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HELMINTH COMMUNITIES OF FISHES FROM THE RIVER DANUBE AND LAKE SREBARNA, BULGARIA

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Abstract

Ecological monitoring from water of the River Danube and Srebarna Lake was performed using freshwater fishes and their parasites and parasite communities as bioindicators. For an ecological evaluation of the situation of the analyzed freshwater ecosystems, principal biotic indexes were fixed. The analysis of the dominant structure of the found taxa was presented to the level of the component communities. During 2012, 16 species and 181 specimens of freshwater and passage fish were examined with standard techniques for parasites. Six species of examined fish (Aspius aspius (L., 1758) , Carassius gibelio (Bloch, 1782), Chondrostoma nasus (L., 1758), Zingel zingel (L., 1758), Cyprinus carpio (L., 1758) and Lepomis gibbosus (L., 1758)) were free of parasites. In ten species of fish (Abramis brama (L., 1758), Alburnus alburnus (L., 1758), Alosa pontica (Eichwald, 1838), Ballerus sapa (Pallas, 1811), Barbus barbus (L., 1758), Romanogobio albipinnatus (Lukasch, 1933), Gymnocephalus schraetser (L., 1758), Neogobius fluviatilis Pallas, 1811, Perca fluviatilis L., 1758, Rutilus rutilus (L., 1758)) seven species of parasites (Gyrodactylus elegans Nordmann, 1832, Diplozoon paradoxum Nordmann, 1832, Nicolla skrjabini (Iwanitzky, 1972), Pomphorhynchus tereticollis (Rudilphi, 1809), Eustrongylides excisus (Jgerskild, 1909) larvae, Hysterothylacium aduncum (Rudolphi, 1802), Camallanus truncatus (Rudophi, 1814)) were fixed. New parasite and host records were determined. All fixed parasite species are core for the parasite communities of examined fishes with the exception of N. skrjabini. Bioindicator significance of parasite species was studied.

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

INTRODUCTION

The Danube River is the second longest river in Europe (about 2 800 km length), connecting Central and South-Eastern Europe and flows through nine countries (Germany, Austria, Slovakia, Ukraine, Hungary, Croatia, Serbia, Bulgaria, Romania). The richness in habitats, flora and fauna determine Danube River as important ecosystem for protection of biodiversity. The river basin, including tributaries and wetlands, is home to around 2 000 plant and 5 000 animal species, including numerous endangered or nearly extinct species. The Bulgarian part of the river and its wetlands on the Lower River, include Lake Srebarna, have important place in the Bulgarian and Europaean 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 fishes and other freshwater organisms (Literathy and Laszlo, 1995, 1999). Parasites of freshwater fishes and their communities are reliable as sensitive indicators of heavy metals in aquatic ecosystems (Baruš et al., 2007; Djikanovic, 2012; Nachev et al., 2010; Marcogliese and Cone, 1997; Oros and Hanzelová, 2009; Overstreet, 1997; Tielen et al., 2004, etc.). 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, Margaritov, Grupcheva, 1978; Margaritov, 1959, 1966; Michalovic, 1954; Moravec et al., 1997; Nachev, 2010; Nache, 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, 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 (villade of Vetren) and the Danube wetland with international importants, Lake Srebarna.

MATERIALS AND METHODS

During June, 2012 sediments, fish and fish parasites were collect and examined from the Lower Danube River (village of Vetren, Bulgarian part) and Lake Srebarna, Fig. 1:

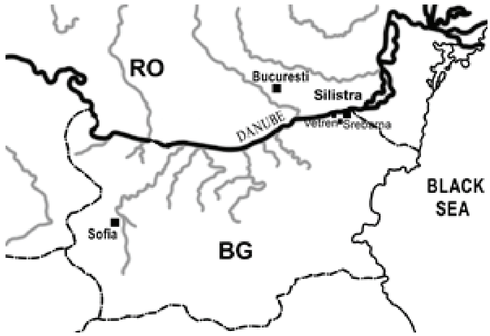

Figure 1. Danube River and Lake Srebarna

Figure 1. Danube River and Lake Srebarna

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 Wetlangs of International Importance and Important Bird site (BirdLife International). The Srebarna Lake is situated in Northeastern Bulgaria (44^{0}7′N, 27^{0}5′E) near to village of Srebarna. It is freshwater euthrophic lake connected throught 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, etc.).

A total of 10 sediment samples and 181 freshwater and passage fish specimens belonging to 5 families and 16 species were collected and examined in June, 2012. The fishes 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 Cd, Cu, Pb and Zn by ICP Spectrometry (Bíreš et al., 1995).

The model of fish species chosen for examination of the heavy metal content in this study were the European perch, *Perca fluviatilis* L. of the Danube River and Lake Srebarna and Common barbel, *Barbus barbus* (L., 1758) of the Danube River.

Helminthological examinations were carried out following recommendations and procedures described by Bykhovskaya-Pavlovskaya (1985), Gusev (1983, 1985), Moravec (1994, 2001), Georgiev et al. (1986), Shigin (1986), 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). Fishes were weighed and measured. Samples of muscles, fat 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 fishes 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 DISCUSSIONS

Fish communities

A total of 55 and 126 fish specimens were collected and examined from the Srebarna Lake and the Danube River, respectively (total 181 specimens). The fish species *Cyprinus carpio* Linnaeus, 1758 and *Lepomis gibbosus* (Linnaeus, 1758) were collected only from the Srebarna Lake. *Ballerussapa* (Pallas, 1811), *Aspius aspius* (Linnaeus, 1758), *Barbus barbus* (Linnaeus, 1758), *Chondrostoma nasus* (Linnaeus, 1758), *Romanogobio albipinnatus* (Lukasch, 1933), *Gymnocephalus schraetser* (Linnaeus, 1758), *Zingel zingel* (Linnaeus, 1758), *Alosa pontica pontica* (Eichwald, 1838) and *Neogobius fluviatilis* Pallas, 1811 were collected and examined only from the Danube River. Common to Srebarna and Danube are the fish species *Abramisbrama* (Linnaeus, 1758), *Carassius gibelio* (Bloch, 1782), *Rutilus rutilus* (Linnaeus, 1758) and *Perca fluviatilis* Linnaeus, 1758. From studied 16 species of fishes, 11 species were estimated as least concern species (LC=Least Concern; IUCN Red List Status), 2 species (*C. carpio*; *A. pontica*) were estimated as vulnerable species (VU=Vulnerable; IUCN Red List Status). Six species (*A. aspius*, *Ch. nasus*, *G. schraetser*, *R. albipinnatus*, Z. *zingel*, *A. pontica*) were included in Appendix 3 of the Bern Convention; 2 species (*Z. zingel*, *A. pontica*) were included in appendices II and V of the Habitats Directive (Directive) and the other two species (*A. aspius*, *R. albipinnatus*) are only included in Appendix II of the Directive. Nine fish species are included in Red Book of Bulgaria (2011), of wich 8 are vulnerable species (VU – *A. aspius*, *B. barbus*, *C. carpio*, *V. melanops*, *R. albipinnatus*, *G. schraetser*, *A. pontica*, *N. fluviatilis*) and one is endangered species (EN – *Z. zingel*). Two species (*G. schraetser*, *A. pontica*) are listen in Anex II and IV of the Biological Diversity Act (BDA, 2002); 2 species (*A. aspius*, *R. albipinnatus*) in Appendix II of the Act; 2 species (*B. barbus*, *Z. zingel*) in Anex IV of the Act. Six species of fish (*Aspius aspius*, *Carassius gibelio*, *Cyprinus carpio*, *Chondrostoma nasus*, *Zingel zingel*

Helminth community structure

A total seven species of helminths were fixed in both biotopes. They are belonging to classes Monogenea (2), Trematoda (1), Nematoda (3) and Acanthocephala (1). The freshwater ecosystem of the Danube River showed significantly larger number of taxa (7) in the parasite communities. *Eustrongylides excisus* (Jägerskiöld, 1909), larvae is common parasite species in freshwater ecosystems of the Srebarna Lake and Danube River. The remaining six types of parasites are found only in fishes from the Danube River (*Gyrodactylus elegans* Nordmann, 1832; *Diplozoon paradoxum* Nordmann, 1832; *Nicolla skrjabini* (Iwanitzky, 1928) Slusarski, 1972; *Pomphorhynchus tereticollis* (Rudolphi, 1809); *Hysterothylacium aduncum* (Rudolphi, 1802) and *Camallanus truncatus* (Rudolphi, 1814)).

Gyrodactyluselegans 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). *Diplozoonparadoxum* 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). The adult *Nicollaskrjabini* are parasite species of *A.acerina*, *S,glanis, G.cernua*, *G. schraetser*, *A.ruthenus*, *A. brama*, *A. ballerus*, *P.cultratus*, *C.carassius*, *C.carpio*, *C.bulgarica*, *C. balcanica*, *S.lucioperca*, *S. volgense*, *P.fluviatilis*, *Z.zingel*, *Z. streber*, *G.kessleri*, *Pr.marmoratus*, *Bl. bjoerkna*, *A. aspius*, *P. cultratus*, *G. gobio*, *C.bulgarica*, *G. fluviatilis*, *G. cephalarges*, *P.minutus, N.kessleri* (Atanasov, 2012; Kakacheva, Margaritov, Grupcheva, 1978; Moravec, 2001). The first intermediate host are the snails *Lithoglyphus* *naticoides* and the secong – *Gammarus* (*Rivulogammarus*) *balcanicus*, *Pontogammarus crassus* and *Dikerogammarus haemobaphes* (Kakacheva-Avramova, 1983; Komarova, 1968; Stenko, 1976). *Pomphorhynchus tereticollis* (Rudolphi, 1809) has intermediate host's amphibians. Definitive hosts are fish species (*Gadus* sp., *Ac.sturio*, *S.fario*, *M.lineata*, *C.regale*, *S.foetens*) (Petrochenko, 1956). The isolated specimens of *Pomphorhynchus* were determined by Špakulová, based on revision of the genus (Špakulová et al., 2011). The specimens *Pomphorhynchus*, established of *A. brama*, *B. sapa*, *B. barbus*, *G. schraetser* and *N. fluviatilis* of the freshwater ecosystem of the Danube (Biotope Vetren) are defined as *P. tereticollis*. *Eustrongylides excisus*, larvae is developed with participation of the first intermediate host oligochets (*Lumbricus variegatus*, *Tubifex tubifex*, *Limnodrilus* sp.) and the second, fish species, amphibians (*R.ridibunda*) and reptiles (*N.tesselata*). The adult nematodes parasitic in the glandular stomach of cormorants (*Ph.carbo* and *Ph. pygmaeus*) (Moravec, 1994). The species was presented of *S. lucioperca* (as paratenic host) and of *Gobius* sp. (as intermediate host) of the Mandra Lake (Margaritov, 1960), of *A. aspius* of the Danube River (Margaritov, 1959); of *P. fluviatilis* (Nedeva, Grupcheva, 1996); of *S.glanis*, *L.lota*, *N.cephalarges*, *N. kessleri*, *P. fluviatilis* of the Danube River (villages of Archar, Dobri dol, Gotomartsi) (Atanasov, 2012), etc. *Hysterothylacium aduncum* was reported of *A.gueldenstaedti*, *A.alosa*, *A. falax*, *A.pontica*, *S.trutta*, *S. salar*, *O.mykiss*, *Oncorhynchus* sp., *C.lavaretus*, *C. nasus*, *O.eperlanus*, *T.tinca*, *Ph.poxinus*, *E.lucius*, *L.lota*, *P.fluviatilis*, *A.anguilla*, *M.quadricornis*, *P.flesus*. Intermediate hosts are pelagic copepods (*Acartia bifilosa*, *Eurytemora affinis*, etc.) (Moravec, 1994). *Camallanus truncatus* was reported of *A. brama*, *A.anguilla*, *A. aspius*, *C.gobio*, *E.lucius*, *G. albipinnatus*, *G.cernuus*, *G. schraetser*, *L.lota*, *P. fluviatilis*, *S.glanis*, *S.lucioperca*, *S. volgense*, *Z. zingel* of the Danube River (Moravec, 2001). Intermediate hosts are cyclops (*Mesocyclops leuckarti*, *Megacyclops viridis*, *Macrocyclops albidus*, *Cyclops strenuus*) (Moravec, 1994).

Six species, parasitic in different fish hosts are generalists and are the most abundant in freshwater ecosystem of the Danube River: two monogenean species (*G. elegans*, *D. paradoxum*), one digenean species (*N. skrjabini*), two nematoda species (*H. aduncum*, *C. truncatus*) and one acanthocephalan species (*P. tereticollis*). *E. excisus*, which use fishes as intermediate hosts represented the allogenic species. In freshwater ecosystem of the Danube River with the highest species diversity were distinguished helminth communities of *A. brama* (3 species). In *A. brama*, the highest prevalence are showed *P. tereticollis* (67%), followed by *G. elegans* and *D. paradoxum* (on 50%). They are core species of the parasite communities of the Danube bream (Biotope Vetren). Helmint communities of the bream are followed by the biodiversity of the helminth communities of the striped ruffe from the same biotope, represented by two helminth species (*N. skrjabini* (7.7%) and *P. tereticollis* (46.2%)). *N. skrjabini* is an accidental species of the helminth communities of the striped ruffe and *P. tereticollis* is a component species for them. All other fish hosts are represented by one species of parasites (Fig. 2, Fig. 3). Each of them is a core species of the studied fish host. From them, with the highest prevalence are distinguished *E. excisus* of the *P. fluviatilis* and *R. rutilus* (on 100%) and *P. tereticollis* of the *B. barbus* (100%), *A. alburnus* (75%) and *B. sapa* (70%). The highest number of specimens for *H. aduncum* (180 specimens) of *A. pontica* and *P. tereticollis* (122 specimens) of *A. alburnus*, followed by number of specimens for *P. tereticollis* of *B. barbus* (32 specimens) are found. All other species range from 1 to 13 specimens. The mean intensity of infection is the highest for *P. tereticollis* (MI=16±2.83) of *B. barbus*, followed by *H. aduncum* (MI=7.5±7.92) of *A. pontica*. With the lowest mean intensity of infection are *C. truncatus* (MI=1±0.70) of *R. albipinnatus* (Fig. 3). *E. excisus* of the parasite communities of *P. fluviatilis* of the Srebarna Lake were distinguished with high prevalence (P=100%), but with low mean intensity (MI=2±1.54). This is a core species of the helminth communities of the perch from the lake freshwater ecosystem. The highest Brillouins biodiversity infex (HB=1.06) are determined for the helminth communities of *A. brama*, followed by this of *G. schraetser* (HB=0.06).

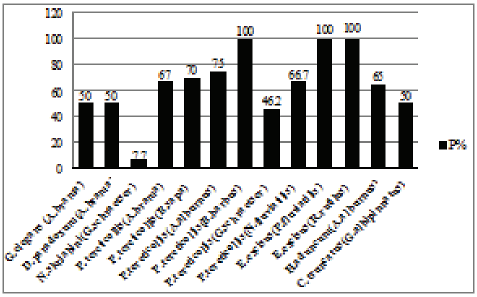

Figure 2. Prevalence (%) of fish parasite species, Danube River, 2012

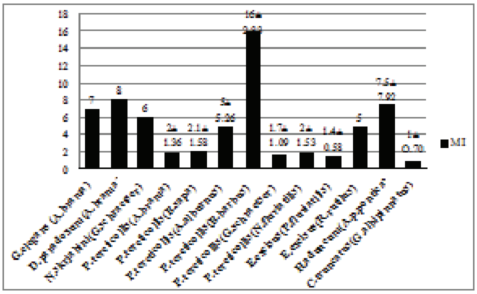

Figure 3. Mean intensity (MI±SD) of fish parasite species, DanubeRiver, 2012

Content of heavy metals insediments, fish and parasites

The result of the chemical analyzes (Pb, Cu and Zn) of 40 samples of muscle, liver, kidneys and bones of *Perca fluviatilis* of the Srebarna Lake; *Perca fluviatilis* and *Barbus barbus* of the Danube River are presented. The content of Pb, Cu and Zn in two parasite species: *Eustrongilides excises* and *Pomphorhynchus tereticollis* were determined. The content of heavy metals in sediments from the two freshwater ecosystems was fixed. 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. For the freshwater ecosystem of the Srebarna Lake was found higher content of zinc and lead in *E.excisus*, than in sediments and in the opposite, higher content of copper in the sediments (Table 1).

From the fish tissues and organs the highest content of copper has liver (15.813 mg/kg). The content of lead was the highest in bone (37.221 mg/kg) and that of zinc – in kidney (145.314 mg/kg). $BCF_{E.\ excisus}$ was the highest for lead (4.850), followed by those for zinc and copper compared to content in the sediments from the lake. With regard to fish organs and tissues, BCF_{Cu} is the highest in liver (0.486); BCF_{Zn} – in liver (1.380) and for lead-in kidney (BCF_{Pb}=1.153). $BCF_{E.\ excisus}$ is higher in muscles for the three heavy metals but is the highest for zinc (11.316), followed by this for copper and lead (Table 1).

The content of copper and zinc was higher in sediments of the Danube River than in *E. excisus* and this of lead was higher in *E. excisus* than in the sediments (Table 2). With regard to fish tissues and organs, the highest concentrations of copper were reported in liver (36.522 mg/kg); of lead-in bone (9.121 mg/kg) and of zinc-in the kidneys (51.634 mg/kg). $BCF_{E.\ excisus}$ was the highest for lead (2.525), followed by these for zinc and copper. BCF_{cu} was the highest of the liver (0.433), for lead – of the bones (BCF_{Pb}=0.211) and for zinc – of the kidneys (BCF_{Zn}=0.325). $BAF_{E.\ excisus}$ was the highest for the muscles and the three trace elements, but was with the highest values in relation to the concentration of lead, followed by these of copper and zinc (Table 2).

According to the results of this study, for the first time was presented the data for content of Pb, Cu and Zn of barbel tissues and organs and of their acanthocephalan parasite *P. tereticollis* of the Danube River (Biotope Vetren). *P. tereticollis*, core helminth species of the helminth communities of barbel of the Danube River, was distinguished with higher content of heavy metals than in sediments. With regards to barbel tissues and organs, the highest contents of copper and zinc were fixed from the liver (38.258 mg/kg, 14.281 mg/kg, respectively) and of lead – from the bones (2.352 mg/kg).

$BCF_{P.\ tereticollis}$ was the highest of lead (8.167), followed by these of copper and zinc (Table 3) with no significant differences between them from the rest. BCF_{Cu} and BCF_{Zn} were the highest from the liver and of lead – from the bones. $BAF_{Ptereticollis}$ was the highest of the muscles for the three trace metals, but was the highest of lead (BCF_{Pb}=2323.993) (Table 3).

Table 1. Bioconcentration factor (BCF= [Chost/parasite tissues]/ [Csediments]) of P. fluviatilis and E. excisus of the Srebarna Lake

Perca fluviatilis	Srebarna		
BCF	Cu	Pb	Zn
$C_{E.excisus}/C_{Sediments}$	0.600	4.850	3.75
$C_{Liver}/C_{Sediments}$	0.486	1.212	1.380
$C_{E.excisus}/C_{Liver}$	1.234	4.000	2.719
$C_{Kidney}/C_{Sediments}$	0.298	1.153	0.595
$C_{E.excisus}/C_{Kidney}$	2.012	4.205	6.303
$C_{Bones}/C_{Sediments}$	0.105	0.962	0.343
$C_{E.excisus}/C_{Bones}$	5.725	5.041	10.916
$C_{Muscles}/C_{Sediments}$	0.083	0.763	0.332
$C_{E.excisus}/C_{Muscles}$	7.198	6.358	11.316
Sediments mg/kg	32.5	30.7	105.3

Table 2. Bioconcentration factor (BCF= [Chost/parasite tissues]/ [Csediments]) of P. fluviatilis and E. excisus of the Danube River

Perca fluviatilis	Danube		
BCF	Cu	Pb	Zn
$C_{E.excisus}/C_{Sediments}$	0.346	2.525	0.566
$C_{Liver}/C_{Sediments}$	0.433	0.095	0.277
$C_{E.excisus}/C_{Liver}$	0.800	26.430	2.045
$C_{Kidney}/C_{Sediments}$	0.040	0.142	0.325
$C_{E.excisus}/C_{Kidney}$	8.633	17.821	1.738
$C_{Bones}/C_{Sediments}$	0.082	0.211	0.260
$C_{E.excisus}/C_{Bones}$	4.239	11.973	2.176
$C_{Muscles}/C_{Sediments}$	0.020	0.0009	0.039
$C_{E.excisus}/C_{Muscles}$	17.303	265.073	14.356
Sediments mg/kg	84.332	43.251	158.612

Table 3. Bioconcentration factor (BCF= [Chost/parasite tissues]/ [Csediments]) of B. barbus and P. tereticollis of the Danube River

B. barbus	Danube		
BCF	Cu	Pb	Zn
$C_{P.\ tereticollis}/C_{Sediments}$	1.603	8.167	1.512
$C_{Liver}/C_{Sediments}$	0.454	0.031	0.090^{s}
$C_{P.\ tereticollis}/C_{Liver}$	3.535	263.224	16.790
$C_{Kidney}/C_{Sediments}$	0.131	0.046^{ns}	0.062
$C_{P.\ tereticollis}/C_{Kidney}$	12.282	60.944	24.243
$C_{Bones}/C_{Sediments}$	0.026	0.054	0.076
$C_{P.\ tereticollis}/C_{Bones}$	60.944	173.927	19.924
$C_{Muscles}/C_{Sediments}$	0.015	0.0004	0.037
$C_{P.\ tereticollis}/C_{Muscles}$	107.585	2323.993	41.342
Sediments mg/kg	84.332	43.251	158.612

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 ($p< 0.001$) are fixed for relationship between $Sediments_{Pb}$-$E.excisus_{Pb}$ of the Danube River and Lake Srebarna. Very significant correlation ($p< 0.001$) were fixed between sediments for the three trace elements and *P. tereticollis*, parasite from helminth communities of *B. barbus*, river Danube ($Sediments_{Cu}$-*P. tereticollis*; $Sediments_{Pb}$-*P. tereticollis*; $Sediments_{Zn}$-*P. tereticollis*; $p< 0.001$).

The major negative anthropogenic impact from Bulgaria of the Danube River and Srebarna Lake ecosystems ar from farm activities (fertilizers, pesticides; wastewater from livestock, etc.) (Dimitrov, 2009; Dimitrov et al., 2012; Stefanov, Dimitrov, 1986). Danube River and Srebarna Lake are included in the National monitoring program (Regulation 1/2011).

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 and Lake Srebarna are slightly higher than those reported by other authors for the same ecosystem, but for another biotopes of the Danube River (Bulgarian part of the river) (Atanassov, 2012; Hristov, 2010; Nachev, 2010).

CONCLUSIONS

As a result of this examination new species a new host records were found for the freshwater ecosystem of the Danube River. *G. elegans* and *D. paradoxum* are presented for the first time for the parasite communities of *A. brama*. *N. skrjabini* was found for the first time in Biotope Vetren. *P. tereticollis* are reported for the first time for helminth communities of *A. brama*, *B. sapa*, *A. alburnus*, *B. barbus*, *G. schraetser* and *N. fluviatilis*. *E. excisus* are reported for the first time for helminth communities of *R. rutilus*. *H. aduncum* are reported for the first time for freshwater ecosystems of the Danube River and helminth communities of *A. pontica*, as well as for the first time for the helminth communities of the fish in Bulgaria *C. truncatus* was found for the first time for the freshwater ecosystem of the Danube River. *B. barbus* is a new host record for this helminth species in Bulgaria.

New data for heavy metal contents in sediments, fish tissues and organs and their parasites from the Danube River and Lake Srebarna are present. From the tissues and organs of the studied species (*P. fluviatilis* and *B. barbus*), the lowest concentrations of Pb, Cu and Zn were found in the muscles. In general, the liver and kidneys of both fish species from both studied ecosystems were found in higher content of heavy metals than the bones and muscles. The high significant correlations determined *E.excisus* as sensitive bioindicator for Pb and *P.tereticollis* as a sensitive indicator of Pb, Cu and Zn.

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REFERENCES

Atanasov G., 2012. Fauna, morphology and biology on the endohelminths of fish from Bulgarian part of the Danube River. PhD these, Sofia.

Baruš V., Jarkovský J., Prokeš M., 2007. Philometra ovate (Nematoda: Philometridae): a potential sentinel species of heavy metal accumulation. Parasitol. Res., 100, 929-933.

Bíreš J., Dianovský J., Bartko P., Juhásová Z., 1995. Effects of enzymes and the genetiv apparatus of sheep after administration of samples from industrial emissions. BioMetals, 8, 53-58.

Bush A., Lafferty K., Lotz J., Shostak A., 1997. Parasitology meets ecology on its own terms. Journal of Parasitology, 83, 575-583.

Bykhovskaya-Pavlovskaya I., 1985. Parasites of fish. Manual on study, Nauka, Leningrad, 121 (in Russian).

Dimitrov Y., 2009. *Proceedings of Jubilee Scientific Conference*, Agricutural University, Plovdiv, v. L?V.

Dimitrov Y., Palagacheva N., 2012. Risky periods of pesticide (insecticidae and fungicidae) pollution of vegetable grown in greenhouses. *Agro-knowledge Journal „?groznanje"*, 13, 1, 153-158.

Djikanovic V., Paunovic M., Nikolic V., Simonovic P., Cakis P., 2012. Parasitofauna of freshwater fishes in the Serbian open waters: a checklist of parasites of freshwater fishes in Serbian open waters. Rev. Fish Biol. Fisheries, 22, 297-324.

Fröse R., Pauly D., 2012. FishBase. World Wide Web electronic publication, www.fishbase.org, version (07/2012).

Gabrashanska M., Nedeva I., Cakic P., Galves-Morros M., Karaivanova E., Atanasov, Lenhardt M., 2004. Heavy metals in fish parasite system from the Danube river (Bulgarian and Serbian parts). Macro and Trace elements, 22 Workshop, Jena Germany, 613-618.

Georgiev B., Biserkov V., Genov T., 1986. In toto staining method for cestodes with iron acetocarmine. Helminthologia, 23, 279–281.

Gusev A., 1983. Methods for collecting and processing of materials of monogenean parasites on freshwater fish. Nauka, Leningrad, 48 (in Russian).

Gusev A., Dubinina M., Pugachev O., Rajkova E., Hotenovskij I., Ergens R., 1985. Guidae to Freshwater fish parasites Fauna SSSR. Nauka, Leningrad, 428 (In Russian).

Hristov S., 2010. Circulation of some heavy metals in the freshwater ecosystem of the Srebarna Biosphere Reserve. J. Ecology& Safety, 4, 2:204-213.

ISO 5667-6:1990. Guidance on sampling of rivers and watercourses. International Organization for Standartization.

IUCN Red List Status (Ref. 90363) 02/2013.

Kakacheva-Avramova D., 1977. Studies on helminths of fishes in the Bulgarian section of the Danube River. Helminthologia, 3, 20-45.

Kakacheva D., Margaritov N., Grupcheva G., 1978. Fish parasites of Bulgarian part of the Danube River. Limnology of Bulgarian part of the Danube River, Bulg. Acad. Sci., 250-271 (In Bulgarian).

Kakacheva-Avramova D., 1983. Helminths of freshwater fishes in Bulgaria. Bul. Acad. Sci., Sofia (In Bulgarian).

Kennedy C., 1993. The dynamics of intestinal helminth communities in eels *Anguilla anguilla* in a small stream: long-term changes in richness and structure. Parasitology, 107, 71-78.

Kennedy C., 1997. Freshwater fish parasites and environmental quality, an overview and caution. Parassitologia, 39, 249-254.

Komarova T., 1968. Metacercarians digenean of benthic invertebrates from water basins of the Danube River. Zool. Journal, 6, 7-14 (In Russian).

Literathy P., Laszlo F., 1995. Harmonization of micropollutant monitoring in a large international river: Danube. Water Sci. Technol., 32, 125-137.

Literathy, P., Laszlo F., 1999. Micropollutants in the Danube river basin. Water Sci. Technol., 40, 17-26.

Magurran A., 1988. *Ecological diversity and its measurement*. Cambridge University Press, London.

Malmberg G., 1970. The excretory systems and marginal hooks as basic for the systematic of Gyrogactylus (Trematoda. Monogenea). Arhiv Zoologie, 2, 1–235.

Marcogliese D., Cone D., 1997. Parasite communities as indicators of ecosystem stress. Parassitologia, 39, 227-232.

Margaritov N., 1959. Parasites of some freshwater fishes. Publishing House NIRRP, Varna.

Margaritov N., 1966. Helminths of the digestive tract and the abdominal cavity of fishes of the Bulgarian section of Danube River. Bulletin de L'institut de Zoologie et Musée, 20, 157-173.

Michalovic M., 1954. Výsledky prupruzkumu parasitu ryb v podunajské oblasti u Komárna. Sbor. VŠZL v Brne, B.2, 67-74.

Michev T., Georgiev B., Petrova A., Stoyneva M., 1998. Biodiversity of the Srebarna Biosphere Reserve. Checklist and Bibliography, Pensoft, Sofia.

Moravec F., 1994. Parasitic nematodes of freshwater Fishes of Europe. Kluwer Academic Publishers, Dordrecht.

Moravec F., 2001. Checklist of the metazoan parasites of fishes of the Czech Republic and the Slovak Republic (1873–2000). Academia, Prague.

Moravec F., Konecny R., Baska F., Rydlo M., Scholz T., Molnar K.. Schiemer F., 1997. Endohelminth fauna of barbel, *Barbus barbus* (L.), under ecological conditions of the Danube basin in Central Europe. Acad. Sci. of the Czech Republic, Praha.

Nachev M., 2010. Bioindication capacity of fish parasites for the assessment of water quality in the Danube River. PhD these, Sofia.

Nachev M., Zimmermann S., Rigaud T., Sures B., 2010. Is metal accumulation in Pomphorhynchus laevis dependent on parasite sex or infrapopulation size? Parasitology, 1-10.

Nachev M., Sures B., 2009. The endohelminth fauna of barbel (*Barbus barbus*) correlates with water quality of the Danube River in Bulgaria. Parasitology, 136, 545–552.

Nedeva I., Atanassov G., Karaivanova E., Cakic P., Lenghardt M., 2003. *Pomphorhynchus laevis* (Müller, 1776) from the River Danube. Experimental Pathology and Parasitology, 6, 14-16.

Oros M., Hanzelová V., 2009. Re-establishment of the fish parasite fauna in the Tisa River system (Slovakia) after a catastrophic pollution event. Parasitol. Res., 104, 1497-1506.

Overstreet R., 1997. Parasitological data as monitors of environmental health. Parassitologia, 39, 169-175.

Pehlivanov L., Tzavkova V., Vasilev V., 2006. Development of the zooplankton community in the Srebarna Lake (nord-eastern Bulgaria) along the process of ecosystem rehabilitation. Proceedings of the 36th International Conference of IAD, Vienna, 280-284.

Petrochenko V., 1956. Acanthocephalans of wild and domestic animals. Acad. Sci. SSSR, Moskwa (In Russian).

Regulation 1/2011 for monitoring of the waters. Ministry of Environment and Water of Bulgaria.

Ricking M., Terytze K., 1999. Trace metals and organic compouds in sediment samples from the River Danube in Russe and Lake Srebarna (Bulgaria). Environ. Geol., 37, 40-46.

Špakulová, M., Perrot-Minnot M.-J., Neuhaus B., 2011. Resurrection of *Pomphorhynchus tereticollis* (Rudolphi, 1809) (Acanthocephala: Pomphorhynchidae) based on new morphological and molecular data. Helminthologia, 48, 3, 268-277.

Shigin A., 1986. Trematode fauna of the USSR. Genus Diplostomum. Metacercariae. Nauka, Moscow.

Stefanov Y., Dimitrov St., 1986. Effective Chemicals for Cotton Thrips Thrips-Tabaci and Aphis-Gossypii Protection. *Rasteniev'd nauki* 23: 72-75.

Shukerova S., 2007. Ecological study of helminthfauna of *Scardinius erythrophthalmus* (Linnaeus, 1758) and accumulation of heavy metals in some helminths and rudd from the protection area Srebarna Biosphere Reserve, Bulgaria Thematic proceedings from International Scientific meeting "Multifunctional Agriculture and Rural Development", Serbia, 1, 515-516.

Shukerova S., Kirin D., 2008. Helminth communities of the rudd, *Scardinius erythrophthalmus* (Cypriniformes, Cyprinidae) from Srebarna Biosphere Reserve, Bulgaria. Journal of Helminthology, 82, 319-323.

Shukerova S., Kirin D., Hanzelova V., 2010. Endohelminth communities of the perch, *Perca fluviatilis* (Perciformes, Percidae) from Srebarna Biosphere Reserve, Bulgaria. Helminthologia, 42, 2, 99-104.

Stenko R., 1976. Life cycle of trematoda *Crowcrocaecumskrjabini* (Iwanitzky, 1928) (Allocreadiata, Opecoelidae). Parasitologia, 10, 1, 9-16 (In Russian).

Sures S., Siddall R., 1999. *Pomphorhynchus laevis*: the intestinal acanthocephalan as a lead sink for its host, chub (*Leuciscus cephalus*). Exp. Parasitol., 93, 66-72.

Thielen F., Zimmermann S., Baska F., Taraschewski H., Sures B., 2004. The intestinal parasite *Pomphorhynchus laevis* (Acanthocephala) from barbel as a bioindicator for metal pollution in the Danube River near Budapest, Hungary. Environmental Pollution, 129, 421-429.

Uzunov Y., Tzavkova V., Todorov I., Varadinova E., 2001. The macrozoobenthos fauna of the Biosphere reserve Srebarna Lake in North-Eastern Bulgaria. Lauterbornia, 40, 43-53.

Woitke P., Wellmitz J., Helm D., Kube P., Lepom P., Litheraty P., 2003. Analysis and assessment of heavy metal pollution in suspended solids and sediments of the river Danube. Chemosphere, 51, 633-642.