

Original Research

**AQUATIC MACROINVERTEBRATE FAUNA AT SELECTED SAMPLING SITES
IN THE GRADAC RIVER**

DOI: 10.5937/EcoTER26530T

**Bojana Tubić^{1,a}, Jelena Đuknić^{1,b}, Nikola Marinković^{1,c}, Nataša Popović^{1,d},
Luka Grbović^{1,e*}, Maja Raković^{1,f}, Momir Paunović^{1,g}**

¹ Department of Hydroecology and Water Protection, Institute for Biological Research
“Siniša Stanković”—National Institute of the Republic of Serbia, University of Belgrade,
Bulevar despota Stefana 142, 11108 Belgrade, SERBIA

^a <https://orcid.org/0000-0003-2050-177X> ^b <https://orcid.org/0000-0003-3635-5463>

^c <https://orcid.org/0000-0002-0360-5393> ^d <https://orcid.org/0000-0001-6136-7867>

^e <https://orcid.org/0009-0004-4298-8229> ^f <https://orcid.org/0000-0001-6899-6113>

^g <https://orcid.org/0000-0002-6432-1191>

Abstract

*Aquatic macroinvertebrates from four sites along the Gradac River, a protected karst watercourse in western Serbia, were studied in June and October 2025 using the Kick and Sweep method. A total of 77 taxa from 14 groups were recorded, with insects (Diptera, Trichoptera, Ephemeroptera, and Coleoptera) dominating both in richness and abundance, comprising nearly 80% of the community, while gastropods accounted for about 11%. The most abundant species were *Lepidostoma hirtum*, *Sericostoma personatum*, and *Holandriana holandrii*, all recorded at the Sunčani vir site. At the Konjički klub site, the invasive snail *Potamopyrgus antipodarum* was recorded. The presence of *Agriotypus armatus*, an aquatic hymenopteran parasitoid of the caddisfly pupa *Silo pallipes*, at the Downstream Konjički klub site was noted. The high proportion of taxa sensitive to moderate organic pollution indicates low organic load and good ecological status. These results confirm the Gradac River as a near-natural, well-preserved watercourse and support its role as a reference site for biomonitoring and freshwater conservation in Serbia.*

Keywords: aquatic macroinvertebrate, diversity, saprobic valence, Gradac River.

INTRODUCTION

Aquatic macroinvertebrates are organisms that, at least during a part of their life cycle, inhabit aquatic ecosystems (such as the bottom, detritus, macrophytes, or filamentous algae) and can be collected with a hand nets mesh size 200–500 µm [1].

Communities of aquatic macroinvertebrates are most commonly used in biomonitoring programs [1] because they possess features that make them reliable indicators of changes in ecological conditions in aquatic ecosystems. The low mobility of these organisms allows spatial analysis of environmental factors and stress impacts, while their relatively long life cycles enable examination of temporal changes caused by environmental shifts. The Gradac Gorge is recognized as a protected area due to its natural and ecological significance. Specifically, the river flows through a karst gorge designated as a nature reserve under state

* luka.grbovic@ibiss.bg.ac.rs

protection. This status helps preserve the Gradac as one of the few nearly natural and well-preserved watercourses in Serbia. The protected status ensures the conservation of the river's high ecological integrity, supporting diverse and sensitive aquatic communities, including aquatic macroinvertebrates, indicators of water quality and ecosystem health. This protection helps maintain the river's role as a reference site for freshwater biodiversity and ecological research in the region, safeguarding it from significant anthropogenic pollution and habitat destruction.

In Serbia, such protected areas are managed according to national conservation laws with the aim of sustaining long-term ecological values, habitat diversity, and associated ecosystem services. The inclusion of the Gradac River in this framework highlights its conservation importance beyond local scales, contributing to regional biodiversity conservation goals as well.

In this study we assess the aquatic macroinvertebrate fauna at selected sampling sites in the Gradac River to evaluate its biological diversity. The study aimed to (i) analyse the composition and diversity of macroinvertebrate communities, and (ii) identify organic pollution-sensitive taxa as indicators of good water quality. Through these analyses, the study seeks to provide insight into the degree of ecological integrity and preservation of the Gradac River ecosystem.

MATERIALS AND METHODS

Sampling of aquatic macroinvertebrates in the Gradac River was conducted in June and October 2025 at four selected sites: Sunčani vir, Peak of Degurić dam, Konjički klub, and Downstream Konjički klub (Figures 1).

Samples were collected using the Kick and Sweep (K&S) sampling method from all available microhabitat types, following established standards [2], with a benthic hand net FBA (25 × 25 cm, with the mesh sizes of 250 and 500 µm). Biological material was collected, transferred to 500 ml containers, and fixed with 96% ethanol in the field. This sampling procedure ensures a representative sample of aquatic macroinvertebrates from various microhabitats within the investigated sector. The samples are considered semi-quantitative because equal effort was applied at each site within a defined time interval. The K&S sampling method follows recognized standards [3] adapted to the specific habitat and research requirements.

Sorting and identification of aquatic macroinvertebrates were performed based on morphological characteristics, using binocular stereoscopic microscopes ZEISS Stemi 2000C (×50) and Nikon SMZ 800N (×120), and a ZEISS Axio Lab. A1 microscope (×630), supported by appropriate identification keys.

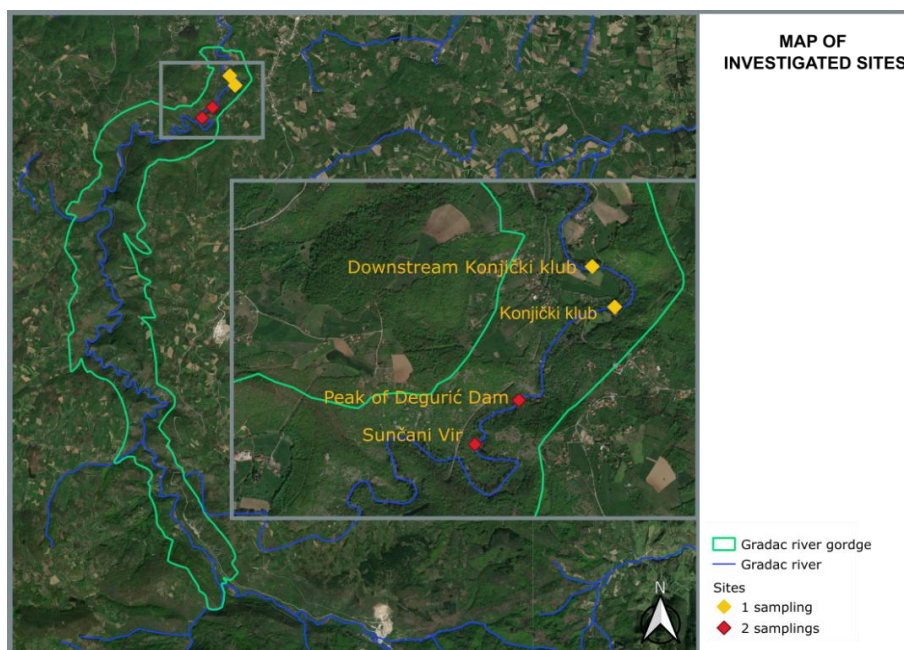


Figure 1 Sampling sites of the Gradac River

RESULTS AND DISCUSSION

A total of 77 taxa from 14 macroinvertebrate groups were recorded at four studied sites on the Gradac River.

The aquatic macroinvertebrate communities at the studied sites were most diverse in the insect groups. The group Diptera (26 taxa), Trichoptera (15 taxa), Ephemeroptera (10 taxa) and Coleoptera (8 taxa) were the most diverse in terms of the number of taxa. Also, among the insect groups, Odonata and Plecoptera were recorded with 2 taxa each, and Colembola with one taxon. The diversity of other macroinvertebrate groups was lower: Gastropoda was recorded with 5 taxa and groups Hirudinea and Crustacea were recorded with 2 taxa each, while Oligochaeta and Hidrachnidia with only one recorded taxa each.

The number of taxa per site was almost even during the sampling in June and October. Highest number of taxa (34) was recorded at site Sunčani vir both in June and in October, while the lowest number of taxa (19) was recorded at Peak of Degurić dam during both sampling campaigns (Figure 2).

Larvae of the aquatic insects were the most abundant groups in the studied community, accounting for almost 80% (Figure 3). The insect groups Trichoptera (37.84%), Diptera (20.74%), and Ephemeroptera (11.23%) had the highest percentage participation. Coleoptera (5.62%) and Plecoptera (2.13%) had lower percentage participation, while the other recorded insect groups each accounted for less than 1%. Gastropoda was also quite abundant group, with 11.02% participation in the total community. The rest of the community consisted of groups with percentage participation under 5%: Crustacea (4.58%), Turbellaria (4.57%), Hirudinea (0.68%) and Oligochaeta (0.16%).

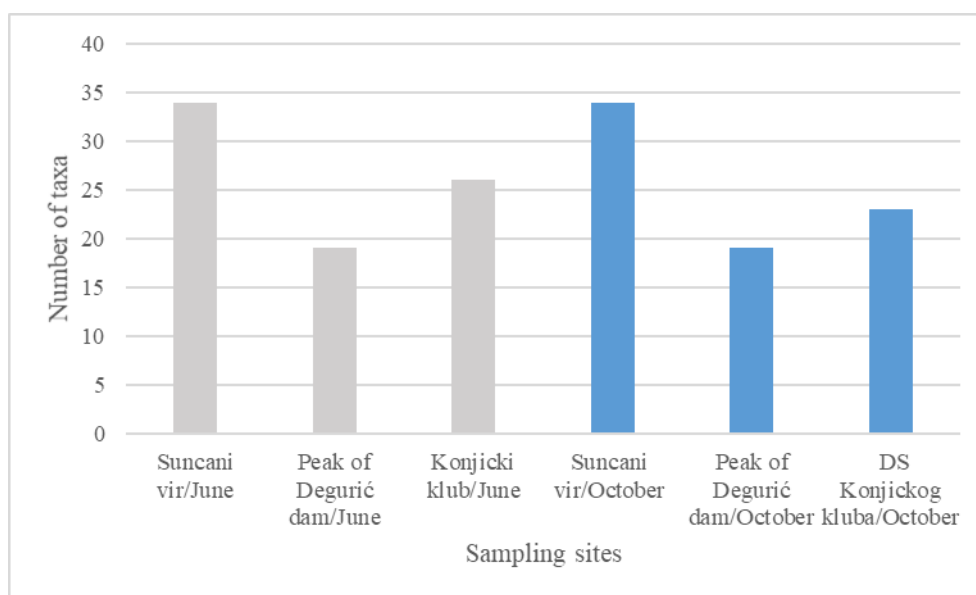


Figure 2 Number of taxa per sampling sites in June and October on the Gradac River

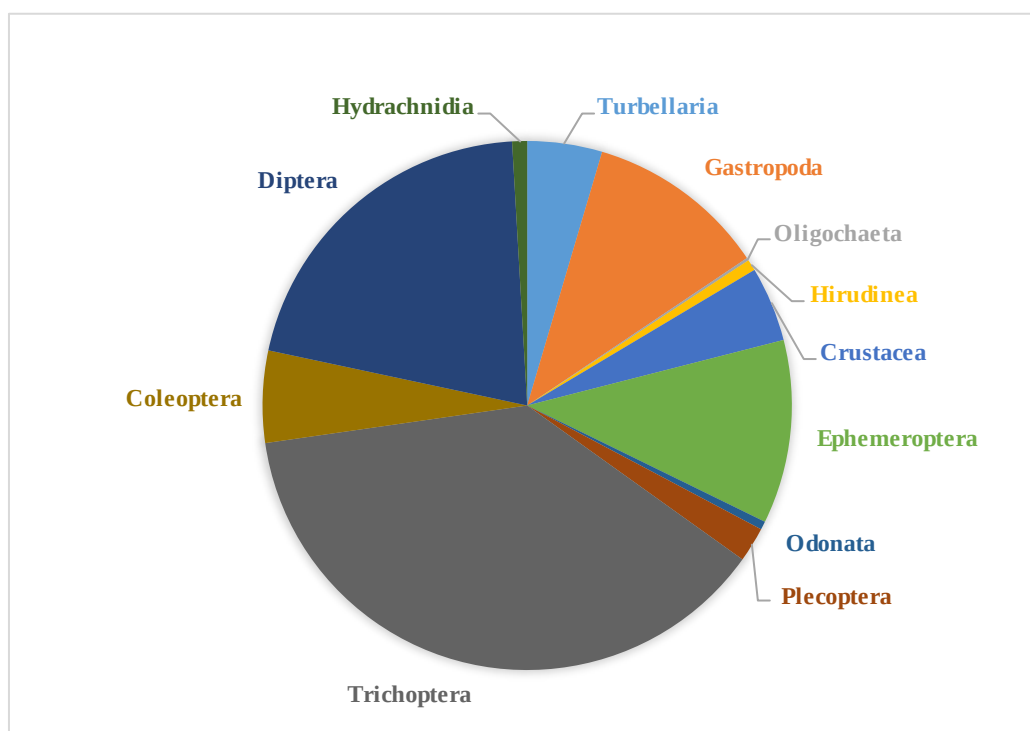


Figure 3 Percentage participation of aquatic macroinvertebrate taxa in the total community of the Gradac River

The most abundant species in the community were *Lepidostoma hirtum* (Fabricius, 1775) and *Sericostoma personatum* (Kirby & Spence, 1826) from the Trichoptera group, as well as *Holandriana holandrii* (C. Pfeiffer, 1828) from the Gastropoda group. All three species were recorded in significant numbers at investigated site Sunčani vir.

The site Konjički klub is hydromorphologically altered section of the Gradac River, affected by flow reduction, sediment deposition, and eutrophication. These conditions directly favor the establishment and persistence of invasive snail *Potamopyrgus antipodarum* (Gray,

1843), a species known for its high ecological tolerance, parthenogenetic reproduction, and ability to rapidly occupy disturbed and degraded habitats [4,5].

During sampling in October, the presence of the species *Agriotypus armatus* Curtis, 1832 was recorded at the sampling site Downstream Konjički klub. *A. armatus* is an aquatic parasitoid hymenopteran known to parasitize the pupae of the caddisfly *Silo pallipes* Fabricius, 1781. This parasitoid is unique because it lays its eggs underwater in the pupal cases of *S. pallipes*, an aquatic insect found in clean, well-oxygenated streams and rivers, such as hilly mountain rivers in Serbia, including the Gradac River. The presence of *A. armatus* indicates good water quality and a healthy aquatic ecosystem, as both the parasitoid and its host require relatively unpolluted habitats to thrive. Thus, *A. armatus* represents a fascinating example of aquatic parasitism within freshwater benthic insect communities in Serbian mountain rivers, highlighting complex biological interactions in these ecologically valuable habitats [6].

Most of the recorded taxa in the research area can be considered sensitive to moderate organic pollution, according to the ecological classification of taxa in relation to saprobic valence according to Moog [7]. A total of 41.28% of β -mesosaprobic organisms and 9.1% of α -mesosaprobic organisms were recorded. Among the identified taxa, 23.72% belong to the oligosaprobic group (taxa tolerant to a low degree of organic pollution), while 5.84% belong to the xenosaprobic group (taxa tolerant to a very low degree of organic pollution). Only a small percentage (0.61%) of the recorded taxa tolerate a high degree of organic pollution (polysaprobic taxa). For a quarter of the examined community (19.43%), there is no data on which to base their classification in terms of organic pollution tolerance.

CONCLUSION

The results show that the Gradac River supports a rich and diverse aquatic macroinvertebrate community, typical for small watercourses in hilly-mountainous watercourses, dominated by sensitive insect groups such as Trichoptera, Diptera, Ephemeroptera, and Coleoptera, with aquatic insects accounting for almost 80% of the total community. The presence of certain pollution-sensitive taxa, a high proportion of β -mesosaprobic and oligosaprobic organisms, and the occurrence of the aquatic parasitoid *Agriotypus armatus* indicate low organic pollution and good ecological status. However, the hydromorphologically altered section at Konjički klub displays signs of disturbance, reflected in habitat degradation and the presence of the invasive snail *Potamopyrgus antipodarum*. Overall, the protected status of the Gradac River has helped preserve its ecological integrity, making it a valuable reference site for biomonitoring and freshwater biodiversity studies in Serbia.

ACKNOWLEDGEMENT

This research was funded by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Contract No. 451-03-33/2026-03/200007) and by the project “Natural Measures for Ecological Gradac” (Project No.: 00147066/00133820/2025/52), implemented within the initiative “EU for Green Agenda in Serbia”, with financial support from Sweden and technical and financial support from the European Union, in partnership with the Ministry of Environmental Protection, and implemented by UNDP in cooperation with Sweden and the European Investment

Bank (EIB), with additional funding from the Governments of Sweden, Switzerland and Serbia. The content of this study is in line with following United Nations (UN) Sustainable Development Goals, under the frame of the UN Agenda for Sustainable Development (*Transforming our World: The 2030 Agenda for Sustainable Development – UN, 2015*): Goals 6, 13, and 15.

REFERENCES

- [1] Rosenberg DM, Resh VH, Kerans BL. Freshwater biomonitoring and benthic macroinvertebrates. 1st ed. Springer New York, NY; 1993.
- [2] Barbour MT, Gerritsen J, Snyder BD, *et al.* Rapid bioassessment protocols for use in streams and wadeable rivers: periphyton, benthic macroinvertebrates and fish. 1999.
- [3] AQEM Consortium. Manual for the application of the AQEM system. A Compr Method to Assess Eur Streams Using Benthic Macroinvertebrates, Dev Purp Water Framew Dir Version 2002;1:2002.
- [4] Marković, V., Gojšina, V., Novaković, B., *et al.* Zootaxa 2021, 5003, 1–64.
- [5] Darden ST, Darden R. Nonindigenous Freshwater Organisms: Vectors, Biology, and Impacts; 2003.
- [6] Bjelanović, K., Živić, I., Petrović, A., *et al.* Knowl. Manag. Aquat. Ecosyst. 2014, 5.
- [7] Moog O. Fauna aquatica austriaca. Wasserwirtschaftskataster, Bundesministerium Für Land-Und Forstwirtschaft, Wien 1995.