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HELMINTHS AND HELMINTH COMMUNITIES OF PERCH (*Perca fluviatilis* Linnaeus, 1758) AS BIOINDICATORS FOR ECOSYSTEM CONDITION OF THE MARITSA RIVER

Dimitrinka KUZMANOVA, Mariya CHUNCHUKOVA, Diana KIRIN

Agricultural University – Plovdiv, Department of Agroecology and Environmental Protection
12 Mendeleev Str, 4000, Plovdiv, Bulgaria

Corresponding author email: dima_kuzmanova@abv.bg

Abstract

Maritsa River is related to the Aegean water collecting region. During 2015, a total amount of 23 specimens of perch (Perca fluviatilis Linnaeus, 1758) are investigated. Identified two species of helminths (Proteocephalus percae (Müller, 1780) and Acantocephalus lucii (Müller, 1776)) are reported for the first time as intestinal parasites of perch from the freshwater ecosystem of the Maritsa River. They are core and authogenic species for the helminth communities of P. fluviatilis. Analyses of the helminth communities were carried out at both levels: infracommunity and component community. Basic ecological characteristics and biotic indices were determined to evaluate ecosystem condition of the river. The results of these studies attest to the importance of perch's helminths and helminth communities as sensitive bioindicators.

Key words*: bioindication, helminths, Maritsa River, Perca fluviatilis.*

INTRODUCTION

The Maritsa River is related to the Aegean Basin. The major negative anthropogenic impact on the Maritsa River ecosystem associated with the changes in the studied freshwater communities are farm activities, industry, power production, irrigation, constructions etc. Maritsa River is included in the National monitoring program (Water Body Type BG3MA350R039– Major rivers; river type R12: Large flat rivers in the Ecoregion 7: Eastern Balkans) (Peev & Gerassimov, 1999; Belkinova et al., 2013).

The Maritsa River is a part of the National Ecological network (Natura 2000). In close connection with the protection of the freshwater communities and natural habitats of the Maritsa River and its riversides were declared protected areas BG 0000578 "River Maritsa" according to Directive 92/43 and protected area BG 0002081 "Maritsa – Parvomaj" according Directive 79/409. There are not a few examinations on species diversity, conservation status and characteristics of the freshwater fish communities of the river and its tributaries (Vassilev and Pehlivanov, 2005; Georgiev, 2006; Kolev, 2013; Kolev, 2016).

Parasites and parasite communities of freshwater fish respond differently to changes in habitat conditions and on the other hand they are defined as complex systems affected by many ecological and environmental factors both in time and space (Lafferty and Kuris, 1999; Valtonen, 2001; Kuhn, 2015; Kadlec et al., 2003; Marcoglise, 2016; Lagrue et al., 2018).

The complex life cycles of the development of a large number of intestinal parasites occurring with the participation of a number of species and groups of intermediate hosts are essential for the endoparasites and characteristics of parasite communities of freshwater fish species to be used as bioindicators for the ecological status of the studied habitats (Sures, 2001; Schludermann et al., 2003; Nachev, 2010; Chapman et al., 2015; De Aquino Moreira et al., 2015; Hofmann et al., 2016).

The paper presents the results of the examinations on the intestinal parasites and its endoparasite communities of European perch (*Perca fluviatilis* Linnaeus, 1758) of the Maritsa River and to evaluate their bioindicator role in the studied freshwater ecosystem (region of the town of Parvomaj; Aegean Basin).

MATERIALS AND METHODS

River Maritsa has a length of 521 km and is the longest river on the Balkan Peninsula. The river springs from Rila Mountains (2°09'40"N, 23°36'00"E, 2378 m altitude, from Maritsa lakes, below Peak Mancho) in Western Bulgaria. It running southeast between the Balkan and Rhodope Mountains, past Plovdiv southeast part of Greece and European Turkey and flows into Aegean Basin (41 m above sea level) (Dakova et al., 2004).

The studied biotope (around the town of Parvomaj, 42.099444N, 25.224167E) is situated on the riverside,about 50 km far away south-eastern from the town of Plovdiv (42.15N, 24.75E). It is characterized by a depth and speedy runningwater, with a sandy bottom. The waterside vegetation is represented mainly by *Salix alba* L., *Populus alba* L., *Populus nigra* L., *Alnus glutinosa* (L.) Gaertn., *Rhobinia pseudoacacia* L. etc.

During 2015, a total of 23 specimens of perch are collected and examined for helminths from the Maritsa River (near the town of Parvomaj; Aegean Basin). Fish names (scientific and common names) are presented according to the FishBase database(Fröse and Pauly, 2018).

Helminthological examinations were carriedout following recommendations described by Zashev and Margaritov (1966), Bykhovskaya-Pavlovskaya (1985), Bauer (1987), Scholz and Hanzelová (1998). Specimens are fixed and preserved in 70% ethyl alcohol. The specimens of Cestoda are studied by methods of Georgiev et al. (1986); Scholz and Hanzelová (1998). The acanthocephalans are studied on temporary mounts with 5% glycerolin 70% ethanol (Petrochenko, 1956; Zashev and Margaritov, 1966). Analyses of helminth community structureare carried out in both levels: infracommunity (total and mean number of species; total and mean number of specimens; Brillouin's index of diversity (HB) and component community (prevalence (P%), mean abundance (MA) and mean intensity (MI) for each species) (Bush et al., 1997; Kennedy, 1993, 1997; Magurran, 1988). The species are classified as core species (P%>20), component species (P%>10) and accidental species (P%<10) (Kennedy, 1993). The diversity measures are calculated by software products Statistica 10 (StatSoft Inc., 2011) and MS Excel (Microsoft 2010).

RESULTS AND DISCUSSIONS

Fish communities

A total of 23 specimens of European perch (*Perca fluviatilis* Linnaeus, 1758; Percidae) are collected and studied for intestinal helminths. *P. fluviatilis* is determined as least concern species (LC=Least Concern; IUCN Red List Status, 2019) and isnot included in Red Data Book of the Republic of Bulgaria (Golemanski, 2011).

The perch is wide spread in Europe, including in Bulgaria, but negative environmental impacts have been reported after its introduction. *P. fluviatilis* prefer rivers, lakes, reservoirs, overgrown with aquatic vegetation. The perch is also found in semi-saline waters and habitats of high acidity, with a high population density. The perch is freshwater, brackish, demersal and anadromous fish species (Kottelat and Freyhof, 2007; Fröse and Pauly, 2018).

The fish species is distinguished by age differences in nutrition: small fish feed on zooplankton; adult fish also add bottom invertebrates to their ration. Only the largest specimens of perch (with size around 12 cm) have been found feeding entirely with small fish (mainly sticklebacks, perches and minnows). *P. fluviatilis* is a predatory fish species. The European perch is an important fish species as food and for game fishing (Karapetkova and Zhivkov, 2006; Fröse and Pauly, 2018).

According to this examination, there are no specimens of perch free of intestinal parasites.

Helminth community structure

23 specimens of perch are infected with two specimens of intestinal helminths: *Proteocephalus percae* (Müller, 1780) and *Acanthocephalus lucii* (Müller, 1776) belonging to two classes, Cestoda and Acanthocephala, respectively.

P. percae grows with the participation of intermediate host copepods (*Cyclops strenuous* Fischer, 1851, *C. vicinus* Ulyanin, 1875, *Eucyclops serrulatus* (Fischer, 1851) etc.). The species is ubiquitously represented in the

habitats of the perch (Bauer, 1987). *P. fluviatilis* is a typical definitive host of *P. percae* (Bauer, 1987; Scholz and Hanzelova, 1998).

Reporting of *P. percae* from other hosts outside of Percidae is considered as erroneous species identification. Detection of parasites in another predatory fish (*Esox lucius*, *Lota lota* etc.) is associated with postcyclic parasitism and obtaining the parasite when these fish species eating infected with *P. percae* specimens of perch (Scholz & Hanzelova, 1998).

In Bulgaria, the species *P. percae* was reported of *Gymnocephalus schraester* (Linnaeus, 1758) (=*Acerina schraester* Linnaeus, 1758), *Sander volgensis* (Gmelin, 1789) (=*Stizostedion volgense* Gmelin, 1789), *Gymnocephalus cernua* (Linnaeus, 1758) (=*Acerina cernua* Linnaeus, 1758) from Danube river (in the region of towns Vidin, Silistra and Tutrakan) (Kakacheva-Avramova et al., 1978) as *Proteocephalus cernuae* (Gmelin, 1790) La Rue, 1911; of *P. fluviatilis* from Lake Srebarna (Shukerova, 2010; Shukerova et al., 2010); etc.

The life cycle of *A. lucii* is carried out with the participation of intermediate host species *Asellus aquaticus* (Linnaeus, 1758). Definitive hosts are many freshwater fish species from Cyprinidae (Linnaeus, 1758) (Kakacheva-Avramova, 1983; Bauer, 1987).

In Bulgaria, the species *A. lucii* was reported of *Silurus glanis* Linnaeus, 1758 and *Squalius cephalus* (Linnaeus, 1758) (=*Leuciscus cephalus* Linnaeus, 1758) (Margaritov, 1959) of *P. fluviatilis* (Margaritov, 1966), of *Ballerus sapa* (Pallas, 1814) (=*Abramis sapa* Pallas, 1814), *Sq. cephalus*, *Rutilus rutilus* (Linnaeus, 1758), *S. glanis*, *P. fluviatilis*, *Lota lota* (Linnaeus, 1758), *G. schraester*, *Benthophilus stellatus* (Sauvage, 1874), *Proterorhinus marmoratus* (Pallas, 1814) (Kakacheva-Avramova et al., 1978); of *Sq. cephalus* (Cakic et al., 2004); of *L. lota*, *Zingel zingel* (Linnaeus, 1766) (Atanasov, 2012); of *Abramis brama* (Linnaeus, 1758) and *Alburnus alburnus* (Linnaeus, 1758) (Chunchukova, 2017); of *A. alburnus* (Chunchukova et al., 2018) of the Danube River; of *A. brama* (Linnaeus, 1758) (Chunchukova et al., 2016); of *P. fluviatilis* (Shukerova, 2010; Shukerova et al., 2010) from Lake Srebarna etc.

Component communities

The two determined species, *P. percae* and *A. lucii* are generalists for the helminth communities of perch of the Maritsa River. With higher prevalence, mean abundance and mean intensity is distinguished *A. lucii* (Table 1). *P. percae* and *A. lucii* are core species of the helminth communities of *P. fluviatilis* from the freshwater ecosystem of the Maritsa River according to on the criterion of Bush et al. (1997). The two determined species of endohelminths are autogenic species of the helminth communities of the perch from the river.

Table 1. Species diversity, prevalence (P%), mean intensity (MI) of the established endohelminth species of *Perca fluviatilis* of the Maritsa River

Species of parasites	Intermediate hosts	Definitive host *P. fluviatilis* (N^1=23)		
		n^2/p^3	^{4}P%	MA^5 MI^6 Rang
Cestoda				
Proteocephalus percae (Müller, 1780)	Copepoda	11/25	47.83	1.09 2.27 1-4
Acanthocephala				
Acanthocephalus lucii (Müller, 1776)	Amphipoda	23/84	100	3.65 3.65 1-7

[1]N = total number of examined fish specimens.
[2]n = total number of infected fish specimens.
[3]p = total number of endoparasite specimens.
[4]P% = prevalence.
[5]MA = mean abundance.
[6]MI = mean intensity

Infracommunities

In 11 of 23, a total examined specimens of perch (47.83%), a mixed invasion was established with the presence of both species of intestinal parasites. All examined fish specimens (23 specimens) were infected with acanthocephalans. The average number of endoparasite specimens found in the total number of studied fish specimens is 4.695±1.717. The minimal number of endoparasite specimens per a fish specimen is 2 and maximal is 9 parasites. The value of Brillouin's diversity index is HB=0.516 (Table 2).

Table 2. Infracomminities data

No. of helminth species		
Total number of species	2	
Number of fish	11	23
Number of helminth species	2	1
Number of helminth specimens		
Total number of specimens	109	
Mean±SD	4.695±1.717	
Range	2-9	
Mean HB±SD	0.395±0.053	

In Bulgaria, 16 endoparasite species were reported as parasites of parasite communities of *P. fluviatilis*. In this study, the fixed two species of helminths (*P. percae* and *A. lucii*) presents for only 12.5% of those established of the perch for the country. The examinations of parasite and parasite communities of perch are mainly from Bulgarian part of the Danube River and its tributaries, Lake Srebarna and some freshwater ecosystems of the Aegean Basin. *P. percae* and *A. lucii* are reported as parasites of perch in Bulgaria, but they are reported for the first time for Maritsa river ecosystem.

Table 3. Species of endoparasites of *P. fluviatilis* in Bulgaria

Trematoda	Authors
Nicollas krjabini	Kakacheva-Avramova et al.,1978
	Kirin, 2005
Ichthyocotylurus pileatus	Nedeva&Grupcheva, 1996
	Shuketrova, 2010
	Shukerova et al., 2010
Bunodera luciopercae	Kirin, 2005
Cestoda	
Caryophyllaeus brachycollis	Nedeva&Grupcheva, 1996
Caryophyllaeides fennica	Shuketrova, 2010
Proteocephalus percae	Shuketrova, 2010
	Shukerova et al., 2010
Acanthocephala	
Acanthocephalus lucii	Margaritov, 1966
	Kakacheva-Avramova et al., 1978
	Shukerova et al., 2010
	Shukerova, 2010
Acanthocephalus anguillae	Shukerova, 2010
	Kirin, 2005
Pomphorhynchus laevis	Kakacheva-Avramova et al., 1978
	Nedeva et al., 2003
	Kirin, 2005
	Shukerova et al., 2010
Neoechinorhynchus rutili	Kirin, 2005
Nematoda	
Eustrongylides excisus	Atanasov, 2012
	Nedeva&Grupcheva, 1996
	Kirin et al, 2013a
	Kirin et al, 2013b
	Shukerova, 2010
	Shukerova et al., 2010
Eustrongylides tubifex	Shukerova, 2010
	Shukerova et al., 2010
Crowcroecoecumskrjabini	Margaritov, 1966
Rhabdochona sp., larvae	Margaritov, 1966
	Kakacheva-Avramova et al., 1978
*Contracoecum*sp., larvae	Kakacheva-Avramova et al., 1978
	Shukerova, 2010
	Shukerova et al., 2010
Rhaphidascaris acus	Nedeva&Grupcheva, 1996
	Shukerova, 2010
	Shukerova et al., 2010

The most commonly was reported *E. excisus* (in 6 scientific studies), followed by *A. lucii* and *P. laevis* (4 studies) (Table 3). *P. percae*; *Bothriocephalus claviceps* (Goeze, 1782) Rudolphi, 1810; *Glanitaenia osculata* (Goeze, 1782); *Triaenophorus nodulosus* (Pallas, 1781) Rudolphi, 1793 (Cestodaspecies), *A. lucii* (Acanthocephala) and *Camallanus lacustris* (Zoega, 1776) (Nematoda) were reported as a commonly parasites of juvenile *P. fluviatilis* (Kuchta et al., 2009). Wierzbicki (1970) established 13 intestinal species, including *P. percae* and *A. lucii*, also determined as common species of perch. Sobeska and Słomínska (2007) determined 8 endoparasite species of perch of the Odra River in Poland, including *P. percae*. Opposite to higher species diversity, *P. percae* from Odra river was distinguished with lower prevalence and mean intensity than those of *P. percae* from Maritsa river ($P\%_{P.\ percae\_\text{river Odra}}=9.09$; $MI_{P.\ percae\_\text{river Odra}}=1.8$; $P\%_{P.\ percae\_\text{river Maritsa}}=47.83$; $MI_{P.\ percae\_\text{riverMaritsa}}=2.27$, Table 1). The examined perch specimens are from brackish water habitats of the Odra River and probably the lower parameters of infection are associated with higher salinity and worse conditions for the development and abundance of intermediate parasite hosts. The prevalence of *P. percae* is even lower of *P. fluviatilis* from Lake Srebarna than this of the Odra River ($P\%_{P.\ percae\_\text{Lake Srebarna}}=3$; Shukerova, 2010; Shukerova et al., 2010) due to the strong anthropogenic impacts, the high euthrophication, the low oxygen content, etc., with negatively affects the interactions and the development of the organisms. Carney & Dick (1999) compared the endohelmints of *P. fluviatilis* from Europe and *Perca flavescens* Mitchill from North America. For *P. fluviatilis*, they have determined four predictable parasitic species, specific for *P. fluviatilis*: *Bunodera luciopercae* (Müller, 1776), *P. percae*, *A. lucii* and *C. lacustris*. The authors point out, that specificity is not a requirement for predictability. They make a conclusion that the predictability of these parasitic groups is closely related to biology, and especially to the nutrition of the perch. Therefore, they established that respectively, parasites of perch are closely related to certain biological elements in the habitats as intermediate hosts and food. Intermediate hosts of *P. percae* (copepods *C. strenuus*, *C. vicinus*, *E. serrulatus* etc.) and of *A. lucii* (amphipods *A. aquaticus*) probably are represented with the highest density in the

studied habitats of the Maritsa River and are predominant in the diet of the examined specimens of perch. *A. aquaticus* is a tolerant species of a range of pollutants (Maltby, 1991).

CONCLUSIONS

As a result of the study of 23 specimens of perch, a total of two species of endoparasites (*P. percae* and *A. lucii*) are determined. They are a core species of the helminth communities of *P. fluviatilis* of the Maritsa River. *P. percae* and *A. lucii* are reported for the first time for the freshwater ecosystem of the Maritsa River in Bulgaria. The poor species diversity of intestinal parasites and the high values of the characteristics of infection indicated poor species diversity of the free-living stages of the river section and negative impacts on the ecosystem.

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