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HELMINTH COMMUNITIES AND ECOLOGICAL APPRAISAL FOR THE CONDITION OF THE VELEKA RIVER, BLACK SEA REGION, BULGARIA

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Abstract

Biodiversity and ecological particularities of the parasite communities of the Black Sea Roach (Rutilus frisii (Nordmann, 1840)) and (Alburnus chalcoides (Güldenstädt, 1772)) from the Veleka River (Black Sea Region) were studied during 2013. 59 specimens of R. frisii and 97 specimens of A. chalcoides 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 both fish host species. Basic physicochemical indicators in fish, some endohelminth species as bioindicators and bottom sediments were analyzed. The obtained results for the parasite communities of R. frisii and A. chalcoides 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 Veleka River condition.

Key words: *parasite communities, bioindication, Rutilus frisii, Alburnus chalcoides, Veleka River.*

INTRODUCTION

The Veleka River is one of the biggest rivers in the Black Sea Water Basin. Veleka River is part of Sredna Gora Tectonic Zone in Nature Park Strandzha (1995). The geological phenomena that can be seen in the protected area have to do with the effects of the so-called Ahtopol Volcanic Structure from the late Cretaceous (70 million years ago). It is responsible for the formation of some of the most picturesque biotopes found on the Bulgarian Black Sea Coast: the volcanic fiord-shaped shores. The mouth of Veleka River is the most scenic spot on the Bulgarian Black Sea Coast. The waters of the Veleka are rich in flora and fauna, with more than 30 species of freshwater fish (*Anguilla anguilla*, *Cyprinus carpio*, *Rutilus rutilus*, *Atherina hepsetus*, *Alburnus chalcoides*, etc) being present. Five endangered animal species (*Phalacrocorax pygmeus*, *Phalacrocorax aristotelis*, *Cygnus olor*, *Falco vespertinus*, *Branta ruficollis*, *Tadorna tadorna*, *Tursiops truncatus*, *Monachus monachus*, etc.) inhabit the river, as well as important regional plants (*Otanthus maritimus*, *Stachys maritima*, *Nuphar lutea* etc.) (Patronov, 2010). Veleka River is included in the National monitoring program (Water Body Type BG2VEL09R001) (Regulation 1/2011).

Parasite species are interesting as indicators of the ecological status of the freshwater ecosystems. One part of parasitological studies discusses the problem for the health of fish species, but more of examinations are about the relationship between pollution and parasitism in the aquatic environment (Baruš et al., 2007, 2012; Cone et al., 1993; Daei et al., 2009; MacKenzie et al., 1995; Mohammad et al., 2011; Sures et al., 1999; Taraschewski, Sures, 1996; Turčekova, Hanzelova, 1996, 1999; Brázová et al., 2012).

This research presents the results from examinations of helminth communities of freshwater fishes and ecological conditions in the studied biocenoses under the influence of the anthropogenic effects (tourism, constructions, motor boats, logging, poaching, etc.) from the Bulgarian border part of the Veleka River (before town of Sinemorets and Black Sea).

MATERIALS AND METHODS

During June - September, 2013 sediments, fish and fish parasites were collect and examined from the Veleka River (before town of Sinemorets). The Veleka River is 147 km long, of which 123 km are in Bulgaria and 25 km are in Turkey. It rises from Demirkapu Peak in Turkey and crosses the Bulgarian border through beautiful gorges. River Veleka takes it sources from a number of Kartst springs in the Turkish part of the Strandzha (İstranca) mountain and flow into the Black Sea at the Bulgarian village of Sinemorets (42°04'10"N, 27°58'06"E). The river's width near the mouth is from 8 to 10 m and its depth ranges from 2 to 4 m. (Patronov, 2010).

The studied biotopes are situated on the riverside, about 2 km far away south-eastern from the town of Sinemorets (Southern Black Sea, Bulgaria). It is distinguished with a depth and slowly running water, with sands and slimes at some places. The waterside vegetation is represented mainly by *Quercus hartwissiana* Stev., *Salix* sp., *Populus* sp. and *Alnus glutinosa* Linnaeus, 1758, *Smilax excelsa* L., *Periploca graeca* L. etc. The region of river basin and the riverside are distinguished with significant diversity of highly protected species and territories declared as protected with national and international nature protective status (Patronov, 2010; Kirin et al., 2011).

A total of 9 sediment samples and 130 freshwater fish specimens belonging to the species *Rutilus frisii* (Nordmann, 1840) (59 specimens) and *Alburnus chalcoides* (Güldenstädt, 1772) (71 specimens) 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 Pb and Zn by atomic absorption spectrometry (Bíreš et al., 1995). Two host-parasite system: The Kutum (*Rutilus frisii* (Nordmann, 1840)), Danube bleak (*Alburnus chalcoides* (Güldenstädt, 1772)) and their nematoda species *Eustrongylides excisus* (Jägerskiöld, 1909) from the Veleka River 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), 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. The sample 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 130 fish specimens from 2 species were collected and examined from the Veleka River: Kutum (*Rutilus frisii* (Nordmann, 1840) and Danube bleak (*Alburnus chalcoides* (Güldenstädt, 1772). In Bulgaria, Kutum is critically endangered (CR) and in International conservation for this fish species are not enough data (DD=Data Deficient; IUCN Red List Status). *Rutilus frisii* is included in Red Data Book of the Republic of Bulgaria (Golemanski (Ed.), 2011) and Bern Convention, Appendix III. Negatively influencing factors for the decline and the spread of *R. frisii* are water pollution, as well as the rapid expansion of tourism infrastructure along the Black Sea coast, leading to loss of the last habitats of the species in the country, introduction of alien species, which directly affect the species as competitors poaching. Kutum occurs mainly in permanent rivers such as dwell and their estuaries. *R. frisii* feeds on algae and small invertebrates (Golemanski, 2011).

Danube bleak is endangered fish species in Bulgaria EN but with no enough data in the world (DD; IUCN). *Alburnus chalcoides* is included in Red Data Book of the Republic of Bulgaria (Golemanski, 2011), in Bern Convention, Appendix III and in Habitats Directive, Appendix II. The species occurs mainly in permanent rivers, but also in estuarine, coastal brackish and freshwater lakes and permanent freshwater marshes. It feeds on zooplankton, insects and small fish. Negatively influencing factors for the decline and the spread of *A. chalcoides* are: pollution and increasing eutrophication, intensive development along the Black Sea coast, introduction of alien species affecting directly the species as competitors and poaching.

Helminth community structure

From the both fish hosts (Kutum (*Rutilus frisii* (Nordmann, 1840) and Danube bleak (*Alburnus chalcoides* (Güldenstädt, 1772)) a total 63 specimens of helminths *Eustrongylides excisus* (Jägerskiöld, 1909) were fixed. They are belonging to class Nematoda and family Eustrongylidae. *Eustrongylides excisus* (Jägerskiöld, 1909), larvae is developed with participation of the first intermediate host oligochets (blackworm *Lumbricus variegatus* Linnaeus, 1758, sludge worm *Tubifex tubifex* (Müller, 1774), *Limnodrilus* sp.) and the second fish species, amphibians (Marsh frog, *Pelophylax ridibundus* (Pallas, 1771) (=*Rana ridibunda* Pallas, 1771) and reptiles (Dice snake, *Natrix tesselata* (Laurenti, 1768)). The adult nematodes parasitic in the glandular stomach of cormorants (Great Black Cormorant *Phalacrocorax carbo* (Linnaeus, 1758) and Pygmy Cormorant *Microcarbo pygmeus* (Pallas, 1773) (=*Ph. pygmaeus* Pallas, 1773)) (Moravec, 1994). In Bulgaria, the species is presented of *Sander lucioperca* (Linnaeus, 1758) (=*Lucioperca lucioperca* Linnaeus, 1758) (as paratenic host) and of *Gobius* sp. (as intermediate host) of *Aspius aspius* (Linnaeus, 1758) from the Danube River (Kakacheva, Margaritov, Grupcheva, 1978; Margaritov, 1959); of *P. fluviatilis* from the Zhrebchevo Reservoir (Nedeva, Grupcheva, 1996) and from the Srebarna Lake (Hristov, 2010; Shukerova, Kirin, 2007; Shukerova et al., 2010); of *Silurus glanis* Linnaeus, 1758; *Lota lota* (Linnaeus, 1758), *Neogobius melanostomus* (Pallas, 1814) (=*Neogobius cephalarges* Pallas, 1814), *N. kessleri* (Günter, 1861), *P. fluviatilis* from the Danube River (Atanasov, 2012), etc.

E. excisus, which use fish as intermediate hosts represented the allogenic species for the helminth communities of the examined freshwater fish species of the Veleka River ecosystem. *E. excisus* of the parasite communities of *A. chalcoides* and of *R. frisii* of the Veleka River were distinguished with high values of prevalence (P=40.84% and P=28.81%, respectively) but with lower value of mean intensity for *E. excisus* (MI=1.47±0.14, 1-3, SE Mean 0.14, C.V. 49.79; MI=1.17±0.39, 1-2, SE Mean 0.09, C.V. 33.40, respectively). The two helminth species are component species of the helminth communities of the Kutum and Danube bleak, respectively.

Content of heavy metals in sediments, fishes and parasites

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

R. frisii	Veleka River	
	Pb	Zn
C $C_{E.excisus}$	24.12±0.27	163.33±0.31
BCF	1.92	0.31
C_{Skin}	2.98±0.23	41.22±0.10
$C_{Skin}/C_{Sediments}$	0.24	0.08
$C_{E.excisus}/C_{Skin}$	8.09	3.96
C_{Bones}	2.88±0.12	39.75±0.47
$C_{Bones}/C_{Sediments}$	0.23	0.07
$C_{E.excisus}/C_{Bones}$	8.37	4.11
$C_{Muscles}$	0.85±0.03	13.62±0.40
$C_{Muscles}/C_{Sediments}$	0.07	0.03
$C_{E.excisus}/C_{Muscles}$	28.37	11.99
Sediments mg/kg	12,55±0.35	534,22±1.23

Bioconcentration factor (BCF=[Chost/parasite tissues]/[Csediments])

Table 2. Content of heavy metals (Cmg/kg±SD) of *A. chalcoides* and *E. excisus*

A. chalcoides	Veleka River	
	Pb	Zn
$C_{E.excisus}$	32.5±0.29	321.22±0.11
BCF	2.59	0.60
C_{Skin}	4.31±0.16	57.42±0.25
$C_{Skin}/C_{Sediments}$	0.97	0.11
$C_{E.excisus}/C_{Skin}$	7.54	5.59
C_{Bones}	4.12±0.02	52.03±0.08
$C_{Bones}/C_{Sediments}$	0.33	0.09
$C_{E.excisus}/C_{Bones}$	7.89	6.17
$C_{Muscles}$	1.07±0.04	17.89±0.16
$C_{Muscles}/C_{Sediments}$	0.09	0.03
$C_{E.excisus}/C_{Muscles}$	30.37	17.96
Sediments mg/kg	12,55±0.35	534,22±1.23

Bioconcentration factor (BCF=[Chost/parasite tissues]/[Csediments])

The result of the chemical analyzes (Pb and Zn) of samples of muscle, skin, bones of *Alburnus chalcoides* and *Rutilus frisii* of the Veleka River were presented (Tables 1 and 2). The content of Pb and Zn in the parasite species *Eustrongilides excisus* 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 and 2).

The highest mean content of Pb are defined in *E. excisus* from *A. chalcoides* (32.5 mg/kg), followed by those in *E. excisus* from *Rutilus frisii* (24.12) and in the sediments (12.55 mg/kg). Of tissues and organs, higher concentrations were obtained for the content of Pb in skin and bones (4.31 and 4.12 mg/kg, respectively). The mean content of Zn showed higher values in the sediments (534.22 mg/kg) than in *E. excisus* from *A. chalcoides* (321,22 mg/kg) and from *Rutilus frisii* (163.33). Of tissues and organs, the highest content was detected for skin (C_{skin}=57.42 mg/kg and 41.22 mg/kg for the both fish species, respectively), followed by those for bones (C_{bones}=52.03; C_{bones}=39.75 mg/kg, respectively). The lowest values of Zn are detected in the muscles of examined fish samples (C_{musles}=17.89 mg/kg and C_{musles}=13.62 mg/kg, respectively) (Table 1).

BCF of *E. excisus*, parasite species of *A. chalcoides* and *Rutilus frisii* of the Veleka River was the highest for Pb ($BCFC_{E.excisus}/C_{SedimentsPb}$=2.59 and $BCFC_{E.excisus}/C_{SedimentsPb}$=1.92). With regard to the examined fish tissues and organs, BCF was the highest for Pb in skin ($BCF_{skin/sedimentsPb}$=0.97 and $BCF_{skin/sedimentsPb}$=0.24) and for Zn in skin ($BCF_{skin/sedimentsZn}$=0.33 and $BCF_{skin/sedimentsZn}$=0.24). BCF was with the lowest values for the two trace heavy metals for fish muscles.

Accumulation of heavy metals in *E. excises* to their content in the fish organs and tissues was the highest of Pb from the muscles ($BCF_{E.excisus/musclesPb}$=30.37 and $BCF_{E.excisus/musclesPb}$=30.37, respectively), followed by those of Pb for bones ($BCF_{E.excisus/lbonesPb}$=7.89 and $BCF_{E.excisus/lbonesPb}$=8.37), of Pb for skin ($BCF_{E.excisus/skinPb}$=7.54; $BCF_{E.excisus/skinPb}$=3.96). Generally, the accumulation of the two trace heavy metals were the highest of fish parasite species *E. excisus*, compared to their contents in muscles.

As a result of this study (Tables 1, 2), the content of Zn was the highest in the sediments of the Veleka River (C_{Zn}=534.22 mg/кg, respectively) and the content of Pb was the highest in *E. excisus* (321.22 mg/кg; 163.33

mg/кg). With regard to organs and tissues, the content was the highest for lead and zink in the samples of skin (C_{skinPb}=4.12/2.98 and C_{skinZn}=57.42/41.22 mg/кg, respectively).

CONCLUSIONS

As a result of this examination a total of 130 fish specimens from 2 species were collected and examined from the Veleka River. *E. excisus*, parasitic in *A. chalcoides* and *R. frisii* is allogenic species. The received data for heavy metal contents in sediments, fish tissues and organs and fish parasites from the Veleka River were presented for the first time for both examined fish species and their parasites *E. excisus*. The highest mean content of Pb are defined in *E. excisus*, followed by those in the sediments. Of tissues and organs, higher concentrations were obtained for the content of lead in skin. Generally, the accumulation of the two trace heavy metals were the highest of fish parasite species *E. excisus*, compared to their contents in muscles of the two fish species, respectively. The high values of the bioconcentration factors and of the significant correlations determined *E. excisus* as sensitive bioindicator for lead.

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