Arch Biol Sci. 2023;75(2):211-220 https://doi.org/10.2298/ABS230306017N

Structure and function of benthic invertebrate assemblages of the Đerekarska River (southwestern Serbia, Pešter plateau)

Melisa Dž. Numanović[1,*], Katarina Z. Stojanović[2], Boris B. Novaković[3], Milica M. Živković[1], Nebojša V. Živić[4] and Branko M. Miljanović[1]

[1]*University of Novi Sad, Faculty of Sciences, Department of Biology and Ecology*, Trg Dositeja Obradovića 2, 21000 Novi Sad, Serbia

[2]*Faculty of Biology, University of Belgrade*, Studentski trg 16, Belgrade, Serbia

[3]*Sector of the National laboratory, Serbian Environmental Protection Agency, Ministry of Environmental Protection of Serbia*, Žabljačka 10a, Belgrade, Serbia

[4]*University of Priština, Department of Biology*, Lole Ribara 29, 38220 Kosovska Mitrovica, Serbia

***Corresponding author**: melisa.numanovic@gmail.com

Received: March 6, 2023; **Revised**: April 17, 2023; **Accepted**: April 19, 2023; **Published online**: May 16, 2023

Abstract: This study is focused on benthic invertebrate communities in the Đerekarska River in Serbia, sampled from June to December 2016. We examined how different microhabitats with various substrates influenced the structure and function of the community. Taxonomic analysis revealed 55 species from 43 genera and 35 families, with the most diverse groups being Trichoptera, Ephemeroptera, and Plecoptera. ANOVA showed that different substrate types impacted the functional feeding groups within the assemblages. Cluster formation was primarily influenced by the substrate structure; this was followed by shredders, gatherers and collectors, passive filters, active filter feeders, the river depth, biochemical oxygen demand, chemical oxygen demand, and the flow velocity; for grazers and scrapers, discharge and saturation did not significantly affect cluster formation. Using two-step cluster analysis, we identified three clusters based on the substrate structure. The first group consisted of organisms that prefer a stone substrate; the second group consisted of organisms that prefer a mixed stone/gravel/sand substrate; the third group consisted of organisms that prefer gravel and sand substrates. Each cluster had a dominant functional feeding group, such as shredders and predators in the first cluster, gatherers and collectors in the second cluster, and predators, grazers, and scrapers in the third cluster.

Keywords: macrozoobenthos, Đerekarska River, karstic river, functional feeding guilds

INTRODUCTION

Macroinvertebrates often show individualistic and continuous responses to environmental conditions [1,2], so that assemblages change gradually in composition, presumably because individual species rather than the whole assemblage respond to environmental gradients. The Balkan Peninsula in Europe is considered one of the most important hotspots of animal diversity [3,4]. It is particularly important for karstic areas, such as the Dinaric region (Western Balkans), which harbor rich communities of aquatic macroinvertebrates [5,6]. Aquatic insects are the most well-known macroinvertebrate species found in Dinaric karstic rivers [7-12].

We concentrated our research on the section of the Đerekarska River in the Pešter area, which is currently a special nature reserve called Peštersko Polje [13]. Our goal was to increase knowledge of the aquatic macroinvertebrate fauna there and to gain a better understanding of the dynamics of aquatic ecosystems and the characteristics of the habitat itself, in order to clarify the interactions between a community and its environment, as well as community change. The use of multimetric indices can potentially reflect multiple impacts of human influence on aquatic ecosystem structure and function. Using functional feeding guild data, we can better assess the ecological potential of the watercourse [14,15]. When the mechanisms that

How to cite this article: Numanović MDž, Stojanović KZ, Novaković BB, Živković MM, Živić NV, Miljanović BM. Structure and function of benthic invertebrate assemblages of the Đerekarska River (southwestern Serbia, Pešter plateau). Arch Biol Sci. 2023;75(2):211-20.

determine whether a species is present in the environment or is not are clearly understood, these methods may be used most effectively to assess the ecological status [16].

In this work, the dynamics of the functional feeding guilds (FFGs) in aquatic ecosystems were evaluated in detail. Special attention was given to the relationship between FFGs and different environmental factors, such as substrate type and physical and chemical parameters.

MATERIALS AND METHODS

Study area

The Pešter plateau is one of the last great preserved and the largest karstic field in Serbia (Supplementary Fig. S1). This area is characterized by unique geomorphological, geological, hydrogeological, hydrological and climatic phenomena and specific landscapes. Pešter is known for its specific microclimate, especially for the severe winters. Here the officially lowest temperature of -39.5°C was measured in Serbia, and this highland is also called "Balkan Siberia". The heterogeneous karst landscape on the Pešter plateau creates a variety of unique habitats and numerous endangered species. The river Đerekarska flows through this field. The source of the river is the wastewater-rich Đerekar spring, which flows from a cave above the village of Đerekare, located on the southern bank of the Pešter highlands (southwestern part of Serbia) at an altitude of 1296 m (as obtained by field measurement using GARMIN ETREX H GPS). The Đerekar spring rises at the junction of limestone and impervious diabase rock. The Đerekarska is a river that sinks at Peštersko Polje. The length of the river is not constant but depends on the amount of water available due to rainfall (season) and the current terrain. Up to the village of Naboje, the river Đerekarska flows through a narrow gorge; from its spring above Đerekare to the village of Boroštica, it bears the name Đerekarska. The river disappears into a sinkhole under the limestone formation of Gorica and reappears again after 18 m as the river Boroštica, which finally drops underneath the limestone formation of the Suka, the longest river that sinkholes in Serbia.

Sampling methods

In the spring, summer and fall of 2016 (June to December) we sampled the fauna of the Đerekarska River on the upper and middle reaches and at the sinkhole (Supplementary Fig. S1). Benthic macroinvertebrate samples were collected using a Surber sampler (500 cm2 area, 250-µm mesh size) with 4 replicates. Considering that the structure and composition of the macrozoobenthos are affected by the morphological characteristics of the substrate, macroinvertebrate sampling was performed in different habitats. First, an analysis of substrate type distribution was done by visual assessment of the substrate as described previously [17]. The composition of the substrate is classified into five classes as follows: fine substrates, sludge (<0.125 mm), sand (0.125-2 mm), gravel (2-64 mm), stone (64-256 mm), rock (>256 mm). Part of each sample was analyzed in its native state and the rest after preservation in 75% ethyl alcohol. Identification of macrozoobenthos was made using taxonomic keys [18-29].

Physical and chemical parameters of the water

The following physical and chemical parameters of the water were measured *in situ*: pH value, electrical conductivity, water temperature using the HI 98130 HANNA COMBO Tester for pH/EC/TDS/T (USA), the concentration of dissolved oxygen and oxygen saturation using the HANNA HI 9146-04/10. Biochemical oxygen demand over 5 days (BOD_5) was determined using the Royal Commission on river pollution methodology [30], and chemical oxygen demand (COD) was determined using the Kubel-Tiemann method (titration with potassium permanganate in acid solution) [31,32]; the alkalinity and carbonate hardness (in German degrees (°dH)) were calculated as the product of alkalinity ($CaCO_3$ mg/L) multiplied by a factor of 2.8 and the concentration of ammonium, nitrite, nitrate, orthophosphate, iron, copper, silicon, chlorine and fluoride with the photometer AQUA-CHECK 2 (Söll, Germany).

Data analysis

The trophic structure of benthic invertebrate assemblages was estimated from the percentage of grazers, collectors (gatherer collectors, active and passive filter feeders), shredders [33], and predators [34]. The Fauna Aquatica Austriaca [35] was used for classifying macroinvertebrates in feeding guilds. The obtained results were processed using SPSS statistics. Using one-way analysis of variance (ANOVA), it was possible to estimate the distributional variations of aquatic macroinvertebrates between sampling sites and seasons. The analysis of differences in feeding groups among all taxa was implemented using one-way ANOVA, Pearson's correlation and cluster analysis.

RESULTS

Benthic invertebrate assemblages in the Đerekarska River

The taxonomic analysis of benthic invertebrates in the Đerekarska River revealed the presence of 55 species from 43 genera belonging to 35 families. The highest diversity was found in the orders Trichoptera (14 species), Ephemeroptera (10 species) and Plecoptera (8 species) (Supplementary Table S1). The upper course was dominated by a rocky substrate, while the middle course was dominated by stones, gravel and sand, and the river sinkhole was dominated by gravel and sand substrates. In the upper course of the river, 10 to 14 benthic invertebrate taxa were found. During the three seasons of investigation, Amphipoda was the most abundant and represented over 46.85% of all individuals in the spring, 84.14% in the summer and 59.28% in the fall. Gammarus balcanicus (Schäferna, 1923) was the most abundant species. The middle course of the Đerekarska River is also characterized by differences in the community structure and composition of macroinvertebrates depending on the season. Thus, 11 taxa of macroinvertebrates were found in the spring, and 25 and 20 in the summer and fall. Ephemeroptera was represented by 62.20% that were collected in spring. The frequency of species of this order decreased to 15.42% during the summer and then increased to 25.28% in the fall. Thus, among the 12-16 macroinvertebrate taxa found at the sinkhole, ephemeropteran species were the most abundant in all samples, comprising over 57.64% of all individuals in the spring, 55.82% in the summer and 51.90% in the fall.

FFGs in relation to different ecological parameters

ANOVA showed that different types of substrates have a different impact on the functional feeding guilds (FFGs) in the assemblage, but that seasonal changes have no influence on the FFGs in the assemblage. As can be seen in Table 1, grazers and scrapers were not significantly affected by the substrate type (P>0.05), as well as predators, while other members of the FFGs are sensitive to the substrate type (P<0.05). The distribution of FFGs according to substrate type is shown in Fig. 1.

Table 1. Analysis of functional feeding guild variance in assemblages and different types of substrates.

ANOVA	Significant
Grazers and scrapers	0.298
Shredders	**0.005***
Gatherers and collectors	**0.013***
Active filter feeders	**0.024***
Passive filter feeders	**0.021***
Predators	0.437

* k – analysis of variance P<0.05. Results of one-way ANOVA in SPSS.

In Table 2, Pearson's correlation analysis shows which FFGs are correlated with physical and chemical parameters. Pearson's correlation results indicate that COD, BOD_5 and water temperature have a significant positive effect on the abundance of grazers and scrapers, while chloride values have a negative effect. The highest correlation with physical and chemical parameters was obtained for shredders for the following parameters:

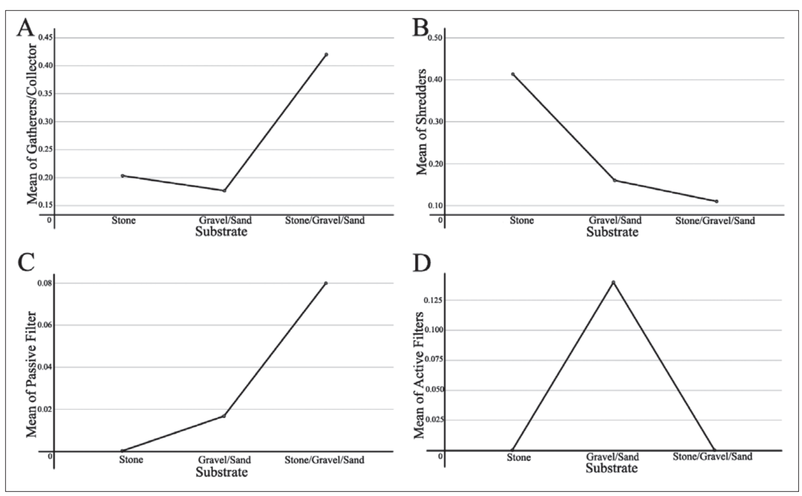


Fig. 1. Distribution of functional feeding guilds according to the type of substrate (mean values). **A** – gatherers/collectors; **B** – shredders; **C** – passive filter; **D** – active filter.

Table 2. Pearson correlation analysis of functional feeding guilds and physicochemical parameters.

Correlations										
		Discharge	**COD**	**BOD$_5$**	**Depth**	**pH**	**Water temperature**	**Si**	**Cl$_2$**	**Total hardness**
Grazers Scrapers	Pearson Correlation	-0.055	**0.683***	**0.673***	0.281	0.311	**0.686***	0.458	**-0.705***	0.642
	Significant	0.889	0.042	0.047	0.463	0.416	0.041	0.215	0.034	0.063
Shredders	Pearson Correlation	-0.356	**-0.747***	**-0.756***	-0.589	-0.663	**-0.751***	**-0.792***	**0.678***	**-0.675***
	Significant	0.347	0.021	0.019	0.095	0.051	0.020	0.011	0.045	0.046
Passive filter feeders	Pearson Correlation	**0.750***	0.305	0.320	**0.754***	**0.805***	0.140	0.437	-0.248	0.271
	Significant	0.020	0.425	0.401	0.019	0.009	0.720	0.239	0.519	0.480

* Significant correlation $P<0.05$. Pearson's correlation analysis of functional feeding guilds and physicochemical parameters is implemented in SPSS software. Chemical oxygen demand (COD); five-day Biochemical oxygen demand (BOD$_5$); The measure of the activity of hydrogen ions (H+) in water – pH; silicon (Si); chlorine (Cl$_2$)

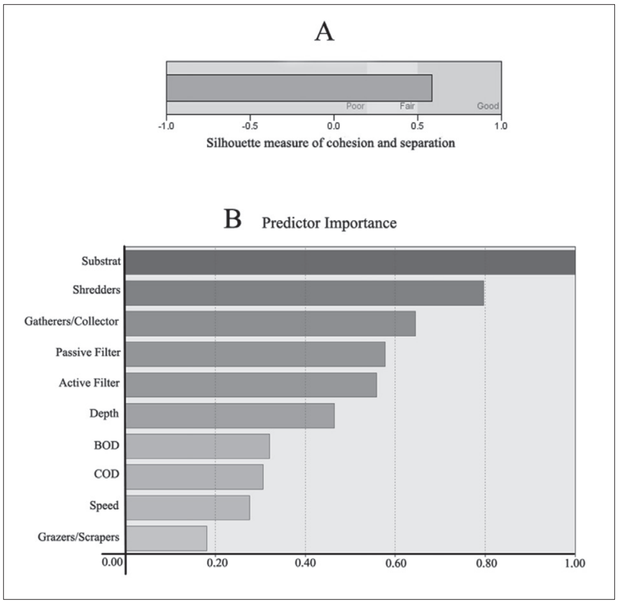


Fig. 2. Graphic representation of cluster analysis quality and parameters contribute to cluster formation. **A** – Cluster quality. **B** – Predictor importance in two-step cluster analysis. Chemical oxygen demand (COD); five-day Biochemical oxygen demand (BOD$_5$).

COD, BOD$_5$, water temperature, silicone, total hardness, and these parameters have a significant negative impact on the abundance of shredders, while chloride has a significant positive impact; the abundance of the passive filter feeders is significantly positively affected by discharge, depth and pH value.

By applying two-step cluster analysis to FFGs, it was established that in the longitudinal profile of the Đerekarska River, three clusters can be distinguished based on the substrate structure in assemblages (Fig. 2B). The analysis showed that cluster formation is most affected by substrate structure, followed by shredders, gatherers and collector, passive filter, active filter feeders, depth, BOD$_5$, COD and flow velocity. For grazers and scrapers, discharge and saturation do not significantly affect cluster formation (Fig. 2B). The first cluster consisted of FFG organisms that prefer a stone substrate, such as shredders and predators, while gatherers and collectors, grazers and scrapers occurred with a lower abundance, and passive and active filter feeders do not prefer this type of substrate (Fig. 3A). The second cluster consists of organisms that prefer a mixed stone/gravel/sand substrate. Gatherers and collectors are the most abundant in this type of substrate, followed by grazers and scrapers, and passive filter feeders, but in lower abundance, while active filter feeders do not prefer this type of substrate. The number of shredders and predators is significantly reduced compared to the stone substrate (Fig. 3B). The third cluster consists of organisms preferring gravel and sand substrates. Predators are dominant on this type of substrate, grazers and scrapers are numerous, and this substrate is preferred by active filter feeders, while other groups of FFG organisms are represented in much lower numbers (Fig. 3C).

DISCUSSION

There is data scarcity on the benthic invertebrates of the Đerekarska River in Serbia. Previously, a single study [36] revealed the presence of two species from the suborder Heteroptera, Hesperocorixa parallela (Fieber, 1860) and Notonecta maculata (Fabricius, 1794) at the Đerekarska spring, which were not recorded during this research. In Croatia, it was established

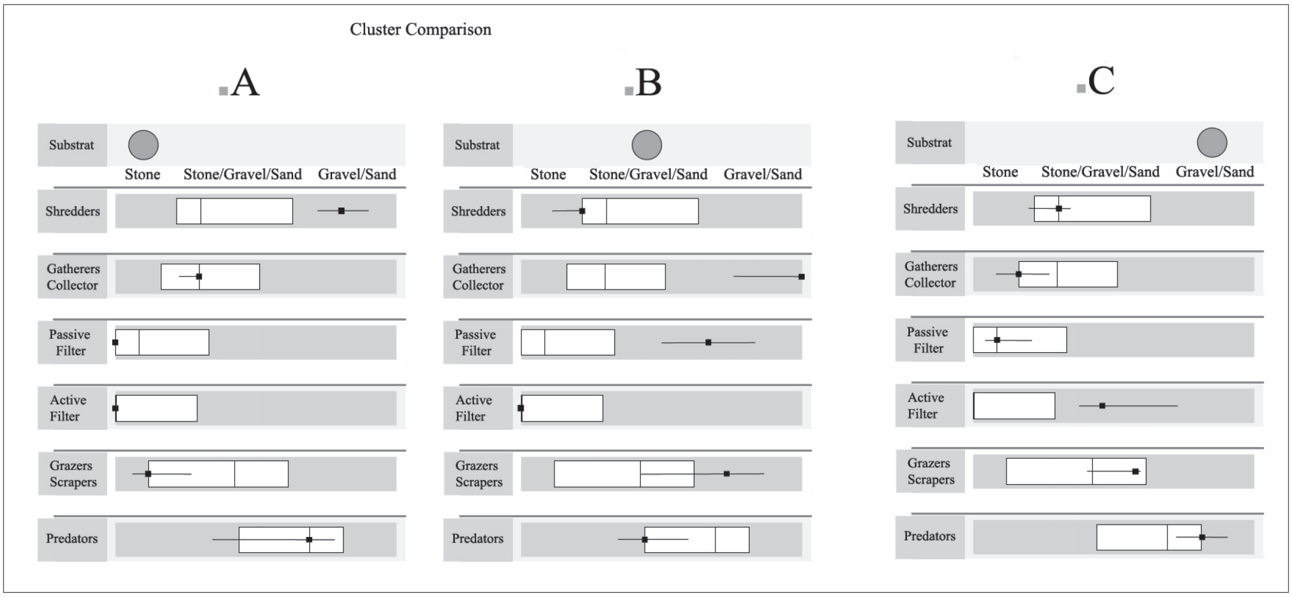


Fig. 3. A graphic representation of the distribution of functional feeding guilds into clusters according to substrate type. **A** – First cluster: stone substrate. **B** – Second cluster: stone/gravel/sand substrate. **C** – Third cluster: gravel/sand substrate.

that the dominant groups among benthic fauna of the karst rivers Cetina and Ruda were Amphipoda and Gastropoda [37]. This differs from our results as well as from the macrozoobenthos study of the Kolubara River [38-41]. It is important to mention that Mollusca, Amphipoda and Insecta were found to be the principal components of macrozoobenthic communities based on the number of identified species and frequency of occurrence in the karstic waters of Croatia [42]. This is a completely different community structure from communities in the continental non-karst area.

Regarding the macrozoobenthos community, streams are an important link in food webs between resources of organic matter (e.g., fallen leaves, algae, and detritus) and fish, and in part because of their diversity and ubiquity, the study of the macrozoobenthos community is a central component of inland water ecology [43]. The high taxonomic diversity of macroinvertebrates is understandable when one considers their great functional diversity [43]. In food chains, macroinvertebrates occupy almost all available sites among consumers, as well as herbivores, detritivores, predators and parasites. This is similar to the manner of movement and the degree of mobility, the manner of breathing, etc. [43]. This diversity as a group makes them sensitive to a variety of abiotic factors and their anthropogenic changes. On the other hand, it was expected that the percentage of grazers would increase downstream and reflect changes in aquatic dynamics [44]. This theory is supported by the data obtained from Pearson's correlation, where we observed that the abundance of grazers and scrapers positively correlates to the values of COD, BOD5 and water temperature. The chloride concentration had negative effects on the abundance of grazers and scrapers in the assemblages and their abundance is not affected by substrate type. The substrate influences the abundance of shredders, which is related to the place of sampling, as in the upper reaches of the Đerekarska River, where a rocky substrate covered with moss prevails, which is also a food source. Gammarus balcanicus was the dominant taxon in the upper reaches of the river. Traditionally, Gammarus spp. were assigned the role of shredders under the FFG classification system [45,46]. We had also expected shredders to decrease from the upper reaches to the middle reaches, and the sinkhole as the input of terrestrial coarse organic matter declined. Pearson' correlation data showed that parameters such as COD, BOD5, water temperature, silicone, chlorides and total hardness are negatively correlated with the abundance of shredders. This can also be explained by the fact that the values of these parameters increased from the upper to the middle reaches and the sinkhole of the river. Analysis of variance showed that the abundance of passive filter feeders depends on the substrate type, and this is referred to as the correlation that shows that at sites where flow, depth and pH are higher, the abundance of passive filter feeders increases. For these parameters, the obtained values increased from the upper to the middle reaches and the sinkhole of the river. From the FFG perspective, we followed the ratio of shredders to passive filter feeders as representative of the two opposite feeding strategies. Shredders are associated with nonimpaired and natural environmental conditions, while passive filter feeders indicate chemical or physical impairment [47]. This was also observed in our study, where shredders are dominant at sites with the lowest hydrological degradation scores. On the other hand, passive filter feeders are expected to thrive in mid-range flows. Therefore, our results confirm the previous observation [48] that shredders are negatively associated with hydrological modification and that their absence favors the abundance of passive filter feeders. A steady decline in the size of detritus particles should cause the number of gatherers that depend on fine organic material to increase along the river continuum. ANOVA showed that the number of gatherers, collectors and active filter feeders depends exclusively on the substrate type, while other physicochemical parameters measured during this study are not significant and do not affect their abundance. The proportion of predators was expected to remain constant along the continuum [44], with which the results of our research are consistent. Also, indices based on community trophic characteristics should be applied as other indices in assessing the ecological status, which are not included in our official gazette RS (74/2011) [49].

CONCLUSIONS

During our examination of the Đerekarska River, we observed that longitudinal abundance, diversity and longitudinal distribution of macroinvertebrates varied from the upper reaches to the river sinkhole. Depending on the substrate type, different functional feeding groups were distributed across all sampling sites. Three major macroinvertebrate clusters (stone, stone/gravel and sand, and gravel and sand) were identified. By analysis of variance, it was determined that the influence of substrate type makes a significant difference in the formation of FFG assemblages. Substrate type also had the highest influence on the formation of clusters in assemblages. We propose an appropriate water management plan based on preserving and improving the quality of the Đerekarska River as well as other waterbodies of the Pešter plateau (southwestern Serbia). This water management plan needs to be based on cooperation between the institutions responsible for Pešter Highland management, water users, local government bodies and the local population in order to make decisions on water protection and raise awareness related to the importance of water in this mountainous area of Serbia.

Funding: This research was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia, Grant Nos. 451-03-47/2023-01/200032, 451-03-47/2023-01/200178.

Author contributions: Melisa Numanović and Branko Miljanović performed the sampling, and identification of macroinvertebrate samples, statistical analysis, and prepared the manuscript; Katarina Stojanović and Boris Novaković performed the statistical analysis, interpreted the data, analyzed the results and prepared the manuscript; Milica Živković analyzed the results, provided the literature review and prepared the manuscript; Nebojša Živić performed physicochemical analysis of the water, interpreted the data and contributed to the study design.

Conflict of interest disclosure: The authors declare no conflict of interest.

Data availability: The data supporting the findings of this study are available at:
https://www.serbiosoc.org.rs/NewUploads/Uploads/Supplementary%20Table%20S1.pdf

REFERENCES

1. Merovich G.T, Petty J.T. Continuous response of benthic macroinvertebrate assemblages to a discrete disturbance gradient: consequences for diagnosing stressors. J North Am Benthol Soc. 2010;29(4):1241-57. https://doi.org/10.1899/09-164.1
2. Heino J, Soininen J. Assembly rules and community models for unicellular organisms: patterns in diatoms of boreal streams. Freshw Biol. 2005;50:567-77. https://doi.org/10.1111/j.1365-2427.2005.01346.x
3. Griffiths H.I, Kryštufek B, Reed JM. Balkan Biodiversity: Pattern and Process in the European Hotspot. Dordrecht: Kluwer Academic Publishers; 2004. 358 p. https://doi.org/10.1007/978-1-4020-2854-0
4. Savic IR. Diversification of the Balkan fauna: its origin, historical development and present status. Adv Arachnol Dev Biol.. 2008;12:57-79.

5. Previsic A, Walton C, Kucinic M, Mitrikeski PT, Kerovec M. Pleistocene divergence of Dinaric Drusus endemics (Trichoptera, Limnephilidae) in multiple microrefugia within the Balkan Peninsula. Mol Ecol. 2009;18(4):634- 47. https://doi.org/10.1111/j.1365-294x.2008.04046.x
6. Klobucar G.I, Podnar M, Jelic M, Franjevic D, Faller M, Stambuk A, Gottstein S, Simic V, Maguire I. Role of the Dinaric Karst (western Balkans) in shaping the phylogeographic structure of the threatened crayfish *Austropotamobius torrentium*. Freshw Biol. 2013;58(6):1089-105. https://doi.org/10.1111/fwb.12110
7. Matonickin I, Pavletic Z. The participation of certain animal and plant groups in the construction of living communities on travertine and erosion waterfalls in Bosnia and Herzegovina. God Biol Inst Sarajevo. 1960;13(1-2):41-62.
8. Habdija I, Radanovic I, Primc-Habdija B. Longitudinal distribution of predatory benthic macroinvertebrates in a karstic river. Arch Hydrobiol. 1997;139(4):527-46. https://doi.org/10.1127/archiv-hydrobiol/139/1997/527
9. Rađa B, Puljas S. Macroinvertebrate diversity in the karst Jadro river (Croatia). Arch Biol Sci. 2008;60(3):437-48. https://doi.org/10.2298/abs0803437r
10. Płóciennik M, Dmitrovic D, Pesic V, Gadawski P. Ecological patterns of Chironomidae assemblages in Dynaric karst springs. Knowl Manag Aquat Ecosyst. 2016;11(417):1-19. https://doi.org/10.1051/kmae/2015044
11. Savic A, Dmitrovic D, Pesic V. Ephemeroptera, Plecoptera, and Trichoptera assemblages of karst springs in relation to some environmental factors: a case study in central Bosnia and Herzegovina. Turk Zool Derg. 2017;41(1):119-29. https://doi.org/10.3906/zoo-1512-31
12. Vilenica M, Stankovic V.M, Sartori M, Kucinic M, Mihaljevic Z. Environmental factors affecting mayfly assemblages in tufa-depositing habitats of the Dinaric Karst. Knowl Manag Aquat Ecosyst. 2017;418:14. https://doi.org/10.1051/kmae/2017005
13. Official Gazette of the RS. Regulation on the proclamation of a special nature reserve “Peštersko polje”. Offic Gazette RS. 2015;114:12
14. Bis B, Zdanowicz A, Zalewski M. Effects of catchment properties on hydrochemistry, habitat complexity and invertebrate community structure in a lowland river. In: Jungwirth M, Muhar S, Schmutz S, editors. Assessing the Ecological Integrity of Running Waters. Vol 149, Developments in Hydrobiology. Dordrecht: Springer; 2000. p. 369-87. https://doi.org/10.1007/978-94-011-4164-2_30
15. Menetrey N, Oertli B, Lachavanne JB. The CIEPT: A macroinvertebrate-based multimetric index for assessing the ecological quality of Swiss lowland ponds. Ecol Indic. 2011;11(2):590-600. https://doi.org/10.1016/j.ecolind.2010.08.005
16. Czerniawska-Kusza I. Comparing modified biological monitoring working party score system and several biological indices based on macroinvertebrates for water-quality assessment. Limnologica. 2005;35(3):169-76. https://doi.org/10.1016/j.limno.2005.05.003
17. Paunović M. The structure of macroinvertebrate communities as an indicator of the types of flowing waters of Serbia [dissertation] [Belgrade]: Faculty of Biology, University of Belgrade;2007. 188 p.
18. Müller-Liebenau I. Revision der europäischen Arten der Gattung Baetis Leach, 1815 (Insecta, Ephemeroptera). Gewässer und Abwässer, 1969;48/49:1-214.
19. Brinkhurst R.O, Jamieson BG. Aquatic Oligochaeta of the World. Edinburgh, United Kingdom: Oliver & Boyd; 1971. 860 p.
20. Rozkošny R. Key for determination of larvae of water insects. Praha: Ceskoslovenska Akademie Ved; 1980.
21. Edington JM, Hildrew AG. A key to the caseless caddis larvae of the British isles: with notes on their ecology. Ambleside: Freshwater Biological Association; 1981. 91 p.
22. Nilsson A.N. Aquatic insects of North Europe: a taxonomic handbook. Volume 1: Ephemeroptera, Plecoptera, Heteroptera, Neuroptera, Megaloptera, Coleoptera, Trichoptera, Lepidoptera. Denmark: Apollo Books; 1996. 274 p.
23. Waringer J, Graf W. Atlas der österreichischen Köcherfliegenlarven: unter Einschluss der angrenzenden Gebiete. Wien: Facultas Universitätsverlag; 1997. 286 p.
24. Glöer P, Meier-Brook C, Ostermann O. Süsswassermollusken: ein Bestimmungssschlüssel für die Bundesrepublik Deutschland. Hamburg, Germany: Deutscher Jugendbuch für Naturbeobachtung; 1998. 136 p.
25. Soldán T, Landa V. A key to the Central European species of the genus Rhithrogena (Ephemeroptera: Heptageniidae). Klapalekiana. 1999;35:25-37.
26. Jacob U. Baetis Leach 1815, sensu stricto oder sensu lato. Ein Beitrag zum Gattungskonzept auf der Grundlage von Artengruppen mit Bestimmungsschlüsseln. Lauterbornia. 2003;47:59-129.
27. Zwick P. Key to the West Palaearctic genera of stoneflies (Plecoptera) in the larval stage. Limnologica. 2004;34(4):315-48. https://doi.org/10.1016/S0075-9511(04)80004-5
28. Lechthaler W, Stockinger W. Trichoptera - Key to larvae from Central Europe (Version CD-Rom-Edition). Vienna: Eutaxa - Technisches Büro für Biologie; 2005.
29. Bauernfeind E, Soldan T. The Mayflies of Europe (Ephemeroptera). Ollerup, Denmark: Apollo Books; 2012. 781 p.
30. Muller M, Bouguelia S, Goy R.A, Yoris A, Berlin J, Meche P, Rocher V, Mertens S, Dudal Y. International cross-validation of a BOD5 surrogate. 2014;(23):13642-5. https://doi.org/10.1007/s11356-014-3202-3
31. Drinking Water Standard Methods for Examination of Hygienic Correctness. In: Feliks R, Škunca-Milovanović S, editors. Beograd: Savezni zavod za zdravstvenu zaštitu & NIP Privredni pregled; 1990. p. 134-6. Serbian.
32. SRPSENISO8467:2007. Water Quality—Determination of CODMn. Belgrade, Serbia: Institute for standardization; 2007.
33. Cummins K. Rapid bioassessment using functional analysis of running water invertebrates. Paper presented at the Proceedings of the First National Workshop on Biological Criteria, EPA-905/9-89/003. Chicago, USA: US Environmental Protection Agency; 1988.
34. Kerans B, Karr JR. A benthic index of biotic integrity (B-IBI) for rivers of the Tennessee Valley. Ecol Appl. 1994;4(4):768-85. https://doi.org/10.2307/1942007

35. Moog O. Fauna Aquatica Austriaca. 2nd ed. A Comprehensive Species Inventory of Austrian Aquatic Organisms with Ecological Notes. Wien, Austria: Austrian Federal Ministry of Agriculture Forestry Environment and Water Management; 2002. 92 p.
36. Protić Lj, Živić M. A contribution to the knowledge of aquatic heteropteran Šar planina mts and Pešter plateau (Serbia). Acta Ent Slov. 2013;21(1):65-76.
37. Vučković I, Božak I, Ivković M, Jelenčić M, Kerovec M, Popijač M, Previšić, A, Širac S, Zrinski I, Kučinić M. Composition and structure of benthic macroinvertebrate communities in the mediterranean karst river the Cetina and its tributary the Ruda, Croatia. Nat Croat. 2009;18(1):49-82.
38. Marković Z, Miljanović B, Mitrović-Tutundzić V. Macrozoobenthos as an indicator of the Kolubara Reka river water quality. Annual proceedings of the Yugoslav society for water protection. 1999:261-6.
39. Živić I, Marković Z, Živić M. Influence of a trout farm on macrozoobenthos communities of the Trešnjica River, Serbia. Arch Biol Sci.. 2009;61(3):483-92. https://doi.org/10.2298/ABS0903483Z
40. Bjelanović K, Živić I, Dulić Z, Živić M, Đorđević J, Marinković S, Marković Z. Water quality assessment in the Raška river based on zoobenthos and zooplankton organisms as bioindicators. In: Conference proceedings VI International conference "Water & Fish". Belgrade, Serbia: Faculty of Agriculture, University of Belgrade. 2013. p. 349-57.
41. Stojanović K. Uticaj pastrmskih ribnjaka na zajednice makrozoobentosa tekućica sa posebnim osvrtom na larve roda Baetis (Ephemeroptera, Insecta) [dissertation] [Belgrade]: Faculty of Biology, University of Belgrade. 2017. 396 p.
42. Rađa B, Puljas S. Do Karst Rivers "deserve" their own biotic index? A ten years study on macrozoobenthos in Croatia. Int J Speleol. 2010;(2):39:137-47. https://doi.org/10.5038/1827-806x.39.2.7
43. Živić I, Ostojić A, Miljanović B, Marković Z. Macroinvertebrates of Serbian streams and their significance as bioindicators in estimation of water quality. In: Petanović R, editor. Proceedings: Ecological and Economic Significance of Fauna of Serbia; 2016 Nov 17; Belgrade, Serbia. Belgrade: SASA; 2018. P. 199-229. (Scientific Meetings; No. 179; Department of Chemical and Biological Sciences; No. 12) Serbian.
44. Vannote RI, Minshall GW, Cummins KW, Sedell JR, Cushing CE. The river continuum concept. Can J Fish Aquat Sci. 1980;37:130-37. https://doi.org/10.1139/f80-017
45. Willoughby LG, Sutcliffe DW. Experiments on feeding and growth of the amphipod *Gammarus pulex* (L.) related to its distribution in the River Duddon. Freshw Biol. 1976;6(6):577-86. https://doi.org/10.1111/j.1365-2427.1976.tb01647.x
46. Cummins KW, Klug MJ. Feeding Ecology of Stream Invertebrates. Annu Rev Ecol Evol Syst. 1979;10:147-72. https://doi.org/10.1146/annurev.es.10.110179.001051
47. Wallace JB, Merritt RW. Filter-feeding ecology of aquatic insects. Annu Rev Entomol. 1980;25(1):103-32. https://doi.org/10.1146/annurev.en.25.010180.000535
48. Chambers DB, Messinger T. Benthic invertebrate communities and their responses to selected environmental factors in the Kanawha River basin, West Virginia, Virginia, and North Carolina. Water-Resources Investigations Report. USGS Publications Warehouse; 2001. 52 p. https://doi.org/10.3133/wri014021
49. Official Gazette of the RS. Regulation on the parameters of ecological and chemical status of surface waters and parameters of chemical status and quantitative status of groundwaters. Official Gazette RS. 2011;74:29.

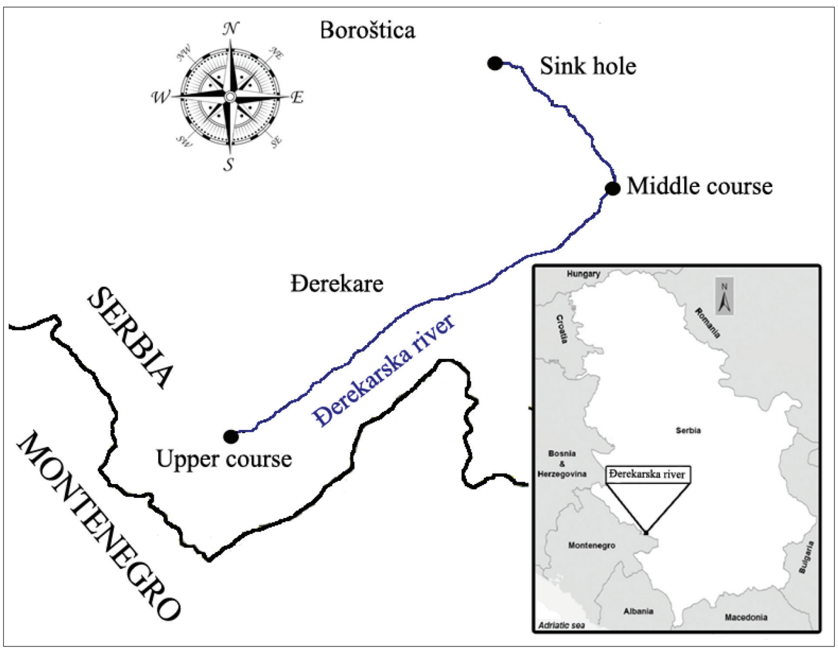
SUPPLEMENTARY MATERIAL

Supplementary Table S1. List of aquatic macroinvertebrate species found on the Đerakarska river in 2016.

TAXON NAME	Upper course Spring	Middle course Spring	The sinkhole Spring	Upper course Summer	Middle course Summer	The sinkhole Summer	Upper course Fall	Middle course Fall	The sinkhole Fall
Tricladida									
Crenobia alpina montenegrina (Mrazek, 1904)	+	-	-	-	-	-	+	-	-
Arhynchobdellida									
Dina lineata (Kenk, 1930)	-	+	-	-	-	-	-	+	-
Unionida									
Unio crassus (Retzius, 1788)	-	+	+	-	+	+	-	+	+
Decapoda									
Austropotamobius torrentium (Schrank, 1803)	-	-	-	-	-	-	-	+	-
Amphipoda									

Gammarus balcanicus (Schaferna, 1922)	+	+	-	+	+	-	+	+	+
Niphargus sp.	+	-	-	+	-	-	+	-	-
Coleoptera									
Agabus bipustulatus (Linnaeus, 1767)	-	-	-	-	+	-	-	+	-
Brychius elevatus Ad. (Panzer, 1793)	-	-	-	-	+	-	-	-	-
Elmis aenea Ad. (Müller, 1806)	+	-	-	+	+	-	+	+	-
Elmis aenea Lv. (Müller, 1806)	+	-	-	+	+	-	+	-	-
Laccophilus sp. Lv.	-	-	-	-	+	+	-	-	+
Oulimnius sp. Lv.	-	-	+	+	+	+	-	+	+
Oulimnius tuberculatus Ad. (Müller, 1806)	-	-	-	-	+	-	-	-	-
Hydraena riparia Ad. (Kugelann, 1794)	-	-	-	-	+	-	-	+	-
Ephemeroptera									
Baetis sp.	-	-	+	-	-	-	-	+	-
Baetis rhodani (Pictet, 1843)	-	-	+	-	+	+	-	-	+
Baetis muticus (Linnaeus, 1758)	-	-	+	-	-	+	-	-	-
Ephemera danica (Müller, 1764)	-	+	-	-	+	-	-	+	-
Ephemera vulgata (Linnaeus, 1758)	-	+	-	-	+		-	+	-
Paraleptophlebia sp.	-	-	-	-	+	+	-	+	+
Serratella ignita (Poda, 1761)	-	+	+	-	-	-	-	-	-
Ecdyonurus dispar (Fabricius, 1775)	-	-	+	-	-	-	-	-	-
Ecdyonurus insignis (Eaton, 1870)	+	-	-	-	-	-	+	-	-
Ecdyonurus venosus (Fabricius, 1775)	-	-	-	-	-	+	-	-	+
Habrophlebia fusca (Curtis, 1834)	-	+	-	-	-	-	-	-	-
Plecoptera									
Isoperla sp.	-	-	-	+	-	-	-	-	-
Isoperla grammatica (Poda, 1761)	+	-	-	-	-	+	+	-	+
Diura bicaudata (Linnaeus, 1758)	+	-	-	+	-	-	-	-	-
Leuctra moselyi (Morton, 1929)	-	-	-	+	-	-	-	-	-
Leuctra nigra (Olivier, 1811)	-	-	-	-	-	-	-	+	-
Nemoura cambrica (Stephens, 1836)	-	-	-	-	-	-	-	-	+
Protonemura montana	+	-	-	-	-	-	+	-	-
Protonemura meyeri (Pictet, 1841)	+	-	-	-	-	-	+	-	-
Protonemura praecox (Morton, 1894)	+	-	-	-	-	-	-	-	-
Trichoptera									
Chaetopteryx villosa (Fabricius, 1798)	-	+	-	-	+	-	-	-	-
Goera pilosa (Fabricius, 1775)	-	-	-	-	+	-	-	-	-
Hydropsyche pellucidula (Curtis, 1834)	-	-	-	-	-	+	-	-	-
Hydropsyche exocellata (Curtis, 1835)	-	-	-	-	-	-	-	-	+
Hydropsyche angustipennis (Curtis, 1834)	-	-	-	-	-	+	-	+	+
Leptocerus tineiformis (Curtis, 1834)	-	-	+	-	-	-	-	-	+
Mystacides azurea (Linnaeus, 1761)	-	-	-	-	+	-	-	-	-
Polycentropus sp.	-	+	-	-	+	-	-	+	-
Psychomyia fragilis (Pictet, 1834)	-	-	-	-	-	-	+	-	-
Psychomyia pusilla (Fabricius, 1781)	-	-	+	-	-	-	-	-	-
Stenophylax sp.	-	-	-	-	-	-	+	-	-
Sericostoma personatum (Kirby&Spence, 1826)	-	-	-	-	-	-	-	-	+
Silo nigricornis (Pictet, 1834)	-	-	-	-	-	-	-	+	-
Rhyacophila sp.	-	-	-	+	+	-	-	-	-
Rhyacophila dorsalis (Curtis, 1834)	-	-	+	-	-	+	-	-	-

Odonata									
Calopteryx splendens (Harris, 1780)	-	-	-	-	+	-	-	-	-
Calopteryx virgo (Linnaeus, 1758)	-	-	-	-	+	-	+	-	-
Onychogomphus forcipatus (Linnaeus, 1758)	-	-	-	-	-	+	-	-	-
Diptera									
Atherix ibis (Fabricus, 1798)	-	-	-	-	+	-	-	-	-
Chironomidae Gen. sp.	+	+	+	+	+	+	+	+	+
Pericoma sp.	+	-	-	-	-	-	-	-	-
Simuliidae Gen. sp.	-	-	+	-	+	+	-	+	+
Tabanidae Gen. sp	-	-	-	-	-	-	-	+	-
Tipula sp.	-	-	-	-	-	-	+	-	-
Megaloptera									
Sialis lutaria (Linnaeus, 1758)	-	+	-	-	+	-	-	+	-



Supplementary Fig. S1. Map of the study area showing the location of sampling sites at the Đerekarska River.