Scientific Papers. Series D. Animal Science. Vol. LX, 2017
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

ACCUMULATION OF LEAD IN *ABRAMIS BRAMA* AND ITS PARASITE *POMPHORHYNCHUS TERETICOLLIS* FROM DANUBE RIVER (VETREN AREA), BULGARIA

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Abstract

During 2016, 45 specimens of freshwater bream (Abramis brama (Linnaeus, 1758)) are collected and examined from the Danube River. Helminth parasites are recorded in 29 freshwater bream specimens (64.44%) from the Danube River. Five species of parasites were identified: one trematode species (Asymphylodora imitans (Mühling, 1898)), three acantocephalans (Acanthocephalus lucii (Müller, 1776), Acanthocephalus anguillae (Müller, 1780), Pomphorhynchus tereticollis (Rudolphi, 1809)) and one nematode species (Raphidascaris acus, (Bloch, 1779), larvae). In the component community of Abramis brama from Danube River A. imitans and A. lucii are core species. P. tereticollis is component parasite species for the helminth communities of freshwater bream. A. lucii and R. acus are accidental parasite species for the helminth communities of A. brama. New data for the lead contents in sediments, parasites, tissues and organs of freshwater bream from the Danube River are presented. From the tissues and organs of the studied fish specimens Abramis brama, the lowest concentrations of lead were found in the muscles. The content of lead in the samples of skin and liver are higher than in the muscles. The acanthocephalan Pomphorhynchus tereticollis showed significantly higher content of lead than its host organs. Bioconcentration factor for lead (Pb) in the tissues and organs of freshwater bream were presented and discussed with respect to their content in sediments. Very significant correlation ($p<0.001$) was fixed for relationship between P. tereticollis $_{Pb}$ -Sediments $_{Pb}$.

***Key words**: Abramis brama, bioindication, Danube River, heavy metals, Pomphorhynchus tereticollis.*

INTRODUCTION

The Bulgarian part of river Danube has important place in the Bulgarian and European ecological network. Fish parasites are sensitive indicators for heavy metals in aquatic ecosystems, due to their ability to accumulate significantly higher concentrations of trace elements than their host (Sures et al., 1994; Sures and Siddall, 1999; Schludermann et al., 2003; Thielen et al., 2004; Nachev et al., 2013). Fish parasite communities, heavy metal content and the state of freshwater ecosystem of the Danube River are studied from different authors (Atanasov, 2012; Djikanovic et al. 2013; Gabrashanska et al., 2004; Kakacheva-Avramova, 1977, 1983; Kakacheva et al., 1978; Kirin et al., 2013; Kirin et al., 2014; Margaritov, 1959, 1966; Moravec et al., 1997; Nachev, 2010; Nachev and Sures, 2009; Nedeva et al., 2003; Ricking and Terytze, 1999; Woitke et al., 2003, etc.). This paper presents the results of examinations of heavy metal contents in sediments, fish parasites, fish tissues and organs from the Bulgarian part of the Lower Danube River (village of Vetren).

MATERIALS AND METHODS

During 2016, sediments, fish and fish parasites are collected and examined from the Lower Danube River (village of Vetren, Bulgarian part) (Fig. 1). The village of Vetren ($44^0$133'N, $27^0$033'E) is situated on the riverside, in the north eastern part of the Danube Valley.
A total of 3 samples of sediment and 45 samples of freshwater bream (*Abramis brama* Linnaeus, 1758) from the Danube River are collected and examined in 2016. The scientific and common names of fish hosts are used according to the FishBase database (Fröse and Pauly, 2016). The freshwater bream *(Abramis brama* Linnaeus, 1758) species chosen for examination of the heavy metal content in this

study were weighed (total weigh from 24-323 g) and measured (total length from 10.5 - 31 cm). Helminthological examinations are carried out following recommendations and procedures described by Bauer et al. (1981), Bykhovskaya-Pavlovskaya (1985), Georgiev et al. (1986), Gusev (1985), Moravec (1994, 2001) etc.

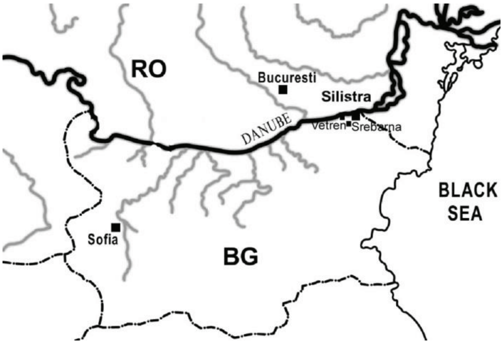

Figure 1. Danube River

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 and Berger-Parker dominance index (d), etc. (Kennedy, 1993, 1997; Magurran, 1988).

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 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 (ISO 8288:1986; BDS EN ISO 17294-2:2016; 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 examine 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, organs and parasites.

RESULTS AND DISCUSSIONS

A total of 45 specimens of freshwater bream *(Abramis brama* Linnaeus, 1758) are collected and examined from the Danube River. *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, 2016).

Helminth parasites are recorded in 29 freshwater bream specimens (64.44%) from the Danube River. Five species of parasites were identified: one trematode species *(Asymphylodora imitans* Mühling, 1898), three acantocephalans (*Acanthocephalus lucii* Müller, 1776), *Acanthocephalus anguillae* Müller, 1780), *Pomphorhynchus tereticollis* Rudolphi, 1809)) and one nematode species (*Raphidascaris acus* Bloch, 1779), larvae) (Table 1). All helminth species occurred as adults with the exception of *R. acus*. *A. imitans*, *A. lucii*, *A. anguillae* and *P. tereticollis* are autogenic species, matured in fish. *R. acus* allogenic species, matured in some species of predatory fishes as *Exos lucius*, *Lota lota*, *Salmo trutta* and others (Moravec, 1994).

Table 1. Helminth parasites of *Abramis brama* from Danube River (N – number of examined hosts, n – number of infected hosts, p – number of parasites, P – prevalence, MA – mean abundance, MI – mean intensity)

Helminth species	N=45					
	n	P	P%	MA±SD	MI±SD	Range
Asymphylodora imitans (Mühling, 1898)	18	544	40.00	12.08±32.93	30.22±46.51	1-198
Acanthocephalus lucii (Müller, 1776)	10	17	22.22	0.37±0.85	1.7±1.004	1-4
Acanthocephalus anguillae (Müller, 1780)	2	2	3.26	0.044±0.206	1	1
Pomphorhynchus tereticollis (Rudolphi, 1809)	5	13	11.11	0.288±1.02	2.6±1.85	1-6
Raphidascaris acus (Bloch, 1779), larvae	3	5	6.67	0.054±0.341	1.67±0.94	1-3

In the component community of *Abramis brama* from Danube River *A. imitans* (P%=40.00) and *A. lucii* (P%=22.22) are core species. *P. tereticollis* (P%=11.11) is component parasite species for the helminth communities of freshwater bream *A. lucii* (P%=3.26) and *R. acus* (P%=6.67) are accidental parasite species for the helminth communities of *Abramis brama* (Table 1).

In the component community of *Abramis brama* from Danube River trematodes are presented with the highest number of specimens, with 1 species and 544 specimens. Acanthocephalans are presented with three species and 32 specimens. Nematodes are represented by one species and 5 specimens.

Asymphylodora imitans was found in the gut of *Blicca bjoerkna* in Bulgarian section of river Danube (Kakacheva-Avramova, 1977). *Acanthocephalus lucii* was found in *Abramis sapa*, *Leuciscus cephalus*, *Rutilus rutilus*, *Gymnocephalus schraetser*, *Benthophylus stellatus*, *Proteorhinus marmoratus*, *Silurus glanis*, *Lota lota* and *Zingel zingel* from Bulgarian section of Danube River (Margaritov, 1959; Kakacheva-Avramova 1977; Atanasov, 2012). *A. anguillae* was found in *Leuciscus idus*, *Blicca bjoerkna*, *Carassius auratus gibelio*, *A. brama* and *Barbus barbus* from Danube River (Margaritov, 1959; Kakacheva-Avramova, 1977; Nachev, 2010; Atanasov, 2012; Chunchukova et al., 2016). Intermediate host of *A. lucii* and *A. anguillae* is *Asellus aguaticus,* and definitive hosts are fish species of different families as Cyprinidae, Salmonidae, Percidae, Anguillidae and others (Kakacheva-Avramova, 1983).

Pomphorhynchus tereticollis was found in *Abramis brama, Ballerus sapa, Barbus barbus, Gymnocephalus schraetser* and *Neogobius fluviatilis* from Bulgarian section of river Danube (Kirin et al. 2013, Kirin et al. 2014). Intermediate hosts of *P. tereticollis* are *Gammarus* sp. (Westram et al., 2011).

Species richness in infracommunity of freshwater bream ranges from 1 to 3 species. With 1 helminth species were infected 22 fishes (75.86 %), with 2 helminth species -5 fishes (17.24%) and with three species-only 2 specimens fish (6.90%). The largest number of helminth specimens established in a single host specimen is 198. The average species richness (mean number of species for fish specimen) in infracommunity of freshwater bream is 0.84 species (Table 2).

Table 2. Infracommunities of *Abramis brama* from Danube River

	Number of endohelminth species				
	0	1	2	3	Mean±SD Range
Abramis brama	16	22	5	2	0.84±0.787 1-3

Average abundance (mean number of helminths in fish) in these infracommunities is 12.64. The parasite communities of *A. brama* from the Danube River showed Berger-Parker dominance index, d=0.706±0.256 (range 0.370-0.990).

The result of the content of lead (Pb) in 3 samples of sediments and 29 samples of muscle, liver and skin of *Abramis brama* and its parasite *P. tereticollis* 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/parasitetissues]/[Csediments]) are defined.

From the fish tissues and organs the highest contents of lead was determined in samples from skin (5.912±5.348 mg.kg^{-1}), followed by those from liver (5.622±4.434 mg.kg^{-1}) and muscles (2.495±1.709 mg.kg^{-1}) (Table 3).

Table 3. Lead concentration (mg.kg^{-1}) in sediments, different organs of *Abramis brama* and its parasites *P. tereticollis*

	Mean±SD	Range
Sediments	45.256±15.958	33.940-67.825
Liver	5.622±4.434	0.552-14.603
Muscles	2.495±1.709	0.652-5.923
Skin	5.912±5.348	1.143-19.147
P. tereticollis	350±104	246-454

The acanthocephalan *P. tereticollis* showed significantly higher content of lead (350 mg.kg^{-1}), than its hosts organs.

This purpose remains regarding the values of BCF, set against the levels of lead in sediments of the Danube River (Biotope Vetren) (Table 4). The highest BCF $_{P.\ tereticollis}$ was for muscles (140.280) followed by those for liver (62.255), skin (59.201) and sediments (7.703).

Table 4. Bioconcentration factor (BCF = [Chost/parasite tissues]/[C Sediments]) of *A. brama* and *P. tereticollis*

Sediments / *A. brama* / *P. tereticollis*	BCF
$C_{P.\ tereticollis}/C_{Sediments}$	7.733
$C_{Liver}/C_{Sediments}$ $C_{P.\ tereticollis}/C_{Liver}$	0.124 62.255
$C_{muscle}/C_{Sediments}$ $C_{P.\ tereticollis}/C_{muscle}$	0.055 140.280
$C_{Skin}/C_{Sediments}$ $C_{P.\ tereticollis}/C_{Skin}$	0.114 59.201

A linear correlation coefficient (Spearman's rank correlation coefficient, r_s) is determined to test the association between the sediments, fish tissues, organs and sediments. Very significant correlation ($p<0.001$) was fixed for relationship between *P. tereticollis* $_{Pb}$-Sediments $_{Pb}$.

The obtained values for the content of Pb in sediments are slightly higher than those reported for the same Biotope (Kirin et al., 2013; Chunchukova et al., 2016). The obtained values for the content of lead in liver, muscles and skin of *A. brama* are slightly lower than those reported for the same Biotope (Chunchukova et al., 2016).

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 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.

CONCLUSIONS

As a result of this study is presented new data for helminthes and helminth communities of *A. brama* from Danube River (Biotope Vetren). New data for heavy metal contents in sediments, fish parasites, fish tissues and organs from the Danube River are presented. From the tissues and organs of the studied fish specimen *A. brama*, the lowest concentrations of lead were found in muscles.

In general, the content of lead in the samples of skin and liver 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.

ACKNOWLEDGEMENTS

This research work was carried out with the support of Agricultural University-Plovdiv, financing PhD thesis NoD31.

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