

## HYDROBIOLOGICAL MONITORING OF THE KAYALIKA RIVER BASED ON THE BIODIVERSITY OF FISH PARASITES AND ECOLOGICAL INDICATORS OF THEIR COMMUNITIES

Radoslava ZAHARIEVA<sup>1,2</sup>, Petya ZAHARIEVA<sup>1,2</sup>, Diana KIRIN<sup>1,2</sup>

<sup>1</sup>National Institute of Geophysics, Geodesy and Geography (NIGGG), Hydrology and Water Management Research Center, Bulgarian Academy of Sciences, Sofia, Bulgaria, Acad. G. Bonchev Str., Sofia, 1113, Bulgaria

<sup>2</sup>Agricultural University - Plovdiv, Department of Chemistry, Phytopharmacy and Ecology, and Environmental Protection, 12 Mendelev Blvd, Plovdiv, 4000, Bulgaria

Corresponding author email: radoslava.zaharieva7@gmail.com

### Abstract

*This study aims to monitor the state of the water of the Kayaliika River, part of the Maritsa River basin in Bulgaria, based on the diversity of parasites and indicators of parasite fish communities. For the purpose of the study, 45 specimens of bleak, *Alburnus alburnus* (Linnaeus, 1758), were caught in the autumn of 2024 from the lower reaches of the Kayaliika River. Infection was found in 77.78% of the studied bleak specimens. The following ecological indices were calculated: mean intensity (MI), mean abundance (MA), prevalence (P%), Brillouin's diversity index (HB), Pielou's evenness index (E), and Simpson's dominance index (C). The circulation of the parasite flow in the studied biotope of the river ecosystem was monitored.*

**Key words:** bleak, Bulgaria, East Aegean region, indices, parasite communities.

### INTRODUCTION

The Maritsa River has the largest watershed in Bulgaria. It rises in the Rila Mountains, drains the northeastern part of Rila, a significant part of the Rhodope Mountains and Sredna Gora, and flows into the Aegean Sea. The river valley in its upper part is narrow, steep, deep, and very wide in the Upper Thracian Lowland. Larger tributaries of the Maritsa River are the Topolnitsa River, Luda Yana River, Sazliyka River, Tundzha River, Stryama River, Vacha River, Chepelarska River, Harmanliyska River, Arda River and others. The Kayaliika River is a right tributary of the Maritsa River, with a length of 39 km. The Kayaliika River rises in the Rhodope Mountains, and in the Upper Thracian Lowland, in the vicinity of the village of Skobelev, it flows into the Maritsa River (Kiradzhiev, 2013). Fish are hosts for a large number of parasites. Fish parasites constitute a significant part of the diversity in aquatic ecosystems. The diversity of fish parasites depends on the species richness of their hosts. Water monitoring can be carried out by monitoring water quality parameters or using

bioindicator organisms, such as fish parasites (Palm, 2011). Parasites play an important role in ecosystems. They can affect the structure of food webs, energy flow, functions and biodiversity in ecosystems, and accumulate heavy metals, thereby indicating the degree of pollution (Hudson et al., 2006; Szopieray & Żbikowska, 2021). Some parasites have complex life cycles, i.e. pathways from invertebrate intermediate hosts to vertebrate definitive hosts, which determines the bioindicator role of parasites and their communities in determining the ecological state of aquatic ecosystems (Kuzmanova et al., 2019). No studies have been conducted on parasites and parasite communities of fish from the Kayaliika River. *Alburnus alburnus* (Linnaeus, 1758) is a freshwater, brackish, schooling fish (Froese & Pauly, 2025). The bleak is a species tolerant to habitat and water quality degradation (Maceda-Veiga & De Sostoa, 2011). It is included in the IUCN Red List with the category "Least Concern" (IUCN, 2025).

This study aims to monitor the waters of the Kayaliika River, part of the Maritsa River basin

in Bulgaria, based on the biodiversity of fish parasites and the ecological indices of the parasite communities.

MATERIALS AND METHODS

Forty-five specimens of the bleak, *Alburnus alburnus* (Linnaeus, 1758), were caught from the lower reaches of the Kayaliika River in the

area of the village of Varbitsa (42°03'07.9"N 25°20'40.2" E), Dimitrovgrad Municipality, Haskovo Province (Figure 1).

Fishing was carried out in the autumn of 2024 with a fishing rod after issuing a ticket for amateur fishing according to the Fisheries and Aquaculture Act (amended 2023). The species of the collected fish was determined according to Froese & Pauly (2025).

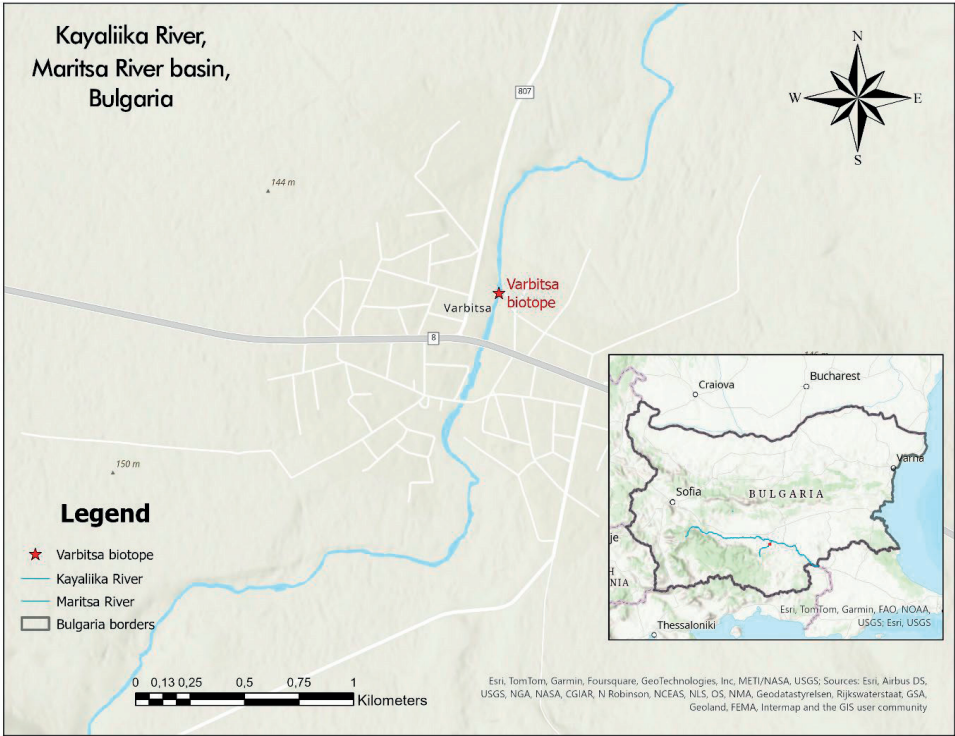


Figure 1. Map of the studied biotope of the Kayaliika River

Parasitological examination was carried out according to standard methods according to Zashev & Margaritov, Bauer (by Zaharieva, 2022), Moravec (2013) under a Micros Austria MZ 1240 stereomicroscope and an XS-213, China microscope. Permanent microscope slides were prepared from the isolated representatives of the classes Trematoda and Cestoda according to Dubinina, Georgiev et al., Scholz & Hanzelová (by Zaharieva, 2022), and temporary microscope slides were prepared from the representatives of the classes Acanthocephala and Nematoda according to Zashev & Margaritov (by Zaharieva, 2022), Moravec (2013). The species affiliation of the

parasites was determined according to Bauer (by Zaharieva, 2022) and Moravec (2013). The basic ecological indices were calculated: mean intensity (MI), mean abundance (MA), prevalence (P%), Brillouin's diversity index (HB), Pielou's evenness index (E), Simpson's dominance index (C) according to Magurran (by Zaharieva, 2022), Bush et al. (by Zaharieva et al., 2025). According to the P% values, parasite species are divided into core (P%>20), component (10<P%<20) and accidental (P%<10) by Kennedy (Zaharieva, 2022). The ecological indices were calculated using the following formulas:

$MI = \frac{p}{n}$ , where:

p - number of parasites;

n - number of infected fish.

$MA = \frac{p}{N}$ , where:

p - number of parasites;

N - number of investigated fish.

$P\% = \left(\frac{n}{N}\right) \times 100$ , where:

n - number of infected fish;

N - number of investigated fish.

$HB = \frac{\ln N! - \sum \ln ni!}{N}$ , where:

ni - number of specimens of each species (i = 1, 2, ... S);

N =  $\sum ni$  - total number of specimens;

S - total number of species.

$E = \frac{H'}{\log_2 N}$ , where:

H' - the Shannon diversity index;

N - number of specimens.

$C = \sum \left(\frac{ni}{N}\right)^2$ , where:

ni - number of specimens in the i<sup>th</sup> species;

N - number of specimens of all species (total number).

## RESULTS AND DISCUSSIONS

### Parasite diversity

In the parasitological examination of 45 specimens of *Alb. alburnus* from Kayaliika River, Varbitsa biotope, 5 parasite species were found - *Clinostomum complanatum* (Rudolphi, 1814) Braun, 1899 and *Posthodiplostomum cuticola* (von Nordmann, 1832) Dubois, 1936 (Trematoda); *Caryophyllaeides fennica*

(Schneider, 1902) Nybelin, 1922 (Cestoda); *Acanthocephalus anguillae* (Müller, 1780) (Acanthocephala) and *Schulmanella petruschewskii* (Schulman, 1948) Ivashkin, 1964 (Nematoda).

### Component community of *Alburnus alburnus*

In the component community of the bleak with the largest number of specimens, trematodes are represented (2 species with more than 3,010 specimens), followed by acanthocephalans (1 species with 60 specimens). One species with two specimens each represents cestodes and nematodes. In the parasite community of the bleak, two core species have been identified - *P. cuticola* (P% = 66.67) and *Ac. anguillae* (P% = 53.33). All other parasite species are accidental. *P. cuticola* had the highest values for MI and MA (Table 1).

### Infracommunity of *Alburnus alburnus*

The present study revealed infection in 77.78% of the studied specimens of bleak. Of these, 14 specimens were infected with one parasite species, 17 specimens - with two parasite species and four specimens - with three species. The number of parasite species in one *Alb. alburnus* specimen varied from 1 to more than 100. Over 3,074 parasite species were isolated. Brillouin's diversity (HB) and Pielou's evenness (E) indices were low. Simpson's dominance index (C) was very high, which was associated with the dominance of *P. cuticola* (Table 2).

Table 1. Component community of *Alburnus alburnus* from the Kayaliika River, Varbitsa biotope (N - number of investigated fish; n - number of infected fish; p - number of fish parasites; MI - mean intensity; MA - mean abundance; P% - prevalence)

N = 45	n	p	MI	MA	P%	Range
<i>Clinostomum complanatum</i> (Rudolphi, 1814) Braun, 1899, metacercariae	2	10	5.00	0.22	4.44	1
<i>Posthodiplostomum cuticola</i> (von Nordmann, 1832) Dubois, 1936, metacercariae	30	> 3,000	100.00	66.67	66.67	> 100
<i>Caryophyllaeides fennica</i> (Schneider, 1902) Nybelin, 1922, adult and juvenile	2	2	1.00	0.04	4.44	1
<i>Acanthocephalus anguillae</i> (Müller, 1780), adult and juvenile	24	60	2.50	1.33	53.33	1-7
<i>Schulmanella petruschewskii</i> (Schulman, 1948) Ivashkin, 1964	2	2	1.00	0.04	4.44	1

Table 2. Infracommunity of *Alburnus alburnus* from the Kayaliika River, Varbitsa biotope

Number of specimens <i>Alburnus alburnus</i>	Number of parasites			
	0	1	2	3
	10	14	17	4
Total number of species (Mean number of species $\pm$ SD)	5 (1.31 $\pm$ 0.92)			
Total number of specimens (Mean number of specimens $\pm$ SD)	> 3074 (68.33 $\pm$ 47.83)			
Range	1 - > 100			
Brillouin's biodiversity index (HB)	0.126			
Pielou's evenness index (E)	0.08			
Simpson's dominance index (C)	0.953			

### Pathways of the parasite flow

The metacercariae of *Cl. complanatum* are found in the muscles at the base of the fins, on the gill covers and on the mouth, surrounded by yellow cysts (Lo et al., 1985; Wang et al., 2017). The definitive hosts of the trematode are fish-eating birds - herons and ducks. The second intermediate host is 12 freshwater fish species, and the first is freshwater snail - *Radix auricularia coreana* (Park et al., 2009; Hara et al., 2014). *Radix auricularia* (Linnaeus, 1758) is a bioindicator for  $\beta$ - $\alpha$ -mesosaprobity (Johnson et al., 1993). The metacercariae of *P. cuticola* are invested in the thick covering of the scales on the skin, fins, gill covers, and muscles according to Zashev & Margaritov; Bauer (by Zaharieva, 2022). Definitive hosts are fish-eating birds - herons. The second intermediate hosts are 39 species of freshwater fish, and the first intermediate hosts are freshwater snail species - *Planorbis planorbis* (Linnaeus, 1758) and *Planorbarius corneus* (Linnaeus, 1758) (syn. *Planorbis corneus*) (Zrnčić et al., 2009). *Pl. planorbis* is a bioindicator for  $\beta$ -mesosaprobity (Johnson et al., 1993). The cestode *C. fennica* is found in the intestines of freshwater fish species. The typical definitive host of the species is *Rutilus rutilus* (Linnaeus, 1758), but the species has also been found in more than 27 species of freshwater fish. Intermediate hosts are *Rhinodrilus proboscideus* Schneider, 1892 (syn. *Nais proboscideus*) and *Stylaria lacustris* (Linnaeus, 1758) according to Kakacheva-Avramova; Protasova et al.; Bauer (by Zaharieva, 2022). *St. lacustris* is a bioindicator for  $\beta$ -mesosaprobity (Kirin et al., 2020). The acanthocephalan *Ac. anguillae* is localized in the intestines of fish. Definitive hosts are freshwater fish species of Cyprinidae,

Salmonidae, Percidae, Anquillidae, Cobitidae, etc. An intermediate host is *Asellus aquaticus* (Linnaeus, 1758) according to Petrochenko; Kakacheva-Avramova; Bauer (by Zaharieva, 2022). *As. aquaticus* is a bioindicator for  $\alpha$ -mesosaprobity (Johnson et al., 1993). The nematode *Sch. petruschewskii* was found in the liver parenchyma. Definitive hosts are fish from Salmonidae, Cyprinidae, Cobitidae, Balitoridae, Siluridae, Centrarchidae, Percidae, Gobiidae. Intermediate hosts are *Eiseniella tetraedra* (Savigny, 1826), *Tubifex* Lamarck, 1816, Gammaridae and Cyclopidae according to Bauer (by Zaharieva, 2022); Moravec (2013). The identified bioindicator organisms (parasites and their intermediate hosts) in the studied biotope indicate  $\beta$ - $\alpha$ -mesosaprobic conditions. The low value of the Pielou's evenness index ( $E = 0.08$ ) and the high value of the Simpson's dominance index ( $C = 0.953$ ) indicate deteriorated environmental conditions. Only two parasite species of *Alb. alburnus* from the present study were reported in the bleak of rivers falling into the East Aegean region. *C. fennica* was reported in the bleak of the Tundzha River (Chunchukova & Kirin, 2020) and the Arda River (Kirin et al., 2002; Kirin, 2003). *Ac. anguillae* was also found in the bleak of the Tundzha River (Chunchukova & Kirin, 2020) and the Arda River (Kirin, 2003). This study identified two parasite species causing fish diseases - *Cl. complanatum* and *P. cuticola*. In fish heavily infected with *Cl. complanatum*, loss of appetite, weight loss due to difficulty in mouth movement and inability to feed, growth retardation, chaotic swimming, etc. are observed (Lo et al., 1985). *Cl. complanatum* is dangerous for humans because it causes parasitic pharyngitis or laryngitis. Entering the

human stomach after consuming raw fish, the metacercariae of the parasite pass through the esophagus and attach to the throat (Hara et al., 2014). *P. cuticola* causes the disease pomphorhynchosis. In heavily infected fish, a decrease in feeding, rapid weight loss, and death are observed (Rusev & Stratev, 2021).

## CONCLUSIONS

The parasitological study revealed infection with five parasite species - *Cl. complanatum*, *P. cuticola*, *C. fennica*, *Ac. anguillae*, *Sch. petruschewskii*. The Varbitsa biotope is a new habitat for the established parasites of bleak. *Cl. complanatum*, *P. cuticola* and *Sch. petruschewskii* are reported for the first time for *Alb. alburnus* from the East Aegean region in Bulgaria. Hydrobiological monitoring shows  $\beta$ - $\alpha$ -mesosaprobic conditions, and the Pielou and Simpson indices point to deteriorated conditions in the studied habitat. Due to the presence of parasites that hurt the state of fish populations and to protect fish resources and human health, it is recommended to monitor the biodiversity of parasites and the ecological indices of parasite communities of fish from the Kayaliika River.

## ACKNOWLEDGEMENTS

This research is supported by the Bulgarian Ministry of Education and Science under the national Program “Young Scientists and Postdoctoral Students-2”. The study was carried out in a laboratory at the Department of Chemistry, Phytopharmacy and Ecology, and Environmental Protection of the Agricultural University-Plovdiv. We thank the university leadership for the opportunity provided.

## REFERENCES

- Churchukova, M. & Kirin, D. (2020). Helminth fauna of some cyprinid fish species from lower stream of River Tundzha, Bulgaria. *International May Conference on Strategic Management – IMCSM20*, XVI(1), 465–473.
- Fisheries and Aquaculture Act, promulgated in the Official Gazette No. 41 of April 24, 2001, amended, OG No. 102 of December 8, 2023.
- Froese, R., & Pauly, D. (Eds) (2025). *FishBase*. World Wide Web electronic publication. www.fishbase.org, version (02/2025).
- Hara, H., Miyauchi, Y., Tahara, S., & Yamashita, H. (2014). Human laryngitis caused by *Clinostomum complanatum*. *Nagoya journal of medical science*, 76(1-2), 181.
- Hudson, P.J., Dobson, A.P., & Lafferty, K.D. (2006). Is a healthy ecosystem one that is rich in parasites? *Trends in ecology & evolution*, 21(7), 381–385.
- IUCN (2025). *The IUCN Red List of Threatened Species*. Version 2024-2. <https://www.iucnredlist.org>
- Johnson, R.K., Wiederholm, T., & Rosenberg, D.M. (1993). Freshwater biomonitoring using individual organisms, populations, and species assemblages of benthic macroinvertebrates. *Freshwater biomonitoring and benthic macroinvertebrates*, 40, 158.
- Kiradzhiev, S. (2013). *Encyclopedic Geographical Dictionary of Bulgaria*. Sofia, BG: “Iztok-zapad” Publishing House (In Bulgarian).
- Kirin, D., Buchvarov, G., & Kuzmanov, N. (2002). Biological diversity and ecological evaluation of the fresh water ecosystems of the Arda River. *Journal of Environmental Protection and Ecology*, 3(2), 449–456.
- Kirin, D.A. (2003). Biodiversity and ecological evaluation of the helminth communities of *Barbus cyclolepis* and *Alburnus alburnus* from Arda River, Bulgaria. *Experimental pathology and helminthology*, 6, 44–50.
- Kirin, D., Churchukova, M., Kuzmanova, D., & Paskaleva, V. (2020). Helminths and helminth communities of round-scaled barbell (*Barbus cyclolepis* Heckel, 1837) and its bioindicator role. *Scientific Papers. Series D. Animal Science*, 63(2), 421–426.
- Kuzmanova, D., Churchukova, M., & Kirin, D. (2019). Helminths and helminth communities of perch (*Perca fluviatilis* Linnaeus, 1758) as bioindicators for ecosystem condition of the Maritsa River. *Scientific Papers. Series D. Animal Science*, LXII(1), 463–468.
- Lo, C.F., Chen, S.C., & Wang, C.H. (1985). The study of *Clinostomum complanatum* (Rud., 1814) V. The influences of metacercaria of *Clinostomum complanatum* on fish. *Fish Pathology*, 20(2-3), 305–312.
- Maceda-Veiga, A., & De Sostoa, A. (2011). Observational evidence of the sensitivity of some fish species to environmental stressors in Mediterranean rivers. *Ecological Indicators*, 11(2), 311–317.
- Moravec, F. (2013). *Parasitic nematodes of freshwater fishes of Europe*. Praha, CZ: Academia Publishing House.
- Palm, H.W. (2011) Fish parasites as biological indicators in a changing world: can we monitor environmental impact and climate change? In: H. Mehlhorn (Ed.), *Progress in Parasitology* (pp. 223–250). Berlin, Heidelberg, GE: Springer Nature.
- Park, C.W., Kim, J.S., Joo, H.S., & Kim, J. (2009). A human case of *Clinostomum complanatum* infection in Korea. *The Korean journal of parasitology*, 47(4), 401.
- Rusev, V., & Stratev, D. (2021). *Fish Diseases*. Stara Zagora, BG: Trakia Press Publishing House (in Bulgarian).

- Szopieray, K., & Żbikowska, E. (2021). Positive role of parasites. *Acta Zoologica Academiae Scientiarum Hungaricae*, 67(3), 289–300.
- Wang, M.L., Chen, H.Y., & Shih, H.H. (2017). Occurrence and distribution of yellow grub trematodes (*Clinostomum complanatum*) infection in Taiwan. *Parasitology research*, 116, 1761–1771.
- Zaharieva, R. (2022). *Parasites and parasite communities of fish from the Danube River – ecology and biodiversity*. PhD Thesis, Plovdiv (in Bulgarian).
- Zaharieva, R., Zaharieva, P., & Kirin, D. (2025). Parasite diversity of *Cyprinus carpio* Linnaeus 1758 from the Danube River, Bulgaria. *Croatian Journal of Fisheries*, 83(1), 21–28. DOI: 10.2478/cjf-2025-0004
- Zrnčić, S., Oraić, D., Mihaljević, Ž., Čaleta, M., Zanella, D., Jelić, D., & Jelić, M. (2009). First observation of *Posthodiplostomum cuticola* (Nordmann, 1832) metacercariae in cypriniformes from Croatia. *Helminthologia*, 46, 112–116.