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HELMINTHS AND HELMINTH COMMUNITIES OFORPHEUS DACE (*Squalius orpheus* Kottelat & Economidis, 2006) FROM STRYAMA RIVER, BULGARIA

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Abstract

During 2018, studies on the biodiversity and biomonitoring by the biological elements for environmental quality: Squalius orpheus (endemic of Balkan Peninsula) and its helminths and helminth communities were carried out. In 59 specimens of Sq.orpheus, four specimens of intestinal helminths are fixed (Allocreadium isoporum (Kowal et Kulakowskaja, 1957); Caryophyllaeus brachycollis (Janiszewska, 1951); Pomphorhynchus laevis (Müller, 1776); Rhabdochona denudata (Dujardin, 1845)). P. laevis is distinguished with the highest prevalence and mean intensity (66.10% and 1.85, respectively). A. isoporum, P. laevis and Rh. denudata are core species for the helminth communities of Orpheus dace, while C. brachycollis is a component species. The eutrophication effects on the pathways of the parasitic flow and the structure of the helminth communities were traced. The bioindicator significance of the parasitic complexes was discussed.

Key words: *eutrophication effects, helminth communities, Stryama River, Squalius orpheus.*

INTRODUCTION

Stryama River (110.1 km long) is one of the largest left tributaries of the Maritsa River in Bulgaria. The freshwater ecosystem and the adjacent areas are characterized by great biodiversity, protected areas (BG0000429 Stryama, BG0000289 Trilistnik, etc.), species and habitats (Natura 2000). The Stryama River valley is an important bio-corridor connecting the Upper Thracian valley with the Balkan Mountains. The aim of the study is to explore the state of endoparasites and parasite communities of *Sq. orpheus* of the Stryama River, as well as to discuss their bioindicator role in the eutrophication processes based on the endoparasitic flow.

MATERIALS AND METHODS

A total of 59 specimens *Squalius orpheus (*Kottelat & Econodimis, 2006) are examined for endohelminths. The scientific and common names of the fish are provided according to the FishBase database (Fröse and Pauly, 2018). Helminthological examinations are implemented following recommendations described by Petrochenko (1956); Zashev and Margaritov, 1966; Bauer, 1987; Moravec, 2013). Specimens are fixed and preserved in 70% ethyl alcohol. The specimens of Trematoda and Cestoda are studied by methods of Zashev and Margaritov (1966); Georgiev et al. (1986); Scholz and Hanzelová (1998) and of Acanthocephala and Nematoda – of Moravec (2013). Analyses of helminth community structure are carried out in both levels: infracommunity (total and mean number of species; total and mean number of specimens; Brillouin's index of diversity (HB)) and component community (prevalence (P%) and mean intensity (MI) for each species) (Bush et al., 1997; Magurran, 1988).The species are divided into core species (P%>20), component species (P%>10) and accidental species (P%<10) (Kennedy, 1997). The diversity measures are calculated by software products Statistica 10 (StatSoft Inc., 2011) and MS Excel (Microsoft 2010).

RESULTS AND DISCUSSIONS

Fish communities

Orpheus dace (*Squalius orpheus* Kottelat & Economidis, 2006) inhabits almost all the rivers and reservoirs in Bulgaria. It is a pelagic

species. The Orpheus dace prefers fast-flowing rivers with sandstone bottom. Young fish feeds on algae and crustaceans, and adults - insects and their larvae, fish, frogs and small rodents (Karapetkova and Zhivkov, 2006; Fröse and Pauly, 2018). *Sq. orpheus* is estimated as least concern species (LC=Least Concern; IUCN Red List Status, 2018) and is not included in Red Data Book of the Republic of Bulgaria (Golemanski (Ed.), 2011). *Sq. orpheus* is an endemic fish species of the Aegean Basin (Kolev, 2013).

Helminth community structure

From studied 59 specimens of Orpheus dace (*Squalius orpheus* Kottelat & Economidis, 2006), a total of 4 species of helminths are determined: *Allocreadium isoporum (*Kowal et Kulakowskaja, 1957); *Caryophyllaeus brachycollis (*Janiszewska, 1951); *Pomphorhynchus laevis* (Müller, 1776); *Rhabdochona denudata* (Dujardin, 1845).

The first intermediate hosts of *A. isoporum* are snails of genus *Sphaerium* and the second – larvae's of insects of genera *Ephemera*, *Anabolia* and *Chaetopterix*. Definitive hosts are many fish species of Cyprinidae, Percidae, Esocidae, Salmonidae, etc. (Kakacheva-Avramova, 1983; Bauer, 1987). *A. isoporum* was reported of *Squalius cephalus* (Linnaeus, 1758) (*Leuciscus cephalus* Linnaeus, 1758), *Alburnoides bipunctatus* (Bloch, 1782), *Barbus barbus* (Linnaeus, 1758) and *Phoxinus phoxinus* (Linnaeus, 1758) from rivers Dokusak and Resovska (Kakacheva-Avramova, 1960); of *Alburnus alburnus* (Linnaeus, 1758) of the Danube River (Kakacheva-Avramova, 1977); of *Gobio gobio* (Linnaeus, 1758) from rivers of Eastern Staraplanina mountain (Kakacheva-Avramova, 1973), from rivers of Strandzha mountain (Kakacheva-Avramova, 1960), from water basins of Trakia (Kakacheva-Avramova, 1965), from rivers Vrabnishka and Nishava (Kakacheva-Avramova, 1969); of *Barbus petenyi* Heckel, 1852 from rivers Mesta and Struma (Kakacheva-Avramova, 1962), from rivers of Western Staraplanina mountain (Kakacheva-Avramova, 1969), from rivers of Central and Eastern Staraplanina mountain (Kakacheva-Avramova, 1973); of *Barbus cyclolepis* Heckel, 1837 of the Vacha River (Margaritov, 1965), from water basins of Trakia (Kakacheva-Avramova, 1965), of the Tundzha River (Kakacheva-Avramova, 1972); etc. Intermediate hosts of *C. brachycollis (*Janiszewska, 1951) are *Limnodrilus hoffmeisteri (*Claparède, 1862) and *Tubifex tubifex* (Müller, 1774). Definitive hosts are fish species of Cyprinidae. Typical definitive hosts are fish species: *B. barbus*, *B. petenyi*, *Sq. cephalus*, *Leucis cusidus* (Kakacheva–Avramova, 1983; Bauer, 1987; Protasova, 1990; Scholz and Hanzelová, 1998; Barčák et al., 2017). *C. brachycollis* was reported of *B. cyclolepis* and *Sq. orpheus* from rivers Asenitsa, Sushitsa, Syuyutlijka, Chepinska, Bedechka and Topolnitsa (Kakacheva-Avramova, 1965); of *Sq. orpheus* from rivers Maritsa, Vacha and Chepinska; of *Vimba melanops* (Heckel, 1837) of the Maritsa River, of *A. alburnus* from rivers Maritsa and Chepinska, of *B. cyclolepis* from rivers Maritsa, Vacha and Topolnitsa, of *Rutilus rutilus* (Linnaeus, 1758) of the Bistritsa River (Margaritov, 1965); of *Sq. cephalus* from rivers Vrabnishka and Nishava, of *B. petenyi* from rivers Mirkovska, Botunya, Ogosta, Iskar, of *B. barbus* of the Bebresh River (Kakacheva-Avramova, 1969); of *Sq. cephalus* of the Palakariya River, of *B. petenyi* from rivers Devinska and Sarneshka, of *Sq. Orpheus* of the Vacha River (Kakacheva-Avramova and Menkova, 1978); of *B. petenyi* of the Blagoevgradska Bistritsa River, of *B. barbus* of the Struma River, of *Sq. cephalus* from rivers Zheleznitsa, Gradevska, Struma (Kakacheva-Avramova and Menkova, 1981); of *Perca fluviatilis* Linnaeus, 1758 of Reservoir Zhrebchevo (Nedeva and Grupcheva, 1996); of *Sq. orpheus* of the Maritsa River (Kirin, 2000, 2001b); of *Sq. cephalus* (*L. cephalus*) and *B. petenyi* of the Mesta River (Kirin, 2001c); of *Sq. orpheus* of Reservoir Kardzhali (Kirin, 2001b); of *B. cyclolepis* of the Luda Yana River (Kirin, 2002c); of *Sq. orpheus* (Kirin, 2002a), of *Sq. orpheus* and *A. alburnus* (Kirin et al., 2002), of *B. cyclolepis* and *A. alburnus* (Kirin, 2003), of *Sq. orpheus* (Kirin et al., 2003) of the Arda River; of *Sq. cephalus* of the Danube River (Cakic et al., 2004); of *Sq. orpheus* of the Stryama River (Kirin et al., 2005), of the Tunja River (Kirin et al., 2013); of *V. melanops* of the Maritsa River (Kirin,

2014), etc. Intermediate host of *P. laevis* is *Gamma ruspulex* (Linnaeus, 1758). Definitive hosts are mainly freshwater fish species of Cyprinidae and less often - of Salmonidae, Percidae, Siluridae, etc. (Petrochenko, 1965; Kakacheva-Avramova, 1983; Bauer, 1987). *P. laevis* was reported of *Sq. cephalus* of the Iskar River, of *B. barbus* of the Danube River (Margaritov, 1959); of *Acipenser ruthenus* Linnaeus, 1758, *G. gobio*, *B. barbus*, *A. alburnus*, *Blicca bjoerkna* (Linnaeus, 1758), *Pelecus cultratus* (Linnaeus, 1758), *Carassius gibelio* (Bloch, 1782), *Cyprinus carpio* Linnaeus, 1758, *Sabanejewia bulgarica* (Drensky, 1928) (*Cobitis bulgarica*), *Silurus glanis* Linnaeus, 1758, *Sander lucioperca* (Linnaeus, 1758) (*Lucioperca lucioperca*), *Zingel zingel* (Linnaeus, 1766) (*Asprozingel* Linnaeus, 1766), *Zingel streber* (Siebold, 1863) (*A. streber* Siebold, 1863), *Gymnocephalus cernua* (Linnaeus, 1758) (*Acerina cernua* (Linnaeus, 1758)), *Gymnocephalus schraetser* (Linnaeus, 1758)), (*A. schraetser* (Linnaeus, 1758)), *Ponticola constructor* (Nordmann, 1840) (*Gobio cephalarges constructor* Nordmann, 1840), *G. gobio* (*G. fluviatilis* Linnaeus, 1758), *Benthophilus stellatus* (Sauvage, 1874) of the Danube River (Matgaritov, 1966); of *Chondrostomanasus* (Linnaeus, 1758) and *Ph. phoxinus*from rivers Ogosta and Nishava (Kakacheva-Avramova, 1969); of *A. ruthenus*, *A. güldenstädtii* Brandt &Ratzeburg, 1833, *Salmo labrax* Pallas, 1814, *Alosaimmaculata* Bennet, 1835 (*Alosapontica* Bennet, 1835), *Anguilla anguilla* Linnaeus, 1758, *C. carpio*, *C. gibelio*, *V. vimba*, *Abramisbrama*(Linnaeus, 1758), *Ballerussapa* (Pallas, 1814) (*Abramissapa* (Pallas, 1814)), *Ballerus ballerus* (Linnaeus, 1758) (*Abramis ballerus* (Linnaeus, 1758)), *P. cultratus*, *A. alburnus*, *B. bjoerkna*, *G. gobio*, *Romanogobio albipinnatus* (Lukasch, 1933) (*G. albipinatus* (Lukasch, 1933)), *B. barbus*, *Ch. nasus*, *L. idus*, *Scardinius erythrophthalmus* (Linnaeus, 1758), *Sq. cephalus*, *Leuciscus aspius* (Linnaeus, 1758) (*Aspius aspius* (Linnaeus, 1758), *Ctenopharyngodon idella* (Valenciennes, 1844), *Proterorhynus marmoratus* (Pallas, 1814), *S. glanis*, *Lota lota* (Linnaeus, 1758), *Esox lucius* Linnaeus, 1758, *S. lucioperca*, *S. volgense*, *P. fluviatilis*, *G. cernua*, *G. schraester*, *Z. zingel*, *Z. streber*, *Ponticola kessleri* (Günther, 1861) (*Gobius kessleri* (Günther, 1861)), *Lepomis gibbosus* (Linnaeus, 1758), *G. gobio*, *B. stellatus* of the Danube River (Kakacheva-Avramova et al., 1978); of *B. barbus* from rivers Struma, Zheleznitsa, Gradevska, of *A. bipunctatus* from rivers Zheleznitsa and Gradevska, of *Sq. cephalus* of the Struma River (Kakacheva-Avramova and Menkova, 1981); of *C. carpio* and *S. lucioperca* (Nedeva and Grupcheva, 1996), of *C. gibelio* of Reservoir Zhrebchevo (Grupcheva and Nedeva, 1999); of *Sq. Orpheus* of the Maritsa River (Kirin, 2000; 2001); of *Sq. cephalus* of the Danube River (Cakis et al., 2004); of *P. fluviatilis* of the Arda River (Kirin, 2005); of *Sq. orpheus* of the Stryama River (Kirinetal., 2005); of *A. brama*, *B. sapa*, *A. ruthenus*, *A. alburnus*, *A. immaculata*, *B. barbus*, *C. gibelio*, *E. lucius*, *G. schraester*, *Sq. cephalus*, *P. cultratus*, *Pomatoschistus minutus* (Pallas, 1770), *S. lucioperca*, *Sc. erythrophthalmus*, *S. glanis*, *Z. zingel* of the Danube River (Atanasov, 2012); of *Sq. cephalus* of the Tunja River (Kirin et al., 2013), etc. Definitive hosts of *R. denudata* are fish species from Cyprinidae. Intermediate hosts are larvaes of the genera *Heptagenia*, *Ephemerella* and *Hydropsyche* (Kakcheva-Avramova, 1983; Bauer, 1987). *R. denudata* was presented of *B. barbus*, *B. petenyi* and *Sq. cephalus* of the Iskar River (Margaritov, 1959); of *Sc. erythrophthalmus* of the Strumeshnitsa River (Kakacheva-Avramova, 1962); of *Sq. cephalus*, *A. alburnus*, *L. aspius*, *B. cyclolepis* from Trakian's freshwater ecosystems (Kakacheva-Avramova, 1965); of *Sq. orpheus* from rivers Maritsa, Vacha, Chepinska, of *V. melanops* of the Maritsa River, of *A. alburnus* from rivers Maritsa and Chepinska, of *B. cyclolepis* from rivers Maritsa, Chepinska, Vacha and Topolnitsa (Margaritov, 1965); *Sq. cephalus* from rivers Ogosta, Vrabnishka, Barziya, Nishava, Botunya, Leva, Archar, Berkovska, Chuprenska, of *B. peteny* from rivers Chuprenska, Barziya and Leva, of *B. barbus* of the Leva River; of *G. gobio* of the Barziya River, of *A. alburnus* from rivers Ogosta, Lomand Leva (Kakacheva-Avramova, 1969); of *Sq. cephalus* of the Shiposhnitsa River and Reservoir Iskar (Margaritov, 1977); of *A. alburnus*, *Z. streber*, *Z. zingel* (Kakacheva-Avramova et al., 1978); of *Sq. cephalus* of the

Palakariya River (Kakacheva-Avramova and Menkova, 1978); of *Cobitis taenia* Linnaeus, 1758 from State Fish Farming Blagoevgrad; of *Sq. cephalus* from rivers Zheleznitsa, Blagoevgradska, Bistritsa, Gradevska and Struma (Kakacheva-Avramova and Menkova, 1981); of *Sq. cephalus* and *B. cyclolepis* of the Struma River (Nedeva, 1991); of *C. carpio* (Kirin, 2001a) of the Mesta River, of *Sq. cephalus* and *A. alburnus* of Reservoir Kardzhali (Kirin, 2001b); of *Sq. orpheus* (Kirin, 2002a), *B. cyclolepis* and *A. alburnus* (Kirin, 2003), *Sq. orpheus* and *A. alburnus* (Kirin et al., 2002) from the Arda River; of *Sq. orpheus* of the Chepelarska River (Kirin, 2002b); of *Sq. orpheus* of the Arda River (Kirin et al., 2003); of *Sq. cephalus* of the Danube River (Cakis et al., 2004); of *Sq. orpheus* of the Stryama River (Kirin et al., 2005); of *S. erythrophthalmus* and *L. aspius* (*A. aspius*) from Srebarna Biosphere Reserve (Shukerova and Kirin, 2008; Shukerova, 2010); of *Sq. cephalus*, *S. erythrophthalmus*, *B. barbus* of the Danube River (Atanasov, 2012); of *Sq. orpheus* of the Tunja River (Kirin et al., 2013), etc. *C. fennica*, *A. lucii* and *R. denudata* are intestinal parasites in the body of fishes. For all reported endoparasite species, *Sq. orpheus* is a definitive host.

Component communities

The found intestinal parasites are generalists for the helminth communities of Orpheus dace of the Stryama River. They are authogenic species for the studied freshwater ecosystem. With the highest prevalence and mean intensity is distinguished *P. laevis* (P%=66.10; MI=1.85), followed by *A. isoporum* (P%=38.98; MI=1.69) (Table 1). *P. laevis*, *A. isoporum* and *Rh. denudata* are core species of the helminth communities of Orpheus dace. Thefourthspecies, *C. brachycollis* is a component species of these communities according to the criterion of Bush et al. (1997).

Infracommunities

The established 4 species of endoparasites are presented a total with 142 specimens. Two fish specimens are free of parasites. With the highest number of parasite species are distinguished 5 specimens of Orpheus dace – 3 species, followed by 20 specimens of fish infected with two species of endohelminths. 30 specimens of examined fish are infected with one species of the reported parasites. Mean number of species and specimens of intestinal parasites per specimen of examined fish are fixed (Table 1). Minimal number of endoparasite specimens per a fish specimen is one and maximal is six (2.37±1.03). Brillouin's diversity index is high (HB=1.13) (Table 1).

Table 1. Biodiversity and ecological indices of the helminth communities of *Sq. Orpheus* from Stryama river

Ecological indices (N = 59) Biodiversity	n p	P% MI Range
Trematoda		
Allocreadium isoporum	23 39	38.98 1.69 1-6
Cestoda		
Caryophyllaeus brachycollis	11 13	18.64 1.18 1-2
Acanthocephala		
Pomphorhynchus laevis	39 72	66.10 1.85 1-4
Nematoda		
Rhabdochona denudata	16 18	27.11 1.12 1-2
Infracommunity data		
Total number of species	4	
Mean±SD	1.75±1.41	
Number of fish	2 30	20 5
Number of helminth species	0 1	2 3
Total number of specimens	142	
Mean±SD	2.37±1.03	
Range	1-6	
HB (Brillouin's diversity index) (Mean±SD)	1.13 (0.409±0.1)	

The parasite communities of Orpheus dace from freshwater ecosystems of Bulgaria, to this time, are represented by 23 species of intestinal parasites. The four species of endoparasites, found in this study, are only 17.39% of the established for the country. The species *C. brachycolis*, *P. laevis* and *Rh. denudata* were reported of *Sq. orpheus* of the Stryama River (Kirin et al., 2005). In 2005, the parasite communities of Orpheus dace were presented a total of 8 species of endohelminths. *A. isoporum* is reported for the first time of *Sq. orpheus* of the Stryama River. The prevalences of *C. brachycolis* and *P. laevis* are higher (2.77 and 1.36 times more, respectively) than those of 2005, but in the opposite, the prevalence of *Rh. denudata* is lower (1.70 times less). Intermediate hosts of *A. isoporum* of genus

Sphaerium are bioindicators of β-α saprobity and the larvae's of insects of genera *Ephemera*, *Anabolia* and *Chaetopterix* are bioindicators of 0-β, 0-α and 0-saprobity, respectively. *L. hoffmeistri* and *T. tubifex*, intermediate hosts of *C brachycolis* are bioindicators of polysaprobity (p). *G. pulex*, intermediate host of *P. laevis* is a bioindicator of χ-β-mesosaprobity and intermediate hosts of *Rh. denudata* (*Ephemerella* sp. and *Hydropsyche* sp.) are bioindicators of 0-α-mesosaprobity (Rosenberg et al., 1997).Most of the intermediate hosts have extensive ecological tolerance. The highest increase is established for the prevalence of *C. brachycolis*, but the highest prevalence is recorded for *P. laevis*. For the three species of parasites, the lower mean intensity was reported than those from the previous study. Probably *G. pulex* is dominant species in the diet of Orpheus dace from the studied habitats. Similar research and dependencies were traced by Brewster (2016), Goga (2016), etc.

CONCLUSIONS

Sq. orpheus and its parasites along the path of nutritional interactions can have an important role in monitoring the effects of eutrophication in taking ecosystem conservation measures.

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