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NEW DATA ON THE HELMINTH FAUNA OF *Alosa immaculata* Bennett, 1835 FROM THE BULGARIAN SECTION OF THE DANUBE RIVER, NORTHWESTERN BULGARIA

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Abstract

During 2019-2021, six specimens of pontic shad (Alosa immaculata Bennett, 1835); family Clupeidae were examined for parasites. The specimens were collected from three biotopes (Kudelin, Yasen, and Koshava) located in the section of the Danube River in the northwestern part of Bulgaria. Infection with 3 species of helminths was found – 1 species of the class Trematoda (Lecithaster confusus Odhner, 1905); 1 species of the class Acanthocephala (Pomphorhynchus laevis (Zoega in Müller, 1776) Porta, 1908) and 1 species of the class Nematoda (Hysterothylacium gadi aduncum (Rudolphi, 1802) Deardorff et Overstreet, 1981 (larvae)). Ecological indices of the found helminth species were examined. The purpose of the study is to provide new data on the helminth fauna of the pontic shad from the freshwater ecosystem of the Danube River in Bulgaria. Al. immaculata is a new host for one endohelminth species (L. confusus). Two of the investigated biotopes (Koshava and Yasen) are new habitats for the found endohelminths in pontic shad.

Key words*: helminths, Koshava, Kudelin, pontic shad, Yasen.*

INTRODUCTION

Pontic shad (*Alosa immaculata* Bennett, 1835) is a passage fish, subject to commercial fishing, and inhabits the Black Sea and the Sea of Azov. The species perform breeding migrations. It spawns in the Danube, Dnieper, Dniester, Don rivers, and others during the period April-August (Karapetkova & Zhivkov, 2006; Visnjic-Jeftic et al., 2010; Đikanović et al., 2018; Smederevac-Lalić et al., 2018; Fröse & Pauly, 2022). The species does not feed during migrations (Ciolac, 2004). The diet of the pontic shad includes fish and crustaceans (Golemanski, 2011; Fröse & Pauly, 2022). Water pollution, overfishing, loss and fragmentation of habitats, construction of dams, low water levels, etc. have a negative impact on the number of species (Golemanski, 2011; Smederevac-Lalić et al., 2018; Grecu et al., 2020; Fröse & Pauly, 2022). Due to the construction of Iron Gate I and Iron Gate II, today the species is found in the Danube River up to the confluence of the Timok River (Visnjic-Jeftic et al., 2010; Smederevac-Lalić et al., 2018). The parasite fauna of the pontic shad from the Bulgarian section of the Danube River is poorly studied (Kakacheva-Avramova et al., 1978; Kirin et al., 2013; Nachev et al., 2022). Few authors provide data on the parasite fauna of *Al. immaculata* (Đikanović et al., 2018; Grecu et al., 2020; Stroe et al., 2021; Stroe et al., 2022). The purpose of the present study is to provide new data on the helminth fauna of *Al. immaculata* from the freshwater ecosystem of the Danube River in Northwestern Bulgaria.

MATERIALS AND METHODS

Six specimens of *Al. immaculata* from three locations (presented as biotopes) from the Bulgarian section of the Danube River were subjected to parasitological examination. Kudelin biotope (44°12'07.9"N, 22°41'28.2"E), Yasen biotope (44°07'26.6"N, 22°52'42.1"E) and Koshava biotope (44°03'59.9"N, 23°02'10.2"E) are located on the right bank of the river, in Vidin Province, Northwestern Bulgaria (Figure 1).

The fish were caught with fishing gear according to permits issued by the Executive Agency for Fisheries and Aquaculture (EAFA). The scientific name of the species was given by Karapetkova & Zhivkov (2006); Fröse & Pauly (2022). Each caught fish specimen was weighed and measured (Table 1).

Figure 1. Location of biotopes from the Bulgarian section of the Danube River, Northwestern Bulgaria (https://www.google.bg/maps/place/Видин)

Table 1. Metric data (total body length (TL; in centimeters); maximum body height (MH; in centimeters); body weight (BW; in grams) of the studied specimens of *Alosa immaculata*

Danube River		TL (cm)	MH (cm)	BW (g)
Alosa immaculata **N = 6**	**min-max**	9.8-30	2.1-5.5	7-146
	Mean±SD	20.20±11.33	3.66±1.71	63.60±73.93

The parasitological examination of the caught specimen's pontic shad was carried out according to standard methods (Zashev & Margaritov, 1966). All found helminth specimens were isolated and stored in 70% ethyl alcohol for further processing. Permanent microscopic preparations were made from the representatives of class Trematoda, and temporary microscopic preparations were made from the representatives of classes Acanthocephala and Nematoda (Dubinina, 1948; Zashev & Margaritov, 1966; Moravec, 2013). The type of all found helminths was determined (Zashev & Margaritov, 1966; Moravec, 2013; and others). Basic ecological indices were calculated and presented: mean intensity (MI); mean abundance (MA) and prevalence (P%) (Bush et al., 1997).

RESULTS AND DISCUSSIONS

Model fish species

Al. immaculata is a pelagic-neritic fish. The species winters in the Black Sea. The fish have a weight of up to 1 kg and a body length of up to 40 cm (Ciolac, 2004). They live up to 7-8 years (Karapetkova & Zhivkov, 2006; Fröse & Pauly, 2022). Pontic shad is protected by national and international legislation. The species is included in the Red Book of the Republic of Bulgaria with the category "VU=Vulnerable", in the Biological Diversity Act, and also in the IUCN Red List with the category "VU=Vulnerable", in the Bern Convention and in the Habitats Directive (Convention on the conservation of European wildlife and natural habitats, 1982; Council Directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora, 1992; Biological Diversity Act, 2002; Freyhof & Brooks, 2011; Golemanski, 2011).

Ecologohelminthological examination

For the period 2019-2021, a total of 6 specimens pontic shad were examined for parasites – two specimens from each of the three biotopes (Kudelin, Koshava, Yasen). Infection with a total of 3 endohelminth species was found: *Lecithaster confusus* Odhner, 1905 (class Trematoda); *Pomphorhynchus laevis* (Zoega in Müller, 1776) Porta, 1908 (class Acanthocephala) and *Hysterothylacium gadi aduncum* (Rudolphi, 1802) Deardorff et Overstreet, 1981 (larvae) (class Nematoda) (Table 2).

Table 2. Taxonomic position, localization, biotopes, season of detection of *Lecithaster confusus*, *Pomphorhynchus laevis*, and *Hysterothylacium gadi aduncum*

Helminth species	***Lecithaster confusus* Odhner, 1905**	***Pomphorhynchus laevis* (Zoega in Müller, 1776) Porta, 1908**	***Hysterothylacium gadi aduncum* (Rudolphi, 1802) Deardorff et Overstreet, 1981**
Taxonomic position	CLASS TREMATODA RUDOLPHI, 1808 Family Lecithasteridae Skrjabin et Guschanskaja, 1954 Genus *Lecithaster* Lühe, 1901	CLASS ACANTHOCEPHALA (RUDOLPHI, 1808) Family Pomphorhynchidae Yamagiti, 1939 Genus *Pomphorhynchus* Monticelli, 1905	CLASS NEMATODA RUDOLPHI, 1808 Family Raphidascarididae Genus *Hysterothylacium* Ward & Magath, 1917
Localization	intestine	intestine	intestine
Biotope	Koshava	Koshava, Yasen	Koshava
Season	spring	spring, summer	spring

In the present study, one helminth species was common to pontic shad from two of the studied biotopes (Koshava and Yasen). The species diversity of the found helminths (3 species) was largest in pontic shad from Koshava biotope, followed by Yasen biotope (1 species). The specimens from Kudelin biotope were not infected. Only one of the examined specimens from Koshava biotope was infected, and 3 endohelminth species were found. Of them, *H. aduncum* had the highest mean intensity and mean abundance (MI = 7.00; MA = 3.50), while all three helminth species had the same prevalence (P% = 50.00). Both examined pontic shad specimens from Yasen biotope were infected with one helminth species - *P. laevis*. This helminth species is common to *Al. immaculata* from Koshava and Yasen biotopes. *P. laevis* had the same mean intensity in both biotopes (MI = 1.00), but higher mean abundance and prevalence in Yasen biotope (MA = 1.00; P% = 100.00) (Table 3).

Table 3. Species diversity and ecological indices in the helminth community of *Alosa immaculata* from the Danube River

***Alosa immaculata* (N = 2 / Koshava)** **Parasite species**	**n**	**p**	**MI**	**MA**	**P%**	**R**
Lecithaster confusus Odhner, 1905	1	1	1.00	0.50	50.00	1
Pomphorhynchus laevis (Zoega in Müller, 1776) Porta, 1908	1	1	1.00	0.50	50.00	1
Hysterothylacium gadi aduncum (Rudolphi, 1802) Deardorff et Overstreet, 1981 (larvae)	1	7	7.00	3.50	50.00	7
***Alosa immaculata* (N = 2 / Yasen)** **Parasite species**	**n**	**p**	**MI**	**MA**	**P%**	**R**
Pomphorhynchus laevis (Zoega in Müller, 1776) Porta, 1908	2	2	1.00	1.00	100.00	1

N - number of investigated fish; n - number of infected fish; p - number of fish parasites; MI - mean intensity; MA - mean abundance; P% - prevalence; R - range

The first intermediate host of *L. confusus* is the snail *Odostomia trifida* (Totten, 1834), and the second host is the crustaceans *Acartia clausi* Giesbrecht, 1889; *Centropages hamatus* (Lilljeborg, 1853). Definitive hosts are *Clupea harengus* Linnaeus, 1758; *Al. immaculata*; *Al. tanaica* (Grimm, 1901); *Clupeonella cultriventris* (Nordmann, 1840); *Atherina boyeri*

Risso, 1810; *Ath. hepsetus* Linnaeus, 1758, others (Bykhovskaya-Pavlovskaya et al., 1962; Gaevskaya et al., 1975; Kakacheva-Avramova, 1983; Bauer (Ed.), 1987). Definitive hosts of *P. laevis* are fish from the families Cyprinidae, Salmonidae, Percidae, Siluridae, etc. The intermediate host of the species is *Gamarus pulex* (Linnaeus, 1758) (Petrochenko, 1956; Kakacheva-Avramova, 1983; Bauer (Ed.), 1987). Typical species of *H. aduncum* are *Alosa alosa* (Linnaeus, 1758); *Alosa fallax* (Lacepède, 1803); *Cl. harengus*. In Europe, the species was reported for the following migratory freshwater fish species: *Al. immaculata*; *Salmo trutta* Linnaeus, 1758; *Salmo salar* Linnaeus, 1758; *Oncorhynchus mykiss* (Walbaum, 1792); *Chondrostoma nasus* (Linnaeus, 1758); *Tinca tinca* (Linnaeus, 1758); *Phoxinus phoxinus* (Linnaeus, 1758); *Esox lucius* Linnaeus, 1758; *Perca fluviatilis* Linnaeus, 1758; others. Intermediate hosts are the marine copepods *Acartia bifilosa* (Giesbrecht, 1881) and *Eurytemora affinis* (Poppe, 1880) (Bauer (Ed.), 1987; Moravec, 2013).

P. laevis and *H. aduncum* found in the present study were reported for pontic shad from the Bulgarian section of the Danube River (Kakacheva-Avramova et al., 1978 and Kirin et al., 2013; Nachev et al., 2022, respectively). *L. confusus* was reported for *Al. immaculata* from the Black Sea (Özer et al., 2013; Sezgin et al., 2017). Đikanović et al. (2018) reported *Contracaecum* sp. in pontic shad from the Danube River (861 river km) in the region of Prahovo, Serbia, and *Contracaecum* sp., *Contracaecum siniperca* Dogiel & Achmerov, 1946 and *Contracaecum bidentatum* (Linstow, 1899) Skrjabin, 1917 in pontic shad from the Danube delta, Romania. Grecu et al. (2020) studied pontic shad from the Romanian section of the Danube River, in the region of Cotul Pisicii and reported four species of parasites - *Mazocreas alosae* (Hermann, 1782); *Allocreadium isoporum* (Looss, 1894); *Nicolla skrjabini* (Iwanitzky, 1928) Dollfus, 1960 (syn. *Coitocoecum skrjabini* (Ivanitzky, 1928)); *Hysterothylacium aduncum* (Rudolphi, 1802) (syn. *Contracaecum aduncum* (Rudolphi, 1802)). The authors indicated that *H. aduncum* (a marine parasite species) had the highest prevalence and mean intensity (P% = 94.44, MI = 55.76 ± 7.65 in 2011; P% = 95.45, MI = 32.38 ± 6.88 in 2018), which is related to the diet of the pontic shad (a migratory species), a diet consisting of intermediate for the nematode crustaceans and fish hosts. *H. aduncum* and *M. alosae* were reported in pontic shad from the Romanian section of the Danube River - in the region of Moldova Nouă (1,048 river km) and Giurgiu (493 river km) (Stroe et al., 2021) and in the section between 169 and 197 river km (Stroe et al., 2022) (Table 4).

Table 4. Distribution of the found helminths (in the present study) of *Alosa immaculata* from the Danube River and its basin

Biotopes / **Helminth species**	Kudelin biotope	Koshava biotope	Yasen biotope	Danube River in other countries	Danube River Basin in other countries	Black Sea Basin	Danube River in Bulgaria	Danube River Basin in Bulgaria
Lecithaster confusus	-	+	-	-	-	+	-	-
Pomphorhynchus laevis	-	+	+	-	-	-	+	-
Hysterothylacium aduncum	-	+	-	+	-	-	+	-

CONCLUSIONS

During the helminthological examination of six specimens pontic shad collected from three biotopes (Kudelin, Yasen, Koshava), infection with 3 helminth species belonging to classes Trematoda, Acanthocephala, and Nematoda was found. The highest mean intensity; mean abundance and range were found for *Hysterothylacium gadi aduncum* (Rudolphi, 1802) Deardorff et Overstreet, 1981 (MI = 7.00; MA = 3.50; R = 7) of *Al. immaculata* from Koshava biotope. The highest prevalence had *Pomphorhynchus laevis* (Zoega in Müller, 1776) Porta, 1908 of *Al. immaculata* from Yasen biotope (P% = 100.00). The pontic shad is

reported for the first time as a host of the trematode *L. confusus* from the Danube River and the river basin, including on Bulgarian territory. The studied biotopes are new habitats for the found endohelminths of *Al. immaculata*.

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