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Original article

GASTROINTESTINAL HELMINTH FAUNA AND HELMINTH COMMUNITIES OF BLEAK (*ALBURNUS ALBURNUS*, L. 1758) FROM LOWER SECTION OF DANUBE RIVER, BULGARIA

M. CHUNCHUKOVA, D. KIRIN & D. KUZMANOVA

Department of Ecology and Environmental Protection,
Agricultural University, Plovdiv, Bulgaria

Summary

Chunchukova, M., D. Kirin & D. Kuzmanova, 2019. Gastrointestinal helminth fauna and helminth communities of bleak (*Alburnus alburnus*, L. 1758) from lower section of Danube River, Bulgaria. *Bulg. J. Vet. Med.,* **22**, No 3, 344–352.

The present study presents the results from examinations of bleak's endohelminth species and structure of helminth communities from the Bulgarian part of the Lower Danube River. In 2015 and 2016, on a seasonal manner, 91 specimens of *Alburnus alburnus* (Linnaeus, 1758) (bleak) were examined with standard techniques for parasites. Five species of parasites: *Nicolla skrjabini* (Ivanitzky, 1928), *Ligula intestinalis* (Linnaeus, 1758), *Acanthocephalus lucii* (Müller, 1776), *Pomphorhynchus laevis* (Müller, 1776) and *Contracoecum microcephalum* (Stossich, 1890)*,* larvae were identified. The analysis of the dominant structure of the found parasite species is presented to the component and infracommunities levels. All parasite species were accidental for the parasitic communities of examined fish with the exception of *P. laevis* and *N. skrjabini*. *P. laevis* was a core parasite species and *N. skrjabini* was a component parasite species for the helminth communities of bleak. The parasite communities of *A. alburnus* were discussed and compared with previous research data on parasite communities of bleak from River Danube in Bulgaria. New data for helminths and helminth communities of *A. alburnus* from Danube River (biotope Vetren) and their seasonal occurrence are presented.

Key words: *Alburnus alburnus,* bleak, Bulgaria Danube, helminths, helminth communities

INTRODUCTION

Danube River has an important place in European ecological network. The fish community in the shoreline zone of the Danube River in Bulgaria is presented by 44 fish species among which *Alburnus alburnus* is one of the most abundant (Polačik *et al.*, 2008). Due to its small size, the bleak has no economical importance and is a subject of sport fishing.

Parasites are well acknowledged as sensitive bioindicators for the aquatic ecosystem state (Marcogliese, 2004). Parasite communities are useful indicators of food web structure and biodiversity (Marcogliese, 2005). Fish parasites communities of *A. alburnus* from the Bulgarian part of the river were reported in few studies (Kakacheva-Avramova, 1977; Atanasov, 2012; Kirin *et al*., 2013). The bleak serves as intermediate and final host of helminth species that might affect the status of fish population. The presence of certain helminth species in this abundant fish species may have consequences for the food web and ecosystem structure. Thus, investigation of bleak parasites is useful for aquatic biomonitoring.

This paper presents the results of examinations of bleak gastrointestinal helminth species, new data for structure of helminth communities, and their seasonal changes from the Bulgarian part of the Lower Danube River (village of Vetren).

MATERIALS AND METHODS

In spring, summer and autumn of 2015 and 2016 fish and fish parasites from the Lower Danube River (village of Vetren, Bulgarian part) were collected and examined. The village of Vetren ($44^0$133'N, $27^0$033'E) is situated on the riverside, in the northeastern part of the Danube Valley.

A total of 91 (spring: 31, summer: 30, autumn: 30) specimens of bleak, *Alburnus alburnus* (Linnaeus, 1758) from the Danube River were collected and examined in 2015 and 2016. Fish were caught by angling under a permit issued by the Ministry of Agriculture and Food of the Republic of Bulgaria. The scientific and common names of fish hosts were used according to the FishBase database (Fröse & Pauly, 2017). The examined fish specimens in this study were weighed (mean total weight 13.99±3.21 g; range 9–24 g) and measured (mean total length 12.29±1.01 cm; range 10.5–15.5 cm).

The fish samples were examined for gastrointestinal parasites (incomplete parasitological study) using standard techniques and immediately after their capture. Trematodes were fixed as permanent slides after their staining with acetic carmine, differentiation in 70% acid ethanol, dehydration in ascending ethanol series, clearing in eugenol and mounting in Canada balm (Bykhovskaya-Pavlovskaya, 1985; Georgiev *et al.*, 1986). The samples were counted and indentified using the keys of Bauer *et al*. (1981) and Bykhovskaya-Pavlovskaya (1985). Acanthocephalans were examined as temporary mounts in ethanol-glycerin and identified (Petrochenko, 1956; Ergens & Lom, 1970; Bykhovskaya-Pavlovskaya, 1985). Nematodes were examined as temporary mounts in glycerin (Moravec, 1994).

The dominant structure of the component helminth communities was determined according to the criteria proposed by Kennedy (1993) on the basis of the prevalence (P%): accidental (P%<10), component (P%<20) and core (P%>20) species. The ecological terms prevalence, mean intensity (MI) and mean abundance (MA) were used and calculated as per Bush *et al*. (1997). Analyses of helminth community structure were carried out during the three seasons and at both infracommunity and component community levels. The seasonal differences were evaluated regardless of the year, thus the data for the two years were combined by seasons. The data were used to calculate the total number of species, mean number of helminths, Shannon diversity index (H'), Brillouin diversity index (HB), Brillouin evenness index (HBe), Pielou evenness index (E), Simpson dominance index (C) and Berger-Parker dominance index (d) (Kennedy, 1993; Magurran, 1988).

Statistical analysis

The Kruskal-Wallis test was used to compare the invasion indices with the established species during the three seasons. To reinforce the argumentation, the results of the two tests were presented. To investigate the relevant factor for the variation (season, year) of the invasion parameters, the nonparametric Friedman test was used. All statistical tests were performed using statistical software (Quantitative Parasitology, version 3, Rozsa et al., 2000; STATISTICA 10.0 program).

RESULTS

Model fish species

A total of 91 specimens of bleak, *Alburnus alburnus* (Linnaeus, 1758) were collected and examined from the Danube River. *Alburnus alburnus* is estimated as least concern species (LC=Least Concern; Anonymous, 2017). Bleak is a freshwater, brackish, benthopelagic, potamodromous fish species. It inhabits open waters of lakes and medium to large rivers. Adult bleaks occur in shoals near the surface. *A. alburnus* larvae live in littoral zone of rivers and lakes, while juveniles leave shores and occupy a pelagic habitat, feeding on plankton, drifting insects or invertebrates fallen on the water surface. The diet of this species includes mainly plankton, as well as crustaceans and insects. Bleak spawns in shallow riffles or along stony shores of lakes and occasionally above submerged vegetation (Fröse & Pauly, 2017).

Helminth community structure

Helminth parasites were recorded in 43 bleaks (47.25%) from the Danube River. Five parasite species presented by 91 specimens were identified: one trematode species, *Nicolla skrjabini* (Ivanitzky, 1928), one cestode species, *Ligula intestinalis* (Linnaeus, 1758), two acantocephalans, *Acanthocephalus lucii* (Müller, 1776), *Pomphorhynchus laevis* (Müller, 1776) and one nematode species: *Contracoecum microcephalum* (Stossich, 1890), larvae. All helminth species occurred as adults with the exception of *C. microcephalum* and *L. intestinalis* (Table 1). *N. skrjabini*, *A. lucii* and *P. laevis* are autogenic species, matured in fish. *C. microcephalum* and *L. intestinalis* are allogenic species, matured in fish-eating birds (Moravec, 1994).

Component community

In the component community of *A. alburnus* from Danube River, acanthocephalans were presented with the highest number of specimens: 2 species and 50 specimens. Trematodes were presented with one species and 32 specimens. Cestodes were represented by one species with only one specimen. Nematodes were represented by one species and 8 specimens. *Pomphorhynchus laevis* was a core species (P%=20.88). *Nicolla skrjabini* (P%=19.78) was a component parasite species for the helminth communities of bleak. *Ligula intestinalis* (P%=1.10), *Acanthocephalus lucii* (P%=4.40) and *Contracoecum microcephalum* (P%=4.40) were accidental parasite species for the helminth communities of bleak (Table 1).

The parasitic species of *A. alburnus* from Danube River (biotope Vetren) appeared in all three sampling seasons with the exception of *L. intestinalis, A. lucii* and *C. microcephalum. L. intestinalis* appeared only in one bleak specimen during the summer. *A. lucii* and *C. microcephalum* appeared only in specimens from summer season. In the component community of *A. alburnus* from Danube

Table 1. Species diversity of helminth parasites of *Alburnus alburnus* from Danube River

Helminth species	N=91					
	n	p	P%	MA±SD	MI±SD	Range
Nicolla skrjabini (Ivanitzky, 1928)	18	32	19.78	0.35±0.79	1.78±0.79	1–3
Ligula intestinalis (Linnaeus, 1758), larvae	1	1	1.10	5.91±23.81	30.22±46.51	1
Acanthocephalus lucii (Müller, 1776)	4	8	4.40	0.09±0.46	2.0±1.0	1–3
Pomphorhynchus laevis (Müller, 1776)	19	42	20.88	0.46±1.11	2.21±1.44	1–6
Contracoecum microcephalum (Stossich, 1890), larvae	4	8	4.40	0.09±0.48	2.00±1.22	1–4

N – number of examined hosts, n – number of infected hosts, p – number of parasites, P% – prevalence , MA – mean abundance, MI – mean intensity.

Table 2. Seasonal species diversity of helminth parasites of *Alburnus alburnus* from Danube River

Season	Spring (N=31)			Summer (N=30)			Autumn (N=30)		
Parasite species	n/p	P%	MI ±SD (Range)	n/p	P%	MI ±SD (Range)	n/p	P%	MI±SD (Range)
Nicolla skrjabini	13/23	41.96	1.77±0.80 (1–3)	4/8	13.33	2.00±0.71 (1–3)	1/1	3.33	1 (1)
Ligula intestinalis	–	–	–	1/1	3.33	1 (1)	–	–	–
Acanthocephalus lucii	–	–	–	4/8	13.33	2±1 (1–3)	–	–	–
Pomphorhynchus laevis	1/1	3.23	1 (1)	8/16	26.67	2.00±1.22 (1–4)	10/ 25	28.8 8	2.5±1.57 (1–6)
Contracoecum microcephalum	–	–	–	4/8	13.33	2.00±1.22 (1–4)	–	–	–

N – number of examined hosts, n – number of infected hosts, p – number of parasites, P% – prevalence , MA – mean abundance, MI – mean intensity.

River *P. laevis* had the highest number of specimens (42), with the highest mean intensity during the autumn (2.5±1.57). *N. skrjabini* exhibited the highest mean summer intensity (2.0±0.71), but higher during the spring (Table 2).

The highest number of helminth species (5 species) and the highest number of specimens (41) were found in summer season. Dominant species in spring season was *N. skrjabini*, while in summer and autumn season it was *P. laevis* (Table 3).

Significant differences for the three seasons were obtained for *Nicolla skrjabini* (P=0.0004), *Acanthocephalus lucii* (P=0.016), *Pomphorhynchus laevis* (P=0.0098) and *C. microcephalum* (P=0.016) with the Kruskal-Wallis test. Significant differences in infestation during the three seasons by means of one-way

Table 3. Basic indices of helminth community of *Alburnus alburnus* from Danube River

Basic indices	Season			Statistical significance
	Spring	Summer	Autumn	
Number of helminth species	2	5	2	P=0.01
Number of helminth specimens	24	41	26	P=0.02
H' (Shannon, diversity)	0.17	1.41	0.16	P=0.0003
HB (Brillouin, diversity index)	0.22	0.87	0.20	P=0.0003
HBe (Brillouin, evenness)	0.13	1.26	0.13	P=0.0066
E (Pielou, evenness)	0.25	0.88	0.24	P=0.0066
C (Simpson, dominance)	0.92	0.27	0.93	P=0.0004
d (Berger-Parker Dominance Index)	0.96	0.39	0.96	P=0.0001
Dominant species	*N. skrjabini*	*P. laevis*	*P. laevis*	

Table 4. Parameters of the infracommunities of *Alburnus alburnus* from Danube River

Alburnus alburnus	Number of endohelminth species				
	0	1	2	Mean±SD	Range
	48	40	3	0.51±0.56	0-2

Alburnus alburnus	Number of endohelminth specimens			
	Total number	Mean±SD	Range	Brillouin's index (HB)
	91	1.00±1.36	1–6	0.43±0.31

ANOVA were established for *Nicolla skrjabini* (P=0.001), *Acanthocephalus lucii* (P=0.035), *Pomphorhynchus laevis* (P=0.018) and *Contracoecum microcephalum* (P=0.048). Friedman Two Way ANOVA showed that the seasons were important for the infestation with *N. skrjabini* (P=0.0019), *A. lucii* (P=0.018), *P. laevis* (P=0.009) and *C. microcephalum* (P=0.018), and for the status of the populations of the species during the three seasons.

Infracommunity

Species richness in bleak infracommunity ranged from 1 to 2 species. Fourty fish (43.96%) were infected with one helminth species and three fish (3.30%) – with 2 helminth species. The largest number of helminth specimens established in a single host was 6. The average species richness (mean number of species for fish specimen) in infracommunity of bleak was 0.51±0.56 species. Average abundance (mean number of helminths in fish) in these infracommunities was 1.00±1.36. The parasite communities of *A. alburnus* from the Danube River showed Brillouin's diversity index HB=0.43±0.31 (range 0.20-0.87) (Table 4).

DISCUSSION

N. skrjabini was reported as a parasite species in *Gymnocephalus cernua, G. schraetser, Acipenser ruthenus, A. aspius, Gobio gobio, Blicca bjoerkna, A. brama, A. ballerus, Pelecus cultratus, Carassius carassius, Cyprinus carpio, Sabanejewia bulgarica, Sabanejewia balcanica, Sander lucioperca, S. volgense, Perca fluviatilis, Zingel zingel, Z. streber, Gobius* *kessleri* (=*Ponticola kessleri*), *Gobius fluviatilis* (=*Neogobius fluviatilis*), *Proterorhinus marmoratus, Gobio gobio, Salmo trutta, Pomatoschistus minutus* и *Neogobius kessleri* (=*Ponticola kessleri*) from Danube River (Margaritov, 1966; Kakacheva-Avramova, 1977; 1983; Atanasov, 2012; Kirin *et al*., 2013). *Nicolla skrjabini* was found in bleak from Danube basin (Moravec, 2001). *N. skrjabini* was found in *A. sapa* from River Latorica in Slovakia (Oros & Hanzelová, 2009). *Alburnus alburnus* from Danube River is a new host for *Nicolla skrjabini*.

L. intestinalis was found in *Scardinius erythrophtalmus* from Danube River (Kakacheva-Avramova, 1983) and in Serbian part of the river (Đjikanović *et al*., 2013). The larval stages of *L. intestinalis* develop in the body cavity of cyprinids *Abramis brama, A. sapa, S. erythrophtalmus, A. alburnus, A. bipunctatus, Gobio gobio, Rutilus rutilus, Barbus barbus, B. m. petenyi, Leuciscus cephalus, L. idus* and *Phoxinus phoxinus* (Kakacheva-Avramova, 1983). In Bulgaria *L. intestinalis* was found in *A. alburnus* from dam lake Iskar, River Shiposhnica and River Palakaria (Kakacheva-Avramova & Naidenov, 1974; Kakacheva-Avramova & Nedeva-Menkova, 1978). *L. intestinalis* was reported as parasite species on bleak from Danube River Basin (Moravec, 2001). *A. alburnus* from Bulgarian section of Danube River is a new host for *L. intestinalis.*

A. lucii was found in *Acipenser ruthenus, Leuciscus cephalus, Abramis sapa, Gymnocephalus schraetser, Benthophilus stellatus, Silurus glanis, Perca fluviatilis, Lota lota* and *Zingel zingel* from Bulgarian section of River Danube (Margaritov, 1959, 1966; Kakacheva-Avramova, 1977; 1983; Atanasov, 2012). *A. lucii* was reported for *Abramis sapa, L. cephalus, R. rutilus, Lota lota, Gymnocephalus schraester, Benthophilus stellatus, Proteoinus marmoratus, Abramis brama, Acipenser ruthenus, Lota lota, Esox lucius, Cyprinus carpio, Leuciscus idus, Tinca tinca, Silurus glanis, Perca fluviatilis, Blicca bjoerkna, Aspius aspius, Barbatula barbatula* from Danube Basin (Kakacheva-Avramova, 1983; Moravec, 2001; Cakic et al., 2008; Shukerova et al., 2010). *A. alburnus* from Danube River is a new host for *A. lucii*.

Intermediate host for *A. lucii* is *Asellus aguaticus,* and definitive hosts are fish from Cyprinidae, Salmonidae, Percidae, Anguillidae and other families (Kakacheva-Avramova, 1983).

P. laevis was found in *A. alburnus* from Danube River (Margaritov, 1966; Kakacheva-Avramova, 1977; Atanasov, 2012). *Gammarus pulex* is an intermediate host of *P. laevis.* Definitive hosts are most often fish from the family Cyprinidae, and less often from families Salmonidae, Percidae, Siluridae etc. (Kakacheva-Avramova, 1983).

C. microcephalum was found in Europe mainly in cyprinids – *Cyprinus carpio, Carassius carassius, Leuciscus idus, A. brama, A. sapa* and *Aspius aspius* (Moravec, 1994). *C. microcephalum* was reported for *C. carpio* and *P. fluviatilis* in the Danube Basin (Moravec, 2001). In Bulgaria it was reported from Lake Srebarna with *C. carpio* (Shukerova, 2006) and *P. fluviatilis* (Shukerova *et al*., 2010) as hosts and from Mesta River with its synonym *Contracaecum squalii* and host *C. carpio* (Kirin, 2001). *A. alburnus* from Danube River is a new host record for *C. microcephalum.*

During this study of helminth communities of *A. alburnus* five parasite species were found (gastrointestinal). This is comparable to some older studies of helminth communities of bleaks from the Bulgarian Danube section, but differs from some more recent studies. In this study of helminth communities of bleak from Bulgarian section of Danube River, the prevalence (P%) was 47.25% which was similar to the established prevalence from Kakacheva-Avramova (1977) of 48.3%, but differs from more recent studies of *A. alburnus* from Bulgarian section of River Danube reporting significantly higher prevalences: 75% (Kirin *et al.,* 2013) and 71.57% (Atanasov, 2012). Kirin *et al.* (2013) reported a single parasite species for bleak (gastrointestinal) in biotope Vetren – *P. laevis* which was reported as *P. tereticollis*, based on the resurrection of the species (Špakulová *et al*., 2011) and redefined as *P. laevis* based on Perrot-Minnot *et al.* (2018). Atanasov (2012) reported 5 parasite species from *A. alburnus* from Bulgarian section of River Danube, two of them gastrointestinal – *Bunodera luciopercae* and *Pomphorhynchus laevis*. Kakacheva-Avramova (1977) reported 9 parasite species from *A. alburnus*, five of them gastrointestinal – *Allocreadium isoporum isoporum*, *Caryophyllaeides fennica*, *Proteocephalus torulosus*, *Rhabdochona denudata* and *Pomphorhynchus laevis*.

CONCLUSION

As a result of the investigation of 91 bleak specimens from the Danube River, five gastrointestinal parasite species were established. Such high gastrointestinal parasite diversity and prevalence have not been recorded for a long period of time. *Alburnus alburnus* from the Bulgarian section of Danube River is a new host for *Nicolla skrjabini*, *Ligula intestinalis, Acanthocephalus luci* and *Contracoecum microcephalum* (larvae). Considering the intermediate hosts of the parasites as well as the differences in the established parasite species in the present and previous studies, it can be concluded that there were changes in the local invertebrate fauna in the studied freshwater ecosystem.

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Correspondence:

Mariya Chunchukova
Department of Ecology and Environmental Protection,
Agricultural University - Plovdiv,
12 Mendeleev blvd, 4000 Plovdiv, Bulgaria
e-mail: m.chunchukova@abv.bg