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Biodiversity, Bioindication and Helminth communities of *Abramis brama* (Linnaeus, 1758) from the Danube River and Lake Srebarna, Bulgaria

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Abstract

Biomonitoring from water of the River Danube and Srebarna Lake was performed using freshwater fish and their parasites and parasite communities as bioindicators. During 2013, 78 specimens of freshwater bream (*Abramis brama* (L., 1758) were examined with standard techniques for parasites. Three species of parasites (*Gyrodactylus elegans* Nordmann, 1832, *Diplozoon paradoxum* Nordmann, 1832 and *Pomphorhynchus tereticollis* (Rudilphi, 1809)) were fixed. The analysis of the dominant structure of the found taxa was presented to the level of the component communities. New parasite and host records were determined. All fixed parasite species are core for the parasite communities of examined fish. Concentration of heavy metals (Pb, Zn, Cu) in fish (muscle, liver, intestine and bones), endohelminth species *Pomphorhynchus tereticollis* as bioindicators and bottom sediments were analyzed. Bioindicator significance of parasite species was studied. For an ecological evaluation of the situation of the analyzed freshwater ecosystems, principal biotic indices were fixed.

Key words: bioindication, *Abramis brama* parasite communities, heavy metals, Lake Srebarna, River Danube.

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. The Bulgarian part of the Danube River and Srebarna Lake are included in the National monitoring program (Regulation 1/2011) and they are important places in European ecological network. The river and adjacent wetlands are under permanent negative anthropogenic impacts of industrial accidents and wastewaters and as a result, pollutions of the water ecosystems killed a lot of fish and other freshwater organisms (Literathy and Laszlo, 1995, 1999). Parasites of freshwater bream *Abramis brama* and their communities are reliable as sensitive indicators of heavy metals in aquatic ecosystems (Baruš et al., 2012; Dzika, 2002; Hayatbakhsh et al., 2011; Marcogliese, Cone, 1997; Palíková et al., 2014; Urdeş, 2010, etc.). The bioaccumulation potential of parasite species represents an important instrument of evaluating the functions of the fish – parasite system and prevents fish intoxication (Sures et al., 1999; Turčekova and Hanzelova 1997). Fish parasite communities, heavy metal content and the state of freshwater ecosystem of the Danube River are studied from different authors (Atanasov, 2012; Djukanovic et al., 2012; Kakacheva-Avramova, 1977, 1983; Kakacheva, Margaritov, Grupcheva, 1978; Lenhardt, 2004; Margaritov, 1959, 1966; Moravec et al., 1997; Nachev, 2010; Nachev, Sures, 2009; Nedeva et al., 2003; Ricking and Terytze, 1999, etc.) but they are comparatively small for *Abramis brama* from the Danube river and Srebarna Lake (Margaritov, 1959; Shukerova 2007; Shukerova, Kirin, 2008; Shukerova, Kirin, Hanzelová, 2009, etc.). This paper presents the results of an examination of heavy metal content in sediments, fish tissues and organs, fish parasites and dominant structure of fish parasite communities from the Bulgarian part of the Lower Danube River (town of Silistra) and the Danube wetland with international importants, the Lake Srebarna.

Materials and Methods

During June, 2013 sediments, fish and fish parasites were collect and examined from the Lower Danube River (town of Silistra, Bulgarian part) and Lake Srebarna (Figure 1). The town of

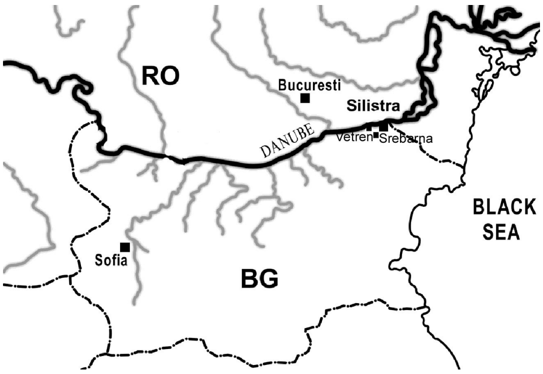

Figure 1. Danube River and Lake Srebarna

Silistra ($44^{0}117'$N, $27^{0}267'$E) is situated on the riverside, in the northeastern part of the Danube Valley, on the last Bulgarian part of the Danube Rive.

Lake Srebarna is situated in Northeastern Bulgaria ($44^{0}7'$N, $27^{0}5'$E) about 16 km far from the town of Silistra. It is declared as a Biosphere Reserve (UNESCO), as site of the World Natural Heritage (Ramsar Convention), as an object in the List of Wetlangs of International Importance and Important Bird site (BirdLife International). It is freshwater euthrophic lake connected through an artificial canal with the Danube River.

A total of 15 sediment samples and 78 freshwater fish specimens were collected and examined in June, 2013. The fish were 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 were used according to the FishBase database (Fröse and Pauly, 2012).

Samples of sediments were collected according to the Guidance on sampling of rivers and watercourses - 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 were carried out according to standard techniques. The samples were analyzed for content of Cu, Pb and Zn by ICP Spectrometry (Bíreš et al., 1995).

The model of fish species chosen for examination of helminth communities and the heavy metal content in this study is the freshwater bream (*Abramis brama* (L., 1758)).

Helminthological examinations were carried out following recommendations and procedures described by Bykhovskaya-Pavlovskaya (1985), Gusev (1983, 1985), Moravec (2001), Malmberg (1970), etc.

The analysis of the dominant structure of the found fish parasite taxa were presented to the level of the component communities. 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 were used to calculate the total number of species, mean number of helminths, Brillouns diversity index (HB), etc. (Kennedy, 1993, 1997; Magurran, 1988). Fish were weighed and measured. Samples of muscles, intestines, bones and liver were 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]]) were calculated (Sures et al., 1999). The bioconcentration factors were 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 were particularly discussed in respect to the bioavailability of trace metals from sediments. A linear correlation coefficient, r_s was used to test associations between the bottom sediments, fish tissues, organs and fish parasites.

Results and Discussion

Fish populations

A total of 29 and 49 fish specimens freshwater bream *Abramis brama* (Linnaeus,

1758) were collected and examined from the Srebarna Lake and the Danube River (Biotope Silistra), respectively (total 78 specimens). Adults freshwater bream inhabit a wide variety of lakes and large to medium sized rivers. *A. brama* is brackish, benthopelagic fish species. Larvae live in still water bodies, feeding on plankton. Juveniles feed on zooplankton and adult are feeding on insects, particularly chironomids, small crustaceans, molluscs and plants (Kottelat and Freyhof, 2007). Larger specimens may feed on small fish. The freshwater bream are estimated as least concern species (LC=Least Concern; IUCN Red List Status).

Helminth community structure

A total three species of helminths were fixed. They are belonging to classes Monogenea (*Gyrodactylus elegans* Nordmann, 1832; *Diplozoon paradoxum* Nordmann, 1832) and Acanthocephala (*Pomphorhynchus tereticollis* (Rudolphi, 1809)). The parasites are found only in fish from the Danube River. Biotope Silistra is a new location for *Pomphorhynchus tereticollis* of *A. brama* from the Bulgarian part of the Danube River.

Gyrodactylus elegans was reported as parasite species on the gills of *A. brama*, *B. sapa*, *A. ballerus*, *A. brama*, *C. carassius*, *C. carpio*, *G. gobio*, *M. fossilis*, *R. rutilus*, *T. tinca*, *V. vimba* (Moravec, 2001; Kakacheva, Margaritov, Grupcheva, 1978). *Diplozoon paradoxum* was reported as parasite species on the gills of *A. brama*, *A. bjoerkna, A. bipunctatus, A. alburnus, A. aspius, B. barbus, C. carassius, Ct. idella, C. carpio, G. gobio, G. cernuus, L. cephalus, L. leuciscus, L. idus, Ph. phoxinus, Rh. sericeus, R. rutilus, Sc. erythrophthalmus, T. tinca*, *V. vimba* of the Danube River (Gusev, 1985; Margaritov, 1959, 1966; Moravec, 2001; Kakacheva, Margaritov, Grupcheva, 1978, Kirin et al., 2013). *Pomphorhynchus tereticollis* (Rudolphi, 1809) has intermediate host's amphipods. According Emde et al. (2012) as amphipods, *Neogobius melanostomus* (Pallas, 1814) could be paratenic host in the life cycle of *P. tereticollis* and adult parasites are most likely to be found in chub (*Leuciscus cephalus* (Linnaeus, 1758) barbel (*Barbus barbus* (Linnaeus, 1758)). Although these final hosts are not known as primarily piscivorous, the authors suppose that especially larger barbels feed on these gobies, as they regularly feed on smaller fish species. Another authors (Dezfuli et al., 2011; Hine and Kennedy, 1974) described that the closely related parasite *Pomphorhynchus laevis* occasionally matures in trout (*Salmo trutta* Linnaeus, 1758), and also catfish (*Silurus glanis* Linnaeus, 1758). They ascertained that if a suitable final host for the parasite does not consume the infected gobies, the life cycle gets interrupted (Emde et al., 2012). Definitive hosts are fish species (*Gadus*, *Ac. sturio*, *S. fario*, *M. lineata*, *C. regale*, *S. foetens*) (Petrochenko, 1956). The isolated specimens of *Pomphorhynchus* were determined by Špakulová, who published revision of the genus (Špakulová et al., 2011). The specimens *Pomphorhynchus*, established of *A. brama* (Biotope Vetren, Kirin et al., 2013) are defined as *P. tereticollis*. It was the first report of *P. tereticollis* for Bulgarian part of the Danube River and *A. brama* – the first host record for this parasite species (Kirin et al., 2013).

Three species, two monogenean species (*G. elegans*, *D. paradoxum*) and one acanthocephalan species (*P. tereticollis*), parasitic in freshwater bream are generalists. In *A. brama*, the highest prevalence is showed *P. tereticollis* (38.78%), followed by *D. paradoxum* and *G. elegans* (20.41% and 10.2%, respectively). Prevalence of the three helminth species of *A. brama* in Biotope Silistra are lower than these in Biotope Vetren (67% and on 50%, respectively). *G. elegans*, *D. paradoxum* are accidental species and *P. tereticollis* is component species for the helminth communities of freshwater bream from the Danube river (Biotope Silistra) (Fig. 2). The highest number of specimens for *P. teraticollis* (106 specimens) of *A. brama* is found. All other species are fixed with 73 and 24 specimens, respectively. The mean intensity of infection is the highest for *P. tereticollis* (2.16±4.54), followed by *D. paradoxum* (MI=1.42±3.08) and *G. elegans* (0.45±1.71). The Brillouin's biodiversity infex (HB=0.475) are determined for the helminth communities of *A. brama*.

A. brama from the Lake Srebarna are free of parasites. It is confirm the previous results of the scientific team (Kirin et al., 2013). These results opposed to the examinations of Shukerova (2010). She was reported 6 parasite species (*D. pseudospathaceum*, *T. clavata*, *P. cuticola*, *P. sapae, C. microcephalum*, *R. acus*) for A. brama from the Srebarna Lake. Freshwater bream is fixed as the first host record for these parasite species in Bulgaria; as the first host record of *D. pseudospathaceum* and *P. cuticola* on the Balkan Peninsula; as the first host record of *D. pseudospathaceum* for Danube Basin and in particular Biotope Srebarna – as a new locality for them. Changes of ecological conditions in this biotope or lower abundance of intermediate and definitive hosts are supposed reasons for the negative results last years.

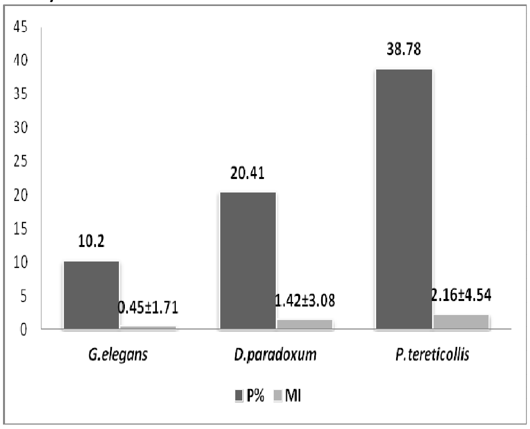

Figure 2. Prevalence (%) and Mean intensity (MI±SD) of fish parasite species, Danube River (Biotope Silistra), 2013

Content of heavy metals in sediments, fish and parasites

The result of the chemical analyzes (Pb, Cu and Zn) of 40 samples of muscle, liver, intestines and bones of *Abramis brama* from the Danube River are presented. The content of Pb, Cu and Zn in parasite species *Pomphorhynchus tereticollis* and bottom sediments were determined. Based on the results of chemical analyzes, mean concentrations (mg/kg) in tissues, organs of the fish, parasites and sediments, as well as the bioconcentration factor (BCF=[Chost/parasite tissues]/[Csediments]) were defined.

According to the results of this study, for the first time was presented the data for content of Pb, Cu and Zn of freshwater bream tissues and organs and of their acanthocephalan parasite *P. tereticollis* of the Danube River (Biotope Silistra). *P. tereticollis*, component helminth species of the helminth communities of *A. brama* of the Danube River, was distinguished with higher content of heavy metals than in sediments. With regards to freshwater bream tissues and organs, the highest contents of lead and zinc were fixed from the intestines and of copper – from the liver. $BCF_{P.\ tereticollis}$ was the highest of lead (6.799), followed by these of copper and zinc (Table 1) with no significant differences between the rest two of them. BCF_{Pb} and BCF_{Zn} were the highest from the intestines and of copper – from the liver. $BAF_{P\ tereticollis}$ was the highest of the muscles for the three trace metals, but was the highest of lead (BCF_{Pb}=1192.295) The order of metal bioaccumulation in the muscles of *A. brama* was Zn > Cu > Pb (Table 1).

A linear correlation coefficient, (r_s, Spearman correlation coefficient) are determined to test associations between the bottom sediments, fish tissues, organs and fish parasites. Very significant correlation (p<0.001) were fixed between sediments for the three trace elements and *P. tereticollis*, parasite from helminth communities of *A. brama*, river Danube ($Sediments_{Cu}$-*P. tereticollis*; $Sediments_{Pb}$-*P. tereticollis*; $Sediments_{Zn}$-*P. tereticollis*; p<0.001).

Table 1. Bioconcentration factor (BCF=[Chost/parasite tissues]/[Csediments]) of *A. brama* and *P. tereticollis*, Danube River

Abramis brama BCF	Indices Cu	Pb	Zn
$C_{P.\ tereticollis}/C_{Sediments}$	1.331	6.799	1.061
$C_{Liver}/C_{Sediments}$	0.306	0.019	0.039
$C_{P.\ tereticollis}/C_{Liver}$	4.339	347.059	27.230
$C_{Intestine}/C_{Sediments}$	0.088	0.034	0.109
$C_{P.sereticollis}/C_{Intestine}$	14.986	196.779	9.734
$C_{Bones}/C_{Sediments}$	0.025	0.011	0.055
$C_{P.\ tereticollis}/C_{Bones}$	54.602	611.954	19.290
$C_{Muscles}/C_{Sediments}$	0.015	0.0004	0.018
$C_{P.\ tereticollis}/C_{Muscles}$	88.423	1192.295	59.063

The obtained values for the content of Pb, Zn and Cu in sediments, freshwater fish organs and tissues and their parasites from the Danube River are slightly higher than those reported by other authors for the same ecosystem, but for another biotopes and freshwater parasite species from the Danube River (Bulgarian part of the river) (Atanassov, 2012; Nachev, 2010).

Conclusions

As a result of this examination new species and a new host records were found for the freshwater ecosystem of the Danube River. *P. tereticollis* are reported for the first time for helminth communities of *A. brama* in Biotope Silistra. New data for heavy metal contents in sediments, fish tissues and organs and parasite species *P. tereticollis* from the Danube River, Biotope Silistra are present. From the tissues and organs of the studied species, the lowest concentrations of Pb, Cu and Zn were found in the muscles. In general, the intestines showed the highest content of lead and zinc and liver is distinguished with the highest content of copper. The high significant correlations were determined for *P. tereticollis* as a sensitive indicator of Pb, Cu and Zn.

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