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Helminth communities of *Silurus glanis* and its bioindicator signification for the condition of the Ivaylovgrad Reservoir, Bulgaria

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Abstract

Biodiversity and ecological particularities of the parasite communities of the wels catfish (*Silurus glanis* L., 1758) from the Ivaylovgrad Reservoir were studied during 2013. Eleven specimens of S. glanis 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 *S. glanis* from the Ivaylovgrad Reservoir. Concentration of heavy metals (Pb, Zn, Cu) 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 *S. glanis* 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 Ivaylovgrad Reservoir condition.

Key words: parasite communities, heavy metals, bioindication, *Silurus glanis*..

Introduction

The Arda River and it’s reservoirs are indicated as a region with middle and high degree of importance in respect to the date about the species richness, the endemic and rare taxa. Basic ecological problems in the region of the Ivaylovgrad Reservoir and Arda River are caused from the negative impact and damages of the environmental components from the performed (Government decree No. 140, Official Newspaper 101/2000) mining-extractive, ore-dressing and metallurgy activity (mining of lead and zinc ores and flotation), leading to a high pollution and aciding of large surfaces with deposited mine masses (Yorova et al., 1992). Arda River is included in the National monitoring program (Regulation 1/2011).

Endoparasite species are interesting as bioindicators of the ecological status of the freshwater ecosystems. Their life cycle require 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; Kennedy, 1997; MacKenzie et al., 1995; Marcogliese and Cone, 1997; Overstreet, 1997; Sures, Siddall, 1999; Thielen et al., 2004; Tieri et al., 2006, etc.).

In the scientific literature, there are relatively few studies on parasites and parasite communities of *Silurus glanis* Linnaeus, 1758 and their role as bioindicators for different load of freshwater ecosystems with heavy metals (Akmirza, Yardimci, 2014; Brázová et al., 2012; Çolak, 2013; Reading et al., 2012; Soylu, 2005, etc.).

This paper presents the first results from of biodiversity helminhs and helminth communities from *Silurus glanis* Linnaeus, 1758 and an examination of heavy metal content in sediments, fish tissues and organs, fish parasites from the Ivaylovgrad Reservoir.

Materials and Methods

During September-October, 2013 sediments, fish and fish parasites were collect and examined from the Reservoir Ivaylovgrad (Aegean Water Basin) (Figure 1).

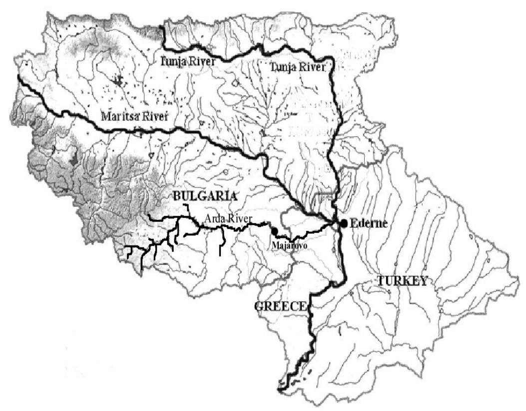

Figure 1. Aegean Water Basin

The Ivaylovgrad Reservoir ($41^0$32.2'N, $26^0$6.27'E) is located in Eastern Rhodopa Mountain, 120 m above sea level. It is situated on the Arda River, 13 km nort-west from town of Ivaylovgrad, Southern Bulgaria. This reservoir is one of the biggest reservoirs in Bulgaria with surface area of 15.2 km^2. Ivaylovgrad hydroelectric power plant is the last stage of the Arda cascade (from 1964 year).

A total of 5 sediment samples and 9 freshwater fish specimens belonging to the species *Silurus glanis* Linnaeus, 1758 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).

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 Wels catfish, *Silurus glanis* (L., 1758) from the Reservoir Ivaylovgrad 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 Bykhovskaya-Pavlovskaya (1985), Dubinina (1987), Georgiev et al. (1986), Gusev (1985), Moravec (1994, 2001), Scholz and Hanzelova (1998), Scholz et al. (1998) 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, etc. (Kennedy, 1993, 1997; Magurran, 1988). Fish 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 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 Spearman correlation coefficient, r_s was used to test associations between the bottom sediments, fish tissues, organs and fish parasites

Results and Discussion

Fish population

Silurus glanis Linnaeus, 1758 (Wels catfish) is native fish species to Eastern Europe and Asia. It has been introduced in several another countries (Germany, France, Spain, Turkey, Greece, England and Netherlands, etc.). The catfish 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. They are protected by Appendix III of Bern Convention. It inhabits large rivers and lakes, deep dams and also brackish waters of Black and Baltic Sea. Catfish is non-migratory fish species. After hatching, young larvae feed on plankton. Larvae and juveniles are benthic, feeding on a wide variety of invertebrates and fish. Adults prey on fish and other aquatic vertebrates. The catfish grow quickly and can reach between 1.5 and 4.5 kg after their first year (Kottelat and Freyhof, 2007). The examined catfish from the Ivaylovgrad Reservoir was measured with length of 52.0 to 85.89 cm and weight from 1.5 – 3.5 kg and age 1-3 years. As a result of this study, two of nine examined fish specimens from the Reservoir Ivaylovgrad are free of parasites.

Helminth community structure

From studied 9 specimens of fishes, 3 parasite species were fixed (*Glanitaenia osculata* (Goeze, 1782) de Chambrier et al. (2004), *Contracaecum bidentatum* (Linstow, 1899), *Eustrongylides excisus* Jägerskiöld, 1909). They are belonging to classes Cestoda (1) and Nematoda (2).

Silurus glanis is specific definitive host of *Glanitaenia osculata* (Goeze, 1782) (= *Proteocephalus osculatus* (Goeze, 1782)). In Bulgaria, the species is presented of *Silurus glanis* Linnaeus, 1758 from the Danube River, near towns of Ruse and Svishov and Lake Shabla (Margaritov, 1959, 1960; Kakacheva, Margaritov, Grupcheva, 1978).

Contracaecum bidentatum (Linstow, 1899), larvae is developed with intermediate hosts amphipod crustaceans (Gammarus). Definitive hosts are Acipenseridae and rarely Cyprinidae or Percidae (Moravec, 1994). In Bulgaria, the species is presented of *Acipenser ruthenus* Linnaeus, 1758, *Zingel zingel* (Linnaeus, 1766), *Zingel streber* (Siebold, 1863), *Gymnocephalus cernus* (Linnaeus, 1758), *Gymnocephalus schraetser* (Linnaeus, 1758), *Ponticola constructor* (Nordman, 1840), *Gobio gobio* (Linnaeus, 1758), *Proterorchinus marmoratus* (Pallas, 1814), *Ballerus sapa* (Pallas, 1814), *Esox lucius* Linnaeus, 1758, *Lota lota* (Linnaeus, 1758), *Silurus glanis* (Linnaeus, 1758), *Alburnus alburnus* (Linnaeus, 1758) from the Danube River, near towns of Vidin, Ruse, Silistra and Svishov (Margaritov, 1959; Kakacheva-Avramova, 1977; Kakacheva, Margaritov, Grupcheva, 1978).

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 (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* (Günther, 1861), *P. fluviatilis* from the Danube River (Atanasov, 2012), etc.

Glanitaenia osculata, parasitic in *S. glanis is* generalists for the helminth communities of the examined freshwater fish species of the Ivaylovgrad Reservoir freshwater ecosystem. *Glanitaenia osculata* of the parasite communities of *S. glanis* are distinguished with high values of prevalence (P=66.94%) and with low values of mean intensities (MI=3±1.89, 1-4). *Gl. osculata* is component species for the helminth communities of *S. glanis*. *C. bidentatum* and *E. excisus*, which use fish as intermediate hosts represented the allogenic species for the helminth communities of the examined freshwater fish species of the Ivaylovgrad Reservoir ecosystem (Aegean Water Basin). *C. bidentatum* and *E. excisus* are component species for the helminth communities of catfish (P=43.27%; MI=4±2.22, 2-5 and P=27.82%; MI=2±0.29, 1-2, respectively).

Content of heavy metals in sediments, fishes and parasites

The content of Pb, Cu and Zn in the Cestoda species *Glanitaenia osculata* 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. The highest mean content of Cu and Zn showed the sediment samples of the reservoir ($C_{SedimentsCu}$=26.110 mg/кg and $C_{SedimentsZn}$=695.402 mg/кg, respectively), followed by these of the parasite species ($C_{G.osculataCu}$=10.417 mg/кg and $C_{G.osculataZn}$=339.583 mg/кg, respectively). The highest mean content of Pb are distinguished parasitological samples ($C_{G.osculataPb}$=31.250 mg/кg) than in sediment samples ($C_{SedimentsPb}$=22.989 mg/кg), and fish tissues. From fish tissues, with the highest content of Cu are the samples of the skin (C_{SkinCu}=9.677 mg/кg). The highest mean content of Pb is defined in bones ($C_{BonesPb}$=12.143 mg/kg). The highest concentrations of Zn was detected also for bones (C_{SkinZn}=19.231

mg/kg), followed by this of skin (C_{SkinZn}=206.272 mg/kg). The lowest values of three trace elements are detected in the muscles of the examined Wels catfish ($C_{MuslesCu}$=2.811 mg/kg; $C_{MuslesPb}$=22.989; $C_{MuslesZn}$=19.231) (Table 1).

Table 1. Bioconcentration factor (BCF=[Chost/parasitetissues]/[Csediment) of *S. glaniss* and *G. osculata*

Silurus glanis	Indices		
	Cu	Pb	Zn
$C_{Gl.osculata}/C_{Sediments}$	0.398	1.359	0.448
$C_{Skin}/C_{Sediments}$	0.370	0.187	0.296
$C_{Gl.osculata}$ / C_{Skin}	1.076	7.265	1.646
$C_{Bones}/C_{Sediments}$	0.260	0.528	0.324
$C_{Gl.osculata}$ /C_{Bones}	1.535	2.573	1.509
$C_{Muscles}/C_{Sediments}$	0.107	0.064	0.028
$C_{Gl.osculata}$ /$C_{Muscles}$	3.705	21.129	17.658
Sediments mg/kg±SD	26.110 ±3.75	22.989 ±2.27	695.402 ±4.92

BCF of *Gl. osculata* was the highest for Pb (BCF$C_{Gl.osculata}/C_{SedimentsPb}$=1.359), followed by those for Zn (BCF $C_{Gl.osculata}/C_{SedimentsZn}$=0.448) and Cu (BCF $C_{Gl.osculata}/C_{SedimentsCu}$=0.389) (Table 1).

With regard to the examined fish tissues, BCF was the highest for Pb in skin ($BCF_{Bones/SedimentsPb}$=0.528), followed by those for Cu in the skin ($BCF_{Skin/SedimentsCu}$=0.370) and for Zn in the bones ($BCF_{Bones/SedimentsZn}$=0.324). BCF was with the lowest values for the tree trace heavy metals for *S. glanis* muscles. Accumulation of heavy metals in *G. osculata* to their content in the fish organs and tissues was the highest of Pb from the muscles ($BCF_{Gl.osculata/musclesPb}$=21.129), followed by those of Zn ($BCF_{Gl.osculata/musclesZn}$=17.658) and of Cu ($BCF_{Gl.osculata/musclesCu}$=3.705). Generally, the accumulation of the tree trace heavy metals are the highest of fish parasite species *Gl. osculata*, 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 $Sediments_{cu}$-$Skin_{cu}$; $Sediments_{Pb}$-*Gl.osculata*$_{Pb}$; $Sediment_{Zn}$-$Bones_{Zn}$.

Conclusion

As a result of this examination a total of 9 fish specimens *Silurus glanis* Linnaeus, 1758 were collected and examined from the Ivaylovgrad Reservoir (Aegean Water Basin). Three parasite species were detected: (*Glanitaenia osculata* (Goeze, 1782) de Chambrier et al. (2004), *Contracaecum bidentatum* (Linstow, 1899), *Eustrongylides excisus* Jägerskiöld, 1909). *Glanitaenia osculata* is a generalist and core helminth species for the helminth communities of *Silurus glanis*. *E. excisus* and *Contracaecum bidentatum* are allogenic and component species. The three parasite species are reported for the first time for the parasite fauna of *Silurus glanis* from Aegean Water Basin and Reservoir Ivaylovgrad. The catfish is a new host record for *C. bidentatum* and *E. excisus* in Bulgaria and Aegean Water Basin. The received data for heavy metal contents in sediments, fish tissues and *Glanitaenia osculata* were presented for the first time. The highest mean content is defined for lead in *Gl. osculata* (31.250 mg/kg), followed by those in the sediments (22.989 mg/kg) and catfish organs. Of tissues and organs, the highest concentrations were obtained for the content of lead in bones. Generally, the accumulation of the tree trace heavy metals were the highest of fish parasite species compared to their contents in muscles. The high values of the bioconcentration factors and of the significant correlations determined *Glanitaenia osculata* as sensitive bioindicators for lead.

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