AGRICULTURAL UNIVERSITY – PLOVDIV
FACULTY OF PLANT PROTECTION AND AGROECOLOGY
Department of Agroecology and Environmental Protection

Radoslava Georgieva Zaharieva

PARASITES AND PARASITE COMMUNITIES OF FISH FROM THE DANUBE RIVER – ECOLOGY AND BIODIVERSITY

ABSTRACT
of a dissertation for awarding educational and scientific degree "Doctor"

Scientific specialty:
"Ecology and ecosystems protection"

Supervisor:
Professor Diana Kirin, PhD

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The dissertation consist of 250 pages including 108 tables and 71 figures. A total of 206 literature sources were used: 45 in Cyrillic, 144 in Latin, and 17 online databases.

The research portion of this dissertation work was carried out in a laboratory at the Department of Agroecology and Environmental Protection, at the Agricultural University – Plovdiv.

The dissertation was discussed and proposed for defense at the Department Council of the Department of Agroecology and Environmental Protection at the Agricultural University – Plovdiv with Protocol No. 2/12.10.2022.

The defense of the dissertation will consist of 21.12.2022 at 13:00 pm in 8 auditorium of the Faculty of Plant Protection and Agroecology at the Agricultural University – Plovdiv at a meeting of the Specialized scientific jury, approved by a decision of the Faculty Council of the Faculty of Plant Protection and Agroecology with Protocol No. 26/25.10.2022 and appointed by the Rector of the Agricultural University with Order No. RD-16-1118/31.10.2022, composed of:

Internal members:

1. Assoc. prof. Penka Stancheva Zapryanova-Aleksieva, PhD – Chairman
2. Prof. Vladislav Haralampiev Popov, PhD – Reviewer

External members:

3. Prof. Diyan Mihailov Georgiev, PhD – Reviewer
4. Prof. Vasil Kostadinov Atanasov, DSc
5. Assoc. prof. Ivelin Aldinov Mollov, PhD

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The materials on the defense are available at the library of the Agricultural University – Plovdiv, 12 Mendeleev Blvd.

I. INTRODUCTION

The Danube River is the second longest river in Europe and spans ten countries for 2,857 km (Juhásová et al., 2019). Numerous islands, lakes, marshes, meadows and forests are located along the Danube River, playing an important role in the protection of the unique nature and habitats of a number of rare and endangered plant and animal species. A number of wetlands, Ramsar sites, reserves, natural parks, national parks, can be found in the adjacent territories of the Danube River and its tributaries.

The river is characterized by a rich ichthyofauna (Juhásová et al., 2019). 102 fish species have been reported for the Danube River, while 115 native fish species have been described for the river basin (Sommerwerk et al., 2009; Tockner et al., 2009; Kováč, 2015). The ichthyofauna is dominated by species from the families Cyprinidae, Percidae, Gobiidae, Cobitidae, Salmonidae and Acipenseridae (Keckeis, Schiemer, 2002). Polačik et al. (2008) reported 44 fish species during researching the ichthyofauna of the Bulgarian section of the Danube River. According to the Environmental Evaluation Report of project of the RBMP of the Danube Region (2016-2021), 66 fish species are identified for the Danube River section on Bulgarian's territory.

Fishing is among the main ways to satisfy the food needs of the population. Fish are largely present in the human diet (Nedić et al., 2018). They are distinguished by high nutritional value; provide omega-3 fatty acids, vitamins, proteins, and other nutrients, which are important for the human body (Petkovšek et al., 2012; Amer, 2014). The Danube River is the object of commercial fishing in Bulgaria.

The most commonly fished species in the Danube River are the common carp (*Cyprinus carpio* Linnaeus, 1758), wels catfish (*Sillurus glanis* Linnaeus, 1758), bighead carp (*Hypophthalmichthys nobilis* (Richardson, 1845)), silver carp (*Hypophthalmichthys molitrix* (Valenciennes, 1844)), freshwater bream (*Abramis brama* (Linnaeus, 1758)), barbel (*Barbus barbus* (Linnaeus, 1758)) (https://www.eufunds.bg/bg/pmdr/node/6441).

Most fish species are hosts of different parasite species. The parasites can be localized in various tissues and organs of fish (Amer, 2014). The Danube River, flowing through the territory of ten European countries, helps to spread a number of parasite species (Radačovská et al., 2019). Parasitism is a widespread way of life. Almost all species on Earth have parasites. It has been established that the parasites have parasites (Lafferty, 2008). The parasite species are present at different trophic levels and in different food webs, thus they can provide information both about the place that their hosts occupy in food chains and about the changes that occur in aquatic ecosystems. The species diversity of parasites in a host shows the diversity of intermediate and definitive hosts involved in their life cycles in the respective ecosystem. The absence of a host may influence the transmission of the parasite through the food chain (Marcogliese, 2004; 2005).

Most of the studies on the fish parasite fauna from the Danube River provide information on the species composition and diversity of the helminths. The data on the ecological indices, the parasite communities structure, their seasonal changes, as well as the circulation paths of the helminth flow in the Danube River, including in Bulgaria are few. Research on the helminthofauna of freshwater fish species from the Bulgarian section of the Danube River is mainly focused on the lower section of the river. Scientific research on the parasites and parasite communities of freshwater fish species from the upper section of the river in Bulgaria are sparse. Single helminthological studies of fish from the freshwater ecosystem of the Danube River after the river entered Bulgarian's territory date only to the 1960s. All this gives rise to the interest and the necessity of conducting the present study.

II. LITERATURE REVIEW

A literature review of research on helminths of the type Plathelminths (classes Trematoda, Cestoda, Acanthocephala, Nematoda) and the helminth communities of freshwater fish species from the Danube River and the river basin from the territory of Bulgaria and those from authors in other countries for the period of 1959 to 2022 is presented. The literature reference includes 101 scientific publications. Studies on the biology, ecology, and other aspects related to helminthological research of freshwater fish species in relation to the topic of the present dissertation are addressed. The results of scientific study on helminths and helminth communities of freshwater fish from the Danube River and the Danube River basin in different countries and those related to research on the territory of Bulgaria have been tracked.

As a result of the literature review, the following main summaries and conclusions are made:

1. The literature reference made for the Bulgarian section of the Danube River shows that few authors carry out research on parasites and parasite communities of freshwater fish, and a significant part of this research includes mainly the lower current of the river (in the vicinities of Silistra, Ruse, Svishtov towns, Vetren village and others). There are even fewer authors studying fish from the upper current of the Danube River on the territory of Bulgaria. For the upper section of the river, the studies were carried out in the area of Vidin town and Archar, Botevo, Koshava, Gomotartsi villages. There are also extremely few authors investigating the parasite fauna of fish from the Danube tributaries flowing through the territory of the country.
2. There is limited information in the scientific literature on the parasite communities of fish from the Danube River and the river basin.
3. The most frequent object of helminthological investigation in other countries is *B. barbus*, followed by: *S. lucioperca*, *P. fluviatilis*, *Abr. brama* and others. For the Bulgarian section of the Danube River and the river basin, *B. barbus*, followed by: *Abr. brama*, *Sq. cephalus*, *P. fluviatilis*, *R. rutilus*, *P. kessleri*, *N. melanostomus* and others are also the most studied fish for helminths.
4. The data on the parasite fauna of freshwater fish from the upper section of the Danube River in Bulgaria date to the 1960s. The most recent studies conducted in the Vidin region (Archar, Botevo, Vidin, Gomotartsi, Koshava, Novo selo) are single publications from 2003, 2010, 2012 and 2022. There are no studies from Kudelin, Yasen, Kutovo biotopes.
5. Based on the scientific publications presented in the literature review, it can be seen that some of the fish species researched in the present dissertation (*Al. immaculata*, *B. ballerus*, *B. gymnotrachelus*, *C. elongata*, *C. taenia*, *P. cultratus*, *Rhodeus amarus* (Bloch, 1782), *S. bulgarica*, *V. vimba* and others) are among the least studied species from the Danube ichthyofauna. Two of the dominant fish species in the present dissertation (*Alb. alburnus* and *Ch. nasus*) are also weakly studied for helminths. No information in the literature was found on parasite communities of *C. elongata*, *H. molitrix*, *P. cultratus*, *Rh. amarus*, *S. bulgarica*, *V. vimba* from the Danube River on the Bulgarian's territory, or on the territory of other countries through which the river flows, or for the Danube River basin. There is limited research on the parasite communities of *Ch. nasus* from the Danube River in other countries.

III. GOAL AND TASKS

The **goal** of the present work is to carry out scientific investigations on the parasite and the parasite communities of fish from the Danube River's freshwater ecosystem.

To realize this goal, the research was carried out on the following **tasks**:

1. Ecologobiological research on the helminths of freshwater fish species from the Danube River (Kudelin, Yasen, Koshava and Kutovo biotopes).
2. Study of the helminth communities of dominant fish species from the Danube River, Kudelin biotope.
   2.1. Study of the helminth communities of *Abramis brama* (Linnaeus, 1758).
   2.2. Study of the helminth communities of *Alburnus alburnus* (Linnaeus, 1758).
   2.3. Study of the helminth communities of *Chondrostoma nasus* (Linnaeus, 1758).
3. Comparative review of the helminth biodiversity of the studied fish species from the Danube River.
4. Comparative review of the helminth communities of the dominant fish species from the Danube River, Kudelin biotope.
5. Seasonal changes of the helminth communities of the dominant fish species from the Danube River, Kudelin biotope.
   5.1. Seasonal changes of the helminth communities of *Abramis brama*.
   5.2. Seasonal changes of the helminth communities of *Alburnus alburnus*.
   5.3. Seasonal changes of the helminth communities of *Chondrostoma nasus*.
   5.4. Comparative review of the seasonal changes of the helminth communities of the dominant fish species from the Danube River, Kudelin biotope.

IV. MATERIALS AND METHODS

IV.1. BRIEF NATURAL AND GEOGRAPHICAL CHARACTERISTICS OF THE DANUBE RIVER, THE DANUBE RIVER BASIN AND THE STUDIED BIOTOPES

IV.1.1. Brief natural and geographical characteristics of the Danube River, the Danube River basin

The Danube River is the second-largest river in Europe (Juhásová et al., 2019) and the longest river flowing through the territory of the European Union, with a length of 2,857 km (https://danube-region.eu/wp-content/uploads/2019/08/eusdr_success_stories_bg.pdf). The Danube River springs from the Black Forest Mountains (Germany) at 1,078 meters above sea level and flows into the Black Sea through an extensive delta, crossing or touching the borders of 10 European countries – Germany, Austria, Slovakia, Hungary, Croatia, Serbia, Bulgaria, Romania, Moldova and Ukraine (https://www.icpdr.org/main/).

The catchment basin of the Danube River covers the territory of 19 European countries. Passing through a large part of Europe, the Danube River is important for the protection of the huge species diversity (https://www.icpdr.org/main/).

IV.1.2. Brief natural and geographical characteristics of studied biotopes from the Danube River

Vidin District is located in the Northwestern region of Bulgaria and covers an area of 3,032.9 km^2. The region includes 11 municipalities. Only the municipalities of Dimovo, Vidin, Novo Selo and Bregovo border on the Danube River. **The village of Koshava** is located at 807 river kilometers of the Danube River. Near the village of Koshava is the island of the same name "Koshava". **The village of Kutovo** is located on the right bank of the Danube River, 801 river kilometers. Near it is the Protected Site "Ostrov Kutovo" (http://old.vidin.bg/wp-content/uploads/2017/06/Общински%20план%20за%20развитие%20на%

20община%20Видин%202014-2020.pdf; https://www.strategy.bg/StrategicDocuments/View.aspx?lang=bg-BG&Id=143). **The village of Novo Selo** is located on the Danube bank, 25 km from the Vidin town. On the territory of the village is located the area "Kamaka", as well as the Protected Site "Nahodishte na Ruzhevidna povetitsa", declared by Ordinance No.RD-34 from 16.01.2013 under the Law on Protected Areas. **The village of Yasen** is located along the Danube River and is 15 km from the Vidin town (https://www.strategy.bg/StrategicDocuments/View.aspx?lang=bg-BG&Id=148; https://vidin.government.bg/upload/files/oblastni_strategii/OPR_Novo_Selo_2014-2020.pdf; http://obshtina-novoselo.com/bg/?p=2039; http://obshtina-novoselo.com/bg/). **The village of Kudelin** is the first settlement on the Bulgarian section of the Danube River (844 river km). The village is located in the Vidin Lowland, surrounded by the rivers Timok and Danube. The lowest parts of the territory of the village are located on the bank of the Danube River (https://vidin.government.bg/upload/files/oblastni_strategii/OPR_Bregovo_2014-2020.pdf).

IV.2. MATERIALS

During the period 2019-2021, 2,367 specimens belonging to 8 families and 31 species of freshwater fish were collected. Fish specimens were collected from the Danube River in the vicinities of 5 settlements: Koshava village, Kudelin village, Kutovo village, Novo selo village and Yasen village, Vidin District, designated as biotopes (Fig. 1; Table 1). The biotopes were visited to collect freshwater fish during the spring (22.03.-22.06.), summer (22.06.-22.09.) and autumn (22.09.-22.11.). The fish were caught using the authorized gear and tools with the permits obtained from the EAFA and MA for catching fish for scientific purposes.

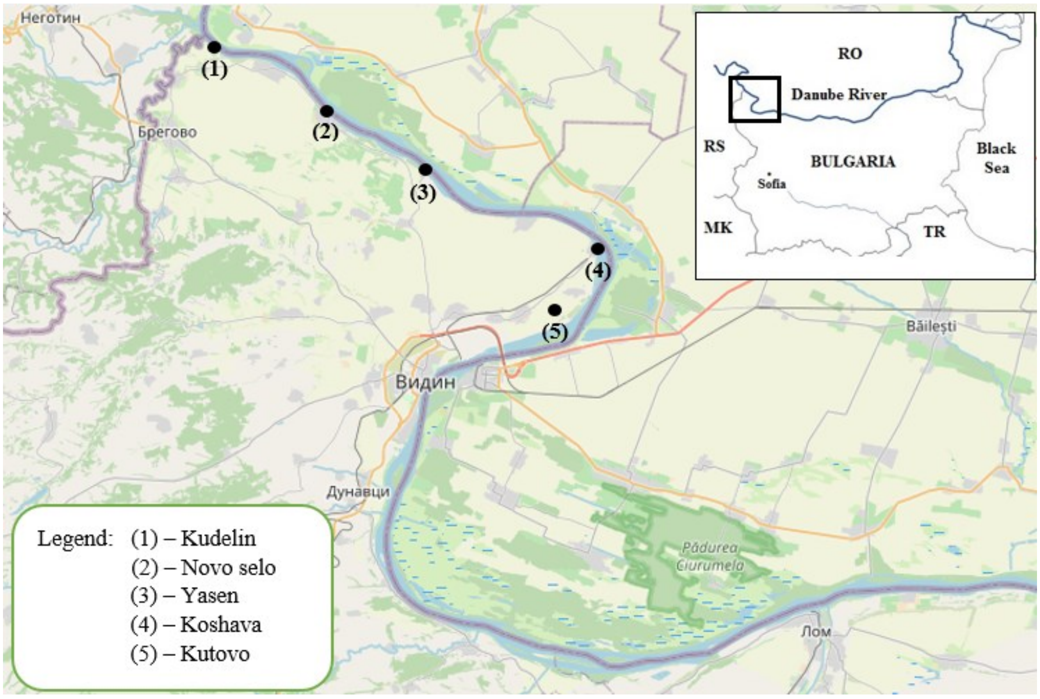


Fig. 1. Location of the Danube River's biotopes from which the fish were caught (by ViewRanger (https://www.viewranger.com/) with changes and addition)

Table 1. Number of studied fish species by biotopes

№	Biotopes Fish species	Koshava	Kudelin	Kutovo	Novo selo	Yasen
	Centrarchidae					
1.	***Lepomis gibbosus*** (Linnaeus, 1758)	2	-	-	-	-
	Clupeidae					
2.	***Alosa immaculata*** Bennett, 1835	2	2	-	-	2
	Cobitidae					
3.	***Cobitis elongata*** Heckel & Kner, 1858	2	2	-	-	-
4.	***Cobitis taenia*** Linnaeus, 1758	6	-	-	-	-
5.	***Sabanejewia bulgarica*** Drensky, 1928	-	1	-	-	-
	Cyprinidae					
6.	***Abramis brama*** (Linnaeus, 1758)	16	332	-	2	1
7.	***Alburnus alburnus*** (Linnaeus, 1758)	81	507	4	139	62
8.	***Ballerus ballerus*** (Linnaeus, 1758)	-	1	-	-	-
9.	***Ballerus sapa*** (Pallas, 1814)	5	-	-	1	-
10.	***Barbus barbus*** (Linnaeus, 1758)	3	12	-	-	-
11.	***Carassius gibelio*** (Bloch, 1782)	-	7	-	2	-
12.	***Chondrostoma nasus*** (Linnaeus, 1758)	42	298	-	-	9
13.	***Ctenopharyngodon idella*** (Valenciennes, 1844)	-	1	-	-	-
14.	***Cyprinus carpio*** Linnaeus, 1758	2	12	-	6	-
15.	***Gobio gobio*** (Linnaeus, 1758)	1	-	-	-	-
16.	***Hypophthalmichthys molitrix*** (Valenciennes, 1844)	-	7	-	-	-
17.	***Leuciscus aspius*** (Linnaeus, 1758)	-	58	-	6	-
18.	***Pelecus cultratus*** (Linnaeus, 1758)	-	44	-	-	-
19.	***Rhodeus amarus*** (Bloch, 1782)	-	1	-	-	-
20.	***Rutilus rutilus*** (Linnaeus, 1758)	6	132	-	14	-
21.	***Scardinius erythrophthalmus*** (Linnaeus, 1758)	-	24	-	2	-
22.	***Squalius cephalus*** (Linnaeus, 1758)	-	7	-	-	-
23.	***Vimba vimba*** (Linnaeus, 1758)	34	234	4	18	-
	Esocidae					
24.	***Esox lucius*** Linnaeus, 1758	-	1	-	-	-
	Gobiidae					
25.	***Babka gymnotrachelus*** (Kessler, 1857)	-	5	-	4	-
26.	***Neogobius fluviatilis*** (Pallas, 1814)	5	31	1	11	-
27.	***Neogobius melanostomus*** (Pallas, 1814)	3	8	-	4	-
	Percidae					
28.	***Gymnocephalus schraetser*** (Linnaeus, 1758)	-	5	-	-	-
29.	***Perca fluviatilis*** Linnaeus, 1758	-	84	-	23	-
30.	***Sander lucioperca*** (Linnaeus, 1758)	-	18	-	1	-
	Siluridae					
31.	***Silurus glanis*** Linnaeus, 1758	1	6	-	-	-
To-tal:	31 species 2367 specimens	16 species 211 speci-mens	28 species 1840 specimens	3 species 9 speci-mens	14 species 233 speci-mens	4 species 74 speci-mens

V.3. METHODS OF RESEARCH
IV.3.1. Field and laboratory examinations

The helminthological examination of the hosts was preceded by the species identification of the fish specimens using basic guides (Karapetkova, Zhivkov, 2006; Kottelat, Freyhof, 2007; Fröse, Pauly, 2022). The scientific name of the species was found in FishBase Database.

Fish were studied for helminths in an open field laboratory immediately after their capture. The analyses were completed in a laboratory of the Department of AEP, Agricultural University – Plovdiv. A stereomicroscope „Micros“, Austria was used. The collected host material was examined by the method of complete helminthological autopsy (Skrjabin, 1946; Zashev, Margaritov, 1966; Moravec, 2013).

IV.3.2. Statistical processing

In the present dissertation main ecological indices were used in accordance with the definitions of Margolis et al. (1982), Magurran (1988), Kennedy (1993, 1997) and others. **The parasite community structure** was analyzed at two levels: component communities and infracommunities. **The component community** was characterized by the indicators mean intensity (MI), mean abundance (MA), prevalence (P%). **The infracommunities** were defined based on the following indicators: total number of species; mean number of species; total number of specimens; mean number of specimens; Brillouin's diversity index (HB); Pielou's evenness index (E); Simpson's dominance index (C) (Magurran, 1988) and others.

Statistical analysis of the data was performed using MS Excel (Microsoft, 2010), BioDiversity Pro (McAleece et al., 1997) and Statistica 10 (StatSoft Inc., 2011).

V. RESULTS
V.1. BIOLOGICAL DIVERSITY OF THE ESTABLISHED HELMINTH SPECIES

In this helminthological study of 2,367 fish specimens belonging to 31 species collected from 5 biotopes from the Danube River during the period 2019-2021 an infection with 20,391 helminth specimens, belonging to the 4 classes (Trematoda, Cestoda, Acanthocephala and Nematoda), 22 families, 27 genus and 34 species was established. Synonyms; taxonomic position; hosts; localization; deposits; prevalence; range of invasion (minimum - maximum); season; brief data on the biology of the species (intermediate and definitive hosts) and notes on the species were recorded for each of the established helminth species.

In the present section the following host species abbreviations are used: Aa – *Alburnus alburnus*; Ab – *Abramis brama*; Ai – *Alosa immaculata*; Bb – *Barbus barbus*; Bg – *Babka gymnotrachelus*; Bs – *Ballerus sapa*; Ce – *Cobitis elongata*; Cg – *Carassius gibelio*; Ci – *Ctenopharyngodon idella*; Cn – *Chondrostoma nasus*; Cyc – *Cyprinus carpio*; El – *Esox lucius*; Gs – *Gymnocephalus schraetser*; La – *Leuciscus aspius*; Nf – *Neogobius fluviatilis*; Nm – *Neogobius melanostomus*; Pc – *Pelecus cultratus*; Pf – *Perca fluviatilis*; Rr – *Rutilus rutilus*; Sb – *Sabanejewia bulgarica*; Sc – *Squalius cephalus*; Se – *Scardinius erythrophthalmus*; Sg – *Silurus glanis*; Sl – *Sander lucioperca*; Vv – *Vimba vimba*.

The symbol "*" indicates the helminth species, for which new hosts were established in Bulgaria; "**" indicates the helminth species, for which new hosts were established for the Danube River and its basin in Bulgaria; "•" indicates the helminth species, for which new hosts were established for the Danube River and its basin; "***" are helminth species that are new for the Danube River and its basin in Bulgaria; "****" are helminth species that are new for the Danube River and its basin.

The presented information is designed based on the example of *Sphaerostoma bramae* (Müller, 1776) Lühe, 1909.

5.2. Genus *Sphaerostomum* Stiles et Hassal, 1898

5.2.1. */**/•/****Sphaerostoma bramae* (Müller, 1776) Lühe, 1909

Synonyms: *Fasciola bramae* Müller, 1776; *Sphaerostoma maius* Janiszewska, 1949; *Stephanostomum maius* (Janiszewska, 1949) Yamaguti, 1953; *Sphaerostomum bramae* (Müller, 1776).

Hosts: */***Abramis brama*, */***Alburnus alburnus*, */**/•*Chondrostoma nasus*, */**/•*Vimba vimba*, */**/•*Barbus barbus*, **/•*Leuciscus aspius*, ***Squalius cephalus*, */**/•*Perca fluviatilis*, */**/•*Silurus glanis*.

Localization: intestine.

Deposits: Kudelin[Aa,Ab,Cn,Vv,La,Sc,Pf,Sg], Koshava[Cn,Vv,Bb], Novo selo[Aa,Vv,La].

Prevalence, minimum and maximum values of invasion by biotopes and hosts are presented in **Table 11**:

Table 11. Prevalence and range of invasion on *Sphaerostoma bramae*

Biotope	Host species	Prevalence Infected/Examined	Range (min. – max.)
Kudelin	*Alburnus alburnus*	13/507	1-6
Novo selo	*Alburnus alburnus*	2/139	1-5
Kudelin	*Abramis brama*	3/332	1-4
Kudelin	*Chondrostoma nasus*	4/298	1-2
Koshava	*Chondrostoma nasus*	1/42	1
Kudelin	*Vimba vimba*	4/234	1-62
Koshava	*Vimba vimba*	1/34	4
Novo selo	*Vimba vimba*	1/18	1
Koshava	*Barbus barbus*	1/3	8
Kudelin	*Leuciscus aspius*	1/58	1
Novo selo	*Leuciscus aspius*	1/6	1
Kudelin	*Squalius cephalus*	1/7	1
Kudelin	*Perca fluviatilis*	2/84	1-2
Kudelin	*Silurus glanis*	2/6	1-10

Season: spring[Aa,Ab,Cn,Sg,Vv,Bb]; summer[Aa,Cn,Vv,La]; autumn[Aa,Ab,Cn,Vv,Sc,Pf,Sg].

Biology of the species: Marita develops in the intestine of Cyprinidae, less often in *P. fluviatilis*; *G. cernua*; *Es. lucius* and others (Bykhovskaya-Pavlovskaya et al., 1962; Gaevskaya et al., 1975; Bauer, 1987). The first intermediate host is *B. tentaculata*, the second – leech from Herpobdella (Bykhovskaya-Pavlovskaya et al., 1962; Gaevskaya et al., 1975; Bauer, 1987).

Notes on the species: *Sphaerostoma* sp. were reported in *Abr. brama* from the Serbian section of the Danube River near Zemun and Višnjica (Đikanović et al., 2011a). *Sph. bramae* was found in *Sq. cephalus* and *Abr. brama* from the Serbian section of the Danube River (Cakic et al., 2004; Đikanović et al., 2011a); in fish from Romanian waters, as well as from the Danube River (Cojocaru, 2007, 2009, 2010). *Sph. bramae* was reported for the Danube River basin in *Sq. cephalus* from the Morava River near Brodské, Czech Republic (Gelnar et al., 1997); in the intestine of *Abr. brama* from the Giroc-Chişoda channel, Banat region, Romania (Cojocaru, 2003); in *Bl. bjoerkna* and *Ct. idella* from Latorica River, Slovakia (Oros, Hanzelová, 2009; Hanzelová et al., 2011); in *Abr. brama*, *Bl. bjoerkna*, *Sq. cephalus*, *R. rutilus* and *Alb. alburnus* from the Danube River basin in Serbia (Djikanovic et al., 2011b).

V.2. BIOLOGICAL DIVERSITY OF THE HELMINTHS BY HOSTS

The section presents the distribution of helminth species (Trematoda, Cestoda, Acanthocephala, Nematoda) by established fish species during the research. Helminths were not detected in six (*L. gibbosus, C. taenia, B. ballerus, G. gobio, H. molitrix* and *R. amarus*) of the 31 examined fish species. For the other 25 fish species, data on their biology, ecology and distribution are discussed (by Muus, Dahlström, 1968; Vostradovsky, 1973; Shireman, Smith, 1983; Miller, 1986; Skelton, 1993; Crossman, 1996; Bianco, 1998; Karapetkova, Zhivkov, 2006; Bogoev, 2007; Kottelat, Freyhof, 2007; Golemanski (Ed.), 2011; Vassilev et al., 2012; Mihov, 2014, Yankov et al., 2015; Margaritova, 2019; Page, Burr, 2011; Fröse, Pauly, 2022; https://natura2000.moew.government.bg; http://ribitebg.free.bg).

V.2.1. HELMINTHS AND HELMINTH COMMUNITIES OF DOMINANT FISH SPECIES

Dominant fish species in the ichthyocenoses of the freshwater ecosystem of the Danube River are *Abr. brama*, *Alb. alburnus* and *Ch. nasus*. The three dominant fish species are selected as models for the analysis of the helminth communities.

The presentation method of helminths and the helminth communities is based on the example of *Chondrostoma nasus* (Linnaeus, 1758).

1.3. Genus Chondrostoma

1.3.1. *Chondrostoma nasus* (Linnaeus, 1758); Common nase

Ch. nasus is a freshwater, benthopelagic, gregarious fish. Adults are found in moderate to fast-flowing rivers with rocky or gravel bottom. Younger specimens inhabit the bottom of coastal areas. With increasing age, they move to fast-flowing water. Young fish feed on invertebrates, and adults use algae for food, which they scrape off stones and rocks. The common nase has an long body slightly flattened laterally covered with large scales. All fins are orange-yellowish, except the dorsal fin, which is smoky grey. A distinguishing marks of the common nase is the shape and location of the mouth. It is lower, located along the entire width of the head. In Bulgaria, it inhabits the water of the Danube River and all rivers flowing into the Danube River.

An ecologoparasitological study on **298 specimens of *Ch. nasus* from Kudelin biotope** was conducted. Parasites were found in 262 specimens (87.92%) of the examined specimens of common nase. 11 helminth species belonging to 4 classes were identified: class Trematoda (3 species – *All. isoporum*, *N. skrjabini*, *Sph. bramae*), class Cestoda (2 species – *Pr. torulosus*, *Sch. acheilognathi*), class Acanthocephala (2 species – *Ac. anguillae*, *P. laevis*) and class Nematoda (4 species – *Contracaecum* sp., *Hysterothylacium* sp., *Ps. tomentosa*, *R. acus*).

The subjects of the study were also **42 specimens of *Ch. nasus* from Koshava biotope**. Helminths were found in 32 specimens of common nase (76.19%). Seven parasite species belonging to 4 classes were identified: class Trematoda (3 species – *All. isoporum*, *N. skrjabini*, *Sph. bramae*), class Cestoda (1 species – *Sch. acheilognathi*), class Acnthocephala (1 species – *P. laevis*) and class Nematoda (2 species – *Contracaecum* sp., *R. acus*).

During the this period (2019-2021) **9 specimens of *Ch. nasus* from Yasen biotope** were also studied for helminths. From them, 3 specimens (33.33%) were infected. Two helminth species, which belong to the classes Acanthocephala (*P. laevis*) and Nematoda (*Contracaecum* sp.) were found.

For the studied period (2019-2021), the greatest helminth diversity was found in common nase from the Danube River, Kudelin biotope (11 species), followed by Koshava biotope (7 species) and Yasen biotope (2 species). Two of the established helminths (*P. laevis* and *Contracaecum* sp.) were found in common nase in all three studied biotopes (Kudelin, Koshava and Yasen). The nematodes *Contracaecum* sp. from the Kudelin biotope have the highest ecological indices (MI=29.28, MA=25.15 and P%=85.91), and those from the Yasen biotope have the lowest (MI=1.50, MA=0.33 and P%=22.22). In regard to *P. laevis*, *P. laevis* in the common nase from the Yasen biotope has the highest prevalence (P%=11.11), and – the one from the Kudelin biotope the lowest (P%=2.01). The mean intensity of acanthocephalan is the same for all three biotopes (MI=1.00). Five helminth species (*All. isoporum*, *N. skrjabini*, *Sph. bramae*, *Sch. acheilognathi*, *R. acus*) were common to Kudelin and Koshava biotopes. Four of the helminths found in the Kudelin biotope (*Pr. torulosus*, *Ac. anguillae*, *Hysterothylacium* sp., *Ps. tomentosa*) were not found in the other biotopes (Koshava and Yasen).

Helminth communities of *Chondrostoma nasus*

The component community and infracommunity of *Ch. nasus* from the Kudelin biotope (2019-2021) represent the largest sample from this biotope and thus are discussed in greater detail.

Component community of Chondrostoma nasus from the Danube River, Kudelin biotope

In the component community of common nase from the Danube River, Kudelin biotope, with the largest number of specimens were helminths belonging to the class Nematoda (4 species with 7,521 specimens), followed by helminths from the class Trematoda (3 species with 14 specimens). Helminths from the classes Acanthocephala and Cestoda have an equal number of species and specimens (2 species with 7 specimens). A core species helminth of the component community of common nase from the Danube River (Kudelin biotope) was *Contracaecum* sp. (P%=85.91). All other parasites were accidental parasite species (P<10%). With the highest ecological indices was *Contracaecum* sp. – MI=29.28, MA=25.15, P%=85.91, respectively. With the lowest ecological indices were *All. isoporum* and *Ac. anguillae* (MI=1.00, MA=0.003, P%=0.34) (**Table 48**).

Table 48. Species diversity and ecological indices of the component community of *Chondrostoma nasus* from the Danube River, Kudelin biotope

Chondrostoma nasus **N=298**	**Egological indices**					
Parasite species	**n**	**p**	**MI±SD**	**MA±SD**	**P%±SD**	**Range**
Allocreadium isoporum (Looss, 1894) Looss, 1902	1	1	1.00	0.003	0.34	1
Nicolla skrjabini (Iwanitzky, 1928) Dollfus, 1960	6	8	1.33±0.28	0.03±0.02	2.01±1.61	1-2
Sphaerostoma bramae (Müller, 1776) Lühe, 1909	4	5	1.25±0.26	0.02±0.03	1.34±2.35	1-2
Proteocephalus torulosus (Batsch, 1786) Nufer, 1905	2	3	1.50±0.57	0.01±0.02	0.67±1.19	1-2
Schyzocotyle acheilognathi (Yamaguti, 1934) Brabec, Waeschenbach, Scholz, Littlewood & Kuchta, 2015	2	4	2.00±0.38	0.01±0.02	0.67±0.85	2
Acanthocephalus anguillae (Müller, 1780) Lühe, 1911	1	1	1.00	0.003	0.34	1
Pomphorhynchus laevis (Zoega in Müller, 1776) Porta, 1908	6	6	1.00±0.57	0.02±0.01	2.01±14.13	1
Contracaecum sp. (larvae)	256	7496	29.28±16.20	25.15±13.88	85.91±8.07	1-315
Hysterothylacium sp. (larvae)	1	3	3.00±0.57	0.01±0.01	0.34±4.27	3
Pseudocapillaria tomentosa (Dujardin, 1843) Moravec, 1987	1	2	2.00±0.38	0.01±0.006	0.34±0.85	2
Raphidascaris acus (Boch, 1779) (larvae)	5	20	4.00±1.39	0.07±0.53	1.68±2.29	1-8

N – number of investigated fish; **n** – number of infected fish; **p** – number of fish parasites; **MI** – mean intensity; **MA** – mean abundance; **P%** – prevalence

Contracaecum sp. is allochthonous species for the endohelminth communities of common nase from the freshwater ecosystem of the Danube River, and the remaining 10 helminth species are autochthonous species.

Infracommunity of Chondrostoma nasus from the Danube River, Kudelin biotope

A total of 298 specimens of common nase were caught and examined for parasites, of which 36 specimen (12.08%) were not infected and 262 specimens (87.92%) were infected. Of the infected common nase specimens, 241 specimens (80.87%) were infected with only 1 helminth species, 19 specimens (6.38%) were infected with 2 helminth species, and 2 specimens (0.67%) with 3 helminth species (**Table 49**).

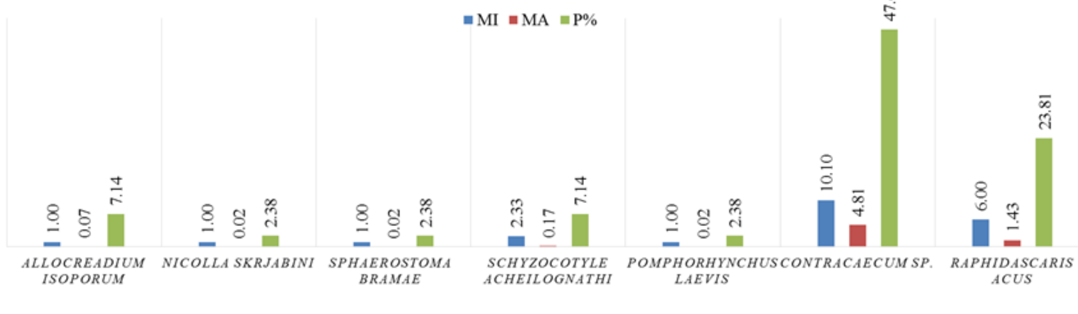
Table 49. Infracommunity of *Chondrostoma nasus* from the Danube River, Kudelin biotope

	Number of parasite specimens			
	0	**1**	**2**	**3**
Number of specimens *Chondrostoma nasus*	36	241	19	2
Total number of species (Mean number of species ± SD)	11 (1.75±1.5)			
Total number of specimens (Mean number of specimens ± SD)	7549 (76.25±110.40)			
Range	1-315			
Brillouin's diversity index (HB)	0.05±0.07			
Pielou's evenness index (E)	0.02±0.03			
Simpson's dominance index (C)	0.98±0.02			

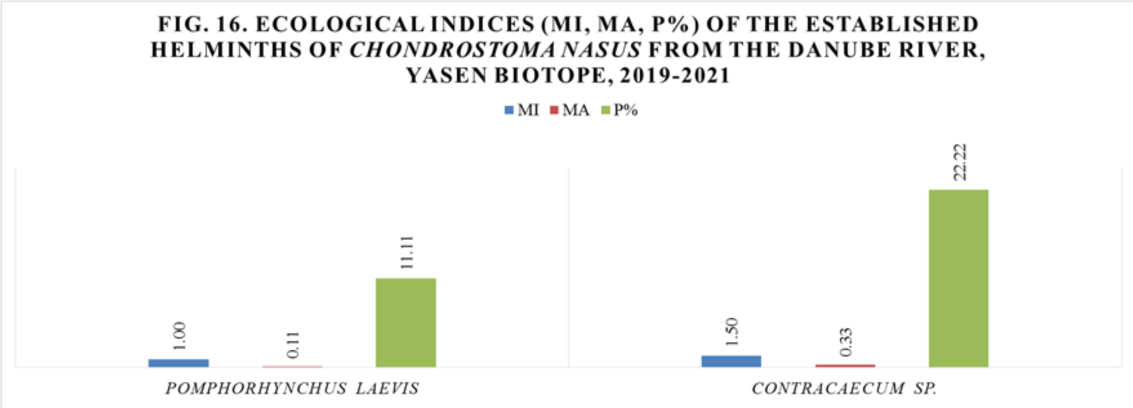
In the infracommunity of common nase from the Danube River (Kudelin biotope), the number of the detected helminths ranged from 1 to 315 in one specimen of *Ch. nasus*. A total of 7,549 helminth specimens were studied. The determined Brillouin's diversity index and Pielou's evenness index were low, due to the highest prevalence of one species, *Contracaecum* sp. The ecological indices of the other helminth species were low, which also determined the very high index of dominance (**Table 49**).

In this study of *Ch. nasus* from Koshava biotope, 7 helminth species were found, with the highest ecological indices were the nematodes *Contracaecum* sp. (MI=10.10, MA=4.81, P%=47.62) and *R. acus* (MI=6.00, MA=1.43, P%=23.81). The lowest values of MI, MA and P% were reported for the trematodes *N. skrjabini* and *Sph. bramae*, and for the acanthocephalan *P. laevis* (MI=1.00, MA=0.02, P%=2.38) (**Fig. 15**).

FIG. 15. ECOLOGICAL INDICES (MI, MA, P%) OF THE ESTABLISHED HELMINTHS OF *CHONDROSTOMA NASUS* FROM THE DANUBE RIVER, KOSHAVA BIOTOPE, 2019-2021



Two parasite species were found in common nase from Yasen biotope during the research period. The highest mean intensity, mean abundance and prevalence were established for *Contracaecum* sp. (MI=1.50, MA=0.33, P%=22.22) (**Fig. 16**).



Discussion

The helminthofauna of common nase from the Danube River and its basin on the territory of other countries, including for the Bulgarian section of the river, as well as from its basin in Bulgaria, is relatively poorly investigated (**Table 50**).

Table 50. Species composition of parasites of *Chondrostoma nasus* from the Danube River and its basin in other countries and in Bulgaria

Authors	Deposite	Species composition of parasites of *Ch. nasus*
for the Danube River (in other countries)		
Đikanović et al., 2013	Danube River, Serbia – near Zemun (1173 river km) and Visnjica (1162 river km)	*Pr. torulosus*; cestode cysts
Jirsa et al., 2011	Danube River, Austria	*C. laticeps*; *P. laevis*
for the Danube River basin (in other countries)		
Oros, Hanzelová, 2009; Hanzelová et al., 2011	Latorica River, Slovakia	*All. markewitschi*
Djikanovic et al., 2011b	Danube River basin, Serbia	*L. intestinalis*; *P. bosniacus*
Jirsa et al., 2011	Drau River, Austria	*C. laticeps*
for the Bulgarian section of the Danube River		
Kakacheva-Avramova et al., 1978	Danube River, Vidin, Svishtov, Ruse, Lom, Tutrakan	*P. laevis*
Atanasov, 2012	Danube River, Simeonovo, Gomotartsi	*Ps. salmonicola*
	Danube River, Archar, Botevo, Svishtov	*C. laticeps*
for the Danube River basin in Bulgaria		
Kakacheva-Avramova, 1969	Ogosta River, Nishava River	*P. laevis*
Kakacheva-Avramova, 1973	Rivers in Stara planina	*Ps. salmonicola*

When comparing the prevalence of ***Pr. torulosus*** in common nase from the Serbian section of the Danube River (P%=0.12) studied by Đikanović et al. (2013) with the prevalence of the same helminth species in common nase from the Bulgarian section of the Danube River, Kudelin biotope (P%=0.67), it was established that in the present study the value for P% is slightly higher. MI and P% of ***P. laevis*** in common nase from four sites along the Danube River in Austria (MI=2, P%=27.3, Danube-Lobau; MI=1, P%=9.6, Danube-Pielach; MI=1, P%=4.4, Melk; MI=13, P%=50.0, Danube-Linz) studied by Jirsa et al. (2011) were compared with those obtained for *P. laevis* in common nase from Kudelin (MI=1, P% =2.01). The established mean intensity of *P. laevis* from Kudelin biotope was similar or lower than that for the Austrian section of the Danube River, while the prevalence was lower.

Ch. nasus, caught from the lower current of the Danube River (Vetren biotope), was subjected to parasitological examination (Kirin et al., 2013). The authors did not reported parasites of common nase.

Conclusion

As a result of the study of 298 specimens of *Ch. nasus* (from Kudelin biotope), 42 (from Kushava biotope) and 9 specimens (from Yasen biotope), 11 taxa of helminths (*All. isoporum*, *N. skrjabini*, *Sph. bramae*, *Pr. torulosus*, *Sch. acheilognathi*, *Ac. anguillae*, *P. laevis*, *Contracaecum* sp., *Hysterothylacium* sp., *Ps. tomentosa*, *R. acus*), 7 (*All. isoporum*, *N. skrjabini*, *Sph. bramae*, *Sch. acheilognathi*, *P. laevis*, *Contracaecum* sp., *R. acus*) and 2 taxa (*P. laevis*, *Contracaecum* sp.) were established, respectively. *Contracaecum* sp. is allochthonous species for the endohelminth communities of common nase from the Danube River's freshwater ecosystem, and the remaining 10 helminth species are autochthonous species.

The species *Contracaecum* sp. had the highest ecological indices in Kudelin, Koshava and Yasen biotopes. In Kudelin biotope, the highest values for mean intensity, mean abundance and prevalence of *Contracaecum* sp. (MI=29.28, MA=25.15, P%=85.91) were found, followed by Koshava biotope (MI=10.10, MA=4.81, P%=47.62) and Yasen biotope (MI=1.50, MA=0.33, P%=22.22). A core helminth species in the component community of common nase from the Danube River (Kudelin biotope) was *Contracaecum* sp. All other 10 species of parasites were accidental. *Contracaecum* sp. had the highest ecological indices (MI=29.28, MA=25.15 and P%=85.91), and *All. isoporum* and *Ac. anguillae* had the lowest (MI=1.00, MA=0.003 and P%=0.34). In the infracommunity of common nase from the Danube River, Kudelin biotope, the number of the established endohelminths was from 1 to 315. The diversity and evenness indeces (HB=0.05 and E=0.02) were low, due to the extremely high number of one species, *Contracaecum* sp. The ecological indices of the other helminth species were low, which determined a very high index of dominance (C=0.98).

The present study determined that Kudelin, Koshava and Yasen biotopes are new habitats for the helminths found in *Ch. nasus*. *Ch. nasus* is a new host for *All. isoporum*, *N. skrjabini*, *Sph. bramae*, *Sch. acheilognathi*, *Ac. anguillae*, *Contracaecum* sp., *Hysterothylacium* sp., *Ps. tomentosa* and *R. acus* in Bulgaria. *All. isoporum*, *N. skrjabini*, *Pr. torulosus*, *Ac. Anguillae* were reported for common nase from other countries (Moravec, 2001). *Pr. torulosus* was reported for common nase from the Serbian section of the Danube River. *Sch. acheilognathi* is a new taxa for the Bulgarian section of the Danube River (**Table 51**).

Table 51. Distribution of the helminths established in the present study in *Chondrostoma nasus* along the current of the Danube River and in its basin

Biotopes **Helminths specimens**	Kudelin biotope	Koshava biotope	Yasen biotope	Danube River in other countries	Danube River basin in other countries	Danube River in Bulgaria	Danube River basin in Bulgaria
Allocreadium isoporum	+	+	-	-	-	-	-
Nicolla skrjabini	+	+	-	-	-	-	-
Sphaerostoma bramae	+	+	-	-	-	-	-
Proteocephalus torulosus	+	-	-	+	-	-	-
Schyzocotyle acheilognathi	+	+	-	-	-	-	-
Acanthocephalus anguillae	+	-	-	-	-	-	-
Pomphorhynchus laevis	+	+	+	+	-	+	+
Contracaecum sp.	+	+	+	-	-	-	-
Hysterothylacium sp.	+	-	-	-	-	-	-
Pseudocapillaria tomentosa	+	-	-	-	-	-	-
Raphidascaris acus	+	+	-	-	-	-	-

V.2.2. HELMINTHS OF NOT DOMINANT FISH SPECIES

In this dissertation the helminths of 22 not dominant fish species are described. The information in the abstract is modeled after the example of *Babka gymnotrachelus* (Kessler, 1857).

5. Family Gobiidae Cuvier, 1816
5.1. Genus *Babka* Iijin, 1927
5.1.1. *Babka gymnotrachelus* (Kessler, 1857) (syn. *Neogobius gymnotrachelus* (Kessler, 1857)); Racer goby

B. gymnotrachelus is a freshwater, brackish and benthopelagic fish. It is found in small and large rivers, lakes and others, rich in aquatic vegetation. There in no air bladder present. It prefers sandy, stony and muddy bottoms. Uses insects, crustaceans, small fish and others for food. The width of the head is larger than its height. There are 8-10 dark stripes along the length of the body, lying slantingly. The species has large eyes. In Bulgaria, it occurs in the Danube River and in the lower currents and mouths of its tributaries, and also in the mouths of rivers flowing into the Black Sea. In addition, the species is also common in coastal lakes.

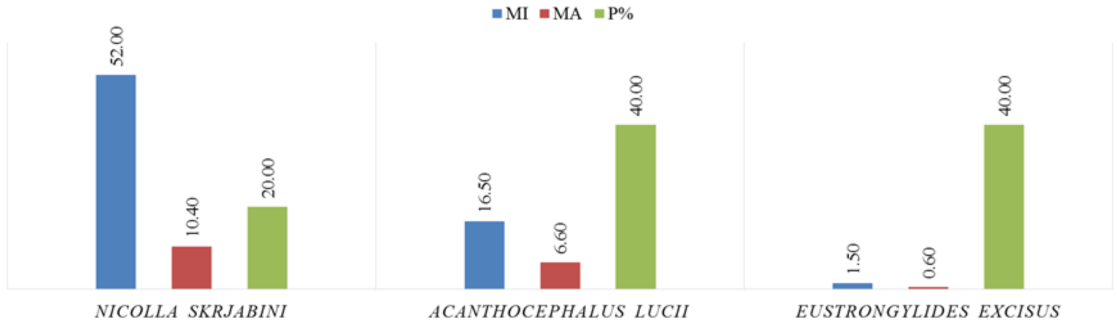
Five specimens of *B. gymnotrachelus* from the Danube River, Kudelin biotope were examined for parasites. Invasion was found in 4 specimens (80%). Three helminth species, belonging to 3 classes were found: class Trematoda (1 species – *N. skrjabini*), class Acanthocephala (1 species – *Ac. lucii*) and class Nematoda (1 species – *E. excisus*).

Four specimens of racer goby from the Danube River, Novo selo biotope were also subjected to a helmintological investigation. Three of the examined racer goby specimens (75%) from Novo selo biotope were infected.

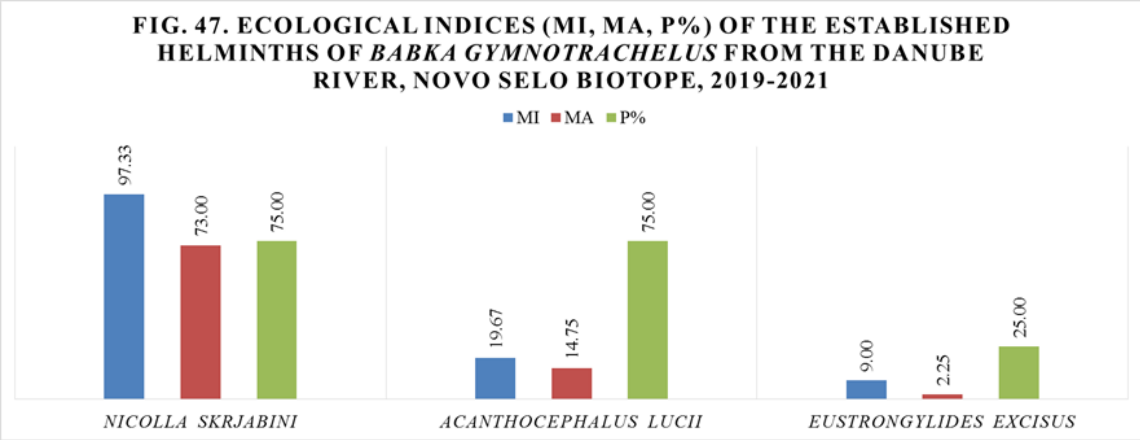
The established 3 helminth species (*N. skrjabini*, *Ac. lucii* and *E. excisus*) were the same as those reported for Kudelin biotope. The trematode *N. skrjabini* and the acanthocephalan *Ac. lucii* had the higher ecological indices in *B. gymnotrachelus* from Novo selo biotope MI=97.33, MA=73.00, P%=75.00 and MI=19.67, MA=14.75, P%=75.00, respectively in comparison with Kudelin biotope. While the nematode *E. excisus* had higher prevalence in Kudelin biotope (P%=40), and higher values for MI and MA in Novo selo biotope (MI=9.00 and MA=2.25).

The highest mean intensity and mean abundance were found for *N. skrjabini* (MI=52.00 and MA=10.40), and the highest prevalence was found for the other two helminths (*Ac. lucii* and *E. excisus*) – P%=40.00, in Kudelin biotope (**Fig. 46**).

FIG. 46. ECOLOGICAL INDICES (MI, MA, P%) OF THE ESTABLISHED HELMINTHS OF *BABKA GYMNOTRACHELUS* FROM THE DANUBE RIVER, KUDELIN BIOTOPE, 2019-2021



In the study of *B. gymnotrachelus* from Novo selo biotope, the highest prevalence was reported for *N. skrjabini* and *Ac. lucii* (P%=75.00). The highest mean intensity and mean abundance had *N. skrjabini* – MI=97.33 and MA=73.00 (**Fig. 47**).



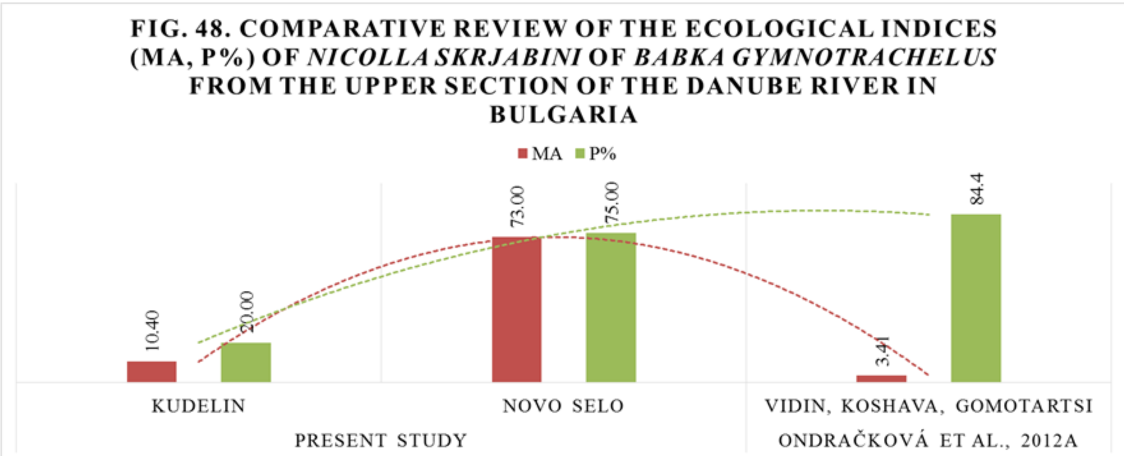
Discussion

There are extremely few investigations on the parasite fauna of the racer goby (**Table 82**).

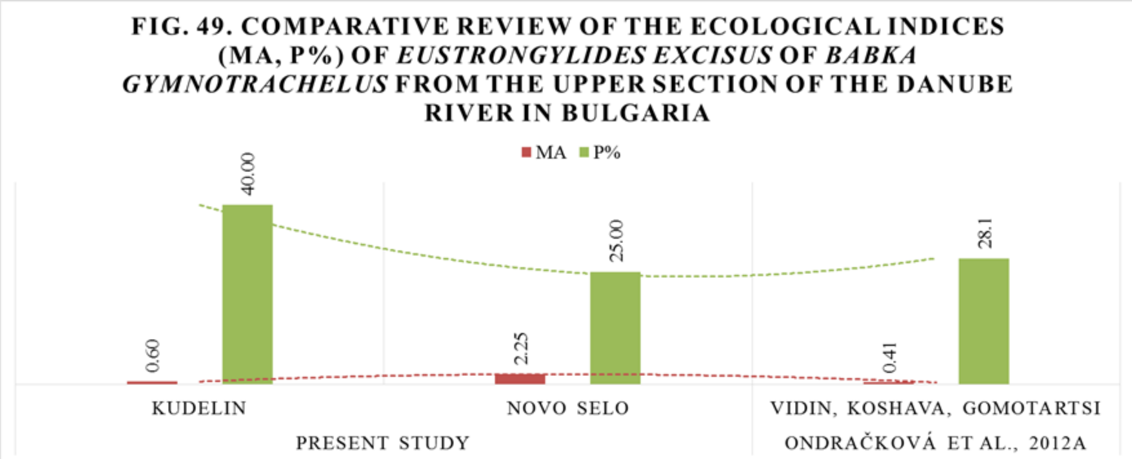
Table 82. Species composition of parasites of *Babka gymnotrachelus* from the Danube River in Bulgaria

Authors	Deposite	Species composition of parasites of *Babka gymnotrachelus*
for the Danube River in Bulgaria		
Ondračková et al., 2012a	Danube River, Vidin town, Koshava and Gomotartsi villages	*N. skrjabini*; *R. acus*; *E. excisus*; *P. laevis*

The values for MA and P% of ***N. skrjabini*** in *B. gymnotrachelus* from the Danube River (MA=3.41, P%=84.4, Vidin, Koshava, Gomotartsi) studied by Ondračková et al. (2012a) were compared with those for Kudelin (MA=10.40, P%=20.00) and Novo selo (MA=73.00, P%=75.00) biotopes. The values for MA reported in the present study were higher and those for P% were lower than those reported by Ondračková et al. (2012a) (**Fig. 48**).



Values for MA and P% of ***E. excisus*** (MA=0.41, P%=28.1, Vidin, Koshava, Gomotartsi) in *B. gymnotrachelus* studied by Ondračková et al. (2012a) were compared with the values for Kudelin (MA=0.60, P%=40.00) and Novo selo (MA=2.25, P%=25.00) biotopes. In the present study, MA and P% were higher than those reported by Ondračková et al. (2012a), except for P% from Novo selo biotope (**Fig. 49**).



Conclusion

As a result of the examination of 5 and 4 specimens *B. gymnotrachelus* from Kudelin and Novo selo biotopes, respectively, invasion with 3 endohelminth species (*N. skrjabini, Ac. lucii, E. excisus*), common to both biotopes, was established. The Kudelin and Novo selo biotopes are new habitats for the three endohelminth species of *B. gymnotrachelus*. *B. gymnotrachelus* is a new host for *Ac. lucii* from the Danube River and the river basin, including in Bulgaria. *Ac. lucii* is reported for the first time for the helminthofauna of *B. gymnotrachelus* in Bulgaria (**Table 83**).

Table 83. Distribution of the helminths established in the present study in *Babka gymnotrachelus* along the current of the Danube River and in its basin

Biotopes / Helminth species	Kudelin biotope	Novo selo biotope	Danube River in other countries	Danube River basin in other countries	Danube River in Bulgaria	Danube River basin in Bulgaria
Nicolla skrjabini	+	+	-	-	+	-
Acanthocephalus lucii	+	+	-	-	-	-
Eustrongylides excisus	+	+	-	-	+	-

V.3. COMPARATIVE REVIEW OF THE HELMINTHS OF THE ESTABLISHED FISH SPECIES FROM THE DANUBE RIVER

During an ecologoparasitological investigation of 31 fish species (from 8 families), caught from 5 biotopes along the Danube River (Kudelin, Novo selo, Yasen, Koshava and Kutovo biotopes), 34 species with 20,391 helminth specimens were found. The established helminths belong to 4 classes – **class Tremadota** (**9 species** – *All. isoporum*, *As. imitans*, *As. tincae*, *B. luciopercae*, *Ichth. pileatus*, *L. confusus*, *N. skrjabini*, *P. incognitus*, *Sph. bramae*), **class Cestoda** (**8 species** – *B. rectangulum*, *C. fennica*, *C. laticeps*, *Gl. osculata*, *N. cheilancristrotus*, *Pr. percae*, *Pr. torulosus*, *Sch. acheilognathi*), **class Acanthocephala** (**4 species** – *Ac. anguillae*, *Ac. lucii*, *Ac. tenuirostris*, *P. laevis*) and **class Nematoda** (**13 species** – *C. lacustris*, *Contracaecum* sp., *E. excisus*, *Hysterothylacium* sp., *H. aduncum*, *K. intestinalis*, *Ph. obturans*, *Ph. ovata*, *Ph. rischta*, *Ps. tomentosa*, *R. acus*, *Rh. denudata*, *Sch. petruschewskii*).

Summaries of the obtained results are presented in the abstract.

During the entire study period, the number of helminth species in all 31 examined fish species varied from 1 to 15 species. The highest number of helminth species were found in *Abr. brama* (15 species), followed by *V. vimba* (13 species) and *Ch. nasus* (11 species). The number of helminth specimens in one fish species varied from 1 to 7,828 specimens. The highest number of helminth specimens were found in *Ch. nasus* (7,828 specimens), followed by *Abr. brama* (6,024 specimens) and *B. barbus* (3,120 specimens).

Helminths were not detected in six (*L. gibbosus, C. taenia, B. ballerus, G. gobio, H. molitrix* and *R. amarus*) fish species.

The largest number of specimens of class Trematoda and class Cestoda were found in *Abr. brama* (5,840 specimens and 114 specimens, respectively); from class Acanthocephala – in *B. barbus* (3,105 specimens); from class Nematoda – in *Ch. nasus* (7,786 specimens). There were 11, 20, 12 and 11 fish species that were not infected with trematodes, cestodes, acanthocephalans and nematodes, respectively.

V.4. COMPARATIVE REVIEW OF THE HELMINTH COMMUNITIES OF THE DOMINANT FISH SPECIES (*ABRAMIS BRAMA*, *ALBURNUS ALBURNUS* AND *CHONDROSTOMA NASUS*) FROM THE DANUBE RIVER, KUDELIN BIOTOPE

For the period 2019-2021, a total of 21 helminth species (**6 species Trematoda** – *All. isoporum*, *As. imitans*, *As. tincae*, *Ichth. pileatus*, *N. skrjabini* and *Sph. bramae*; **6 species Cestoda** – *B. rectangulum*, *C. fennica*, *C. laticeps*, *N. cheilancristrotus*, *Pr. torulosus* and *Sch. acheilognathi*; **2 species Acanthocephala** – *Ac. anguillae* and *P. laevis*, and **7 species Nematoda** – *Contracaecum* sp., *E. excisus*, *Hysterothylacium* sp., *Ph. rischta*, *Ps. tomentosa*, *R. acus* and *Sch. petruschewskii*) were found in the three dominant fish species.

In the dissertation a comparative review of the helminth communities of *Abr. brama*, *Alb. alburnus* and *Ch. nasus* from Kudelin biotope at the level of the component communities and infracommunities is presented.

A summary of the results is presented in the abstract.

The largest number of endohelminth species were found in *Abr. brama* (15 species); followed by *Ch. nasus* (11 species) and *Alb. alburnus* (9 species). The largest number of helminth specimens were found in *Ch. nasus* (7,549 specimens), followed by *Abr. brama* (5,423 specimens) and *Alb. alburnus* (104 specimens). The number of helminth specimens of the classes Trematoda, Cestoda and Acanthocephala was the largest in *Abr. brama*, while of class Nematoda in *Ch. nasus*.

Common helminth species for *Abr. brama*, *Alb. alburnus* and *Ch. nasus* were *N. skrjabini*, *Sph. bramae*, *P. laevis*, *Contracaecum* sp. *N. skrjabini* and *P. laevis* had the highest ecological indices (MI=6.61, MA=0.62, P%=9.34 and MI=1.63, MA=0.16, P%=9.64, respectively) in *Abr. brama*; *Sph. bramae* had the highest ecological indices in *Alb. alburnus* (MI=2.08, MA=0.05, P%=2.56); *Contracaecum* sp. had the highest values for MI, MA and P% in *Ch. nasus* (MI=29.28, MA=25.15, P%=85.91).

In the component community of *Ch. nasus*, one core helminth species (*Contracaecum* sp.) and 10 accidental species; of *Abr. brama* – one component species (*As. imitans*) and 14 accidental species; of *Alb. alburnus* – 9 accidental species were found.

The Brillouin's diversity and Pielou's evenness indices were highest in the infracommunity of *Alb. alburnus* (HB=1.52 and E=0.75, respectively) due to the presence of species with close, low ecological indices, while Simpson's dominance indices were highest at *Abr. brama* and *Ch. nasus* (C=0.87 and C=0.98, respectively) due to the presence of species with high number (*As. imitans* (with 5,023 specimens) and *Contracaecum* sp. (with 7,496 specimens), respectively).

V.5. SEASONAL CHANGES OF THE HELMINTH COMMUNITIES OF DOMINANT FISH SPECIES FROM THE DANUBE RIVER, KUDELIN BIOTOPE

Seasonal changes in the ecological indices of the dominant fish species (*Abr. brama*, *Alb. alburnus*, *Ch. nasus*) were examined. The analysis of the component communities by season was based on the determination of the main ecological indices (MI, MA and P%) for each endohelminth species. In the section also included were indicators reporting the species composition and diversity, and the quantitative structure of the endohelminth complexes by host species. The infracommunities are described by the indicators: total number of species; total number of specimens; mean number of species; mean number of specimens; diversity of faunal complexes represented by the Brillouin's diversity index (HB); Pielou's evenness index (E) and Simpson's dominance index (C).

In the dissertation the seasonal changes of the helminth communities are presented by fish species. In the abstract the seasonal changes of the helminth communities are presented of the example of *Ch. nasus* from the Danube River, Kudelin biotope.

V.5.3. Seasonal changes of the helminth communities of *Chondrostoma nasus* from the Danube River, Kudelin biotope

A total of 298 specimens of *Ch. nasus* from Kudelin biotope were collected and subjected to a helminthological study for the period 2019-2021. Of these, 107 specimens of common nase were examined in the spring, 99 specimens in the summer and 92 specimens in the autumn.

Infection was observed in 262 specimens out of the 298 examined common nase specimens for the entire period. During the spring season, helminths were found in 100 specimens (93.46%) out of the 107 examined common nase specimens, in the summer – in 83 specimens (83.84%) out of the 99 examined common nase specimens, and in autumn – in 79 specimens (85.87%) out of the 92 examined common nase specimens.

In all seasons (for the entire period of the study) 11 helminth species were established. During the **spring season**, 9 species were established – 2 species of class Trematoda (*All. isoporum*, *N. skrjabini*), 2 species of class Cestoda (*Pr. torulosus*, *Sch. acheilognathi*), 1 species of class Acanthocephala (*P. laevis*) and 4 species of class Nematoda (*Contracaecum* sp., *Hysterothylacium* sp., *Ps. tomentosa*, *R. acus*). During the **summer season**, **6 species** were found – 1 species of class Trematoda (*Sph. bramae*), 1 species of class Cestoda (*Pr. torulosus*), 2 species of class Acanthocephala (*Ac. anguillae*, *P. laevis*) and 2 species of class Nematoda (*Contracaecum* sp., *R. acus*). For the **autumn season**, **3 species** were established – 1 species of classes Trematoda (*Sph. bramae*), Acanthocephala (*P. laevis*) and Nematoda (*Contracaecum* sp.). The largest number of trematode (2), cestode (2) and nematode (4) species were found in the spring, and the largest number of acanthocephalan (2) species were found in the summer (**Table 105**).

Table 105. Species diversity and ecological indices (MI, MA, P%) of the component communities of *Chondrostoma nasus* from Kudelin biotope by season

Season	Spring (N=107)			Summer (N=99)			Autumn (N=92)		
Helminth species	MI±SD	MA±SD	P%±SD (range)	MI±SD	MA±SD	P%±SD (range)	MI±SD	MA±SD	P%±SD (range)
Allocreadium isoporum	1.00±0.57	0.01±0.01	0.93±12.83 (1)	-	-	-	-	-	-
Nicolla skrjabini	1.33±0.84	0.07±0.06	5.61±4.82 (1-2)	-	-	-	-	-	-
Sphaerostoma bramae	-	-	-	1.00±0.57	0.01±0.02	1.01±1.65 (1)	1.33±0.76	0.04±0.07	3.26±5.41 (1-2)
Proteocephalus torulosus	2.00±1.15	0.02±0.04	0.93±1.92 (2)	1.00±0.02	0.01±0.02	1.01±1.65 (1)	-	-	-
Schyzocotyle acheilognathi	2.00±1.15	0.04±0.05	1.87±2.56 (2)	-	-	-	-	-	-
Acanthocephalus anguillae	-	-	-	1.00±0.57	0.01±0.02	1.01±1.86 (1)	-	-	-
Pomphorhynchus laevis	1.00±1.15	0.02±0.02	1.87±12.03 (1)	1.00	0.03	3.03±0.18 (1)	1.00±0.57	0.01±0.02	1.09±1.92 (1)
Contracaecum sp.	27.49±10.54	24.66±11.89	89.72±6.23 (1-315)	23.74±6.03	19.42±6.51	81.82±7.92 (1-173)	37.14±32.03	31.89±23.25	85.87±10.08 (1-232)
Hysterothylacium sp.	3.00±1.73	0.03±0.04	0.93±12.83 (3)	-	-	-	-	-	-
Pseudocapillaria tomentosa	2.00±1.15	0.02±0.02	0.93±2.56 (2)	-	-	-	-	-	-
Raphidascaris acus	4.25±2.45	0.16±1.53	3.74±5.13 (1-8)	3.00±1.73	0.03±0.05	1.01±1.75 (3)	-	-	-

N – number of investigated fish; **n** – number of infected fish; **p** – number of fish parasites; **MI** – mean intensity; **MA** – mean abundance; **P%** – prevalence

Infection of *Ch. nasus* from Kudelin biotope was observed in all three seasons and in all three years. Only two helminth species (*P. laevis* and *Contracaecum* sp.) were found in common nase during all studied seasons. *P. laevis* had the highest mean abundance and prevalence (MA=0.03 and P%=3.03) during the summer season and the same mean intensity (MI=1.00) in all three seasons. *Contracaecum* sp. had the highest values for MI and MA (MI=37.14 and MA=31.89) during the authumn, and the highest prevalence (P%=89.72) during the spring season. *Pr. torulosus* and *R. acus* were found in common nase during two of the seasons (spring and summer). *Pr. torulosus* had slightly higher mean intensity and mean abundance (MI=2.00 and MA=0.02) in the spring and a slightly higher prevalence (P%=1.01) in the summer. *R. acus* had higher ecological indices (MI=4.25, MA=0.16 and P%=3.74) during the spring. *Sph. bramae* was also detected in only two of the seasons (summer and autumn), with higher ecological indices (MI=1.33, MA=0.04 and P%=3.26) in autumn. Five of the helminths (*All. isoporum*, *N. skrjabini*, *Sch. acheilognathi*, *Hysterothylacium* sp. and *Ps. tomentosa*) found in common nase occured only in the spring. Of them, the one with the highest mean intensity was *Hysterothylacium* sp. (MI=3.00), and the one with the highest mean abundance and prevalence was *N. skrjabini* (MA=0.07 and P%=5.61). One specimen of *Ac. anguillae* was found in 1 specimen of common nase during summer. In the component community of common nase from Kudelin biotope, *Contracaecum* sp. was presented with the largest number of specimens (2,934) in autumn and with the largest number of specimens (7,496) throughout the period.

The largest number of helminth species (9 species) were found in the spring season, and the smallest number (3 species) in the autumn season. On the other hand, the largest number of helminth specimens (2,939) in common nase were found during the autumn season, and the lowest (1,932) in the summer season of research (**Table 106**). The only core species for the component communities of *Ch. nasus* was *Contracaecum* sp. All other species were accidental during the three seasons of the studied period.

Table 106. Infracommunities of *Chondrostoma nasus* from Kudelin biotope by season

***Chondrostoma nasus* from the Danube River**	**SPRING (Mean±SD)**	**SUMMER (Mean±SD)**	**AUTUMN (Mean±SD)**
Total number of species (Mean number of species ± SD)	9 (4.33±3.21)	6 (3.33±0.58)	3 (1.67±0.58)
Total number of specimens (Mean number of specimens ± SD)	2678 (23.69±40.01)	1932 (21.95±26.33)	2939 (35.41±43.72)
Range	1-315	1-173	1-232
Brillouin's diversity index (HB)	0.01±0.09	0.11±0.02	0.04±0.03
Pielou's evenness index (E)	0.01±0.04	0.05±0.02	0.02±0.04
Simpson's dominance index (C)	0.99±0.03	0.97±0.006	0.98±0.01

The Brillouin's diversity index and the Pielou's evenness index were highest for the summer (HB=0.11; E=0.05) and lowest for the spring (HB=0.01; E=0.01). In general, these indices had very low values. Significant differences were obtained for diversity index values in the spring and summer seasons (Bootstrap 2-sample t-test, t=36.57, df=2, p=0.02<0.05). The remaining dependencies for this index (HB) did not have statistically significant differences with respect to spring and autumn values (t=11.83, df=2, p=0.07>0.05); summer and autumn (t=3.09, df=2, p=0.24>0.05). Regarding the evenness index, no significant differences in values were obtained in the three seasons (Kruskal-Wallis One-Way Nonparametric AOV test, H=1.47, p=0.48>0.05). The Simpson's dominance index was highest for the spring (C=0.99), associated with the highest ecological indices of the core species *Contracaecum* sp. The differences in the indicators of this index were close and statistically insignificant for the values of the index in the spring and autumn periods (t=12.0, df=2, p=0.07>0.05); summer and autumn (t=3.0, df=2, p=0.25>0.05), but were statistically significant for spring and summer values (t=36.0, df=2, p=0.02<0.05).

A significant positive correlation was found between MI and P% with endohelminths in all three seasons (Spearman correlation coefficient, r_s; r_s>0.05, n=3-9, p=0.00001<0.05).

Mean intensity (MI) was statistically significant between the spring and autumn seasons (Bootstrap 2-sample t-test, t=2.85, df=32, p=0.002<0.05); summer and autumn (t=4.0, df=32, p=0.0001<0.05), and was not statistically significant for spring and summer (t=1.40, df=32, p=0.17>0.05). Significant differences in P% of helminths were established between the three seasonal samples (Kruskal-Wallis test, H=3.07, p=0.04<0.05). In general, the prevalence of the endohelminths in the helminth complexes of *Ch. nasus* were at maximum in the spring (χ^2=63.68, df=32, p=0.0007<0.05), were best expressed in the core species *Contracaecum* sp., where a decrease was established in the summer and a second peak was observed in the autumn. The season was a determining factor for variation in the invasion parameters (Friedman test; F=10.31, df=2, p=0.005<0.05). As most of the established species were accidental in the component communities of *Ch. nasus* except *Contracaecum* sp., to remove the effect of variation in the invasion parameters associated with them, only species present in all seasonal samples (*Contracaecum* sp. and *P. laevis*) were subjected to further analysis. *Contracaecum* sp. was a core species and *P. laevis* was an accidental species for the helminth complexes of the common nase during the three seasons of the studied period. The highest values of mean intensity and abundance were obtained in the autumn, followed by those of the spring and the lowest in the summer. Significant differences in the mean intensity were found for *Contracaecum* sp. between the summer and autumn values (t=28.25, df=2, p=0.03<0.05). The obtained distributions of the values for the indicator were not significant for spring and summer (t=3.06, df=2, p=0.25>0.05); spring and autumn (t=9.24, df=2, p=0.09>0.05). The prevalences (P%) of *Contracaecum* sp. in the three seasons were high, but the differences between them were not statistically significant (Kruskal-Wallis One-Way NAOV test, H=1.68, p=0.43>0.05). In *P. laevis*, the ecological indices were very low in all three seasons. The mean intensity (MI) and mean abundance (MA) had the higher values in summer, followed by those in spring and were the lowest in autumn. The difference in the distributions of the values for these indicators in the three seasons was not significant (Kruskal-Wallis One-Way NAOV test, H=2.67, p=0.26>0.05). In contrast, the prevalence (P%) showed the highest values in spring and decreased approximately twofold from spring to autumn (t=4460.19, df=2, p=0.0002<0.05 for spring and summer; t=39.14, df=2, p=0.02<0.05 for spring and autumn; t=113.97, df=2, p=0.008<0.05 for summer and autumn).

The results of this study show that regardless of the number of established species, the ecological indices were low. The helminth complexes of *Ch. nasus* from the Danube River, Kudelin biotope, included species with complex development cycles, carried out through the participation of the intermediate hosts. Two main elements were distinguished: (1) a group of helminths that were established in the three seasons of the studied period (*P. laevis* and *Contracaecum* sp.) and (2) a group of helminths that represent a variable part of the complexes (the remaining 9 helminth species). Regardless of the established variations in the species composition and parameters of invasion of endohelminths, the obtained results indicated the existence of seasonal dynamics in the distribution of species.

Discussion

Demir, Karakişi, (2014) studied the parasite fauna of *Ch. nasus* from Tahtalı Dam in Turkey during the period 07.2007 – 06.2008, and found the highest prevalence of *Contracaecum* sp. (larvae) in March, followed by that in November, April and May, and the species was not found in common nase during the July, August, December and February. In the present study of common nase from the Danube River, Kudelin biotope, the prevalence of *Contracaecum* sp. was high in all seasons, but was the highest in the spring.

V.5.4. Comparative review of the seasonal changes of the endohelminth complexes of *Abramis brama*, *Alburnus alburnus* and *Chondrostoma nasus* from the Danube River, Kudelin biotope

In the dissertation, a comparative review of the seasonal changes of the endohelminth complexes of *Abramis brama*, *Alburnus alburnus* and *Chondrostoma nasus* from the Danube River, Kudelin biotope is presented. A summary of the results is presented in the abstract.

The infection in *Abr. brama* and *Ch. nasus* was the highest in the spring season, 57.78% and 93.46%, respectively, while in *Alb. alburnus* it was the highest in the autumn (15.39%). For the studied period, the most helminth species were found in freshwater bream (15 species), followed by common nase (11 species) and bleak (9 species). In all three dominant fish species, the most parasite species were found in the spring season (12 species of freshwater bream; 6 species of bleak; 9 species of common nase). Four helminth species were found in freshwater bream during all three studied seasons (*As. imitans*, *N. skrjabini*, *C. fennica* and *P. laevis*); four helminth species were also found in bleak during the three seasons (*N. skrjabini*, *Sph. bramae*, *P. laevis* and *Contracaecum* sp.). In common nase only two helminth species were common to the three seasons (*P. laevis* and *Contracaecum* sp.).

In **the component communities of *Abr. brama* and *Alb. alburnus* from the Kudelin biotope**, with the highest number of specimens were *As. imitans* (with 5,040 specimens for the entire studied period) and *N. skrjabini* (with 41 specimens for the entire period), respectively. While in the **component community of *Ch. nasus* from Kudelin biotope**, *Contracaecum* sp. was with the highest number of specimens (with 7,496 specimens for the entire studied period). **In the spring season**: *As. imitans* was a core species (P%=35.56), *N. skrjabini* was a component species (P%=14.81), and the remaining 10 species were accidental for the helminth community of the freshwater bream; all 6 helminth species were accidental for the helminth community of the bleak; *Contracaecum* sp. was a core species (P%=89.72) and all other 8 species were accidental for the helminth community of the common nase. **During the summer season**: *P. laevis* was a component species (P%=13.04) and the remaining 4 helminth species were accidental for the helminth community of the freshwater bream; all 6 established helminth species were accidental for the helminth community of the bleak; *Contracaecum* sp. was a core species (P%=81.82) and all other 5 species were accidental for the helminth community of the common nase. **In the autumn season**: 10 established helminth species in freshwater bream and respectively 5 helminth species in bleak were accidental for the helminth communities; *Contracaecum* sp. was a core species (P%=85.87) and the remaining 2 species were accidental for the helminth community of the common nase.

The infracommunities of *Abr. brama* had the highest **Brillouin's diversity index** in the spring (HB=1.54), while the infracommunities of *Alb. alburnus* and *Ch. nasus* had the highest Brillouin's index in the summer (HB=1.23 and HB=0.11, respectively). **The Pielou's evenness index** of the freshwater bream was the highest in the autumn (E=0.75); of the bleak – in the spring (E=0.85); and of the common nase – in the summer (E=0.05). **The Simpson's dominance index** was the highest in the summer for *Abr. brama* (C=0.91); for *Alb. alburnus* – in the autumn (C=0.31), and for *Ch. nasus* – in the spring (C=0.99).

Of the freshwater bream and bleak, a significant positive correlation was found between MI, MA and P% ($r_s>0.18$, n=7-11, $p=0.00001<0.05$ and $r_s>0.30$, n=5-7, $p=0.00001<0.05$, respectively) with endohelminths in the three seasons, and of common nase a significant positive correlation was found between MI and P% with endohelminths in the spring, summer and autumn ($r_s>0.05$, n=3-9, $p=0.00001<0.05$).

For *Abr. brama* and *Ch. nasus*, regardless of the established variations in the species composition and invasion parameters with helminth, the obtained results showed the existence of seasonal dynamics in the distribution of species, while for *Alb. alburnus* seasonal dependence existed only in the distribution of *N. skrjabini* and *Sph. bramae*.

Development processes of endohelminth complexes from the spring to the autumn were closely related to the development cycles of helminth species. The identification of three clusters for each of the host species (better expressed in regards to Ics) was associated with differences in the lifestyle and diet of the hosts.

The circulation paths of the endohelminth flow in the three dominant species (*Abr. brama*, *Alb. alburnus*, *Ch. nasus*) of Kudelin biotope were extremely diverse, which was closely related to the differences in the nutritional spectrum of these species and their lifestyle.

SUMMARY AND CONCLUSIONS

For the period 2019-2021, 2,367 fish specimens belonging to 8 families and 31 species: *L. gibbosus*, *Al. immaculata*, *C. elongata*, *C. taenia*, *S. bulgarica*, *Abr. brama*, *Alb. alburnus*, *B. ballerus*, *B. sapa*, *B. barbus*, *C. gibelio*, *Ch. nasus*, *Ct. idella*, *C. carpio*, *G. gobio*, *H. molitrix*, *L. aspius*, *P. cultratus*, *Rh. amarus*, *R. rutilus*, *Sc. erythrophthalmus*, *Sq. cephalus*, *V. vimba*, *Es. lucius*, *B. gymnotrachelus*, *N. fluviatilis*, *N. melanostomus*, *G. schraetser*, *P. fluviatilis*, *S. lucioperca*, *S. glanis* were subjected to an ecologohelminthological study. The fish were caught from 5 biotopes (Kudelin, Novo selo, Yasen, Koshava and Kutovo) along the Danube River. Endohelminths were not found in *L. gibbosus*, *C. taenia*, *B. ballerus*, *G. gobio*, *H. molitrix* and *R. amarus*. Based on the obtained results, the following summaries and conclusions can be made:

1. An invasion with **20,391 helminth specimens**, belonging to the **4 classes (Trematoda, Cestoda, Acanthocephala and Nematoda), 22 families, 27 genus and 34 species** was established. **Nine species of class Trematoda** (*All. isoporum*, *B. luciopercae*, *L. confusus*, *As. imitans*, *As. tincae*, *P. incognitus*, *N. skrjabini*, *Sph. bramae*, *Ichth. pileatus* (larvae)); **8 species of class Cestoda** (*B. rectangulum*, *Sch. acheilognathi*, *C. laticeps*, *N. cheilancristrotus* (larvae), *C. fennica*, *Gl. osculata*, *Pr. percae*, *Pr. torulosus*); **4 species of class Acanthocephala** (*Ac. anguillae*, *Ac. lucii*, *Ac. tenuirostris*, *P. laevis*) and **13 species of class Nematoda** (*Contracaecum* sp. (larvae), *C. lacustris*, *Ps. tomentosa*, *Sch. petruschewskii*, *E. excisus* (larvae), *Ph. obturans*, *Ph. ovata*, *Ph. rischta* (larvae), *Hysterothylacium* sp. (larvae), *H. g. aduncum* (larvae), *R. acus* (larvae), *Rh. denudata*, *K. intestinalis*) were isolated.
2. The Kudelin, Yasen, Novo selo, Koshava and Kutovo biotopes are **new habitats** for all established helminth species.
3. *Sch. acheilognathi* is a **new taxa** for the helminthofauna and the helminth communities of the freshwater fish from the Danube River in Bulgaria. Six helminth species (*L. confusus, Sph. bramae, N. cheilancristrotus* (larvae)*, C. lacustris, Ph. obturans, K. intestinalis*) are **new for the Danube River and its basin in Bulgaria**. Three helminth species (*L. confusus, N. cheilancristrotus* (larvae)*, Ph. obturans*) are **new for the Danube River and its basin**.
4. For 25 helminth species **new hosts** are established **in Bulgaria**:
   - 4.1. ***Abr. brama*** – for *Icht. pileatus*, *Sph bramae*, *N. cheilancristrotus, Sch. acheilognathi, E. excisus*.
   - 4.2. ***Alb. alburnus*** – for *As. imitans*, *Sph. bramae*, *B. rectangulum* and *Ph. rischta*.
   - 4.3. ***B. gymnotrachelus*** – for *Ac. lucii*.
   - 4.4. ***B. sapa*** – for *As. imitans*, *N. skrjabini* and *Contracaecum* sp.
   - 4.5. ***B. barbus*** – for *Sph. bramae*.
   - 4.6. ***Ch. nasus*** – for *All. isoporum*, *N. skrjabini*, *Sph. bramae*, *Pr. torulosus*, *Sch. acheilognathi*, *Ac. anguillae*, *Contracaecum* sp., *Hysterothylacium* sp., *Ps. tomentosa* and *R. acus*.

4.7. ***C. elongata*** – for *Ps. tomentosa.*
4.8. ***C. carpio*** – for *Sch. acheilognathi* and *Ac. lucii.*
4.9. ***Es. lucius*** – for *Ac. anguillae.*
4.10. ***N. fluviatilis*** – for *Ac. lucii.*
4.11. ***N. melanostomus*** – for *Ac. lucii*, *Ac. anguillae* and *Contracaecum* sp.
4.12. ***P. fluviatilis*** – for *Sph. bramae.*
4.13. ***R. rutilus*** – for *All. isoporum*, *As. tincae*, *P. incognitus*, *C. fennica*, *P. laevis* and *Rh. denudata.*
4.14. ***S. bulgarica*** – for *As. tincae.*
4.15. ***S. lucioperca*** – for *Ph. obturans.*
4.16. ***S. glanis*** – for *Sph. bramae, Contracaecum* sp. and *C. lacustris.*
4.17. ***Sq. cephalus*** – for *E. excisus* and *Ps. tomentosa.*
4.18. ***V. vimba*** – for *All. isoporum*, *As. imitans*, *N. skrjabini*, *Sph. bramae*, *P. incognitus*, *Contracaecum* sp., *E. excisus*, *Ph. ovata*, *Ph. rischta* and *R. acus.*

5. For 29 helminth species **new hosts for the Danube River and its basin in Bulgaria** are established:

5.1. ***Abr. brama*** – for *Icht. pileatus*, *Sph bramae*, *N. cheilancristrotus, Sch. acheilognathi, E. excisus* and *Sch. petruschewski.*
5.2. ***Alb. alburnus*** – for *As. imitans*, *Sph. bramae*, *B. rectangulum*, *N. cheilancristrotus* and *Ph. rischta.*
5.3. ***Al. immaculata*** – for *L. confusus.*
5.4. ***B. gymnotrachelus*** – for *Ac. lucii.*
5.5. ***B. sapa*** – for *As. imitans*, *N. skrjabini* and *Contracaecum* sp.
5.6. ***B. barbus*** – for *Sph. bramae.*
5.7. ***Ch. nasus*** – for *All. isoporum*, *N. skrjabini*, *Sph. bramae*, *Pr. torulosus*, *Sch. acheilognathi*, *Ac. anguillae*, *Contracaecum* sp., *Hysterothylacium* sp., *Ps. tomentosa* and *R. acus.*
5.8. ***C. elongata*** – for *Ps. tomentosa.*
5.9. ***C. carpio*** – for *Sch. acheilognathi* and *Ac. lucii.*
5.10. ***Es. lucius*** – for *Ac. anguillae.*
5.11. ***L. aspius*** – for *Sph. bramae*, *C. fennica*, *Pr. torulosus*, *Ac. anguillae* and *R. acus.*
5.12. ***N. fluviatilis*** – for *Ac. lucii.*
5.13. ***N. melanostomus*** – for *Ac. lucii*, *Ac. anguillae* and *Contracaecum* sp.
5.14. ***P. cultratus*** – for *Pr. torulosus*, *Contracaecum* sp. and *Ps. tomentosa.*
5.15. ***P. fluviatilis*** – for *Sph. bramae.*
5.16. ***R. rutilus*** – for *All. isoporum*, *As. tincae*, *P. incognitus*, *C. fennica*, *P. laevis* and *Rh. denudata.*
5.17. ***S. bulgarica*** – for *As. tincae.*
5.18. ***S. lucioperca*** – for *Ph. obturans.*
5.19. ***Sc. erythrophthalmus*** – for *As. imitans*, *N. skrjabini*, *Ac. tenuirostris*, *Contracaecum* sp. and *K. intestinalis.*
5.20. ***S. glanis*** – for *Sph. bramae*, *Contracaecum* sp. and *C. lacustris.*
5.21. ***Sq. cephalus*** – for *Sph. bramae*, *E. excisus* and *Ps. tomentosa.*
5.22. ***V. vimba*** – for *All. isoporum*, *As. imitans*, *N. skrjabini*, *Sph. bramae*, *P. incognitus*, *Contracaecum* sp., *E. excisus*, *Ph. ovata*, *Ph. rischta* and *R. acus.*

6. For 26 helminth species **new hosts for the Danube River and its basin** are established:

6.1. ***Abr. brama*** – for *Icht. pileatus*, *N. cheilancristrotus, Sch. acheilognathi, E. excisus*.
6.2. ***Alb. alburnus*** – for *As. imitans*, *B. rectangulum*, *N. cheilancristrotus*.
6.3. ***Al. immaculata*** – for *L. confusus*.
6.4. ***B. gymnotrachelus*** – for *Ac. lucii*.
6.5. ***B. sapa*** – for *Contracaecum* sp.
6.6. ***B. barbus*** – for *Sph. bramae*.
6.7. ***Ch. nasus*** – for *All. isoporum*, *N. skrjabini*, *Sph. bramae*, *Sch. acheilognathi*, *Ac. anguillae*, *Contracaecum* sp., *Hysterothylacium* sp., *Ps. tomentosa* and *R. acus*
6.8. ***C. elongata*** – for *Ps. tomentosa*.
6.9. ***C. carpio*** – for *Sch. acheilognathi*.
6.10. ***Es. lucius*** – for *Ac. anguillae*.
6.11. ***L. aspius*** – for *Sph. bramae*, *C. fennica* and *R. acus*.
6.12. ***N. fluviatilis*** – for *Ac. lucii*.
6.13. ***N. melanostomus*** – for *Ac. anguillae* and *Contracaecum* sp.
6.14. ***P. cultratus*** – for *Pr. torulosus*, *Contracaecum* sp. and *Ps. tomentosa*.
6.15. ***P. fluviatilis*** – for *Sph. bramae*.
6.16. ***R. rutilus*** – for *P. laevis*.
6.17. ***S. bulgarica*** – for *As. tincae*.
6.18. ***S. lucioperca*** – for *Ph. obturans*.
6.19. ***Sc. erythrophthalmus*** – for *As. imitans*, *N. skrjabini*, *Ac. tenuirostris*, *Contracaecum* sp. and *K. intestinalis*.
6.20. ***S. glanis*** – for *Sph. bramae* and *Contracaecum* sp.
6.21. ***Sq. cephalus*** – for *E. excisus* and *Ps. tomentosa*.
6.22. ***V. vimba*** – for *All. isoporum*, *As. imitans*, *N. skrjabini*, *Sph. bramae*, *P. incognitus*, *Contracaecum* sp., *E. excisus*, *Ph. ovata*, *Ph. rischta* and *R. acus*.

7. Of the 31 established fish species from 5 biotopes, the largest number of helminth species were found in *Abr. brama* (15 species), *V. vimba* (13 species) and *Ch. nasus* (11 species). The largest number of the endohelminth specimens were isolated from *Ch. nasus* (7,828 specimens), *Abr. brama* (6,024 specimens) and *B. barbus* (3,120 specimens). A total of 21 helminth species in the three dominant fish species (*Abr. brama*, *Alb. alburnus*, *Ch. nasus*) from Kudelin biotope were found. The largest number of helminth species were established at the freshwater bream (15 species), and the largest number of specimens at the common nase (7,549 specimens). Four helminth species (*N. skrjabini*, *Sph. bramae*, *P. laevis* and *Contracaecum* sp.) were common for the three dominat fish species. *N. skrjabini* and *P. laevis* had the highest ecological indices at *Abr. brama*; *Sph. bramae* at *Alb. alburnus*; *Contracaecum* sp. at *Ch. nasus*.
8. **The highest value for MI** was established of *As. imitans* (MI=700) from *B. sapa* (Koshava); **the highest value for MA** of *P. laevis* (MA=237.33) from *B. barbus* (Koshava); **the highest values for P%** (P%=100) were established of *P. laevis* and *Ps. tomentosa* from *B. barbus* (Koshava); *P. laevis* from *Al. immaculata* (Yasen); *As. tincae* from *S. bulgarica* (Kudelin); *P. laevis* from *Ct. idella* (Kudelin); *Ac. anguillae* from *Es. lucius* (Kudelin); *N. skrjabini* from *N. fluviatilis* (Kutovo); *P. laevis* and *E. excisus* from *S. glanis* (Koshava). At the dominant fish species the helminths with the highest ecological indices (MI, MA and P%) were *As. imitans* (MI=78.75, MA=15.18, P% =19.28; MI=48.42, MA=36.31, P%=75.00) from *Abr. brama* (Kudelin; Koshava,

respectively) and *Contracaecum* sp. (MI=29.28, MA=25.15, P%=85.91) from *Ch. nasus* (Kudelin). For MI, MA and P%, the higher values were established of *P. laevis* from *B. barbus*, Koshava biotope, relative to Vetren biotope and of *Pr. torulosus* from *R. rutilus*, Kudelin biotope, relative to Srebarna lake. Independantly from the huge diversity of the established parasite species, the ecological indices were low.

9. The component community of ***Abr. brama*** was presented by one component species (*As. imitans*; P%=19.28) and 14 accidental species. Four species of them (*Ichth. pileatus*, *N. cheilancristrotus*, *Contracaecum* sp. and *E. excisus*) were allochthonous. The component community of ***Alb. alburnus*** was presented by 9 accidental helminth species. Two species of them (*N. cheilancristrotus* and *Contracaecum* sp.) were allochthonous. The component community of ***Ch. nasus*** was presented by one core (*Contracaecum* sp.; P% =85.91) and 10 accidental helminth species. *Contracaecum* sp. was an allochthonous species.
10. In the **infracommunities of *Abr. brama* and *Alb. alburnus*** from **Kudelin biotope**, with the largest number of specimens were *As. imitans* (a total of 5,040 specimens) and *N. skrjabini* (a total of 41 specimens), respectively, while in the **infracommunity of *Ch. nasus*** it was *Contracaecum* sp. (a total of 7,496 specimens). The infracommunities of *Abr. brama* and *Ch. nasus* were distinguished by high indices of dominance (C=0.87; C=0.98, respectively) and low indices of diversity (HB=0.36; HB=0.05, respectively) and evenness (E=0.13; E=0.02, respectively). In the infracommunity of *Alb. alburnus*, high indices of diversity (HB=1.52) and evenness (E=0.75) and a low index of dominance (C=0.25) were established.
11. The infection in *Abr. brama* and *Ch. nasus* was the highest in the spring, 57.78% and 93.46%, respectively, while in *Alb. alburnus* it was the highest in the autumn (15.39%). In all three dominant fish species, the highest number of parasite species was established in the spring (12, 6 and 9 species at the freshwater bream, bleak and common nase, respectively). Four helminth species were present in the three seasons of the studied period in freshwater bream (*As. imitans*, *N. skrjabini*, *C. fennica* and *P. laevis*) and in bleak (*N. skrjabini*, *Sph. bramae*, *P. laevis* and *Contracaecum* sp.), while only two species of helminths were common in common nase (*P. laevis* and *Contracaecum* sp.).
12. In the spring, one core species in the component communities of freshawater bream and common nase (*As. imitans* (P%=35.56) and *Contracaecum* sp. (P%=89.72), respectively); one component species in the community of the freshwater bream (*N. skrjabini* (P%=14.81)), as well as 10, 6, and 8 accidental helminth species in the communities of the freshwater bream, bleak and common nase, respectively, were found. In the summer, one core species in the component community of the common nase (*Contracaecum* sp. (P%=81.82); one component species in the community of the freshwater bream (*P. laevis* (P%=13.04)), as well as 4, 6, and 5 accidental helminth species in the communities of the freshwater bream, bleak and common nase, respectively, were found. In the autumn, one core species in the component community of the common nase (*Contracaecum* sp. (P%=85.87)), as well as 10, 5 and 2 accidental helminth species in the communities of the freshwater bream, bleak and common nase, respectively, were found.
13. The highest Brillouin's diversity index was established at *Abr. brama* (HB=1.54); the highest Pielou's evenness index at *Alb. alburnus* (E=0.85) and the highest Simpsons's dominance index at *Ch. nasus* (C=0.99) during the spring.

14. In the freshwater bream and bleak, a significant positive correlation was found between MI, MA and P% (r_s>0.18, n=7-11, p=0.00001<0.05 and r_s>0.30, n=5-7, p=0.00001<0.05, respectively), and in common nase - between MI and P% (r_s>0.05, n=3 -9, p=0.00001<0.05) with endohelminths in the three seasons.
15. The invasion parameters of *Abr. brama* showed **a maximum during spring, reduction in summer and a second peak in autumn** (χ^2=31.18, df=12, p=0.002<0.05); of *Alb. alburnus* – **a maximum during autumn, reduction in summer and a second peak in spring** (χ^2=47.46, df=12, p=0.0000). The helminth complexes of *Ch. nasus* (by P%) were **highest in spring** (χ^2=63.68, df=32, p=0.0007<0.05), were best expressed in the core species *Contracaecum* sp., where **a decrease was established during summer and a second peak in autumn**.
16. The seasonal dynamics in the distribution of species invasion parameters for *Abr. brama* and *Ch. nasus* was established. For *Alb. alburnus* seasonal dependence was established only in the distribution of *N. skrjabini* and *Sph. bramae*.
17. The development of helminth complexes and the circulation paths of the endohelminth flow from spring to autumn were closely related to the development cycles of helminth species; with differences in the nutritional spectrum of the hosts species and their lifestyle.
18. The pathogenic for the fish helminth species (*Sch. acheilognathi*, *P. laevis*, *Contracaecum* sp., *E. excisus*, *R. acus*) and for the human helminth species (*Contracaecum* sp., *E. excisus*) are established.

SCIENTIFIC AND SCIENTIFIC-APPLIED CONTRIBUTIONS

1. A new taxa for the helminthofauna and the helminth communities of the freshwater fish from the Bulgarian section of the Danube River (*Sch. acheilognathi*) is established.
2. New six endohelminth species (*L. confusus, Sph. bramae, N. cheilancristrotus* (larvae)*, C. lacustris, Ph. obturans, K. intestinalis*) for the Danube River and its basin in Bulgaria are established.
3. New three endohelminth species (*L. confusus, N. cheilancristrotus* (larvae)*, Ph. obturans*) for the Danube River and its basin are established.
4. New hosts for 25 helminth species in Bulgaria are established. New hosts for 29 helminth species for the Danube River and its basin in Bulgaria are established. New hosts for 26 helminth species for the Danube River and its basin are established.
5. New 22 host species of helminths are established for the Danube River and the river basin, including Bulgaria, as well as new 18 host species of helminths are established in Bulgaria.
6. The helminth communites of *Ch. nasus* from the Bulgarian section of the Danube River and in Bulgaria are studied for the first time. The data for the helminth communites of *Alb. alburnus* and *Abr. brama* from the Danube River are updated.
7. The helminth communites of *Abr. brama*, *Alb. alburnus* and *Ch. nasus* from the Bulgarian section of the Danube River, Kudelin biotope are compared for the first time.
8. The seasonal changes of helminth complexes of *Abr. brama*, *Alb. alburnus* and *Ch. nasus* from the Danube River, Kudelin biotope are considered for the first time.
9. The scientific literature on research on the parasites and the parasite communities of fish from the Danube River's freshwater ecosystem is enriched.

10. The data for the species composition of helminths for 25 fish species from the Danube River are enriched.
11. New data for the invasion parameters of pathogenic for the studied fish species helminths – *Sch. acheilognathi*, *P. laevis*, *Contracaecum* sp., *E. excisus* and *R. acus* are presented.
12. New data for the invasion parameters of pathogenic for the human helminths – *Contracaecum* sp. and *E. excisus* are presented.

RECOMMENDATIONS

1. Visual inspection on the surface body and body cavity of the fish before treatment and subsequend consumption is recommended.
2. Removal of the fish skin and careful examination of the fish muscles (for example at *Abr. brama*, *V. vimba*, *L. aspius*, *Sq. cephalus*, *N. fluviatilis*, *B. gymnotrachelus*, *P. fluviatilis*, *S. lucioperca*, *S. glanis*) when cleaning and before processing of the fish meat for consumption is recommended, due to the localization of *E. excisus* (a pathogenic helminth species for humans).
3. A good heat treatment of the fish and avoiding the consumption of raw fish (for example *Abr. brama*, *Alb. alburnus*, *Ch. nasus*, *V. vimba*, *B. sapa*, *C. gibelio*, *C. carpio*, *L. aspius*, *P. cultratus*, *Sc. erythophthalmus*, *N. melanostomus*, *P. fluviatilis*, *S. glanis*) is recommended, due to the possibility of the presence of *Contracaecum* sp. (a pathogenic helminth species for humans).
4. A permanent tracking of the ecological indices with *Sch. acheilognathi*, *P. laevis*, *Contracaecum* sp., *E. excisus*, *R. acus* as pathogenic species for freshwater fish is recommended, to minimize fish mortality.
5. Small fish species (for example *Alb. alburnus*, *B. gymnotrachelus*, *N. fluviatilis*, *N. melanostomus* and others) should be consumed obligatory after removing the internal organs due to presence of helminths, some of them with high ecological indices.
6. The ecologoparasitological research (Ministry of Agriculture, Ministry of Health) on the parasites and the parasite communities of fish, including those that often exist in the human diet should be continued.
7. Permanent investigations (Ministry of Environment and Water, Ministry of Agriculture) on freshwater fish for tracking of the parasites and the parasites complexes are recommended, to protect the biodiversity of the fish and the fish resources.
8. The strict veterinary-medical control (Ministry of Agriculture, Bulgarian Food Safety Agency) for the fish parasites imported into trade network is recommended.

LIST WITH SCIENTIFIC PAPERS IN RELATION TO THE DISSERTATION

1. **Zaharieva R., Kirin D., 2020**. New data on parasites and parasite communities of *Alburnus alburnus* (Linnaeus, 1758) from the Danube River. Book of Proceedings, Scientific Papers. Series D. Animal Science. LXIII(2), 397-404. ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750 http://animalsciencejournal.usamv.ro/pdf/2020/issue_2/Art61.pdf
2. **Zaharieva R., Kirin D., 2020**. Parasites and parasite communities of the Common nase (*Chondrostoma nasus* (Linnaeus, 1758) from the Danube River. Book of Proceedings, Scientific Papers. Series D. Animal Science. LXIII(2), 413-420. ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750 http://animalsciencejournal.usamv.ro/pdf/2020/issue_2/Art63.pdf

PARTICIPATION IN INTERNATIONAL SCIENTIFIC CONFERENCES

1. Zaharieva P., Zaharieva R. 2020. Ecologohelminthological investigations and circulation of arsenic in the system Water – Sediments – *Chondrostoma nasus* – *Contracaecum* sp., larvae from the Danube River. Book of Abstracts of 16th International May Conference on Strategic Management - IMCSM20, 29. (EBSCOHost Database; Web of Science) http://media.sjm06.com/2020/09/IMCSM20-Book-of-Abstracts.pdf
2. Zaharieva P., Zaharieva R., 2021. Parasite communities and a content of copper in *Chondrostoma nasus* and *Alburnus alburnus* from the Danube River, Bulgaria. Book of Abstracts of 17th International May Conference on Strategic Management - IMCSM21, 23. (EBSCOHost Database; Web of Science) ISBN 978-86-6305-114-0 https://drive.google.com/file/d/1dm8vGyrgYJosbIYfQvZ_EkRgZZximrk9/view
3. Zaharieva R., Zaharieva P., 2021. Parasite communities and a content of arsenic in *Alburnus alburnus* and *Abramis brama* from the Danube River, Bulgaria. – In: International May Conference on Strategic Management (IMCSM21): Book of Abstracts of 17th International May Conference on Strategic Management - IMCSM21, 24. (EBSCOHost Database; Web of Science) ISBN 978-86-6305-114-0 https://drive.google.com/file/d/1dm8vGyrgYJosbIYfQvZ_EkRgZZximrk9/view

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