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HELMINTHS AND HELMINTH COMMUNITIES OF THE BROWN TROUT (*Salmo trutta fario*, Linnaeus, 1758) FROM THE TAMRASHKA RIVER, BULGARIA

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Abstract

Ecoparasitological examinations of brown trout from the Tamrashka River, Aegean Water Basin, Bulgaria were carried out. Five species of helminths, one Trematoda species (Nicolla skryabini (Iwanitzky, 1928) Ślusarski, 1972) and four Nematoda species (Rhabdochona hellichi (Šramek, 1901) Chitwood, 1933; Raphidascaris acus (Bloch, 1779); Salmonema ephemeridarum (Linstow, 1872) Moravec, Santos et Brasil-Sato, 2008; Schulmanela petruschewskii (Shulman, 1948) Ivashkin, 1964) are determined. S. ephemeridarum was distinguished with the highest prevalence (50%). It is a core species for the helminth communities of the brown trout from the studied river ecosystem. *Sch. petruschewskii is a new parasite species of brown trout and S. t. fario is a new host record for Sch. petruschewskii in Bulgaria. The Tamrashka River is a new habitat for N. skryabini, Rh. hellichi, R. acus, S. ephemeridarum and Sch. petruschewskii as parasites of the brown trout in Bulgaria.*

Key words*: Aegean Water Basin, helminths, helminth communities, Salmo trutta fario.*

INTRODUCTION

The Tamrashka River is related to the Aegean Water Basin. The river and its adjacent terrestrial ecosystems (predominantly forest ecosystems) are located in one of the most beautiful sections of the Rhodopa Mountain - the Chernatitsa ridge, Bulgaria. In the past geological times, the ridge was one of the first continental droughts in the Balkans. The Tamrashka River springs from 1816 meters west of Modar Peak (Valchev, 2015). The river ecosystem refers to type R3: Mountain, Rock Type Rivers in Ecoregion 7 - Eastern Balkans (Belkinova et al., 2013). The territory is distinguished by its extremely biodiversity and the most natural complexes unaffected by human activity. At the same time, there is very little data in the scientific literature about the biodiversity, nature conservation significance and ecological status of the freshwater ecosystem of the Tamrashka River. Fish are an essential element of biodiversity in the river ecosystems. Elements of biodiversity are the parasites and the parasite communities formed in them. The most endoparasites have complex development cycles and thus reflect the state of the biodiversity and the environment. The study aims to present the results of the examinations of the gastrointestinal helminths and their helminth communities of the brown trout (*Salmo trutta fario* Linnaeus, 1758), as a typical and dominant fish species of the ichthyocenoses in the studied river ecosystem.

MATERIALS AND METHODS

During 2018, a total of 6 specimens *Salmo trutta fario* Linnaeus, 1758 are studied for gastrointestinal helminths. The fish are caught by angling under a permit issued by the Ministry of Agriculture, Food and Forestry of the Republic of Bulgaria. Ecologo-helminthological studies are performed according to Zashev & Margaritov (1966); Bauer (Ed.) (1987); Moravec (2013). All parasite specimens are fixed and stored in 70% ethyl alcohol. For species determination, the trematode specimens are included in permanent microscope slides by the methods of Georgiev et al. (1986); Scholz & Hanzelova (1998) and the specimens of nematodes - in temporary microscope slides by the method of Moravec (2013). Helminth community structure is

studied in both levels: infracommunity (total and mean number of species; total and mean number of specimens; Brillouin's diversity index; Pielou's evenness index) and component community (prevalence - P%; mean intensity - MI, for each species) (Bush et al., 1997; Magurran, 1988). According to the criteria of Kennedy (1997), the species of the component community are classified as core species (P% > 20), component (P% > 10) and accidental species (P% < 10). Calculation of the diversity measures is performed by software products Statistica 10 (StatSoft Inc., 2011) and MS Excel (Microsoft 2010).

RESULTS AND DISCUSSIONS

Fish communities

The brown trout (*Salmo trutta fario* Linnaeus, 1758; Salmonidae) inhabits clean, oxygen-rich, fast-flowing and cold waters. Brown trout reach 40 cm in length and 800 g in weight, in rare cases and more, growing relatively slowly. Young specimens feed mainly on insect larvae - daydreams, brooks, lower crustaceans, and adults - with water beetles, and small fish. The species breeds from late September to early December at 6-8^{0}C and always migrates upstream during reproduction. Sexual maturity reaches 2-4 years, and individuals of 14-21 cm length are most important for the reproduction of populations. The brown trout is a native fish species for Europe (including Bulgaria) and North Asia. The species is subject to commercial and sport fishing. *S. t. fario* is a Least concern (LC) species by the IUCN (Freyhof, 2012; Karapetkova & Zhivkov, 2006). The brown trout is a bioindicator species in freshwater ecosystems as sensitive species. *S. t. fario* is also a well-established 'model organism' for heavy metal bioaccumulation (Dvorak et al., 2020).

Helminth community structure

A total of five species of parasites belonging to two classes, four orders and four families were found in the six examined specimens of the brown trout. A total of 91 specimens of gastrointestinal helminths were studied (Table 1). The Trematoda species *Nicolla skrjabini* (Iwanitzy, 1928) Ślusarski, 1972, are distinguished by a one year developmental cycle, involving two intermediate hosts. The first intermediate hosts (for sporocysts) are the snails *Lithoglyphus naticoides* (Pfeiffer, 1828). They are localizing to the liver, glands and gills. Second intermediate hosts (for the metacercariaes) are the crustaceans *Gammarus balcanicus* Schäferna, 1923; *Obesogammarus crassus* (Sars, 1894) and *Dikerogammarus haemobaphes* (Eichwald, 1841). In them, the metacercariaes are located in the musculature of the dorsal side of the body and limbs, also in the body cavity.

Table1. Biological diversity and basic ecological characteristics of the helminth communities of *S. trutta fario*

Helminth species	N[1]	P[2]	P%[3]	MI[4]
Trematoda Class Order Fasciolida Family Opecoelidae				
Nicolla skrjabini (Iwanitzky, 1928) Ślusarski, 1972	2	7 2-5	33.34	3.5
Nematoda Class Order Spirurida Family Rhabdochonidae				
Rhabdochona hellichi (Šramek, 1901) Chitwood, 1933	2	28 6-22	33.34	14
Order Ascaridida Family Anisakidae				
Raphidascaris acus (Bloch, 1779)	1	40	16.67	40
Order Trichinellida Family Capillariidae				
Salmonema ephemeridarum (Linstow, 1872) Moravec, Santos et Brasil-Sato, 2008	3	9 1-4	50	3
Schulmanela petruschewskii (Shulman, 1948) Ivashkin, 1964	1	7	16.67	7
Total number of species	5			
Mean ± SD	1.50 ± 0.55			
Number of fish	3		3	
Number of helminth species	3		1	
Number of fish	1	1	1	3
Number of helminth specimens	11	22	40	6
Total number of specimens	91			
Mean ± SD	10.12 ± 12.80			
HB (Brillouin's diversity index)	1.26			
E (Pielou's evenness index)	0.837			

[1]N = total number of infected fish specimens.
[2]P = total number of endoparasite specimens.
[3]P% = prevalence.
[4]MI = mean intensity.

Metacercariaes are encysted, with oval or round, dark, unevenly pigmented cysts (Bauer, 1987). *G. balcanicus* is a bioindicator for χ–saprobityand refers to the relatively tolerant forms (group C) (Rusev, 1993; Peev & Gerasimov, 1999; Belkinova et al., 2013).

Definitive hosts of *N. skryabini* are different fish species of the families Cyprinidae, Percidae, Gobiidae, Siluridae, Gadidae, Esocidae, Acipenceridae, Salmonidae (Bauer, 1987). *N. skrjabini* was reported by *S. trutta fario*, including from the rivers in the Rhodopa Mountain (from Trigradska and Vacha rivers – Kakacheva-Avramova and Nedeva, 1978; from Chuprenska, Trigradska, Vacha, Shirokolashka rivers – Kakacheva-Avramova and Nedeva, 1979; from Trigradska, Vacha, Chuprenska rivers; rivers from Rhodopa Mountain in the district of the town Devin-Kakacheva-Avramova and Nedeva, 1982; Table 2). According to this study, the Tamrashka River is a new habitat of *N. skryabini* as a helminth species on the brown trout.

Rhabdochona hellichi (Šramek, 1901) Chitwood, 1933 is an intestinal parasite of many fish species of the families Cyprinidae, Salmonidae, Acipenseridae, Siluridae etc., which are the final hosts of the species (Bauer, 1987; Kakacheva-Avramova, 1983; Moravec, 2013). *Rh. hellichi* has been reported as a parasite of *S. t. fario* including from rivers of the Rhodopa Mountain (from Trigradska, Devinska, Vacha, Mugla, Shirokolashka rivers – Kakacheva-Avramova & Nedeva, 1979; Table 2). The Tamrashka River is a new habitat of *R. hellichi* as a parasite species of *S. trutta fario*.

Raphidascaris acus (Bloch, 1779) as an adult form is found in the intestine and stomach of various species of freshwater fish (*Esox lucius* Linnaeus, 1758, *Salmo trutta fario* Linnaeus, 1758, *Lota lota* (Linnaeus, 1758), *Anquilla anquilla* (Linnaeus, 1758) *Perca fluviatilis* (Linnaeus, 1758), *Sander lucioperca* (Linnaeus, 1758), *Hucho hucho* (Linnaeus, 1758), *Onchorhynchus mykiss* (Suckley, 1859), *Thymallus thymallus* (Linnaeus, 1758) etc.). Free or encapsulated larvae of *R. acus* have been established in different internal organs of freshwater fish from different families, acting as intermediate or paratenic hosts for the parasite species. The species *R. acus* has been reported as a parasite of different species of fish in Bulgaria (Bauer, 1987; Kakacheva-Avramova, 1983; Moravec, 2013). The species has been reported from *S. t. fario*, including from rivers in the Rhodopa Mountain (from the Arda River - Kirin, 2002; Table 2). The Tamrashka River is reported for the first time as a new habitat of *R. acus* as a parasite species of *S. t. fario*.

Table 2. Species of endoparasites of *S. t. fario* in Bulgaria

Species of endoparasites	Authors
Trematoda	
Bunodera lucioperca (Müller, 1776)	1, 8
Crepidistomum farionis (Müller, 1784)	10, 4, 5, 7, 8
Crepidostomum metoecus (Braun, 1900)	2, 3, 4, 6, 7, 8
Nicolla skrjabiny (Iwanitzky, 1928)	4, 5, 7
Nicolla proavita (Wišnewski, 1934) (syn. *Crowcrocaecum proavitum*)	2, 3, 4, 5, 6, 7
Nicolla wisniewski (Ślusarski, 1958)	4, 5, 6, 7, 8
Nicolla testobliqua (Wišnewski, 1933)	4, 6, 7
Cestoda	
Tetraonchus sp.	4
Acanthocephala	
Acanthocephalus anguillae (Müller, 1776)	1, 5, 7, 8
Pomphorhynchus laevis (Müller, 1776)	5
Metechinorhynchus truttae (Schrank, 1788)	4, 6, 7, 8
Nematoda	
Rhabdochona hellichi (Šramek, 1901) Chitwood, 1933	5, 8
Cucullanus truttae (Fabricius, 1794)	5, 6, 7
Raphidascaris acus (Bloch, 1779)	8
Salmonema ephemeridarum (Linstow, 1872) Moravec, Santos et Brasil-Sato, 2008 (Syn. *Ichthyobronema tennuissima*; *Cystidicoloides ephemeridarum*)	9, 10, 1, 2, 3, 4, 5, 6, 7, 8

[1]Kakacheva-Avramova, 1969.
[2]Kakacheva-Avramova, 1972.
[3]Kakacheva-Avramova, 1973.
[4]Kakacheva-Avramova & Nedeva, 1978.
[5]Kakacheva-Avramova&Nedeva, 1979.
[6]Kakacheva-Avramova & Nedeva, 1981.
[7]Kakacheva-Avramova & Nedeva, 1982.
[8]Kirin, 2002.
[9]Margaritov, 1959.
[10]Margaritov, 1964.

The life cycle of *Salmonema ephemeridarum* (Linstow, 1872) Moravec, Santos et Brasil-Sato, 2008 was carried out with the participation of the intermediate hosts *Habroflebia lauta* Eaton, 1884, *Habroleptoides modesta* (Hagen, 1864), *Ephemera danica* Müller, 1764. *H. lauta* and *E. danica* are bioindicators for 0-β-mesosaprobity of the water ecosystem. *H. modesta* is a bioindicator for 0-saprobity. Intermediate hosts refer to less sensitive forms (group B) (Rusev, 1993; Peev and Gerasimov, 1999; Belkinova et al., 2013). The adult parasite develops in definitive hosts of different species of fish from the families

Salmonidae, Thymalidae, Acipenseridae, Percidae, Esocidae, Angillidae. The species *S. ephemeridarum* was reported from *S. t. fario* in Bulgaria (Bauer, 1987; Kakacheva-Avramova, 1983; Moravec, 2013). The species was reported from the rivers in the Rhodopa Mountain (from the Vasil Kolarov Reservoir and the Yadenitsa River - Margaritov, 1959; 1964, respectively; from the Arda River - Kirin, 2002; Table 2). The helminth species was also reported as endoparasite species of brown trout from the Tundzha River and rivers of the Balkan Mountain (Kakacheva-Avramova, 1972 and Kakacheva-Avramova, 1973, respectively). The Tamrashka River is a new habitat of *S. ephemeridarum* as a parasite species of *S. t. fario*.

Schulmanela petruschewskii (Shulman, 1948) Ivashkin, 1964, accomplishes its development with the participation of intermediate hosts of oligochaetes of the species *Eisenia tetraedra* Jackson, 1931. The specific localization of the parasite is oligochaetes body cavity until it reaches an invasive stage. The final hosts for the parasite species are freshwater fish species (*Gymnocephalus cernua* (Linnaeus, 1758), *Cobitis taenia* (Linnaeus, 1758), *Lepomis gibbosus* (Linnaeus, 1758), *Sander lucioperca* (Linnaeus, 1758), *Perca fluviatilis* (Linnaeus, 1758), *Salmo trutta fario*, Linnaeus, 1758 and others). The adult form of the parasite inhabits the liver parenchyma of fish (Bauer, 1987; Kakacheva–Avramova, 1983; Moravec, 2013). The species *Sch. petruschewskii* has been reported from other freshwater fish species in Bulgaria (Kakacheva-Avramova, 1983). *S. t. fario* is a new host record for *Sch. petruschewskii* in Bulgaria. The Tamrashka River is reported for the first time as a new habitat of *Sch. petruschewskii*.

Component communities

Salmonema ephemeridarum (Linstow, 1872) Moravec, Santos et Brasil–Sato, 2008 is distinguished with the highest prevalence (P% = 50). It is the core species for the endoparasite communities of the brown trout. The species *Nicolla skryabini* (Iwanitzky, 1928) Ślusarski, 1972 and *Rhabdochona hellichi* (Šramek, 1901) Chitwood, 1933 are also core species for the helminth communities of *S. t. fario* from the Tamrashka River, but they are represented with lower prevalences (on P% = 33.34, respectively). *Raphidascaris acus* (Bloch, 1779) and *Schulmanela petruschewskii* (Shulman, 1948) Ivashkin, 1964 are component species for the endoparasite communities of the brown trout (on P% = 16.67, respectively). According to the results of the study, *Raphidascaris acus* is distinguished with the highest mean intensity (40 specimens in one specimen of fish), followed by *Rhabdochona hellichi*, MI = 14 (28 helminth specimens in two specimens of fish). The remaining species have significantly lower mean intensity (Table 1).

Infracommunities

The helminths of class Nematoda are represented by a significantly higher number of parasite species and specimens (four species with a total of 84 specimens) than those of the class Trematoda (one species with seven specimens). Three specimens of fish have two species of endoparasites, and the other three specimens of examined fish have on one endoparasite species. There are 40 specimens of *R. acus*, found in one specimen of brown trout; 22 specimens of *Rh. hellichi*, found in one specimen of fish and six specimens of some helminth species, found in another examined fish specimen. Mixed infection was detected in three specimens of fish: two specimens of *N. skrjabini* and four specimens of *S. ephemeridarum*; 5 specimens *N. skrjabini* and one specimens *S. ephemeridarum*; seven specimens *Sch. Petruschewskii* and four specimens *S. ephemeridarum*. Determined species of parasites of the brown trout (*S. trutta fario*), total number of taxa and specimens of them, peculiarities of the life cycles of the parasites, determined indices of diversity (HB = 1.26, Brillouin's diversity index) and evenness (E = 0.837, Pielou's evenness index) testify for the 0-β-mesosaprobty conditions and for the very good ecological status of the investigated freshwater ecosystem (Rusev, 1993; Peev & Gerasimov, 1999; Belkinova et al., 2013). No parasites causing dangerous diseases to fish, humans or other hosts have been identified, which cases and diseases (suspected pathogens) have been reported to other parasites by other authors (Kakacheva-Avramova and Menkova, 1979; Pekova et al.,

2017; Pekova et al., 2017a; Mitev et al., 2020). The endoparasites, identified in this study, account for 33.34% of the registered endoparasites of the brown trout in the country (Table 2). All helminth species of the brown trout identified in this study were reported by other countries and authors (Bauer, 1987; Moravec, 2013). *Salmo trutta fario* has been the subject of a number of helminthological studies in other countries. Hanzelová et al. (2001) were studied *S. t. fario* from the Morskeoko Lake in Eastern Slovakia. The authors reported for five species of helminths: *B. luciopercae*, *P. longicollis*, *N. rutili*, *P. laevis*, *C. ephemeridarum*. With highest prevalence, mean abundance and mean intensity they were fixed *N. rutili* and *P. longicollis* (P% = 86, MA = 13, MI = 15.2; P% = 53, MA = 12, MI = 22.4, respectively). Moravec (2002) studied the morphological differences between *Crepidostomum farionis* and *C. metoecus* by the methods of a scanning electron microscopy. Pellitero (1979) studied *S. t. fario* from different water ecosystems in Spain. The author established eight species of helminths belonging to classes of Nematoda and Trematoda, but he does not identify helminths belonging to classes Cestoda and Acanthocephala. The author determined the prevalences of the fixed helminth species: *Cystidicoloides tennuissima* (P% = 79.47); *Crepidostomum metoecus* (P% = 78.96); *C. farionis* (P% = 63.10); *Raphidascaris acus* (P% = 44.10); *Spinitectus gordoni* (P% = 39.47); *Capillaria coregoni* (P% = 25.96); *Nicolla* sp. (P% = 18.33); *Rhabdochona sulaki* (P% = 7.69). *C. tennuissima*, *R. acus*, *C. coregoni*, *Nicolla* sp. and *Rh sulaki* Pellitero reported as new species records for Spain and Portugal. The infection with *Rh. sulaki* was determined as accidental. The author proves the links for the following infections: *C. farionis* and *C. metoecus*; *C. farionis* and *C. tenuissima*; *C. metoecus* and *C. tenuissima*. The biocenotic relations, Pellitero explained with seasonal differences and different ecological characteristics in studied freshwater ecosystems. Moravec (2004) was studied the transmission and the seasonality of infection of the helminth species *R. acus* in *S. t. fario* from a small stream in North Bohemia, Czech Republic. The author points to *Gammarus fossarum* Koch, 1836 as an intermediate host for this parasite species and highest infection (prevalence and intensity) in August and October. Mladineo et al. (2009) were examined *S. t. fario* from the Cetina River (Croatia). The authors reported for two species of parasites: *Cyathocephalus truncatus* (Cestoda, Spathebothriidae) and *Echinorhynchus trutae* (Acanthocephala, Echinorhynchidae), parasitizing in the intestine of the examined fish. They point out that the heavy load on the body of the trout with intestinal parasites could cause their exhaustion and even death.

CONCLUSIONS

As a result of this study, *S. t. fario* is a new host record for *Sch. Petruschewskii* in Bulgaria.
The Tamrashka River is a new habitat of *N. skryabini*, *Rh. hellichi*, *R. acus*, *S. ephemeridarum*, *Sch. petruschewskii* as parasite species of *S. t.fario*. *S. ephemeridarum*, *N. Skryabini* and *Rh. hellichi* are core species in the helminth communities of brown trout.
R. acus and *Sch. petruschewskii* are component helminth species in these communities.

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