Аграрен университет – Пловдив, Научни трудове, т. LXVII, кн. 1, 2025 г.
Юбилейна научна конференция *„80 години Аграрен университет – Пловдив: Традиции срещат иновации “*
Anniversary Scientific Conference
"80 Years Agricultural University – Plovdiv:Traditions Meet Innovations"
Agricultural University – Plovdiv, Scientific Works, vol. LXVII, book 1, 2025

DOI: 10.22620/sciworks.2025.02.014

BIODIVERSITY AND ECOLOGICAL ASSESSMENT OF THE FRESHWATER ECOSYSTEM OF THE KRUMOVITSA RIVER

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Abstract

The study aims to present the results obtained from the conducted physicochemical and biological monitoring to establish potential anthropogenic impacts on the biodiversity and ecological state of the freshwater ecosystem of the Krumovitsa River, located in the Eastern Aegean Basin and Southern Bulgaria. The Krumovitsa River belongs to river typeR14c: Sub-Mediterranean, temporary (drying-up) small and medium-sized rivers and streams. The study is conducted in the middle part of the Krumovitsa River catchment, near the town of Krumovgrad, in the spring of 2025. Standard research methods are applied. The main physicochemical quality elements are monitored. The biological diversity of the ichthyologic complexes are described. The main ecological characteristics (distribution, number of species, abundance in percentages (A%), weight (Wg), weight in percentages (W%), and absolute dominance (Di)) are analysed. Biotic indices for ecological assessment are presented as Diversity indices for statistical representations of different aspects of biodiversity (Brillouin's diversity index (HB), Simpson's dominance index (S), Pielou's evenness index (E), etc.). Ichthiocomplexes are represented by two eudominant species (*Alburnus alburnus* (Linnaeus, 1758), *Barbus cyclolepis* Heckel, 1837) and one subrecident species (*Cobitis elongatoides* Băcescu & Mayer, 1969). The parasite component communities and infracommunities of the identified fish species and the indicator significance are discussed. A component species for the parasite communities is *Rhabdochona denudata* (Dujardin, 1845) Raillet, 1916 (P%=30.62). The ecological assessment is based on a complex analysis of the obtained results and statistical processing of the data.

Keywords: bioindication, bioticindices, physicochemical monitoring, comprehensive assessment, Bulgaria

INTRODUCTION

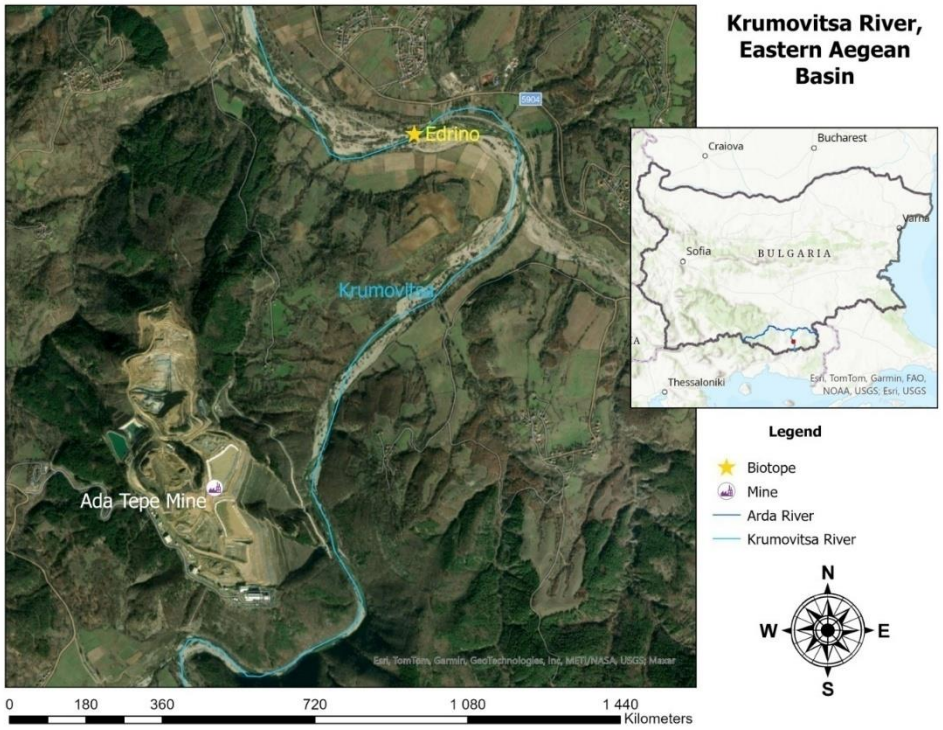
The Krumovitsa River is located in the Eastern Rhodopes, within the Arda River Basin and the Eastern Aegean Basin in Bulgaria. The river and its adjacent territories are distinguished by rich biodiversity and protected habitats, studied by a number of authors (Beron & Popov, 2005 (eds.)). For their protection, according to the Biodiversity Act (2002), the following protected areas were declared: PA BG0001032 Eastern Rhodopes under the Habitats Directive (Directive 92/43/EEC), PA BG0002012 Krumovitsa under the Birds Directive (Directive 2009/147/EC) and others. There are no domestic wastewater treatment plants built along the Krumovitsa River. Since 2019, a wastewater treatment plant at AD Ada Tepe has been constructed and put into operation, which is integrated into the Company's recycling cycle and thus does not discharge into the environment. AD Ada Tepe carries out permanent monitoring of air, water and soil, has an environmental management system and guarantees zero negative impact. Pandakov & Hudek (2000) carried out partial ecological monitoring on the territory of AD Ada Tepe to assess the impact on the ecological state of the Krumovitsa River by the biological element macrozoobenthos. According to the authors, the river ecosystem is not significantly affected. They highlight the new modern technologies and the work of the gold mining company. Stefanov & Trichkova (2017) describe the biodiversity and distribution of fish in the Eastern Rhodopes. Complex scientific studies on the ecological state of the river ecosystem, based on biological quality elements (BQEs) such as macrophytes, macrozoobenthos, fish, and their parasites, have not been conducted to date. This study aims to study the biodiversity and to make an ecological assessment of the state of the river ecosystem in the studied biotope by the BQEs macrophytes, macrozoobenthos, fish and their parasites.

MATERIAL AND METHODS

During the period May-June 2025, ecological monitoring studies were carried out on the Krumovitsa River in the vicinity of the village of Edrino (a section of the river designated as the Edrino biotope with coordinates 41°26'57.32"N 25°40'23.46"E; Map 1; Photos 1-2). The studies were based on biological quality elements (BQEs), including macrophytes, macrozoobenthos, fish, and their parasites.

The studies on BQE macrophytes were carried out in accordance with the requirements of the European Water Framework Directive (Directive 2000/60/EC), adapted for Bulgaria (Gecheva et al., 2010; Belkinova et al., 2013; Regulation No.H-4, 2012). The studies based on the BQE macrozoobenthos were based on the BDS EN ISO 8689-1:2001 standard. The samples were collected using a standard hydrobiological network in accordance with BDS EN ISO 10870:2012 and EN 16150:2012. Shortened scales were used to determine the BI, adapted for river type R14 (Belkinova et al., 2013). The fish were collected after permits for scientific fishing were issued byExecutive Agency for Fisheries and Aquaculture (EAFA) and Ministry of Agriculture and Food (MAF) in compliance with the BDS EN 14011:2004 standard. The ecological structure, condition and assessment of the condition of the ichthyocomplexes were analyzed based on the indicators number of specimens, abundance (%), weight (W g, %), maximum body length (L cm, %), absolute

dominance (Belkinova et al., 2013; Regulation No. H-4, 2012), Fulton's condition factor (Golub et al., 2019). Some basic physicochemical quality elements were determined: temperature (0C), pH, electrical conductivity (μS/cm), and hardness (ppm) in water samples according to Regulation No. H-4, 2012. The ecological condition was assessed according to the legislative documents (Directive 2000/60/EC; Water Act; Regulation No. H-4, 2012).



Map 1. Edrino Biotope

Photos 1-2. Edrino Biotope

RESULTSAND DISCUSSION

General characteristics of the Edrino biotope, river basin and macrophytes

The Krumovitsa River (length 58 km; catchment area 497.6 km2), with the studied biotope, is a natural water body. It belongs to the river type R14c Sub-Mediterranean, temporary (drying up) small and medium-sized rivers and streams, Ecoregion 7: Eastern Balkans. The river is part of the Arda River basin. In the middle part, from the catchment of the Krumovitsa River to the town of Krumovgrad, the river loses its flow during the low-water season. Isolated pools remain, formed as a result of connections with groundwater. The river is characterised by drying up during the summer period, pronounced spring high water and autumn low water. The river bed is composed of sand, gravel, and stones (more than 40%). The width of the river is less than 30 m. Typical macrophytes are *Typha latifolia* L. from the heliophytes, *Alisma lanceolatum* With. and *Lithrum salicaria* L. from the amphiphytes (<2%). *L. salicaria* is included in the IUCN Red List as LC (Least Concern). The total abundance of macrophytes is two according to Kohler (1978). The total coverage is 1-2% (Belkinova et al., 2013; BDS EN 14184:2014; Naredba H-4, 2012). Areas without macrophytes, as well as those typically dominated by river macrophytes, are also typically dominated by *T. latifolia*. Due to the variability of the water level and, accordingly, of the growths, the group of macrophytes must be supplemented with the results of other BQEs for assessing the ecological state. The river basin is dominated by *Salix alba* Linnaeus, 1753 and *Tamarix tetrandra* Pall, ex Bieb. The natural habitat 31F9 Riparian communities of Tamarix spp. is listed in the Red Book of the Republic of Bulgaria, item 3. Natural habitats with a conservation status of EN (Endangered, IUCN). The natural habitat is subject to protection by the Biological Diversity Act (2002), the Bern Conventionandthe Habitats Directive.

Fish species

In the studied biotope of the Krumovitsa River, the biological diversity characterising the ichthyofauna is represented by three species of freshwater fish belonging to the Cyprinidae family: Bleak *Alburnus alburnus* (Linnaeus, 1758); Round-scaled barbell *Barbus cyclolepis* Heckel, 1837; *Cobitis elongatoides* Băcescu & Mayer, 1969. *A. alburnus* is a brackish, benthopelagic, potamodromous and herd type fish species. It is distributed in almost all of Europe, as well as everywhere in Bulgaria. It prefers clean, shallow waters, inhabiting the surface layers. The main food of *A. alburnus* is zooplankton and insects that have fallen into the water. *B. cyclolepis* is a benthopelagic, rheophilic fish species, endemic to Bulgaria and the Balkan Peninsula. *B. cyclolepis* is protected and is subject to limited use according to the Biological Diversity Act. The species is distributed in Bulgaria, Greece, and Turkey, in the Aegean basin. It inhabits the upper and middle reaches of rivers with fast-flowing water and sandy-gravel bottoms. The main food sources are the larvae and pupae of aquatic insects, molluscs, and vegetation. *C. elongatoides* is a benthopelagic, non-migratory species. The three species of fish are included in the IUCN Red List as Least Concern (LC) (Karapetkova & Zhivkov, 2006; Kottelat & Freyhof, 2007). The three species of fish have been reported for the fauna of the Eastern Rhodopes (Stefanov & Trichkova, 2017).

Ecological structure, state, and assessment of the state of ichthyocomplexes

The fish communities established in the studied biotope of the Krumovitsa River, in the region of the village of Edrino, are dominated by *A. alburnus* (Di%=64.84), followed by *B. cyclolepis* (Di=34.25%). They are eudominants for the ichthyocomplexes. The third species, *C. elongatoides*, is distinguished by low values of the dominance coefficient (Di=0.93) and is a subrecident. *A. alburnus* and *B. cyclolepis* are less tolerant species, while *C. elongatoides* is a tolerant species (Belkinova et al., 2013; Regulation No. H-4, 2012) (Table 1).

Table 1. Biodiversity and basic ecological indices of fish complexes

Fish species	***Number of specimens Abundance, %***	***Weihgt range, W g (Mean±SD), W%***	***Length range, L cm (Mean±SD) L%***	***Absolute dominance, Di %***
Bleak *Alburnus alburnus* (Linnaeus, 1758)	49 65.34%	1-5 (1.78±1.01) 64.33%	2.2-7.8 (5.07±1.37) 68,08%	64.84 Eudominant
Round-scaled barbell *Barbus cyclolepis* Heckel, 1837	25 33.34%	1-3 (1.36±0.57) 35.16%	4.0-6.8 (5.43±0.81) 26.60%	34.25 Eudominant
Cobitis elongatoides Băcescu & Mayer, 1969	1 1.34%	2.0 0.52%	6.8 5.32%	0.93 Subrecedent

The obtained values for Fulton's condition factor (K): *A. alburnus* K=0.46-18.52 (2.79±4.53), *B. cyclolepis* K=0.32-1.56 (0.86±0.30), and *C. elongatoides* K=0.64, indicated a normal condition of fish and the studied biotope of the Krumovitsa River.

Parasites and parasite communities of the studied fish species

A total of 49 specimens of *A. alburnus*, 25 specimens of *B. cyclolepis* and one specimen of *C. elongatoides*weresubjectedto ecoparasitologicalstudies.Infections were found only in specimens of Bleak (Table 2).

Invasion by two species of parasites was registered, respectively, from the class Cestoda and the phylum Nematoda. *Rh. denudata* is a component species for the parasitic communities of *A. alburnus* (P%=30.61), and *Pr. torulosus* is anaccidental species (Table 2). A total of 9 specimens of *A. alburnus* were invaded by 16 specimens of parasites. The number of parasite specimens in the infected fish varied from 1 to 3. In 40 specimens of *A. alburnus*, no infection was detected (Table 3).

The Brillouin's diversity index (BI) characterises species richness with emphasis on the number of species and sample size. The Simpson's dominance index is low, likely due to the small number of specimens and the absence of pronounced dominance. The Pielou index (E) is close to unity, indicating an approach to the optimal state.

Table 2. Parasite species and component community of *Alburnus alburnus* from the Krumovitsa River

Alburnus alburnus (Linnaeus, 1758) ***N=49*** ***Type of parasite***	***n/p (range)***	***P% (Mean±SD)***	***MA (Mean±SD)***	***MI (Mean±SD)***
Class Cestoda **Order Tetraphyllidea (Beneden, 1849) Carus, 1863** **Family Proteocephalidae La Rue, 1911** **Genus *Proteocephalus* Weinland, 1858**				
Proteocephalustorulosus(Batsch, 1786) Nufer, 1905	1/1	2.04	0.02	1.00
Phylum Nematoda **Class Adenophorea** **Order Spirurida Chitwood, 1933** **Family Rhabdochonidae Travassos, Artigas et Pereira, 1928** **Genus *Rhabdochona* Railliet, 1916**				
Rhabdochona denudata (Dujardin, 1845) Raillet, 1916	8/15	30.61 (20±7.07)	0.31 (0.16±0.014)	1.88 (1.34±0.47)

Table3. Infracommunity data

No. of parasite species				
Number of fish (*A.alburnus*)	40	4	3	2
Number of parasite species	0	1	2	3
Number of parasite specimens				
Total number	16			
Mean ± SD	0.30±0.07			
Range	1-3			
Brillouin's diversity index (HB) (Mean±SD)	1.15 (0.64±0.06)			
Simpson's dominance index (C) (Mean±SD)	0.19 (0.35±0.01)			
Pielou's evenness index (E) (Mean±SD)	0.96 (0.98±0.03)			

Proteocephalus torulosus (Batsch, 1786) Nufer, 1905 was reported as a parasite of *A. alburnus* by Kakacheva-Avramova (1977) andKakacheva-Avramova et al. (1978). *Rhabdochona denudata* (Dujardin, 1845) Raillet, 1916 was reported for *A. alburnus* by Kakacheva-Avramova (1962), Kakacheva-Avramova (1965), Kakacheva-Avramova (1969), Kakacheva-Avramova (1972), Kakacheva-Avramova (1973), Kakacheva-Avramova (1977) for the Struma River, reservoirs of Thrace, the Ogosta River, the Lom River, the Leva River, the Tundzha River, the Eleshnitsa River, and the Danube River. The adult form of *Pr. torulosus* parasitises mainly in the intestines of Cyprinidae. Intermediate hosts are representatives of the crustaceans of the genera *Cyclops* and *Diatomus*. *R. denudata* is an intestinal

endoparasite of freshwater species of Cyprinidae. The development takes place with the participation of an intermediate host from the genera *Heptagenia*, *Hydropsiche* and *Ephemerella* (Kakacheva-Avramova, 1983; Bauer (ed.), 1987; Moravec, 2013). The three species of parasites are reported for the first time from the Bleakof the Krumovitsa River, Edrino biotope. The studied biotope is a new location for the distribution of the established species of parasites.

Ecological assessment based on the biological quality element macrozoobenthos

In the freshwater ecosystem of the Krumovitsa River, Edrino biotope, 23 taxa of bioindicator macrozoobenthos have been identified (Table 4). A total of 194 specimens have been studied. Representatives of Chironomidae (60 specimens) dominate, followed by *Serratella ignita*, larvae (28 specimens), *Hydropsyche ornatula*, larvae (24 specimens), *Limnephilus rhombicus*, larvae (10 specimens). The number of other taxa is less than 10 specimens. 0-β-mesosaprobic organisms (7 taxa) predominate, followed by the groups 0-α-mesosaprobic organisms and β-α-mesosaprobic organisms, represented by two taxa each. All other saprobity groups (χ-0 saprobic; χ-β-mesosaprobic; 0 saprobic; β-mesosaprobic; α-mesosaprobic) have one taxon each. Of the sensitivity groups, group C (relatively tolerant forms) is best represented – 14 taxa with 71 specimens. Group A (sensitive forms) and group B (less sensitive forms) are represented by two taxa each, with 11 and 30 specimens, respectively. Chironomidae (68 specimens) from sensitivity group D (tolerant forms) are well represented.

Table 4. Metrix of ecological state

Metrix	***Values***	***Ecological state***
Total number of taxa	23	very good
EPT	9	good
Margalef's species richness index (Dmg)(Mean±SD)	4.21 (2.39±0.75)	4.21<8
Shannon-Weaver's species diversity index (H') (Mean±SD)	2.35 (1.77±0.22)	β-mesosaprobic conditions
Pielou's evenness index (E) (Mean±SD)	0.649 (0.62±0.04)	β-mesosaprobic conditions
SPUB	1.88	very good
BI R14	3	good (EQR 0,857)

The metrics, including the Total number of taxa and the SPUB (Pantle & Buck Saprobic Index), in the study indicate a very good ecological state. The EPT (%) and BI (Adapted Biotic Index) indices indicate a good ecological state in the studied biotope (Belkinova et al., 2013). TheMargalef's species richness index (Dmg) is determined based on the total number of species and the total number of specimens. It is believed that values of Dmg>8 indicate optimal development of ecosystems. The Shannon-Weaver's species diversity index(H') is high when the ecosystem is in optimal condition, i.e. when the number of species is large, and the number of

specimens of each species is relatively small and even. The Pielou's evenness index (E) reflects the evenness in the distribution of the total number between the individual species (Roussev, 1993). The results obtained for E and H' indicate β-mesosaprobic conditions.

Physicochemical quality elements

The main physicochemical quality elements were determined.The measurements were carried out at a temperature of 18.17-19.8^{0}C (18.77±0.89^{0}C).The pH values ranged from 8.13-8.35 (8.22±0.12; good state), the electrical conductivity - from 602-738 µS/cm (664.67±68.62 µS/cm; good state), and the hardness - from 301-342 ppm (322.34±20.55);class 5;Environmental Quality Standards (EQS) Regulation).According to Regulation No. H-4/2012, for rivers of type R14, the obtained quality elements indicate good water state in the studied biotope.

CONCLUSIONS

Based on the results obtained, the integrated ecological assessment indicates a good ecological state of the Krumovitsa River's ecosystem in the Edrino biotope.

ACKNOWLEDGEMENTS

This work has been carried out in the framework of the National Science Program "Critical and strategic raw materials for a green transition and sustainable development", approved by the Resolution of the Council of Ministers № 508/18.07.2024 and funded by the Ministry of Education and Science (MES) of Bulgaria.

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