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HELMINTH COMMUNITIES AND HEAVY METAL CONTAMINATION IN MACEDONIAN VIMBA AND FISH PARASITES OF THE MARITSA RIVER, BULGARIA

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Abstract

Biodiversity and ecological particularities of the parasite communities of the Macedonian Vimba (Vimba melanops (Heckel, 1837)) from the Maritsa River were studied during 2013. Thirty two specimens of V. melanops 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 V. melanops from the Maritsa River. Concentration of heavy metals in fish (muscle, liver, intestines and bones), some endohelminth species as bioindicators and bottom sediments were analyzed. The obtained results for the parasite communities of V. melanops 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.

Keywords: *parasite communities, heavy metals, bioindication, Vimba melanops, river Maritsa.*

INTRODUCTION

The Maritsa River is related to the Aegean water collecting region. The Tunja and Arda 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, constructions, industry, etc. 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.).

Different studies show higher concentrations of heavy metals in some intestinal fish helmithes than in tissues and organs of their final host Macedonian Vimba (*Vimba melanops* (Heckel, 1837)). In Gabrashanska and Nedeva (1996) examined the contents of Cu, Cr and Zn in the host parasite system *Vimba melanops* – *Caryophyllaeus brachicollis* and report for higher concentrations of determined heavy metals in cestods than in the fish host. Kirin et al., 2013 reports the higest values of Cu, Pb and Zn in helminths *Caryophyllaeus laticeps* than in muscles in its host Macedonian Vimba (Arda River, Bulgaria), 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 part of the Maritsa River (after town of Plovdiv).

MATERIALS AND METHODS

During April - September, 2013 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 15 sediment samples and 69 freshwater fish specimens belonging to the species *Vimba melanops* (Heckel, 1837) were collected and examined. The fish were caught by angling. The scientific and common names of fish host was used according to the FishBase database (Fröse and Pauly, 2012). Fish were weighed and measured.
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 Macedonian Vimba *Vimba melanops* (Heckel, 1837) from the Maritsa River and its cestoda species *Caryophyllaeus brachycollis* Janiszewska, 1951 were chosen as a model fish species and as a model helminth species for parasitological examination and for 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. The parasites, dissected organs and tissues of fish samples were weighed, packed in polyethylene bags and kept at -30 ^{0}C until analysis. Samples of muscle, liver, kidneys and bones were collected from all individuals.
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. 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.

RESULTS AND DISCUSSION

Fish communities

A total of 69 fish specimens from the species *Vimba melanops* (Heckel, 1837) were collected and examined from the Maritsa River. *V. melanops* is included in Red Data Book of the Republic of Bulgaria (Golemanski (Ed.), 2011) as Vulnerable species (VU=Vulnerable). It is demersal fish species.

Macedonian Vimba distributed Northern Aegean basin from Maritza to Pinios drainages (Turkey, Greece, Bulgaria, Macedonia). Inhabits rivers and streams, usually with relatively swift current. Also occurs in lakes and lowland water courses with little current. Feeds on invertebrates and plants. Spawns among stones in large rivers (Kottelat, Freyhof, 2007).

Helminth community structure

A total 79 specimens of helminths belonging to species *Caryophyllaeus brachycollis* Janiszewska, 1951 (Platyhelminthes, Cestoda, Caryophyllidea) were fixed from 36 infected fish speciment.

Caryophyllidean (Platyhelminthes:Eucestoda) parasites represent a widely distributed group of intestinal helminthes of Cyprinidae and Siluridae fishes occurring in all zoogeographical regions except the Neotropics. Some cariophyllideans may be pathogenic for their fish hosts (Mackiewicz, 1994; Oros et al., 2010; Scholz and Hanzelová, 1998).

Caryophyllaeus brachycollis Janiszewska, 1951 is developed with participarion of the first intermediate host *Limnodrilus hoffmeisteri* Claparède, 1862 and *T. tubifex* and the second different fish secies. In Bulgaria caryophyllidean tapeworms were presented from different fish species and freshwater ecosystems: as *C. laticeps* (Pallas, 1781) - of *Barbus barbus* (Linnaeus, 1758) (Margaritov, 1959; 1966) from the Danube River; of *B. cyclolepis* Heckel, 1837 (Margaritov, 1959) from the Iskar River; of *A. alburnus* (Margaritov, 1959) of the Tunja River; of *B. barbus*, *Vimba carinata* (Pallas, 1814), *Abramis brama* (Linnaeus, 1758) and *Ballerus sapa* (Pallas, 1811) (=*A. sapa* Pallas, 1811) (Kakacheva, Margaritov, Grupcheva, 1978) from the Danube River; of *Leuciscus cephalus* (Linnaeus, 1758) (Cakic et al., 2004) from the Danube River; as *C. fennica* (Schneider, 1902) – of *B. barbus* (Margaritov, 1959; 1966) from the rivers Iskar and Tunja; of *B. cyclolepis* and *L. cephalus* (Margaritov, 1959) from the Iskar River; of *L. cephalus*, *V. melanops* and *B. cyclolepis* (Margaritov, 1963/64) from the rivers Maritsa and Topolnitsa; of *B. cyclolepis*, *L. cephalus*, *V. melanops* (Kakacheva, 1965) from the rivers Asenitsa, Harmanlijska, Topolnitsa, Syuyutlijka, Sushenitsa and Bedechka; of *B. barbus* and *S. lucioperca* (Margaritov, 1966) from the Danube River; of *B. petenyi* (Kakakcheva, 1969) from the rivers Nishava, Ogosta, Vodomerka, Buchinska, Vrabnishka, Barsiya, Chuprenska, Iskrecka, Botunya, Bebresh; of *L. cephalus* and *R. rutilus* (Margaritov, 1977) from the Shiposhnitsa River and Reservoir Iskar; of *V. carinata*, *A. brama*, *B. sapa*, *Ballerus ballerus* (Linnaeus, 1758) (=*Abramis ballerus*), *Blicca bjoerkna* (Linnaeus, 1758), *A. alburnus*, *B. barbus*, *S. lucioperca*, *Sc. erythrophthalmus* and *Pelecus cultratus* (Linnaeus, 1758) (Kakacheva, Margaritov, Grupcheva, 1978) from the Danube River; of *L. cephalus* and *R. rutilus* (Kakacheva, Menkova, 1978) from the Palakariya River; of *B. barbus* (Kakacheva, Menkova, 1981) from the Struma River; of *B. cyclolepis*, *A. alburnus*, *Sq. orpheus*(=*L. cephalus*) (Kirin, 2002b, 2003) from the Arda River; of *L. cephalus* (Cakic et al., 2004) from the Danube River; as *C. brachycollis* Janiszweska, 1953 - of *B. cyclolepis* and *L. cephalus* (Kakacheva, 1965) from the rivers Asenitsa, Sjujutlijka, Chepinska, Bedechka and Topolnitsa; of *L. cephalus*, *V. melanops*, *A. alburnus*, *B. cyclolepis*, *R. rutilus* (Margaritov, 63/64) from the rivers Maritsa, Vacha, Chepinska, Topolnitsa, Bistritsa; of *L. cephalus*, *Barbus petenyi* Heckel, 1852 and *B. barbus* (Kakacheva, 1969) from the rivers Vrabnishka, Nishava, Mirkowska, Botunya, Ogosta, Malak iskar; of *L. cephalus* (Kakacheva, Menkova, 1978) from the Palakariya River; of *B. petenyi*, *L. cephalus* (Kakacheva, Menkova, 1978) from the rivers Devinska, Sarneshka and Vacha; of *B. petenyi*, *B. barbus*, *L. cephalus* (Kakacheva, Menkova, 1981) from the rivers Blagoevgradska Bistritsa, Struma, Zheleznitsa and Gradevska; of *P. fluviatilis* (Nedeva, Grupcheva, 1996) from the Zhrebchevo reservoir; of *B. cyclolepis*, *A. alburnus*, *Sq. orpheus*(=*L. cephalus*) (Kirin, 2002b, 2003) from the Arda River; and of *L. cephalus* (Cakic et al., 2004) from the Danube River; as *Caryophyllaeus* sp. – of *L. cephalus* and *A. alburnus* (Kakacheva, 1965) from the rivers Maritsa, Syuyutlijka and Harmanlijska; of *Cyprinus carpio* Linnaeus, 1758 (Margaritov, 1975, 1976) from the Fish Farming–Yambol; of *C. carpio* (Kakacheva, Menkova, 1981) from the Fish Farming–Blagoevgrad; of *V. melanops* (Kakacheva, 1965) from the Harmanlijska River; of *Cobitis bulgarica* (Drensky, 1928) (Margaritov, 1966) from the Danube River; as as *Caryophyllaeus* sp. juv. - of *Gobio gobio* (Linnaeus, 1758), *B. cyclolepis*, *V. melanops* (Kakacheva, 1965) from the river Maritsa, Chepinska and Harmanlijska; of *B. petenyi* (Kakacheva, Menkova, 1978) from the Palakariya River; of *C. carpio* (Margaritov, 1992) from the Fish farms-Yambol, Blagoevgrad, etc.

C. brachycollis of *V. melanops* of the Arda River were distinguished with prevalence P=55.35% and mean intensity MI=2.19±1.95, 1-9. *C. brachycollis* is a core species of the helminth communities of the Macedonian Vimba.

Content of heavy metals in sediments, fishes and parasites

The result of the chemical analyzes (Pb, Cu and Zn) of 60 samples of muscle, liver, kidneys and bones of *Vimba melanops* of the Maritsa River were presented (Table 1 and 2). The content of Pb, Cu and Zn in *Caryophyllaeus brachycollis* was determined. The content of heavy metals in sediments 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 1, 2).

Table 1. Content of heavy metals (Cmg/kg±SD) of *Vimba melanops* and *Caryophyllaeus brachycollis*

Vimba melanops	River		
	Cu	Pb	Zn
$C_{C.brachycollis}$	52.04± 1.02	72.15± 1.26	22.31± 1.16
C_{Liver}	36.64± 1.18	3.76± 0.07	7.77± 1.38
C_{Kidney}	3.26± 1.15	4.81± 0.02	28.31± 0.20
C_{Bones}	8.61± 0.29	5.26± 0.22	6.25± 0.03
$C_{Muscles}$	1.26± 0.12	0.12± 0.02	2.21± 0.09
Sediments mg/kg	109.2± 2.95	35.88± 1.23	69,22± 1.33

The highest mean content of Cu showed the sediment samples of river (109.2 mg/кg), followed by those of the parasite species *R. acus* (52.04 mg/кg). From fish tissues and organs, with the highest content of Cu were distinguished the liver (36.64 mg/кg). The highest mean content of Pb are defined in *C. brachycollis* (72.15 mg/kg), followed by those in the sediments (35.88 mg/kg). Of tissues and organs, higher concentrations were obtained for the content of Pb in bones and kidneys (5.26 and 4.81 mg/kg, respectively). The mean content of Zn showed higher values in the sediments (69.22 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 liver and bones (C_{liver}=7.77; C_{bones}=6.25 mg/kg, respectively). The lowest values of Zn are detected in the muscles of examined vimba (C_{musles}=2.21 mg/kg) (Table 1).

Table 2. Bioconcentration factor of *Vimba melanops* and *Caryophyllaeus brachycollis*

Vimba melanops BCF	Arda River		
	Cu	Pb	Zn
$C_{C.br.}/C_{Sediments}$	0.476	2.020	0.322
$C_{Liver}/C_{Sediments}$ $C_{C.br.}/C_{Liver}$	0.335 1.420	2.011 19.188	0.112 2.871
$C_{Kidney}/C_{Sediments}$ $C_{C.br/}\ C_{Kidney}$	0.029 15.963	0.134 15.00	0.409 0.788
$C_{Bones}/C_{Sediments}$ $C_{C.br.}/C_{Bones}$	0.079 6.044	0.146 13.716	0.090 3.569
$C_{Muscles}/C_{Sediments}$ $C_{C.br/}C_{Muscles}$	0.012 41.302	0.003 601.25	0.032 10.095
Sediments mg/kg	109.2± 2.95	35.88± 1.23	69,22± 1.33

BCF of *C. brachycollis*, parasite species of *V. melanops* of the Maritsa River was the highest for Pb (BCF $C_{C.br}/C_{SedimentsPb}$=2.020), followed by those for Cu (BCF $C_{C.br.}/C_{SedimentsCu}$=0.476) and Zn (BCF $C_{C.br.}/C_{SedimentsZn}$=0.322) (Table 2). With regard to the examined fish tissues and organs, BCF was the highest for Cu in liver ($BCF_{liver/sedimentsCu}$=0.335), followed by those for Zn in kidneys ($BCF_{kidneys/sedimentsZn}$=0.409) and for Pb in bones ($BCF_{bones/sedimentsPb}$=0.146). BCF was with the lowest values for the tree trace heavy metals for vimba's 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 ($BCF_{C.br./musclesPb}$=601.25), followed by those of Pb for liver ($BCF_{C.br./liverPb}$=19.188), of Pb for kidneys and of Pb for bones ($BCF_{R.acus/kidneysPb}$ = 14.923; $BCF_{R.acus/bonesPb}$ = 15.0). Generally, the accumulation of the tree trace heavy metals were the highest of fish parasite species *C. brachycollis*, compared to their contents in muscles.

CONCLUSIONS

As a result of this examination a total of 69 fish specimens *Vimba melanops*, collected from the Maritsa River. The determined helminth species *Caryophyllaeus brachycollis* is a core species for the helminth communities of *Vimba melanops* 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 *V. melanops* and parasite species *C. brachycollis*. The highest mean content of Pb was defined in *C. brachycollis* (72.15 mg/kg) than these in sediments, tissues and organs. From tissues and organs, the highest concentrations were obtained for the content of Cu in liver of the vimba. Generally, the accumulation of lead was higher of fish parasite species *C. brachycollis*, compared to their contents in sediments. The high values of the bioconcentration factors and of the significant correlations determined *C. brachycollis* as sensitive helminth species for heavy metal (lead, copper and zinc) content of freshwater ecosystems.

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