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BOOK OF PROCEEDINGS

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THE MACROINVERTEBRATE COMMUNITY COMPOSITION IN SOME WATER ECOSYSTEMS IN STARA PLANINA MOUNTAINS (SOUTHEAST SERBIA)

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ABSTRACT:
Due to its geographical location and paleogeographic history, the Stara Planina Mountains are one of the six biodiversity hotspots in Europe. This study aims to depict the composition of the aquatic macroinvertebrates situated on the Serbian side of the Stara Planina Mountains. Aquatic macroinvertebrates were collected at six sites: Jelovičko Spring, Dojkinačka, Visočica, Temštica, and Rakitska rivers, and the spring of Bigar Stream. We recorded 1974 specimens within 97 taxa and ten systematic groups. Insects dominated the macroinvertebrate community, comprising 82.5% of the total density. Specifically, Diptera (23.5%), Ephemeroptera (19.8%), and Trichoptera (19.3%) were the most abundant. Trichoptera was the most diverse group, represented by 27 taxa, followed by Diptera (25 taxa) and Ephemeroptera (15 taxa). The diversity of macroinvertebrate communities, expressed as the Shannon index of general diversity, varied spatially from 3.17 (Dojkinačka River) to 1.72 (spring of Bigar Stream), while Simpson's Diversity Index ranged from 0.73 (spring of Bigar Stream) to 0.95 (Rakitska River). This study contributes to filling knowledge gaps about benthic communities in rivers and streams in the Stara Planina Mountains, which is essential for evaluating the vulnerability of freshwater ecosystems.

Keywords: *macroinvertebrate diversity, sensitive taxa, rivers, springs*

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1. INTRODUCTION

Freshwater ecosystems are considered one of the most important providers of ecosystem services in terms of economic value, culture, science, and education. They also contain about 10% of all described animal species, making these habitats among the most productive ecosystems on earth (Nieto et al., 2017). At the same time, these ecosystems are the most threatened worldwide; therefore, freshwater organisms require more attention for their conservation. Macroinvertebrates are a part of every freshwater ecosystem in the world and often exhibit high diversity. Aquatic macroinvertebrates can have an important impact on nutrient cycling, primary productivity, decomposition, and translocation of material (Wallace and Webster, 1966). Because of their long life cycles (generally one year, most of which is spent in the water) and relative immobility, aquatic macroinvertebrates are good indicators of stream health, and therefore, their survival is directly linked to their habitat (Agouridis et al., 2015; Parmar et al., 2016).

Regions of the Stara Planina Mountains have always attracted attention because of the vast diversity of landforms and heterogeneous habitats. At the suggestion of the Serbian Institute for Nature Conservation, the western Stara Planina Mountains were placed under strict protection in 1997 as a "natural merit of the first class". Due to their geographical position and paleogeographical history, the Stara Planina Mountains are one of the six biodiversity hotspots in Europe (Stojanović et al., 2017).

A literature survey of the biota on the Serbian side of this mountain yielded a high number of different scientific works. However, there have been limited and sporadic investigations concerning aquatic macroinvertebrates (Živić et al., 2005). Therefore, the study focused on the macroinvertebrate assemblage in the selected localities of the Stara Planina Mountains to expand our knowledge of macroinvertebrate diversity in this biodiversity hotspot.

2. MATERIALS AND METHODS

2.1. Study area

Field research of aquatic macroinvertebrates of Stara Planina Mountain was performed at six sites. A detailed description of the sites, including geographical coordinates, altitudes, and the date of sampling, is provided in Table 1.

Table 1. Characteristics of the studied sites.

Name of sites	Site code	Date of sampling	Geographic coordinates	Elevation
Jelovičko Spring	**JEL**	**28.05.2022.**	**43°11'00.2"N 22°50'00.1"E**	756 m
Dojkinačka River	**DOJ**	**29.05.2022.**	**43°14'27.1"N 22°46'42.2"E**	866 m
Visočica River	VIS	29.05.2022.	43°09'23.9"N 22°48'27.2"E	705 m
Rakitska River	RAK	18.11.2022.	43°20'39.0"N 22°40'38.9"E	729 m
Temštica River	**TEM**	**22.03.2023.**	**43°15'47.6"N 22°33'01.8"E**	388 m
Bigar Stream	BIG	22.03.2023.	43°20'42.7"N 22°26'21.7"E	537 m

2.2. Sample collection and processing

At each site, three subsamples of macrozoobenthos were gathered from the most dominant substrate types using a Surber sampler with a 250 mm mesh. These subsamples were combined into a single sample, promptly preserved in a 96% alcohol solution, transported, and stored at the Institute of Biology and Ecology, Faculty of Science, University of Kragujevac, Republic of Serbia. The collected materials were analyzed using a Nikon Szm 800 stereomicroscope equipped with a Leica camera and a Nikon Eclipse E100 microscope. Taxonomic identification was conducted utilizing available literature (Rozkošný, 1980; Nilson, 1997; Eiseler, 2005; Waringer & Graf, 2011). Macroinvertebrate community composition and diversity were assessed across sampling sites, including the number of individuals, taxon richness, and Shannon (Shannon, 1948) and Simpson (Simpson, 1949) diversity indices.

3. RESULTS AND DISCUSSION

During our study, we recorded 1974 individuals and 97 taxa, of which 73 were identified at the species level, 24 at the genus level, and one at the family level. The highest numbers of taxa were collected at the Temštica River (45 taxa), followed by the Dojkinačka River (40 taxa). Trichoptera was the most diverse group, represented by 27 taxa, followed by Diptera (24 taxa), Ephemeroptera (15 taxa), and Plecoptera (12 taxa) (Table 2).

Table 2. Qualitative and quantitative analysis (absolute number of individuals) of macroinvertebrates from the investigated water ecosystems

TAXA	**JEL**	**DOJ**	**VIS**	**RAK**	**TEM**	**BIG**
Turbellaria						
Dugesia gonocephala (Duges, 1830)	0	0	0	0	2	0
Dugesia sp.	13	0	0	4	0	0
Polycelis felina (Dalyell, 1814)	0	0	0	0	0	8
Polycelis sp.	27	0	0	0	0	0
Oligochaeta						
Eiseniella tetraedra (Savigny, 1826)	0	0	0	0	1	0
Lumbriculus sp.	0	0	0	0	0	0
Psamoryctides barbatus (Grube, 1860)	0	0	2	0	0	0
Stylodrilus heringianus Claparède, 1862	0	1	1	0	0	1
Gastropoda						
Ancylus fluviatilis (O. F. Müller, 1774)	6	2	6	8	1	0
Bythinella dispersa Radoman, 1976.	0	0	0	0	0	136
Amphipoda						
Gammarus balcanicus Schaferna, 1922	19	0	0	0	18	85
Decapoda						
Austropotamobius torrentium (Schrank, 1803)	2	2	1	1	0	0
Ephemeroptera						
Baetis alpinus (Pictet, 1843)	0	0	0	0	4	0
Baetis lutheri Müller-Liebenau, 1967.	2	8	9	0	0	0
Baetis rhodani (Pictet, 1843)	7	27	59	0	19	6
Baetis sp.	2	3	0	0	0	1

Ecdyonurus helveticus Gr. Eaton, 1883	0	0	0	0	0	7
Ecdyonurus sp.	8	7	6	4	3	0
Epeorus assimilis Eaton, 1865	0	21	46	9	29	0
Ephemera danica Müller, 1764	0	4	1	5	0	0
Ephemerella mucronata (Bengtsson, 1909)	0	9	6	0	0	0
Ephemerella sp.	0	1	0	0	0	0
Paraleptophlebia submarginata (Stephens, 1835)	0	0	0	0	3	0
Paraleptophlebia werneri Ulmer, 1919	0	0	4	0	0	0
Rhithrogena semicolorata (Curtis, 1834)	0	15	35	0	24	0
Seratella ignita (Poda, 1761)	0	0	0	0	2	0
Torleya major (Klapálek, 1905)	0	0	2	0	0	0
Plecoptera						
Brachyptera risi (Morton, 1896)	1	0	0	0	10	0
Dinocras megacephala (Klapálek, 1907)	0	3	0	0	0	0
Isoperla grammatica (Poda, 1761)	0	0	5	0	1	0
Isoperla tripartita Illies, 1954	51	6	0	0	0	0
Leuctra pseudosignifera Aubert, 1954	0	0	0	0	3	0
Leuctra sp.	0	0	0	9	0	0
Nemoura erratica Claassen, 1936	2	0	0	0	0	14
Perla burmeisteriana Claassen, 1936	0	0	0	0	5	0
Perla marginata (Panzer, 1799)	0	11	8	7	12	0
Protonemura nitida (Pictet, 1836)	95	3	0	0	2	0
Protonemura sp.	0	0	1	0	0	0
Taeniopteryx schoenemundi (Mertens, 1923)	0	0	0	0	1	0
Trichoptera						
Athripsodes albifrons (Linnaeus, 1758)	0	0	1	0	0	0
Chaetopteryx villosa (Fabricius, 1798)	0	0	0	0	0	8
Cheumatopsyche lepida (Pictet 1834)	0	2	29	0	0	0
Drusus sp.	1	0	0	0	0	0
Glossosoma sp.	0	2	0	0	1	0
Lepidostoma basale (Kolenati, 1848)	0	0	0	0	4	0
Oligoplectrum maculatum (Fourcroy, 1785)	0	0	0	0	1	0
Odontocerum albicorne (Scopoli, 1763)	0	1	0	0	1	0
Helicopsyche bacescui Orghidan and Botosaneanu, 1953	0	0	0	0	0	6
Halesus digitatus Curtis, 1834	0	17	4	0	0	0
Hydropsyche botosaneanui Marinković-Gospodnetić (1966)	0	0	0	0	6	0
Hydropsyche instabilis (Curtis, 1834)	0	2	24	14	0	0
Hydropsyche sp.	0	4	4	0	0	0
Micrasema morosum (McLachlan, 1868)	0	0	0	0	6	0
Potamophylax luctuosus (Piller & Mitterpacher, 1783)	3	41	5	0	4	0
Polycentropus sp.	0	0	0	5	0	0
Philopotamus montanus (Donovan 1813)	0	0	0	13	1	0
Psychomyia pusilla (Fabricius, 1781)	0	0	41	0	0	0
Rhyacophila laevis Pictet, 1834	0	0	0	0	3	0

Rhyacophila tristis Pictet, 1834	0	0	0	0	1	0
Rhyacophila sp.	12	2	13	0	1	1
Silo piceus (Brauer, 1857)	0	2	0	9	30	0
Silo sp.	0	0	1	1	0	0
Sericostoma flavicorne Schneider, 1845	0	2	1	0	0	0
Sericostoma personatum (Kirby & Spence, 1826)	0	3	2	0	11	0
Thremma anomalum McLachlan, 1876.	0	0	0	0	0	2
Wormaldia occipitalis (Pictet, 1834)	0	0	0	0	1	0
Diptera						
Antocha vitripennis (Meigen, 1830)	0	2	7	1	0	0
Atherix ibis (Fabricius, 1798)	0	1	0	0	0	0
Berdeniella sp.	5	0	0	0	0	0
Bezzia sp.	0	0	2	0	0	0
Dicronata bimaculata (Schummel, 1829)	0	0	0	0	2	0
Dixa sp.	0	0	0	1	0	0
Ibisia marginata (Fabricius, 1781)	0	0	0	3	0	0
Chironomidae	0	10	0	7	0	3
Brillia flavifrons (Johannsen 1905)	0	0	0	0	2	0
Brillia modesta (Meigen, 1830)	0	0	0	0	1	0
Diamesa insignipes Kieffer, 1908	0	0	0	0	9	0
Diamesa sp.	17	0	0	0	1	0
Eukiefferiella fittkaui Lehmann, 1972	116	12	0	0	0	0
Eukieferiella sp.	0	0	0	2	0	0
Micropsectra sp.	0	0	0	0	0	5
Nilotanypus dubius (Meigen, 1804)	0	5	0	0	0	0
Orthocladius thienemanni Kieffer, 1906	0	0	0	0	187	0
Orthocladius frigidus (Zetterstedt 1838)	0	0	0	0	2	0
Orthocladius sp.	20	0	0	0	13	1
Parametriocnemus stylatus (Spärck, 1923).	0	1	0	3	0	0
Polypedilum convictum (Walker, 1856)	0	15	0	0	0	0
Thienemanniella majuscula (Edwards, 1924)	0	2	0	0	0	0
Tvetenia calvescens (Edwards, 1929)	3	0	0	0	3	1
Tvetenia discoloripes (Goetghebuer & Thienemann, 1936)	2	21	3	9	1	0
Tanytarsus sp.	0	4	0	0	0	0
Coleoptera						
Pomatinus substriatus (Müller, 1806)	0	0	1	3	1	0
Hydraena gracilis Germar, 1823	0	0	2	11	0	0
Elmis sp.	3	5	15	12	3	29
Limnius volckmari (Panzer, 1793)	0	0	3	0	0	0
Limnius sp.	0	2	7	0	0	0
Orectochilus villosus (O. F. Müller, 1776)	0	3	21	0	1	0
Number of taxa	**23**	**40**	**37**	**23**	**45**	**17**
Number of individuals	**417**	**284**	**381**	**141**	**437**	**314**

Looking at the absolute number of individuals, the most representative groups are Diptera (25.6%), Ephemeroptera (20.2%), Trichoptera (17.8%), and Plecoptera (12.7%). Other groups were numerically poorer, with an average percentage of less than 10% (Figure 4). The Chironomidae family (18 taxa) accounts for 95.2% of all individuals within the Diptera order.

Diversity indices for studied sites are shown in Table 2. The diversity of macroinvertebrate communities, measured by the Shannon index of general diversity, showed spatial variation ranging from 3.17 (Dojkinačka River) to 1.72 (Bigar Stream spring). Meanwhile, Simpson's Diversity Index exhibited a range from 0.73 (Bigar Stream spring) to 0.95 (Rakitska River) (Table 3).

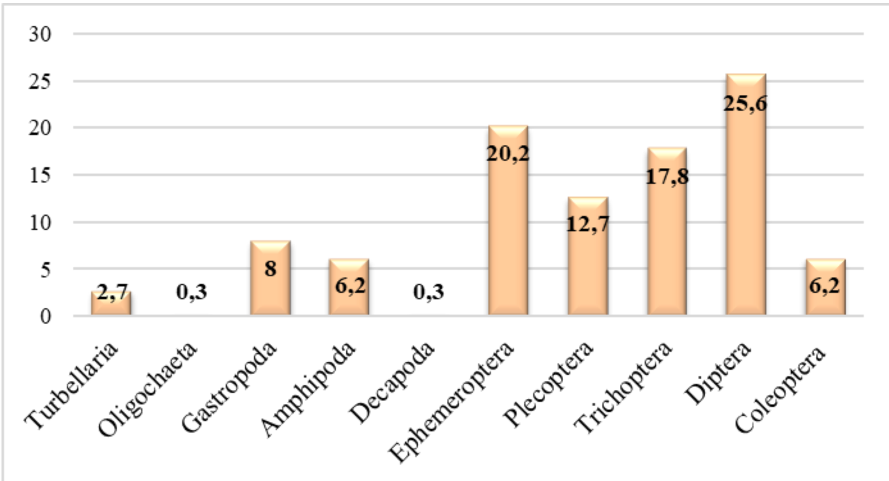

Figure 1. Percentage of individuals in each taxonomic group at the studied rivers and streams.

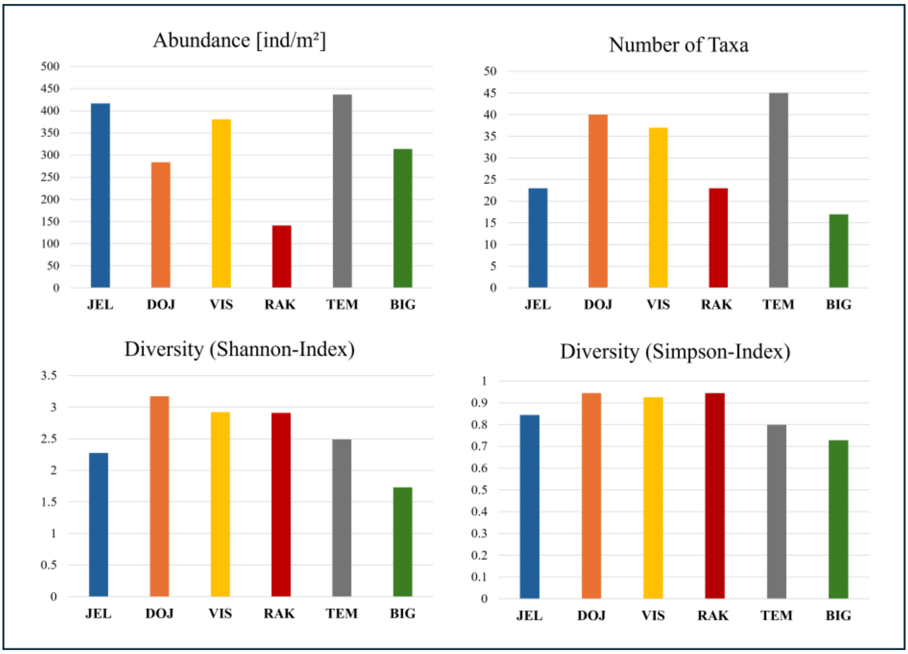

Figure 2. Macroinvertebrate diversity in the investigated sites is presented as the number of individuals, taxa richness, Shannon and Simpson's diversity indeces.

The Stara Planina Mountains are recognized as one of Europe's most important biodiversity hotspots. However, the biogeographic patterns of its freshwater fauna, especially for macroinvertebrates, are poorly understood due to the lack of basic data on distribution and ecology, particularly for the Serbian side. We investigated the diversity of aquatic macroinvertebrates in the six watercourses on the Serbian side, recording 97 taxa. The observed community composition was as expected for this type of watercourse and is not significantly different from that typically found in hilly-mountainous rivers in the Balkan Peninsula region (Živić et al., 2005; Paunović et al., 2006; Petrović et al., 2015). The bottom fauna of these investigated watercourses is characterized by taxonomic richness and a vast diversity of insects. This is quite typical because these macrozoobenthos groups dominate highland rivers and streams. The water resources of the Stara Planina Nature Park are mostly preserved and represent water sources of high quality (class I or II), which are of national and regional importance for Serbia. This is supported by the recorded high level of Ephemeroptera, Plecoptera, and Trichoptera (EPT groups) taxa, which are sensitive to physical, chemical, and hydromorphological degradation (Moog et al., 2017). In the spring of the Jelovička River, insects dominated, such as *Protonemura nitida*, *Isoperla triprtita*, and *Eukiefferiella fittkaui*, which are mostly cold-water stenothermal species that normally inhabit these habitats. In addition to insects, the benthic fauna in the studied springs was dominated by amphipods (*Gammarus balcanicus*) and gastropod (*Bythinella dispersa*) taxa, such as was the case in many other springs in Serbia and neighbors' regions (Marković et al., 1999; Dumnicka et al., 2007) The recorded abundant populations of *B. dispersa*, pollution-sensitive snails at the spring of Bigar Stream, indicate favorable habitat conditions and a low level of anthropogenic pressures. The great diversity of insect larvae indicates that habitat conditions at the investigated sites (shallow and fast streams with a rocky and stony bed cover and minimum human impact) are more favourable for insects than for any other macrozoobenthic groups. This significant diversity is matched by relatively uniform and high Shannon and Simpson indices values on the Temštica, Visočica, Dojkinačka, and Rakitska rivers. The dominant species in the upper reaches of these four rivers were *Epeorus assimilis*, *Baetis rhodani*, *Rhithrogena semicolorata*, *Perla marginata*, as well as species of the genus *Rhyacophila* and *Hydropsyche*. Váncsa et al. (2011) examined the Ephemeroptera fauna on the Bulgarian side of the Stara Planina Mountain, and their findings align with ours. Following the Appendix of the Book of Regulations regarding the identification and conservation of protected and strictly protected wild species of plants, animals, and fungi (Anonymous, 2010), we recorded the presence of three strictly protected species (*Austropotamobius torrentium, Thremma anomalum, Helichopsyche bacescui*).

The taxonomic richness and high diversity of macroinvertebrates in the Visočica and Temšica rivers were previously documented by Živić et al. (2005), recording the presence of 102 taxa. Compared to our study, the greater number of recorded taxa is attributed to sampling macroinvertebrates across a larger number of localities and throughout more seasons. Therefore, this one-time study should be extended to encompass a broader range of habitats and a longer observation period.

3. CONCLUSION

During our investigation, we documented a fauna typical of hilly-mountainous rivers and streams in the Balkan Peninsula region, with the domination of insects in both qualitative and quantitative community composition. Understanding the composition, distribution, and abundance of macrozoobenthos in mountain rivers is essential for effective river management and conservation efforts. Monitoring these organisms provides valuable data for assessing the impacts of human activities and climate change on freshwater ecosystems.

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