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BIODIVERSITY AND STRUCTURE OF THE HELMINTH COMMUNITIES OF *CARASSIUS GIBELIO* (BLOCH, 1782) FROM THE TUNDZHA RIVER, BULGARIA

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Abstract

In 2021, ecologoparasitological research was done based on the helminths and helminth communities of Prussian carp (Carassius gibelio (Bloch, 1782)) from the freshwater ecosystem of the Tundzha River, Aegean Water Basin. As a result of the examined twenty-one specimens of Prussian carp, three taxa of helminths were found: Nicolla skrjabini (Iwanitzky, 1928) Dollfus, 1960; Pomphorhynchus laevis (Müller, 1776) Porta, 1908; Contracaecum sp. The dominant structure of the helminth communities was determined. N. skrjabini is a core species for helminth communities of C. gibelio (P% = 23.81). New data on the helminth communities of the Prussian carp from the studied area of the freshwater ecosystem are presented. The basic ecological indices of the helminth populations and communities were determined. The bioindication role of the established helminth species as well as an assessment of the ecological status of the studied biocenoses was presented.

***Key words**: bioindication, Carassius gibelio, helminth communities, river Tundzha.*

INTRODUCTION

After the Danube and Iskar rivers, the Tundzha River is the third largest river in Bulgaria (390 km) and the Maritsa River's largest tributary, Aegean Water Basin. The river springs from the Balkan Mountain (2083 m altitude) and flows into the Maritsa River before Edirne (32 m altitude). The river ecosystem and its adjacent territories are distinguished by great biological diversity, including rich ichthyofauna, related to the declaration of a number of protected areas. Helminths and helminth communities are elements of biodiversity. In most cases, helminths are characterized by complex life cycles involving intermediate hosts. Therefore, the invasion indices with them reflect the integrity of food chains and the state of ecosystems in general. *Carassius gibelio* (Bloch, 1782) has been the subject of helminthological research by various authors in a number of countries (Shukerova, 2005; Koyun & Altunl, 2007; Cojocaru, 2010; Őktener, 2014; İnnal et al.,2020; Stroe et al., 2021). In Bulgaria, the Prussian carp from the Tundzha River Basin has been the subject of ecologo-helminthological research by a few authors (Grupcheva & Nedeva, 1999; Chunchukova & Kirin, 2021). The study presents data on the endohelminths and helminth communities of Prussian carp from the Tundzha River (middle section) and discusses the condition of the communities from the studied part of the river.

MATERIALS AND METHODS

In 2021, twenty-one specimens of Prussian carp from the Tundzha River were examined for helminths. The examined fish were caught by angling according to permission from the Ministry of Agriculture, Food and Forestry of the Republic of Bulgaria. According to Froese & Pauly (Eds.) (2020), the fish's scientific name was present. The fish were collected in the section of the river located between the Balkan Mountain and the Mountain range Sredna Gora, Central Southern Bulgaria (42°33′12″N, and 25°38′21″ E; 309 m). The helminthological study was carried out according to Petrochenko (1956); Zashev & Margaritov (1966); Bauer (Ed.) (1987); Moravec (2013). Helminth specimens were fixed in 70% of ethyl alcohol. Species diversity was determined on permanent slides according to the method of staining with alum carmine (Dubinina, 1948) and on temporary slides carried out by the methods of Moravec (2013) and Petrochenko (1956). Helminth community structure was analysed on the two levels: on the level of component community (prevalence (P%); mean intensity (MI) for the determined species) and on the level of infracommunity (total number of fish species; total and mean number of fish specimens; Brillouin's diversity index (HB)). In the component community, the found species were divided into three groups: core species (P% > 20), component species (P% > 10) and accidental species (P% < 10), according to the criteria of Magurran (1988); Bush et al. (1997) and Kennedy (1997). The obtained results were statistically processed using Statistica 10 (StatSoft Inc., 2011) and MS Exel (Microsoft 2010).

RESULTS AND DISCUSSIONS

Characteristics of the studied fish species

Carassius gibelio (Bloch, 1782) (Cyprinidae) is freshwater, benthopelagic and brackish fish species. The Prussian carp is considered to be an omnivorous species. It feeds mainly on worms, insect larvae, and even small fish during the cold months. During the summer months, the main food is plant food. The Prussian carp grows best in gullies, swamps, mortuaries, old riverbeds, micro-dams, gravel pits, lower slow rivers and large dams. It is characterized by a specific method of reproduction unique to it (Froese & Pauly, 2020; Karapetkova & Zhivkov, 2006). *C. gibelio* has an unclear conservation status on a European scale as a native or non-native species. The species is defined as not endangered in the International Red Book (LC; IUCN). The Prussian carp is not a protected species on the territory of Bulgaria. The species is widespread in the country.

Helminths and helminth community structure

In 2021, as a result of the ecologoparasitological examinations of 21 specimens of *C. gibelio* from the Tundja River, three taxa of endohelminths was established: Nicolla skrjabini (Iwanitzky, 1928) Dollfus, 1960; *Pomphorhynchus laevis* (Müller, 1776) Porta, 1908 and *Contracaecum* sp., larvae, belonging to three classes, three orders, three families and three genera (Table 1).

Nicolla skrjabini **(Iwanitzky, 1928) Dollfus, 1960** is an intestinal parasite of many species of fish from Cyprinidae, Percidae, Gobiidae, Cobitidae, Siluridae, Gadidae, Esocidae, Acipenseridae, Salmonidae. The development cycle of the species is carried out with the participation of two types of intermediate hosts.

Table 1. Biodiversity and ecological indices of helminths and helminth communities of *Carassius gibelio* from the Tundja River

Silurus glanis (N[1] = 21) Helminth species	n[2]	p[3]	P%[4]	MI[5] (min-max)
Class Trematoda Rudolphi, 1808 **Order** Plagiorchiida La Rue, 1957 **Family** Opecoelidae Ozaki, 1925 **Genus***Nicolla* Wisniewski, 1944				
Nicolla skrjabini (Iwanitzky, 1928) Dollfus, 1960	5	11	23,81	2,2 (1-5)
Class Acanthocephala Rudolphi, 1808 **Order** Echinorhynchida Southwell & Macfie, 1925 **Family** Pomphorhynchidae Yamaguti, 1939 **Genus** *Pomphorhynchus* Monticelli, 1905				
Pomphorhynchus laevis (Zoega in Müller, 1776) Porta, 1908	2	5	9,52	2,5 1-3
Class Nematoda Rudolphi, 1808 **Order** Ascaridida Skrjabin et Schulz, 1940 **Family** Anisakidae Skrjabin et Karokhin, 1945 **Genus** *Contracaecum* Railliet et Henry, 1912				
Contracaecum sp., larvae	1	2	4,76	2,0 2

Legend: [1]N = total number of examined fish specimens.
[2]n = total number of infected fish specimens.
[3]p = total number of helminth specimens.
[4]P% = prevalence.
[5]MI = mean intensity.

The first intermediate host is the snail *Lithoglyphus naticoides* (Pfeiffer, 1828) (Class Gastropoda). Sporocysts of parasites are localized in the liver, gonads and gills. The second intermediate hosts are crustaceans *Gammarus balcanicus* Schäferna, 1923 (Class Malacostraca) with localization of metacercariae in the back muscles and limbs (Bauer, 1987; Kakacheva-Avramova, 1983). In Bulgaria, *N. skrjabini* (as *Crowcrocoecum skrjabini*) is reported as endohelminth species of *Gobio gobio* (Linnaeus, 1758); *Pelecus cultratus* (Linnaeus, 1758); *Cyprinus carpio* Linnaeus, 1758; *Sabanejewia bulgarica* (Drenski, 1928) (*Cobitis bulgarica*); *Silurus glanis* Linnaeus, 1758; *Sander lucioperca* (Linnaeus, 1758) (*Lucioperca lucioperca*); *Perca fluviatilis* Linnaeus, 1758; *Zingel zingel* (Linnaeus, 1766) (*Aspro zingel*); *Gymnocephalus cernua* (Linnaeus, 1758) (*Acerina cernus*); *G. schraester* (Linnaeus, 1758) (*A. schraester*); *Neogobius melanostomus* (Pallas, 1814) (*Gobius cephalarges contructor*); *Neogobius fluviatilis* (Pallas, 1814) (*G. fluviatilis*) of the Danube River (Margaritov, 1966); of *Salmo trutta fario* Linnaeus, 1758 of the Chuprenska River (Kakacheva-Avramova, 1969); of *C. carpio* of Fisheries Belene (Margaritov, 1975, 1976, 1992). *N. skrjabini* is reported also of *Acipenser ruthenus* Linnaeus, 1758; *P. fluviatilis*; *S. lucioperca*; *S. volgensis* (Gmelin, 1789); *G. schraetser*; *Z. zingel*; *Z. streber*; *C. carpio*; *Carassius carassius* (Linnaeus, 1758); *Abramis brama* (Linnaeus, 1758); *Ballerus ballerus* (Linnaeus, 1758) (*Abramis ballerus*); *Blicca bjoerkna* (Linnaeus, 1758); *Leuciscus aspius* (Linnaeus, 1758) (*Aspius aspius*); *P. cultratus*; *G. gobio*; *S. bulgarica* (*C. bulgarica*); *Gobio gobio* (Linnaeus, 1758) (*Gobio fluviatilis*); *Neogobius melanostomus* (Pallas, 1814) (*Gobio cephalarges*); *Proterorhinus marmoratus* (Pallas, 1814) (*Proterorinchus marmoratus*) of the Danube River (Kakacheva-Avramova et al., 1978); of *Salmo trutta fario* Linnaeus, 1758 of the rivers Trigradska and Vacha (Kakacheva-Avramova & Menkova, 1978); of *S. t. fario* of the rivers Chuprenska, Trigradska, Vacha, Shirokolashka (Kakacheva-Avramova & Menkova, 1978); of *Alburnus alburnus* (Linaneus, 1758) of the Danube River (Chunchukova et al., 2019); of *A. alburnus* of the Danube River (Zaharieva & Kirin, 2020a); of *A. brama* of the Tundja River (Kirin & Chunchukova, 2021); of *A. alburnus* and *A. brama* of the Danube River (Zaharieva & Zaharieva, 2021); of *Vimba vimba* (Linnaeus, 1758) of the Danube River (Zaharieva & Kirin, 2021), etc.

Pomphorhynchus laevis **(Müller, 1776)** develops as a marita in a lot of freshwater fish species of Cyprinidae, Salmonidae, Percidae, Siluridae, etc. The developmental cycle isrelated to the participation of an intermediate host *Gammarus pulex* (Linnaeus, 1758) (Bauer, 1987; Kakacheva–Avramova, 1983). *G. pulex* is a bioindicator forx–β–mesosaprobity as well as relatively tolerant forms (Group C) in terms of environmental conditions in habitats (Belkinova et al., 2013). Smallfish species of Cyprinidae have been established as reservoir hosts. The species was reported of *Sq. cephalus* of the Iskar River, of *B. barbus* of the Danube River (Margaritov, 1959); of *A. ruthenus*, *G. gobio*, *B. barbus*, *A. alburnus*, *B. bjoerkna*, *P. cultratus*, *C. gibelio*, *C. carpio*, *S. bulgarica*, *Silurus glanis* Linnaeus, 1758, *S. lucioperca*, *Z. zingel*, *Z. streber*, *G. cernua*, *G. schraetser*, *P. constructor*, *G. gobio*, *Benthophilus stellatus* (Sauvage, 1874) of the Danube River (Matgaritov, 1966); of *Chondrostoma nasus* (Linnaeus, 1758) and *Phohinus phoxinus* from rivers Ogosta and Nishava (Kakacheva-Avramova, 1969); of *B. cyclolepis* in Bulgaria of the Tundzha River (Kakacheva–Avramova, 1972); of *A. ruthenus*, *A. güldenstädtii* Brandt & Ratzeburg, 1833, *Salmo labrax* Pallas, 1814, *Alosa immaculata* Bennet, 1835 (*Alosa pontica* Bennet, 1835), *Anguilla anguilla* Linnaeus, 1758,
C. carpio, *C. gibelio*, *V. vimba*, *A. brama*, *Ballerus sapa* (Pallas, 1814) (*Abramis sapa*), *Ballerus ballerus* (Linnaeus, 1758) (*Abramis ballerus*), *P. cultratus*, *A. alburnus*, *B. bjoerkna*, *G. gobio*, *Romanogobio albipinnatus* (Lukasch, 1933) (*G. albipinatus*), *B. barbus*, *Ch. nasus*, *L. idus*, *Scardinius erythrophthalmus* (Linnaeus, 1758), *Sq. cephalus*, *Leuciscus aspius* (Linnaeus, 1758) (*Aspius aspius*), *Ctenopharyngodon idella* (Valenciennes, 1844), *Proterorhynus marmoratus* (Pallas, 1814), *S. glanis*, *Lota lota* (Linnaeus, 1758), *Esox lucius* Linnaeus, 1758, *S. lucioperca*, *S. volgense*, *P. fluviatilis*, *G. cernua*, *G. schraester*, *Z. zingel*, *Z. streber*, *Ponticola kessleri* (Günther, 1861) (*Gobius kessleri*), *Lepomis gibbosus* (Linnaeus, 1758), *G. gobio*, *B. stellatus* of the Danube River (Kakacheva-Avramova et al., 1978); of *B. barbus* from rivers Struma, Zheleznitsa, Gradevska, of *A. bipunctatus* from rivers Zheleznitsa and Gradevska, of *Sq. cephalus* of the Struma River (Kakacheva-Avramova & Menkova, 1981); of *C. carpio* and *S. lucioperca* (Nedeva & Grupcheva, 1996); of *C. gibelio* of Reservoir Zhrebchevo (Grupcheva & Nedeva, 1999); of *Sq. cephalus* of the Danube River (Cakis et al., 2004); of *P. fluviatilis* of the Arda River (Kirin, 2005); of *P. fluviatilis* of the Adra River (Kirin, 2005); of *A. brama*, *B. sapa*, *A. ruthenus*, *A. alburnus*, *A. immaculata*, *B. barbus*, *C. gibelio*, *E. lucius*, *G. schraester*, *Sq. cephalus*, *P. cultratus*, *S. lucioperca*, *Sc. erythrophthalmus*, *S. glanis*, *Z. zingel* of the Danube River (Atanasov, 2012); of *Sq. sephalus* of the Tunja River (Kirin et al., 2013); of *B. barbus* of the Danube River (Chunchukova & Kirin, 2018); of *A. alburnus* of the Danube River (Chunchukova et al., 2019); of *Sq. orpheus* of the Stryama River (Kirin et al., 2019); of *A.*

brama of the Danube River (Chunchukova & Kirin, 2020); of *B. cyclolepis* and *Sq. orpheus* of the Topolnitsa River (Chunchukova et al., 2020); of *V. vimba* of the Danube River (Zaharieva & Kirin, 2021), etc. ***Contacaecum* sp.** is reported of *A. alburnus* (Chunchukova et al., 2019);of *Ch. nasus* (Zaharieva & Zaharieva, 2020a, b; Zaharieva & Kirin, 2020a, b; Zaharieva & Kirin, 2021, respectively;) of the Danube River; of *Scardinius erythrophthalmus* (Linnaeus, 1758) of the Maritsa River (Chunchukova et al., 2019), etc.

Component community

The presented helminth taxa were found in 8 of the studied twenty-one Prussians carp specimens (30.09%). Prevalence (P%), mean intensity (MI) and rank were determined for each taxon. *N. skrjabini* (P%=23.81) is a core species of the endohelminth communities of *C. gibelio* from the Tundzha River. The other two species are accidental ($P\%_{P.lavis}$=9.52; $P\%_{Contr.sp.}$=4.76). *P. laevis*is distinguished with the highest mean intensity (MI=2.5), followed by *N. skrjabini* and *Contracaecum* sp. (MI=2.2 and MI=2.0, respectively). Only two specimens of *Contracaecum* sp. was fixed in the infected specimen of *C. gibelio*. *Contracaecum* sp. is an allogenic species. *N. skrjabini* and *Contracaecum* sp. *P. laevis* are autogenic species. Therefore, the established taxa are generalists for the helminth communities of *C. gibelio* from the Tundzha River, Bulgaria (Table 1).

Infracommunity. A total of thirteen examined specimens of *C. gibelio* are free of helminths (61.90%). In this study detected no mixed invasion. The maximum number of parasites found in a single specimen by the host is five (*N. skrjabini*). The average number of all endohelminth specimens is low (0.86±1.35), as well as the value of Brillouin's diversity index (HB) (Table 2).

Table 2. Infracommunity data

Number of helminth species		
Number of infected fish	13	8
Number of helminth species	0	1
Number of helminth specimens		
Total number	18	
Mean±SD	0,86±1,35	
Range	1-5	
Mean HB±SD	0,41±1,49	

A total of seven endohelminth taxa of Prussian carp were reported in Bulgaria. According to the study, only three taxa were reported (42.86%). In the country, *P. laevis* was reported in previous studies as endohelminths of *C. gibelio.*

Detected specimens of the genus *Contraceaceum* and *N. skrjabini* have not been identified. Research on Prussians carp parasites are mainly related to the Danube and Tundja River Basins (Tables 1, 3).

Table 3. Endohelminths of *Carassius gibelio* from freshwater ecosystems of Bulgaria

Species diversity	**Authors**	**Freshwater ecosystems (Biotopes)**
Trematoda		
Trematoda sp. metacercaria	Grupcheva, Nedeva, 1999	reservoir Zhrebchevo, Tundja River Basin
Trematoda sp. cysts	Grupcheva, Nedeva, 1999	reservoir Zhrebchevo, Tundja River Basin
Cestoda		
Cysticercus Paradilepis scolecina (Weld, 1855)	Grupcheva, Nedeva, 1999	reservoir Zhrebchevo, Tundja River Basin
Acanthocephala		
Pomphorhynchus laevis (Müller, 1776)	Margaritov, 1966	river Danube
	Kakacheva, Margaritov, Grupcheva, 1978	river Danube (t. Svishov, t. Ruse, t. Vidin, t. Lom t. Tutrakan)
	Atanasov, 2012	river Danube (v. Archar, v. Dobri dol, t. Svishov, v. Botevo, v. Gomotarci, v. Vardim, v. Novo selo, v. Simeonovo, t. Kozloduj)
Acanthocephalus anguillae (Müller, 1780)	Atanasov, 2012	river Danube (v. Archar, t. Svishov, v. Vardim,)
	Chunchukova, Kirin (2021)	river Tundja
Nematoda		
Raphidascaris acus (Bloch, 1799), larvae	Shukerova, 2005	Biosphere Reserve Srebarna
Contracaecum microcephalum (Rudolphi, 1809), larvae	Shukerova, 2005	Biosphere Reserve Srebarna

CONCLUSIONS

The study presents the first data on the helminths and helminth communities of the *C. gibelio* of the Tundzja River, middle section. Of the three found helminth species, *N. skrjabini* is a core species, and the other two are accidental species for the helminth communities of *C. gibelio*. Only *Contracaecum* sp. is an allogenic species in communities. The values of the prevalence and mean intensity are closely related to the intensity of the intermediate host populations and food chains' integrity.

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