ИЗСЛЕДВАНЕ НА ВЛИЯНИЕТО НА РЕКА ДУНАВ ВЪРХУ БИОСФЕРЕН РЕЗЕРВАТ "СРЕБЪРНА" ЧРЕЗ МОДЕЛНАТА ЕКОСИСТЕМА *ABRAMIS BRAMA* – МАКРОБЕЗГРЪБНАЧНИ – СЕДИМЕНТИ
RESEARCH OF THE IMPACT OF THE RIVER DANUBE ON THE *SREBARNA* BIOSPHERE RESERVE BY THE MODEL ECOSYSTEM *ABRAMIS BRAMA* – MACROINVERTEBRATES - SEDIMENTS

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Abstract

During 2015, 76 specimens of freshwater bream (*Abramis brama* (Linnaeus, 1758)) were examined by means of standard techniques for parasites. Seven species of parasites (*Paradiplozoon homoion* (Bychowsky & Nagibina, 1959), *Dactylogyrus yinwenyingae* (Gusev, 1962), *Asymphylodora tincae* (Modeer, 1790), *Caryophyllaeus fimbriceps* (Annenkova-Khlopina, 1919), *Acanthocephalus lucii* (Miiller, 1776), *Acanthocephalus anguillae* (Müller, 1780), *Contracaecum microcephalum* (Stossich, 1890), larvae) were fixed. New parasite and host records were determined. The analysis of the dominant structure of the found parasite taxa were presented to the level of the component communities. All fixed parasite species were component for the parasite communities of examined fish with the exception of *A. lucii* and *A. anguillae*. *A. lucii* is a core parasite species and *A. anguillae* is an accidental parasite species for the helminth communities of freshwater bream. New data for the lead content in sediments, tissues and organs of bream from the Danube River are presented. From the tissues and organs of the studied fish specimens of *A. brama*, the lowest concentrations of lead were found in the muscles.

Key words: bioindication, fish parasite communities, heavy metals, Lake *Srebarna*, the Danube River.

INTRODUCTION

The Bulgarian part of the river and its wetlands on the Lower River, include Lake Srebarna, have important place in the Bulgarian and European ecological network. While the river and adjacent wetlands are under permanent negative anthropogenic impacts of industrial accidents and wastewaters. As a result, pollutions of the water ecosystems killed a lot of fish and other freshwater organisms (Literathy and Laszlo, 1995; 1999). Fish parasite communities, heavy metal content and the state of freshwater ecosystem of the Danube River are studied from different authors (Atanasov, 2012; Gabrashanska et al., 2004; Kakacheva-Avramova, 1977; 1983; Kakacheva et al., 1978; Kirin et al., 2013; Kirin et al., 2014; Margaritov, 1959; 1966; Michalovič, 1954; Moravec et al., 1997; Nachev, 2010; Nachev and Sures, 2009; Nedeva et al., 2003; Ricking and Terytze, 1999; Woitke et al., 2003, etc.) but they are comparatively small from the Srebarna Lake (Hristov, 2010; Margaritov, 1959; Shukerova 2007; Shukerova, 2010; Shukerova and Kirin, 2008; Shukerova et al., 2009; Kirin et al., 2013; Kirin et al., 2014, etc.). The aim of this study is to present the results of an examinations of fish parasite species, structure of parasite communities and heavy metal contents in sediments, fish tissues and organs from the Bulgarian part of the Lower Danube River (village of Vetren) and the Danube wetland with international importance, Lake Srebarna.

MATERIALS AND METHODS

During 2015, sediments, fish and fish parasites are collected and examined from the Lower Danube River (village of Vetren, Bulgarian part) and Lake Srebarna (Fig. 1). 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. About 5 km from the village of Vetren is located Lake Srebarna. It is declared as a Biosphere Reserve (UNESCO), as site of the World Natural Heritage (Ramsar Convention), as an object in the List of Wetlands of International Importance and Important

Bird site (BirdLife International). The Srebarna Lake is situated in Northeastern Bulgaria (44⁰7'N, 27⁰5'E) near to the village of Srebarna. It is freshwater eutrophic lake connected through an artificial canal with the Danube River. The lake is distinguished, as well as the Danube River, with significant diversity of highly protected species (Michev et al., 1998; Uzunov et al., 2001; Pehlivanov et al., 2006; Uzunov et al., 2012, etc.).

A total of 10 sediment samples, 47 specimens Freshwater bream (*Abramis brama* (Linnaeus, 1758)) from the Danube River and 29 fish specimens from Lake Srebarna are collected and examined in 2015. The fish are caught by nets, by angling and eloctrofishing under a permit issued by the Ministry of Agriculture and food and Ministry of Environment and waters of Bulgaria. The scientific and common names of fish hosts are used according to the FishBase database (Fröse and Pauly, 2015).

The model of fish species chosen for examination of the heavy metal content in this study is the Freshwater bream, *Abramis brama* (L., 1758). Fish specimens are weighed (total weigh from 91 - 383 g) and measured (total length from 17.5-30.5 cm).

Helminthological examinations are carried out following recommendations and procedures described by Bauer et al. (1981), Bykhovskaya-Pavlovskaya (1985), Gusev (1983; 1985), Moravec (1994; 2001), Georgiev et al. (1986), Malmberg (1970), Protasova et al. (1990), etc.

The analysis of the dominant structure of the found fish parasite taxa were presented to the level of the component communities. 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 (10 < P% < 20) and core (P% >20) species. The ecological terms prevalence, mean intensity are used, based on the terminology of Bush et al. (1997). Analyses of helminth community structure were carried out during the three seasons and in both levels: infracommunity and component community. The infracommunity data are used to calculate the total number of species, mean number of helminths, Brillouns diversity index (HB) and Berger-Parker dominance index (d), etc. (Kennedy, 1993; 1997; Magurran, 1988; Marcogliese and Cone, 1997).

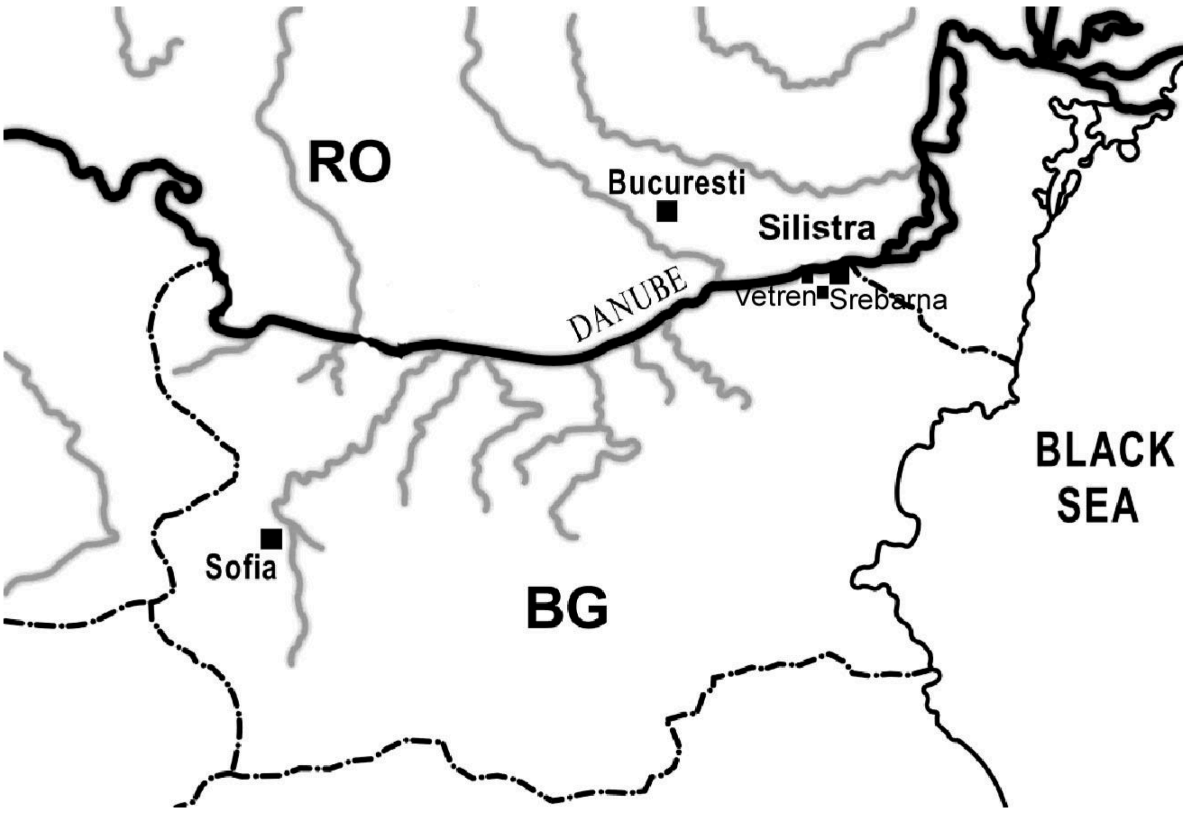

Fig. 1. *Danube River and La ke Srebarna*

Samples of sediments are collected according to the Guidance on sampling of rivers and watercourses – BSS ISO 5667-6:1990, introduced as a Bulgarian standard in 2002. Heavy metal concentration of the water and sediment samples, fish tissues, organs and parasites are carried out according to standard techniques. The samples are analyzed for content of Pb by ICP Spectrometry (Bíreš et al., 1995).

Samples of muscles, skin and liver are collected from all individuals. In order to determine the relative accumulation capability of the fish tissues in comparison to the sediments, bioconcentration factor (BCF=[Chost tissues]/[Csediments]]) are calculated (Sures et al., 1999). The bioconcentration factors are computed to establish the accumulation order and to examined fish for use as biomonitors of trace metal pollutants in freshwater environments. The differences in concentration factors are particularly discussed in respect to the bioavailability of trace metals from sediments. A Spearman's rank correlation coefficient, r_s is used to test associations between the bottom sediments, fish tissues and organs.

RESULTS AND DISCUSSION

Model fish species

A total of 47 and 29 specimens of Freshwater bream *Abramis brama* (Linnaeus, 1758) are collected and examined from the Danube River and the Srebarna Lake, respectively (total 76 specimens). *Abramis brama* is estimated as least concern species (LC=Least Concern; IUCN Red List Status). Freshwater bream is brackish, benthopelagic, potamodromous fish species. Adults inhabit a wide variety of lakes and large to medium sized rivers. Fish species is the most abundant in backwaters, lower parts of slow-flowing rivers, brackish estuaries and warm and shallow lakes. Adults occur usually in still and slow-running waters where they travel in large shoals. Larvae and juveniles live in still water bodies, feeding on plankton. Adults feed on insects, particularly chironomids, small crustaceans, molluscs and plants. Larger specimens may feed on small fish (Fröse and Pauly, 2015).

Helminth community structure

Helminth parasites are recorded in 26 bream specimens (55.3%) from the Danube River and in one infected bream (3.4%) from Srebarna Lake. Seven parasite species are identified: two monogenean (*Paradiplozoon homoion* (Bychowsky & Nagibina, 1959), *Dactylogyrus yinwenyingae* (Gusev, 1962)); one digenean (*Asymphylodora tincae* (Modeer, 1790)); one cestoda species (*Caryophyllaeus fimbriceps* (Annenkova-Khlopina, 1919)), two acantocephalans (*Acanthocephalus lucii* (Miiller, 1776), *Acanthocephalus anguillae* (Müller, 1780)) and one nematode species (*Contracaecum microcephalum*, (Stossich, 1890), larvae. All helminth species are occurred as adults excepting *C. microcephalum* (Table 1). All helminth species are the autogenic species, matured in fish and only *C. microcephalum* is allogenic species, matured in fish-eating birds (Moravec, 1994).

Table 1. Species diversity of helminth parasites of *Abramis brama* from the Danube River (Biotop Vetren) (p - number of parasites; P - prevalence; MI - mean intensity)

	N	n	p	P%	MI±SD	range
Asymphylodora tincae (Modeer, 1790)	47	8	18	17,02	2.25±2.12	1-7
Contracaecum microcephalum Stossich, 1890*, larvae*	47	7	17	14,89	2.43±1.90	1-6
Caryophyllaeus fimbriceps Annenkova-Khlopina, 1919	47	6	14	12,77	2.33±2.80	1-8
Dactylogyrus yinwenyingae Gusev, 1962	47	7	44	14,89	6.29±6.34	1-19
Paradiplozoon homoion Bychowsky & Nagibina, 1959	47	8	16	17.02	2.00±1.77	1-6
Acanthocephalus lucii (Miiller, 1776)	47	10	28	21.28	2.80±2.15	1-7
Acanthocephalus anguillae (Müller, 1780)	47	1	1	2.13	1.00±00	1

Component community

In component community of bream from Danube River all found parasite species are component species with the exception of *A. lucii* and *A. anguillae. A. lucii is* a core parasite species (P%=21.28) and *A. anguillae* is an accidental parasite species (P%=2.13) for the helminth communities of Freshwater bream (Table 1). The parasite communities of *A. brama* from the Danube River (Biotope Vetren) showed Brillouin's diversity indices, HB=0.487±0.168 (range 0.322-0.837) and Berger-Parker dominance indices, d=0.810±0.219 (range 0.5-1.0).

P. homoion is recorded as parasite species on the gills of *R.rutilus* from Danube and as parasite species on the gills of *R. rutilus, Alburnus alburnus, Leuciscus cephalus, Chondrostoma nasus, Cyprinus carpio, Gobio gobio, Barbus cyclolepis* (Kakacheva-Avramova, 1977; Kakacheva-Avramova et al., 1978). *Paradiplozoon homoion* was recorded as parasite species of Freshwater bream from the Danube Basin (Moravec, 2001).

Dactylogyrus yinwenyingae was found in *Scardinius erythrophtalmus* and *Rhodeus amarus* from the Danube Basin (Dávidová et al., 2008).

A. lucii is found in *Abramis sapa*, *L. cephalus, R. rutilus*, *Lota lota*, *Gymnocephalus schraetser*, *Benthophilus stellatus*, *Proteorhinus 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 in the Danube Basin* (Kakacheva-Avramova, 1983; Moravec, 2001; Djikanovic et al., 2012; Shukerova et al., 2010).

A. anguillae is found in *S. cephalus*, *L. idus*, *Blicca bjoerkna, R. rutilus, S. fario*, *A. alburnus*, *B. cyclolepis*, *Carassius carassius*, *P. fluviatilis*, *A. brama, Barbus barbus* from Danube Basin (Margaritov, 1959; 1966; Kakacheva-Avramova, 1969; 1977; Kakacheva-Avramova at al., 1978; Shukerova et al., 2010; Atanasov, 2012; Djikanovic et al., 2012).

Intermediate host of *A. lucii* and *A. anguillae* is *Asellus aguaticus,* and definitive host are fish from families Cyprinidae, Salminidae, Percidae, Anguillidae*, etc. (Kakacheva-Avramova, 1983).*

A. tincae is reported of *Tinca tinca* and *R. rutilus* from the Danube Basin (Andric, 1984; Moravec, 2001). *A. tincae* is reported as parasite species of *S. erythrophtalmus* from Srebarna Lake (Margaritov, 1959). Intermediate hosts of *A. tincae* are snails (*Bithynia tentaculata* and *Radix auriculata*), and definitive host fish from family Cyprinidae*.*

C. fimbriceps is found in *Abramis brama* from the Danube Basin (Georgescu, 1984; Scholz, 1989; Moravec, 2001; Oros and Hanzelová, 2009; Djikanovic et al., 2012; Atanasov, 2012).

Intermediate hosts of *C. fimbriceps* are *Tibifex tubifex* and *Psametrictes albicola* and definitive hosts are freshwater fish*.*

C. microcephalum is reported from *C. carpio*, *P. fluviatilis, A. brama* from the Danube Basin (Margaritov, 1959; 1976; Shukerova et al., 2010; Shukerova, 2010; Kirin et al., 2013; Kirin et al., 2014).

Definitive host of *C. microcephalum* are different fish-eating birds (*Ardea cinerea*, *A. purpurea*, *A. ralloides*, *Nycticorax nycticorax*, *Pelecanus onocrotalus*, *Ciconia ciconia, C. nigra),* where adult nematodes are localized in intestine. First intermediate hosts of this parasite species are copepods, and the second intermediate hosts are fish species (Moravec, 1994).

The monogeneas species *P. homoion* and *D. yinwenyingae* are with direct life cycles (Gusev at al., 1985; Moravec, 1994; Jarkovsky´ et al., 2004).

All parasitic species of *A. brama* from Danube River (Biotop Vetren) are generalist with exception of *C. fimbriceps,* which is specialist of Freshwater Bream.

The parasite communities of bream from the Danube River showed significantly larger number of taxa (7).The common helminth species of bream from Srebarna Lake and from the Danube River (Biotop Verten) is the nematioda *C. microcephalum*. Seven fish hosts from the Danube River are infected with 17 individuals of *C. microcephalum* and from Srebarna Lake a one fish are infected with 4 individuals of *C. microcephalum.* The remaining six types of parasites are found only in fishes from the Danube River (*P. homoion*, *D. yinwenyingae*, *A. tincae*, *C. fimbriceps, A. lucii*, *A. anguillae*).

This study of parasite communities of *A. brama* from Danube River (Biotop Vetren) showed the highest species diversity (7 helminth species) than previous researches of parasite communities of Freshwater Bream from Danube River (Biotop Silistra, 3 parasite species (*Gyrodactylus elegans* Nordmann, 1832; *Diplozoon paradoxum* Nordmann, 1832 and *Pomphorhynchus tereticollis*) Kirin et al., 2013; Kirin et al., 2014). In this research of parasite community of *A. brama* from Srebarna Lake is found only *C. microcephalum*, this is opposite of the previous results of the scientific team in which *A. brama* was not infected (Kirin et al., 2013; Kirin et al., 2014). These results opposed also to the examinations of Shukerova (2010), when was reported 6 parasites (*D. pseudospathaceum*, *T. clavata*, *P. cuticola*, *P. sapae*, *C. microcephalum*, *R. acus*).

Content of heavy metals in sediments, fish and parasites

The result of the content of lead (Pb) in 10 samples of sediments and 47 samples of muscle, liver and skin of *Abramis brama* from the Danube River are presented. Based on the results of chemical analyzes, mean concentrations (mg.kg^{-1}) in tissues, organs of the fish, parasites and sediments, as well as the bioconcentration factor (BCF=[Chost/parasite tissues]/[Csediments]) are defined.

The contents of lead are the highest in samples of sediments from the both examined freshwater ecosystems – Biotope Vetren on the Danube River and the Srebarna Lake ($C_{Sed/Danube}$=43.251 and $C_{Sed/Srebarna}$=30.7 mg.kg^{-1}, respectively). From the fish tissues and organs the highest contents of lead are determined in samples of skin (6.883±3.599 mg.kg^{-1}), followed by these of liver (6.077±5.512) and muscles (4.038±1.154). This purpose remains regarding the values of BCF, set against the levels of lead in sediments of the Danube River (Biotope Vetren) (Table 2).

Linear correlation coefficients (r_s, Spearman correlation coefficient) are determined to test associations between the bottom sediments, fish tissues and organs. Very significant correlations (p<0.001) are fixed for relationship between $C_{SedimentsPb}$-$C_{LiverPb}$ (r_s=0.990), $C_{SedimentsPb}$-C_{SkinPb} (r_s=0.999), $C_{SedimentsPb}$-$C_{MusclePb}$ (r_s=0.991) of the Danube River (Table 2).

The maximum lead level permitted for fish is 0.2 mg.kg^{-1} according the EU and Bulgarian food codex (Anonymus, 2004); 2.0 mg.kg^{-1} for WHO and 0.5 mg.kg^{-1} for FAO. Lead content in analyzed fish organs and tissues of *A. brama* are found to be 1.5-7 times higher than limits. These results showed human health risk with respect to the concentrations of lead in analyzed samples of Freshwater bream from the Bulgarian part of the Danube River.

The major negative anthropogenic impact of the Danube River and Srebarna Lake ecosystems are from industrial and farm activities (fertilizers, pesticides; wastewater from livestock, etc.). Danube River and Srebarna Lake are included in the National monitoring programe (Regulation 1/2011).

The obtained values for the content of Pb in sediments, freshwater fish organs and tissues from the Danube River and Lake Srebarna are slightly higher than those reported by other examinations or authors for the same ecosystem, but for another biotopes of the Danube River (Bulgarian part of the river) (Atanassov, 2012; Hristov, 2010; Kirin et al., 2014; Nachev, 2010).

CONCLUSIONS

1. As a result of these examinations of helminthes and helminth communities of *A. brama* from Danube River (Biotop Vetren) and from Srebarna Lake *A. brama* is a new host record for *P. homoion* in Bulgarian part of Danube River. *A brama* is a new host record of *Dactylogyrus yinwenyingae* in the Danube Basin. *Dactylogyrus yinwenyingae* is a new parasite species for parasitofauna of fish in Bulgaria. *A brama* is a new host record for *A. lucii* in Bulgaria. *Abramis brama* is reported as a new host record of *A. tincae* in Danibe River.

2. New data for heavy metal contents in sediments, fish tissues and organs from the Danube River are presented. From the tissues and organs of the studied fish specimens *A. brama*, the lowest concentrations of lead are found in the muscles. In general, the content of lead in the samples of liver and skins are higher than in the muscles. These results showed human health risk with respect to the concentrations of lead in analyzed samples of Freshwater bream from the Bulgarian part of the Danube River.

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Table 2. Content of lead (mg.kg^{-1}), bioconcentration factor (BCF) and Spearman correlation coefficients (r_s) determined for the relationships between the content of lead in *A. brama* and in the bottom sediments

A. brama tissues/organs	Mean±SD (Min - Max) [mg.kg^{-1}]	*A. brama* relationships	BCF	r_s
C_{Liver}	6.077±5.512 (1.321-13.894)	$C_{Liver}/C_{Sediments}$	0.140	0.990
C_{Skins}	6.883±3.599 (3.214-10.235)	$C_{Skins}/C_{Sediments}$	0.159	0.999
$C_{Muscles}$	4.038±1.154 (2.384-5.067)	$C_{Muscles}/C_{Sediments}$	0.093	0.991

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