (2, N = 180) = 6.582291 $p = 0.0372$). Seasonal changes in mean intensity of infection were found being negligible. More prominent differences were observed only in mean intensity of infection of *T. clavata* between summer and autumn (Bootstrap t-test $p < 0.05$) (Table 2). The species diversity and dominance were alike in the different season periods (Table 3).

Table 3. Seasonal variations of species diversity and dominance within helminth communities in the perch from the Srebarna Lake

Season	Number of helminth species	Number of helminth individuals	Brillouin's Diversity Index	Berger-Parker Dominance Index
Spring	7	536	0.165	0.970
Summer	7	486	0.596	0.858
Autumn	7	1100	0.185	0.965

Infracommunity

Out of 180 examined fish hosts, 143 specimens (79.4 %) were infected with helminths. The infracommunities consisted of 1 – 3 helminth species (mean value 1.3); 102 hosts (71.3 %) were infected with only one helminth species, 33 perch (26.6 %) with two species and 8 perch (5.6 %) with 3 helminth species. The maximum number of helminth individuals recorded in a single perch was 122 (in two fish). Perch were mostly infected with one and five helminths (17.0 % and 9.4 %, respectively). Fifteen (8.3 %) fishes harboured two helminths and 14 (7.8 %) perch hosted 4 worms. The total number of helminths ranged from 1 to 122 specimens per one fish (11.8 on average).

Discussion

Last 50 years, the helminth fauna of perch was not subjected to serious complex research in the Srebarna Lake. Margaritov (1959), within his study of fish fauna of this

lake reported only 3 helminth species. In our study, 13 helminth species have been identified and all but one (*B. confusus*) are recorded for the first time from perch in the Srebarna Lake.

To date, helminth parasites of perch have been studied from various rivers and water reservoirs in Bulgaria. In Bulgarian part of the Danube River, Margaritov (1966) found only three helminths parasitic in perch, namely *Acanthocephalus lucii*, the digenean *Nicolla skrjabini* (Iwanitzky, 1928) and *Rhabdochona* sp. nematodes. Kakacheva-Avramova (1977) and Kakacheva-Avramova *et al.* (1978) discovered more varied diversity of perch parasites from the Danube River.

They found digeneans *Bunodera luciupercae* (Müller, 1776), *Diplostomum baeri* Dubois, 1937 (metacercariae), *N. skrjabini*, acanthocephalans *A. lucii* and *Pomphorhynchus laevis* (Müller, 1776) and also specimens of *Rhabdochona* and *Contracaecum* (larvae).

More recent papers dealing with perch parasites by Nedeva & Grupcheva (1996), Nedeva & Babacheva (1999), Nedeva *et al.* (2003) and Kirin (2005) described the occurrence of some other helminths of perch from Bulgarian rivers and water reservoirs. Except from previously mentioned species, they gave an account of findings of two monogeneans *Gyrodactylus gasterostei* Glaser, 1974 and *Dactylogyrus anchoratus* (Dujardin, 1845), digeneans *Diplostomum chromatophorum* (reported as *D. volvens*), *Ichthyocotylurus pileatus* and *Tylodelphys clavata*, a cestode *Caryophyllaeus brachycollis* Janiszewski, 1953, acanthocephalans *Acanthocephalus anguillae* and *Neoechinorhin-*

chus rutili (Müller, 1870) and nematodes *Eustrongilides excisus* and *Raphidascaris acus*.

The present findings of *Eustrongilides tubifex* is reported for territory of Bulgaria for the first time and thus represent the new geographical records and new species of Bulgarian helminth fauna. *Diplostomum pseudospathaceum*, *Posthodiplostomum cuticola*, the cestode *Proteocephalus percae*, the acanthocephalan *Acanthocephalus anguillae* and the nematodes *Contracaecum microcephalum* and *E. tubifex* are reported from the first time for the helminth communities of *P. fluviatilis* from the territory of Bulgaria. *P. fluviatilis* was reported as a new host record for *D. pseudospathaceum*, *P. cuticola*, *C. microcephalum* and *E. tubifex* from Balkan peninsula.

Srebarna Lake is an oxbow lake, last 15 years connected with the Danube via an artificial canal. Since fish populations in the lake and the river are believed to be closely associated (frequently exchanging individuals), the species composition of the component communities of perch were

expected to be very much alike. However, there was only one species, the acanthocephalan *A. lucii* which was found parasitic in perch in both the Danube River and the Srebarna Lake.

A possible reason of this phenomenon could be the different ecological conditions of these localities. The parasite species with complex life cycles which were mostly recorded in the Danube River use benthic molluscs and crustaceans as intermediate hosts. The Srebarna Lake bottom is covered by a thick layer of detritus which is generally associated with low oxygen concentrations and low density of benthic organisms. As a result of this, the composition of the benthic communities involve mostly dipterans (chironomids) of the Srebarna Lake (Uzunov *et al.*, 2001). Molluscs are almost entirely lacking in benthic samples from the central part of the lake which is probably due to the thick detritus layer and the absence of aquatic vegetation (Angelov, 2000; Uzunov *et al.*, 2001). One of the most abundant mollusc species, *Radix ovata* (L.) serves as the intermediate host of *T. clavata*, representing the only core species parasitizing perch in the Srebarna Lake. The great number of metacercariae of *Diplostomum* recorded in the course of the present study can be explained with the large diversity of fish-eating birds (Michev *et al.*, 1998; Angelov, 2000) and rather high abundance of snails occurring in the shore region of the lake.

Halmetoja *et al.* (2000) who studied parasites of perch from a Vetämajärvi natural lake in Finland recorded 18 parasite species. Nine core species with the prevalence exceeded 20 % were recorded: *Bunodera luciopercae* (48.6 %), *Diplostomum* spp. (97.3 %), *Diplostomum spathaceum* (48.6 %), *T. clavata* (metacercariae; 75.5 %), *Ichthyocotylurus variegatus* (43.2 %), *Trienophorus nodulosus* (29.7 %), *P. percae* (21.6 %), *Camallanus lacustris* (91.9 %) and *Acanthocephalus lucii* (62.2 %). Concerning seasonal dynamics, the prevalence of core species was the highest in autumn and the lowest in spring (Halmetoja *et al.*, 2000), which correlates well with results of Nedeva & Grupcheva (1996) also with present results.

Based on several most recent parasitological studies (Halmetoja *et al.*, 2000; Kirin, 2005; Sobecka & Slomińska, 2007; Morozińska-Gogol, 2009; present study), the helminth species diversity is not very high in perch (13 – 42) and digeneans always represent a dominant group of its component communities which was also found during our studies in the Srebarna Lake.

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