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HELMINTH COMMUNITIES AND ECOLOGICAL APPRAISAL FOR THE CONDITION OF THE MARITSA RIVER, BULGARIA

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Abstract

Biodiversity and ecological particularities of the parasite communities of the northern pike (Esox lucius L., 1758) from the Maritsa River were studied during 2012. Nine specimens of E. lucius were examined with standard techniques for parasites and heavy metal contamination. The purpose of this research is to represent new data for the biodiversity, prevalence, intensity and mean intensity, mean abundance of parasite communities of E. lucius from the Maritsa River. Concentration of heavy metals (Pb, Zn, Cu) in fish (muscle, liver, kidneys and bones), some endohelminth species as bioindicators and bottom sediments were analyzed. The obtained results for the parasite communities of E. lucius correspond and are in close connection with dependence of the biology and ecology of the determined species of helminthes and the place of the intermediate hosts as bioindicators for the status of the studied natural freshwater ecosystems. The results may be applied in the various monitoring systems for assessment and forecast of the Maritsa River condition.

Key words: *parasite communities, heavy metals, bioindication, Esox lucius, Maritsa River.*

INTRODUCTION

The Maritsa River is related to the Aegean water collecting region. East of Svilengrad, Bulgaria, the river flows eastwards, forming the border between Bulgaria (on the north bank) and Greece (on the south bank), and then between Turkey and Greece. At Edirne, the river flows through Turkish territory on both banks, then turns towards the south and forms the border between Greece on the west bank and Turkey on the east bank to the Aegean Sea. Turkey was given a small sector on the west bank opposite the city of Edirne. The river enters the Aegean Sea near Enez (14.5 km far from town of Alexandroupolis), where it forms a delta ($41^0$42'30N, $26^0$21'40E). The Tunja and Maritsa rivers are its chief tributaries. The lower course of the Maritsa River forms part of the Bulgarian-Greek border and most of the Greek-Turkish border. The upper Maritsa valley is a principal east-west route in Bulgaria (Dakova et al., 2004).

The unnavigable river is used for power production and irrigation. The major negative anthropogenic impact on the Maritsa River ecosystem associated with the changes of the studied freshwater communities are farm activities (using of fertilizers, pesticides; wastewater from livestock, etc.; 70% of the region are agricultural lands), constructions, industry, etc. (Dimitrov, 2009; Dimitrov, Palagacheva, 2012; Stefanov, Dimitrov, 1986). Maritsa River is included in the National monitoring program (Water Body Type BG3MA350R039 – Major rivers) (Regulation 1/2011).

Parasite species are particularly interesting as indicators of the ecological status of the freshwater ecosystems because the completion of their life cycle requires interactions with several host vertebrates and invertebrates, and the effects on each of the hosts differ according to the pollution level of the habitat in question (Baruš et al., 2007; Cone et al., 1993; Gelnar et al., 1997; Kennedy, 1997; MacKenzie et al., 1995; Marcogliese and Cone, 1997; Overstreet, 1997; Sures, Siddall, 1999; Thielen et al., 2004; Tieri et al., 2006, etc.).

Northern Pike parasites and its tissues, organs and parasites as heavy metal bioindicators were studied from different countries and authors (Atanasov, 2012; Djikanovic et al., 2012; Kakacheva-Avramova, 1965, 1976, 1977, 1983; Kakacheva, Margaritov, Grupcheva, 1978; Kirin, 2006; Margaritov, 1959, 1964;

Molnár, 1980; Novohatskaya, 2008; Oros and Hanzelová, 2009; Poulin, 2006; Shostak and Dick, 1989; Shukerova, 2010; Tekin-Özan and Kir, 2007, etc.).

This paper presents the results from an examination of heavy metal content in sediments, fish tissues and organs, fish parasites and dominant structure of fish parasite communities from the Bulgarian border part of the Maritsa River (after town of Plovdiv).

MATERIALS AND METHODS

During September-October, 2012 sediments, fish and fish parasites were collect and examined from the Maritsa River (after town of Plovdiv).

The Maritsa River is with a length of 521 km and is the longest river that runs solely in the interior of the Balkans. It has its origin in the Rila Mountains (2°09'40'N, 23°36'00'E, 2378 m altitude, from Maritsa lakes and below Peak Mancho) in Western Bulgaria, flowing southeast between the Balkan and Rhodope Mountains, past Plovdiv to Edirne, Turkey and to Aegean Basin (41 m above sea level) (Dakova et al., 2004).

The studied biotope (village of Manole, 42.183N, 24.933E) is situated on the riverside, about 18 km far away north-eastern from the town of Plovdiv (42.15N, 24.75E). It is distinguished with a depth and speady running water, with sands and slimes at some places. The waterside vegetation is represented mainly by *Salix* sp., *Populus* sp. and *Alnus glutinosa* Linnaeus, 1758. The region of the town and the riverside are distinguished with significant diversity of highly protected species and territories declared as protected with national and international nature protective status (Assyov, 2012; Kirin et al., 2006).

A total of 5 sediment samples and 9 freshwater fish specimens belonging to the species *Esox lucius* Linnaeus, 1758 were collected and examined. The fish were caught by angling. The scientific and common names of fish host 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 atomic absorpsion spectrometry (Bíreš et al., 1995). The Northern pike, *Esox lucius* (L., 1758) from the Maritsa River was chosen as a model fish species for parasitological examination and for the heavy metal content in this study.

Helminthological examinations were carried out following recommendations and procedures described by Byhovskaya-Pavlovskaya (1985), Dubinina (1987), Gusev (1983, 1985), Moravec (1994, 2001), etc.

Specimens were fixed and preserved in 70% ethyl alcohol. The Monogenea was studied with Methods of Gusev (1983). The nematodes were studied on temporary mounts with 5% glycerol in 70% ethanol (Zashev, Margaritov, 1966; Moravec, 1994).

The ecological terms prevalence (P%), mean intensity (MI) are presented for each species. Analyses of helminth community structure were carried out in both levels: infracommunity and component community. The infracommunity data were used to calculate the total number of species, mean number of helminths, etc. (Kennedy, 1993, 1997; Magurran, 1988). The infracommunity data were used to calculate the total number of species, mean number of helminth worms, the Brillouin's diversity index (HB) and evenness index of Brillouin (Maguran, 1988). The analysis of the dominant structure of the found fish parasite taxa were presented to the level of the component communities. The criterion of Bush at al. (1997) was used for determining the dominant structure of the component helminth communities. Fish were weighed and measured. Samples of muscle, liver, kidneys and bones were collected from all individuals. In order to determine the relative accumulation capability of the fish tissues in comparison to the sediments, the bioconcentration factor (BCF= [Chost tissues]/[Csediments]]) was 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 DISCUSSIONS

Fish communities

A total of 9 fish specimens from the species *Esox lucius* Linnaeus, 1758 were collected and examined from the Maritsa River. *E. lucius* is estimated as least concern species (LC=Least Concern; IUCN Red List Status, 2012) and is no included in Red Data Book of the Republic of Bulgaria (Golemanski (Ed.), 2011). The Northern pike is occurs in clear vegetated lakes, quiet pools and backwaters of creeks and small to large rivers. It is freshwater, brackish, demersal, potamodromous and predatory fish species. Usually it is solitary and highly territorial. Adults feed mainly on fishes, but at times feed heavily on frogs and crayfish. Cannibalism for Northern pike is common (Fröse and Pauly, 2012; Grossman, 1996). Two of examined fish specimens are free of parasites.

Helminth community structure

From studied 9 specimens of fishes, 2 parasite species were fixed (*Tetraonchus monenteron* Diesing, 1858 and *Raphidascaris acus* (Bloch, 1779)). They are belonging to classes Monogenea (1) and Nematoda (1).

Gill's parasite *Tetraonchus monenteron* Diesing, 1858 are developing in freshwater and marine ecosystems (Bray, 2001). The species is from special fish parasites which are using as a source of information about the aquatic communities (Kennedy, 1975). *T. monenteron* are developing with participation of their specific fish hosts, *Esox lucius* (Canning et al., 1973; Gusev et al., 1985).

In Bulgaria, the species was presented from *Esox lucius* Linnaeus, 1758 and the Danube River (Kakacheva, Margaritov, Grupcheva, 1978; Margaritov, 1959) and from the Maritsa River (Kirin, 2006), etc. *Raphidascaris acus* (Bloch, 1779) is developing ussing different ways: exogenous development; with participarion of the first intermediate hosts crustaceans (*Macrocyclops*, *Asellus*), tubificids (*Tibifex templetoni* Souther, 1909) or vertebrates. The fishes in which frequently occurred encysted or free larvae of *R. acus*, are the obligate intermediate hosts. In them the larvae attain the infective stage for the definitive hosts, picsivorous fishes (Moravec, 1994). In Bulgaria, the larval stage of the species was presented of *Chondrostoma nasus* Agassiz, 1832 from the Maritsa River (Margaritov, 1964); of *Rutilus rutilus* Rafinesque, 1820 from the Bistritsa River (Margaritov, 1964); *Carassius gibelio* (Bloch, 1782) from the Lake Srebarna (Shukerova, 2005); of *Cyprinus carpio* (Linnaeus, 1758) from the Lake Srebarna (Shukerova, 2006). The adult worms were reported of *Perca fluviatilis* Linnaeus, 1758 from the Zhrebchevo Reservoir (Nedeva, Grupcheva, 1996) and from the Lake Srebarna (Shukerova et al., 2010); of *Leuciscus cephalus* (Linnaeus, 1758) from the Danube River (Sakis et al., 2004), etc. The two determined species, *Tetraonchus monenteron* (Wagener, 1857) and *Raphidascaris acus* (Bloch, 1779), parasitic in *E. lucius* are generalists for the helminth communities of the examined freshwater fish species of the Maritsa River ecosystem. *T. monenteron* and *R. acus* of the parasite communities of *E. lucius* of the Maritsa River were distinguished with high values of prevalence (P=33.34% and P=77.78%, respectively) and with low values of mean intensities for the both helminth species (MI=2±0.82, 1-3; MI=2±0.94, 1-3, respectively). *T. monenteron* is component species and *R. acus* is core species for the helminth communities of the Northern pike.

Content of heavy metals in sediments, fishes and parasites

The result of the chemical analyzes (Pb, Cu and Zn) of 20 samples of muscle, liver, kidneys and bones of *Esox lucius* of the Maritsa River were presented (Table 1).

The content of Pb, Cu and Zn in the nematoda species *Raphidascaris acus* was determined. The content of heavy metals in sediments from the studied biotope of the freshwater ecosystem 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 (Table 2).

Table 1. Content of heavy metals (Cmg/kg±SD) of E. lucius and *R. acus*

Esox lucius	Maritsa River		
	Cu	Pb	Zn
$C_{R.acus}$	60.04± 1.02	103.12± 2.06	32.31± 1.16
C_{Liver}	56.64± 1.18	4.46± 0.17	7.77± 1.38
C_{Kidney}	5.76± 1.15	6.91± 0.22	28.31± 0.20
C_{Bones}	10.81± 0.40	9.36± 0.32	7.85± 0.03
$C_{Muscles}$	3.76± 0.12	0.04± 0.02	3.21± 0.11
Sediments mg/kg	117.5± 3.75	39.48± 4.24	71.32± 1.66

The highest mean content of Cu showed the sediment samples of river (117.5 mg/kg), followed by those of the parasite species *R. acus* (60.04 mg/kg). From fish tissues and organs, with the highest content of Cu were distinguished the liver (56.64 mg/kg).

Table 2. Bioconcentration factor (BCF= [Chost/parasite tissues]/ [Csediments]) of *E. lucius* and *R. acus*

Esox lucius	Maritsa River		
BCF	Cu	Pb	Zn
$C_{R.acus}/C_{Sediments}$	0.511	2.612	0.453
$C_{Liver}/C_{Sediments}$	0.482	0.113	0.109
$C_{R.acus}/C_{Liver}$	1.060	23.121	4.158
$C_{Kidney}/C_{Sediments}$	0.049	0.175	0.397
$C_{R.acus}/C_{Kidney}$	10.424	14.923	1.141
$C_{Bones}/C_{Sediments}$	0.092	0.237	0.110
$C_{R.acus}/C_{Bones}$	5.554	11.017	4.116
$C_{Muscles}/C_{Sediments}$	0.032	0.001	0.045
$C_{R.acus}/C_{Muscles}$	15.968	2578	10.065
Sediments mg/kg	117.5± 3.75	39.48± 4.24	71.32± 1.66

The highest mean content of Pb are defined in *R. acus* (103.12 mg/kg), followed by those in the sediments (39.48 mg/kg). Of tissues and organs, higher concentrations were obtained for the content of Pb in bones and kidneys (9.36 and 6.91 mg/kg, respectively). The mean content of Zn showed higher values in the sediments (71.32 mg/kg) than of *R. acus* (32.31 mg/kg). Of tissues and organs, the highest concentrations were differed of Zn. The highest content of this trace heavy metal was detected for kidneys (C_{kidney}=28.31 mg/kg), followed by those for bones and liver (C_{bones}=7.86; C_{liver}=7.77 mg/kg, respectively). The lowest values of Zn are detected in the muscles of examined pike (C_{musles}=3.21 mg/kg) (Table 1).

BCF of *R. acus*, parasite species of *E. lucius* of the Maritsa River was the highest for Pb (BCF $C_{R.acus}/C_{SedimentsPb}$=2.612), followed by those for Cu (BCF $C_{R.acus}/C_{SedimentsCu}$=0.511) and Zn (BCF $C_{R.acus}/C_{SedimentsZn}$=0.453) (Table 2). With regard to the examined fish tissues and organs, BCF was the highest for Cu in liver ($BCF_{liver/sedimentsCu}$=0.482), followed by those for Zn in kidneys ($BCF_{kidneys/sedimentsZn}$=0.397) and for Pb in bones ($BCF_{bones/sedimentsPb}$=0.237). BCF was with the lowest values for the tree trace heavy metals for pike muscles. Accumulation of heavy metals in *R. acus* to their content in the fish organs and tissues was the highest of Pb from the muscles ($BCF_{R.acus/musclesPb}$=2578), followed by those of Pb for liver ($BCF_{R.acus/liverPb}$=23.121), of Pb for kidneys and of Pb for bones ($BCF_{R.acus/kidneysPb}$=14.923; $BCF_{R.acus/bonesPb}$ =11.017). Generally, the accumulation of the tree trace heavy metals were the highest of fish parasite species *R. acus*, compared to their contents in muscles.

A linear correlation coefficient, (r_s, Spearman correlation coefficient) were determined to test associations between the bottom sediments, fish tissues, organs and fish parasites. Very significant correlation (r_s=0.999; $p<0.001$) were fixed for relationship between $Sediments_{Pb}$-$Bones_{Pb}$. Highly significant correlations were fixed for relationship between $Sediment_{cu}$-$R.acis_{cu}$; $Sediment_{cu}$-$Bones_{cu}$; $Sediments_{Pb}$-$R.acus_{Pb}$; $Sediment_{zn}$-$Kidneys_{zn}$. Significant correlation was determined for content of zinc in fish bones and sediments of the Maritsa River.

The results from this study were higher then these for *R. acus* and *E. lucius* reported by another studies (for example, Tekin-Özan and Kiri, 2007).

CONCLUSIONS

As a result of this examination a total of 9 fish specimens *Esox lucius*, collected from the Maritsa River. The determined helminth species *Tetraonchus monenteron* and *Raphidascaris acus* are generalists for the helminth communities of *Esox lucius* from the studied ecosystems.

The received data for heavy metal contents in sediments, fish tissues and organs and fish

parasites from the Maritsa River were presented for the first time for *E. lucius* and their parasite species *R. acus*. The highest mean content of Pb was defined in *R. acus* (103.12 mg/kg), followed by those in the sediments (39.48 mg/kg). Of tissues and organs, higher concentrations were obtained for the content of lead in bones and kidneys of the pike. Generally, the accumulation of the tree trace heavy metals was higher of fish parasite species *R. acus*, compared to their contents in muscles. The high values of the bioconcentration factors and of the significant correlations determined *R. acus* as sensitive helminth species for heavy metal (lead, copper and zinc) content of freshwater ecosystems.

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